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Phylogenetic Analyses of Cretaceous Fossils Related to Chloranthaceae and their Evolutionary Implications

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Abstract Chloranthaceae were one of the first common lines during the early radiation of angiosperms, possibly reflecting adaptation to more open habitats. Phylogenetic analyses clarify the position of Cretaceous mesofossils in molecular trees of Recent taxa. Plants that produced *Asteropollis* pollen, with tepals adnate to a single carpel, are nested in crown group Chloranthaceae with *Hedyosmum*; *Canrightiopsis*, with three stamens and no perianth, is sister to *Sarcandra* and *Chloranthus*; and *Canrightia* is a stem relative that illustrates a still bisexual stage in floral reduction. Plants that produced *Pennipollis* pollen are related to Chloranthaceae and/or *Ceratophyllum* rather than monocots. *Appomattoxia*, which produced *Tucanopollis* pollen, has equivocal affinities, but *Pseudoasterophyllites*, with similar pollen and stems with reduced leaves, may be a link between Chloranthaceae and *Ceratophyllum*. These results imply that flowers became unisexual before losing the perianth, while bisexual flowers in *Canrightiopsis*, *Sarcandra*, and *Chloranthus* are secondarily derived from unisexual flowers.

Keywords Angiosperms · Chloranthaceae · Cretaceous · Paleobotany · Phylogeny · Evolution

Introduction

Of the living angiosperm lines recognized in the Early Cretaceous fossil record, one of the most common but least familiar to botanists is the small family Chloranthaceae, which consists today of four genera and about 75 species of herbs, shrubs, and small trees (Swamy, 1953; Endress, 1987; Todzia, 1993; Eklund et al., 2004). These plants show a curious combination of presumed primitive features, such as monosulcate pollen and vessels with scalariform perforations, and unusually simple flowers (Fig. 1). Some *Ascarina* species have the simplest possible unisexual flowers, which consist of one stamen or one carpel containing one ovule, borne in the axils of bracts in spicate inflorescences, although others have stamen numbers ranging from two to five, lateral bracts associated with the stamen and/or carpel, groups of two carpels, or both a stamen and a carpel (Swamy, 1953; Smith, 1976, 1981; Moore, 1977; Jérémie, 1980).

Hedyosmum differs in having three tepals adnate to the ovary, which is therefore inferior; the female flowers are borne in thyrses of monochasial cymes in the axils of bracts, whereas the male flowers are single stamens borne in spikes with no subtending bracts. Doria et al. (2012) described the tepals as free from the ovary, but this was apparently a result of misinterpretation of anatomical sections (Doyle & Endress, 2014, p. 577). Both *Ascarina* and *Hedyosmum* are inferred to be wind pollinated, although there are few direct observations. *Sarcandra* has the simplest possible bisexual flowers, with one stamen adnate to the back of one carpel, whereas *Chloranthus* has an adnate three-lobed androecium (with only one theca on each lateral lobe) that has been variously interpreted as three fused stamens or one subdivided stamen (Swamy, 1953; Endress, 1987; Doyle et al., 2003); in both genera the flowers are borne on spikes in the axils of bracts and are insect pollinated. *Hedyosmum* is disjunct between East Asia (*H. orientale*) and tropical America; *Ascarina* is concentrated in the southwest Pacific, with one species in Madagascar; and *Sarcandra* and *Chloranthus* occur in East and South Asia. Although the morphological cladistic analyses of Loconte and Stevenson (1991) and Eklund (1999) postulated a basal split into *Hedyosmum* plus *Ascarina* and *Sarcandra* plus *Chloranthus*, all more recent morphological and molecular phylogenetic analyses (Qiu et al., 1999; Zhang & Renner, 2003; Eklund et al., 2004; Zhang et al., 2011, 2015) have indicated that *Hedyosmum* is basal (sister to the rest of the family) and *Ascarina* is sister to *Sarcandra* and *Chloranthus* (Fig. 1).
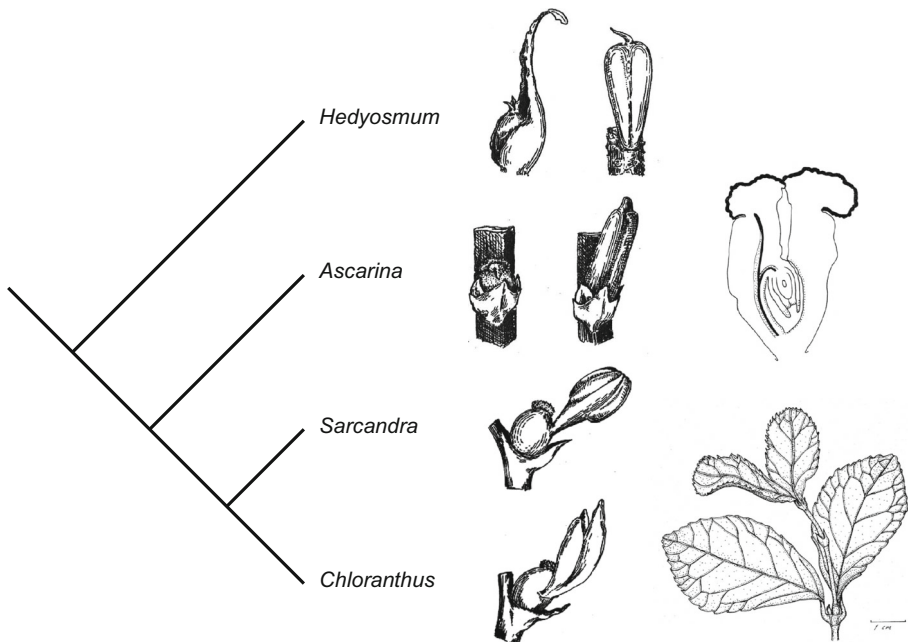
Fig. 1 Relationships of the four extant genera of Chloranthaceae based on morphological and molecular phylogenetic analyses. Drawings of flowers of *Hedyosmum orientale*, *Ascarina diffusa*, *Sarcandra glabra*, and *Chloranthus henryi* from Swamy (1953); longitudinal section of carpel of *Ascarina lucida*, showing ventral vascular bundle and extent of the stigma with thick black lines and the single pendent orthotropous ovule, from Endress (1987); drawing of vegetative branch of *Chloranthus spicatus* from Eklund et al. (2004)

The idea that Chloranthaceae were an important early angiosperm group dates back to Couper (1958), who described *Clavatipollenites hughesii* from the Barremian of England and compared it with pollen of *Ascarina lucida* in New Zealand. These grains are monosulcate and have reticulate-columellar exine structure; Couper and other early authors (e.g., Brenner, 1963; Doyle, 1969) described the columellae as varying from free to fused at their heads to form a tectal reticulum, but SEM studies showed that they are typically reticulate (Doyle et al., 1975; Hughes et al., 1979). Pollen of the general reticulate monosulcate type makes up most of the angiosperm record until the rise of tricolpate pollen (representing early eudicots) in the Albian. SEM studies by Hughes et al. (1979) and Hughes (1994) showed that pollen from the type sample of *C. hughesii* consists of several distinct types, as might be suspected from close examination of light micrographs of the three grains figured by Couper (1958, pl. 31, figs. 19–22), which vary in shape, coarseness of the reticulum, and exine thickness. Walker and Walker (1984) showed that similarities between *Ascarina* and one Aptian pollen type identified as *C. hughesii*, from the Potomac Group of Maryland, extend to the SEM and TEM level, such as the presence of a thick nexine made up of foot layer (plus laminated endexine under the sulcus), supratectal spinules, and a sculptured sulcus membrane. As discussed further below, these similarities do not necessarily imply that *Clavatipollenites* was more closely related to *Ascarina* than the other genera, since they probably represent ancestral states for the whole family.

There is additional evidence for the early presence of Chloranthaceae in the leaf and floral records. Upchurch (1984) showed that early Albian leaves from the Potomac Group of Virginia have chloranthoid marginal teeth (Hickey & Wolfe, 1975) and cuticular features like those of Chloranthaceae (Doyle & Upchurch, 2014), and Upchurch and Dilcher (1990) described additional leaves with chloranthaceous features from the latest Albian of the Dakota Formation of Nebraska. One Potomac specimen shows a stem with apparently opposite leaf attachment, another characteristic of Chloranthaceae (Doyle & Upchurch, 2014). Most significant because of the number of systematically informative characters are fossil flowers and fruits in the several millimeter size range (mesofossils) with in situ pollen in the stamens or adhering to the carpels, mostly from Cretaceous sediments of the eastern USA, Sweden, and Portugal (Friis et al., 1986, 1994b, 1999, 2011, 2015; Herendeen et al., 1993; Eklund et al., 1997; Friis & Pedersen, 2011). Some of these fossils are similar to the living genera, but others show quite different combinations of characters and thus have potential to provide novel insights on the origin and evolution of the family.

Partly because of their abundance in the early angiosperm fossil record, there was formerly much speculation that Chloranthaceae might provide an alternative model for the ancestral flower (Meeuse, 1972; Burger, 1977; Leroy, 1983; Endress, 1986), a view supported by some morphological cladistic analyses (Taylor & Hickey, 1992; Nixon et al., 1994). However, this conflicts with molecular analyses of combined sequences from multiple genes (beginning with Mathews & Donoghue, 1999; Parkinson et al., 1999; Qiu et al., 1999; Soltis et al., 1999; reviewed in Soltis et al., 2005), which identified *Amborella*, Nymphaeales, and Austrobaileyales (the "ANITA lines") as the first three branches below the vast bulk (ca. 99.9% of species) of angiosperms, named *Mesangiospermae* by Cantino et al. (2007). Chloranthaceae are one of five clades making up mesangiosperms, along with eudicots, magnoliids (in a restricted mono-phyletic sense, including Magnoliales, Laurales, Canellales, and Piperales), monocots,

and the rootless aquatic genus *Ceratophyllum*. Since most ANITA groups (except the highly reduced Hydatellaceae in Nymphaeales: Saarela et al., 2007) have multi-parted flowers, it is most parsimonious to assume that the flowers of Chloranthaceae are reduced rather than primitively simple (Endress & Doyle, 2009; Doyle & Endress, 2011).

Although assignment of Chloranthaceae to the mesangiosperms is strongly supported by molecular data, their position among the five clades is not resolved. In trees based on nearly complete chloroplast genomes (Jansen et al., 2007; Moore et al., 2007), Chloranthaceae are sister to magnoliids and *Ceratophyllum* is sister to eudicots. Of the several arrangements found in other studies, the most intriguing are those in which Chloranthaceae and *Ceratophyllum* form a clade. In an analysis of three genes and morphology (Doyle & Endress, 2000), which did not include *Ceratophyllum*, Chloranthaceae were sister to all other mesangiosperms, based in part on their retention of ascidiate carpels like those of the ANITA lines. When Endress and Doyle (2009) added *Ceratophyllum* to a revised version of this morphological data set, with other taxa constrained to an arrangement based mainly on Doyle and Endress (2000), *Ceratophyllum* and Chloranthaceae formed a clade supported by five synapomorphies: sessile flowers, one stamen, embedded pollen sacs, one carpel, and orthotropous ovule. Interestingly, this clade was anticipated by Cordemoy (1863), who included *Ceratophyllum* (and *Platanus*) in his tribe Chloranthacées. Linking *Ceratophyllum* with eudicots was nine steps less parsimonious. This result would imply that *Ceratophyllum* was derived from an ancestor of Chloranthaceae with reduced flowers that moved into an aquatic environment. A Chloranthaceae-*Ceratophyllum* clade 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; Sun et al., 2015); chloroplast genes from the inverted repeat region, thought to be more reliable in detecting ancient splits because of their many informative sites but low rates of substitution (Moore et al., 2011); and nuclear genes (Zhang et al., 2012; Zeng et al., 2014; Sun et al., 2015). These studies varied as to the sister group of the Chloranthaceae-*Ceratophyllum* clade: eudicots (Antonov et al., 2000; Moore et al., 2011; Zhang et al., 2012; Zeng et al., 2014), magnoliids (Sun et al., 2015, nuclear genes), monocots (Sun et al., 2015, mitochondrial genes), magnoliids and monocots (Duvall et al., 2006, 2008), or all other mesangiosperms (Qiu et al., 2010).

It should be noted that one of the characters uniting *Ceratophyllum* and Chloranthaceae, one stamen per flower, depends on the assumption that the multistaminate male structures of *Ceratophyllum* are spikes of unistaminate flowers that lack subtending bracts, rather than flowers with numerous stamens. The flower interpretation was supported by Strasburger (1902), Endress (1994), and more recently Iwamoto et al. (2003), but rejected by Endress (2004) and Endress and Doyle (2009) in favor of the inflorescence interpretation. Arguments for the latter were the fact that the stamens have highly variable phyllotaxis and show a distinct acropetal polarity in development, as is typical of inflorescences, rather than developing nearly simultaneously, as in normal multistaminate flowers. It may be noted that the same two hypotheses have been proposed for *Hedyosmum*, where Leroy (1983) argued that the male structures are multistaminate flowers. This interpretation was rejected by Endress (1987) on the grounds that male flowers of some *Ascarina* species are also unistaminate and are always arranged in spikes. The only difference is that in *Ascarina*

each unistaminate unit has a subtending bract, which clearly marks it as a flower, whereas in *Hedyosmum* a subtending bract is not present. However, although in most species the top of the cuneate stamen is broad and thick, functioning for protection in bud, in some species (especially *H. orientale*) the top of the anther forms a pointed tip curved toward the apex of the inflorescence. This tip has normally been interpreted as an extension of the connective, but the possibility that it is a reduced bract amalgamated with the stamen should be investigated.

The chloroplast genome tree (Jansen et al., 2007; Moore et al., 2007) has been widely accepted, but a reason for caution in accepting its results concerning Chloranthaceae is the fact that only one member of the family (*Chloranthus*) was sampled, which raises the possibility that its position was affected by long branch attraction. As discussed below, inclusion of *Hedyosmum* would reduce the time represented by this branch by at least 111 Ma. By contrast, the analysis of the chloroplast inverted repeat (Moore et al., 2011), which linked Chloranthaceae with *Ceratophyllum*, included *Hedyosmum* and *Sarcandra* as well as *Chloranthus*. Further study is needed to determine whether the different results of Moore et al. (2007) and (2011) are due to taxon sampling or biases in one or the other set of genes.

In this article we review current fossil evidence on the early evolution of Chloranthaceae, focusing on phylogenetic analyses of Doyle and Endress (2014) and more recent authors, and exploring the general significance of Chloranthaceae in the Cretaceous angiosperm radiation. Most of the basic data are from descriptions of mesofossils by Friis, Crane, Pedersen, and collaborators (reviewed in Friis et al., 2011). These studies are exemplary in their meticulous and consistent morphological observations, which make it unusually easy to evaluate what is and is not known about characters of the fossils. Our analyses have used a molecular scaffold approach (Springer et al., 2001; Hermsen & Hendricks, 2008), which involves parsimony analysis of a morphological matrix of living and fossil angiosperms with the arrangement of living taxa fixed by a backbone constraint tree based on molecular data. To deal with the uncertainties on the arrangement of the five mesangiosperm clades, we used the two trees just discussed – J/M based on analyses of chloroplast genomes by Jansen et al. (2007) and Moore et al. (2007), D&E based on Doyle and Endress (2000) and Endress and Doyle (2009) – on the view that these cover the range of currently viable alternatives. This approach does not address the possibility that addition of fossils might affect inferred relationships among living taxa; it only asks what is the best position of a fossil if the backbone tree is correct. One justification for this approach is the fact that in most cases of conflict between the morphological and molecular analyses of Doyle and Endress (2000), their combined analysis agreed with the molecular result. In addition, progress in molecular systematics gives increased confidence that molecular results are accurate. In most cases, analyses of sequences of new genes from all three plant genomes have continued to give congruent results, and support statistics for clades (bootstrap values, posterior probabilities) have increased as sequences are combined. Both continued congruence and increasing support suggest that fossils are unlikely to overturn most molecular results. Finally, unconstrained analyses of living taxa using our morphological data set (Doyle & Endress, 2014) retrieved many of the clades found with molecular data and generally gave relationships of fossils to living groups that are consistent with analyses

using backbone constraint trees. However, these are all points that can and should be tested in the future.

In most cases our earlier analyses of Early Cretaceous fossils confirmed suggestions of the original authors on their systematic position. For example, Doyle and Endress (2010) found that *Sapindopsis* (Crane et al., 1993) and *Spanomera* (Drinnan et al., 1991) belong near the base of the eudicots, in Proteales and Buxales, respectively, whereas *Virginianthus* (Friis et al., 1994a) and *Mauldinia* (Drinnan et al., 1990) are near Calycanthaceae and Lauraceae plus Hernandiaceae, respectively, in the magnoliid order Laurales, and Doyle and Endress (2014) confirmed that *Monetianthus* (Friis et al., 2009) is nested within Nymphaeales. In analyses of fossils related to the ANITA lines, magnoliids, and eudicots, the choice of backbone tree had little or no effect on the results, presumably because the related modern groups are nested well within major clades of mesangiosperms or the ANITA lines. However, it did have an effect on placement of most of the fossils discussed here, since one of the main differences between the two trees is the position of Chloranthaceae, and assumptions concerning the closest living relatives of the family can have a substantial effect on the most parsimonious position of potentially related fossils. Also, we often obtained different results when we added several fossils to the backbone trees rather than one at a time. In some cases we discuss the use of fossils for age constraints (calibrations) in molecular dating analyses; some earlier dates need revision in light of advances in understanding of the stratigraphy of the fossil-bearing deposits (Heimhofer et al., 2007; Doyle & Endress, 2014; Massoni et al., 2015; Tanrikulu et al., 2017). Statements on the relative parsimony of or character support for arrangements not discussed in previous publications are based on analysis of the relevant data sets with MacClade (Maddison & Maddison, 2003). It is our hope that this discussion will be of general interest in illustrating the problems and potentials of integrating fossils into phylogenies.

In this paper we consider two taxa described since Doyle and Endress (2014): *Canrightiopsis*, based on flowers from the Albian of Portugal (Friis et al., 2015), and *Pseudoasterophyllites*, known as whole plants with reproductive structures from the Cenomanian of Bohemia (Kvaček et al., 2016). We have analyzed *Canrightiopsis* using the data set of Kvaček et al. (2016), which incorporated a new potential synapomorphy of *Pseudoasterophyllites* and *Ceratophyllum* and several minor changes in scoring designed to correct inconsistences in treatment of characters as applicable or inapplicable. We document decisions made in scoring *Canrightiopsis* and present the data matrix in the Appendix.

Fossil Taxa

Couperites

A major advance in angiosperm paleobotany was the description by Pedersen et al. (1991) of carpels (in the fruit stage) from the early Cenomanian of Maryland with pollen of the *Clavatipollenites* type on the stigma, named *Couperites*. This was the first direct evidence that such fossil columellar monosulcate pollen was angiospermous, which had been questioned by some early workers (e.g., Brenner, 1963). It must be noted that the pollen of *Couperites* differs from the type identified as *C. hughesii* that

was studied with SEM and TEM and compared with *Ascarina* (Doyle et al., 1975; Walker & Walker, 1984). It is more like pollen described as *Retimonocolpites dividuus* (Cenomanian; Pierce, 1961) and *Clavatipollenites rotundus* (Albian; Kemp, 1968) in being somewhat larger and having a narrower sulcus, with thickened margins, and fewer columellae supporting the reticulum, which tends to detach from the nexine. The fruits resemble those of Chloranthaceae in having a single pendent seed and a barrel-like shape suggestive of ascidiate development, but the seed is anatropous, the most common type in angiosperms, rather than orthotropous as in Chloranthaceae. As Pedersen et al. (1991) recognized, this could mean that *Couperites* was outside the living clade, or crown group; i.e., that it was a stem relative of Chloranthaceae. Furthermore, the seed is unlike Chloranthaceae but like Nymphaeales and Austrobaileyales in having a palisade exotesta (lignified layer derived from the outer epidermis of the outer integument), although it has an inner seed coat layer interpreted as a fibrous exotegmen (from the outer epidermis of the inner integument), as in *Ascarina*.

These ambiguities remained in our analyses (Doyle & Endress 2014; Fig. 2a). With the J/M tree, where *Ceratophyllum* is sister to eudicots, well removed from Chloranthaceae, the most parsimonious position for *Couperites* is linked with Chloranthaceae, supported by its thick nexine, but attached below the crown group, because of its anatropous ovule. Positions within Chloranthaceae are at least one step less parsimonious, since they require an extra step in ovule curvature (either a reversal to anatropous or two origins of orthotropous). However, with the D&E tree, where *Ceratophyllum* is sister to Chloranthaceae, *Couperites* has four equally parsimonious positions: one sister to Chloranthaceae and *Ceratophyllum*, two nested in Chloranthaceae, and one attached to the stem lineage of mesangiosperms as a whole. Many other positions are only one step less parsimonious, notably among the ANITA lines with a palisade exotesta (e.g., sister to *Trimenia*, which has similar carpels with one pendent anatropous ovule). Until evidence is found on organization of the flowers (e.g., whether the carpels represent whole female flowers or were from a flower with many parts, as in *Trimenia*), it may remain unclear whether *Couperites* is related to Chloranthaceae.

These results do not necessarily apply to all dispersed pollen that has been called *Clavatipollenites*, since as already noted such pollen is diverse at the SEM level and may well be heterogeneous. To clarify this situation, we treated the pollen of *Couperites* as a separate taxon in the data set of Kvaček et al. (2016) and analyzed its position on the D&E tree (Fig. 2b). All its most parsimonious positions are related to Chloranthaceae: on the stem lineage of Chloranthaceae and *Ceratophyllum*, with *Ceratophyllum*, and at all positions in Chloranthaceae except nested within the clade consisting of *Sarcandra* and *Chloranthus*, which is united by loss of supratectal spinules. Because of its thick nexine, all positions outside the Chloranthaceae-*Ceratophyllum* line are at least one step less parsimonious. However, if nexine characters are scored as unknown (as would be necessary for most pollen studied only with SEM), it becomes equally parsimonious to attach this pollen type to all branches between the divergence of Nymphaeales and the clade consisting of magnoliids, monocots, and eudicots, except nested in the *Schisandra-Illicium* and *Sarcandra-Chloranthus* clades, and with the monocot *Aponogeton*. Whether or not *Couperites* is related to Chloranthaceae, it is of no
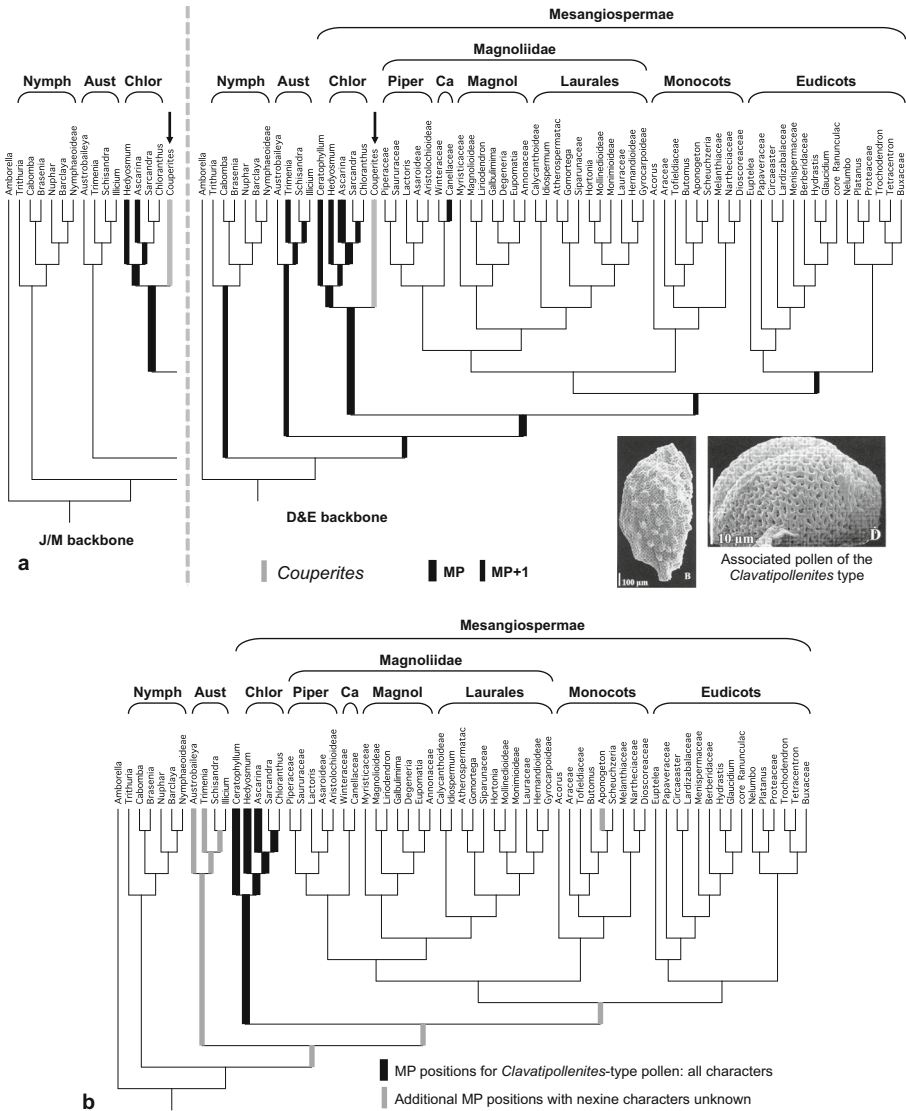
Fig. 2 a Relationships of *Couperites* obtained after addition to the J/M and D&E backbone trees (modified from Doyle & Endress, 2014). Thick lines indicate most parsimonious (MP) and one step less parsimonious (MP+1) positions for *Couperites* (for MP+2 positions in this and subsequent figures, see Doyle & Endress, 2014). Unless otherwise stated, illustrations of fossil flowers and pollen in this and subsequent figures are reproduced from Friis et al. (2011), with permission of Cambridge University Press. **b** Most parsimonious positions of the pollen of *Couperites* (which corresponds to the *Clavatipollenites* type of many authors) on the D&E tree when treated as a separate taxon. Thicker black lines: positions found with all pollen characters; thinner gray lines: additional positions when the two nexine characters are treated as unknown. Abbreviations: Nymph = Nymphaeales, Aust = Austrobaileyales, Chlor = Chloranthaceae, Piper = Piperales, Ca = Canellales, Magnol = Magnoliales

value for calibration in molecular dating studies, since it is younger than other fossils that are nested within Chloranthaceae. These include the early Albian

flower *Canrightiopsis* (Friis et al., 2015), which also has pollen of the *Clavatipollenites* type.

Asteropollis Plant

An important group of floral structures from the probable early Albian of Portugal (Friis et al., 1994b, 1999, 2011; Eklund et al., 2004) and middle Albian of Virginia (Puddledock: Friis et al., 2011) is characterized by *Clavatipollenites*-like pollen with a four- or five-branched sulcus. Such pollen is known in the dispersed record as *Asteropollis*, which was first described from the middle Albian of Oklahoma by Hedlund and Norris (1968) and compared with pollen of *Hedyosmum* by Doyle (1969), Muller (1981), and Walker and Walker (1984). One of the Portuguese localities, Torres Vedras, has been considered Barremian or early Aptian (Friis et al., 2010, 2011), but it is almost surely post-Barremian and may be as young as earliest Albian (Doyle & Endress, 2014; Tanrikulu et al., 2017). Dispersed *Asteropollis* pollen occurs widely in Albian sediments (Eklund et al., 2004); as noted by Friis et al. (2011), it has also been reported from the Barremian-Aptian, but many such reports are poorly dated, and most if not all well-dated pre-Albian grains have either a sulcus with three branches (trichotomosulcate) or irregular apertures, which suggests that they may or may not be related to typical *Asteropollis* (Doyle & Endress, 2014). To our knowledge the only Recent group with a four- or five-branched sulcus is *Hedyosmum*, but trichotomosulcate pollen is more widespread; for example, it occurs as a variant in the predominantly monosulcate pollen of *Ascarina* (Eklund et al., 2004). As in *Hedyosmum*, the female flowers consist of one carpel with three adnate tepals and three peculiar "windows" on the sides, and the male structures appear to be spikes of flowers that consist of one stamen with no subtending bract. These spikes differ from those of living *Hedyosmum* mainly in having more stamens per whorl and being nearly spherical rather than more or less elongate.

The *Asteropollis* plant was linked with *Hedyosmum* both in the morphological cladistic analysis of Eklund et al. (2004), where it was either sister to *Hedyosmum* or nested among its basal lines (subgenus *Hedyosmum* of Todzia, 1988), and in the analyses of Doyle and Endress (2014), using both backbone trees (Fig. 3). The windows may provide additional support; they were not included as a character by Doyle and Endress (2014) because their presence depends on adnation of the perianth to the gynoecium, which would make the character inapplicable in other living Chloranthaceae and most outgroup taxa. Since *Hedyosmum* is sister to the remaining Chloranthaceae, the *Asteropollis* plant provides a firm minimum age of early Albian (ca. 111–113 Ma: Ogg & Hinnov, 2013) for the basal split in the family; this is somewhat more conservative than the 120 Ma (mid-Aptian) age assumed by Zhang and Renner (2003) based on earlier dates for *Asteropollis*. Although it is quite possible that some earlier trichotomosulcate forms represent more plesiomorphic relatives of *Hedyosmum*, this is not established, and it would be premature to use them as a basis for an older minimum age. It is likely that the real age of crown group Chloranthaceae is considerably older, since, as discussed below, the early Albian fossil *Canrightiopsis* appears to be a stem relative of the *Sarcandra-Chloranthus* clade and therefore provides an early Albian minimum age for the split between this clade and *Ascarina*, one node above the base of the family.
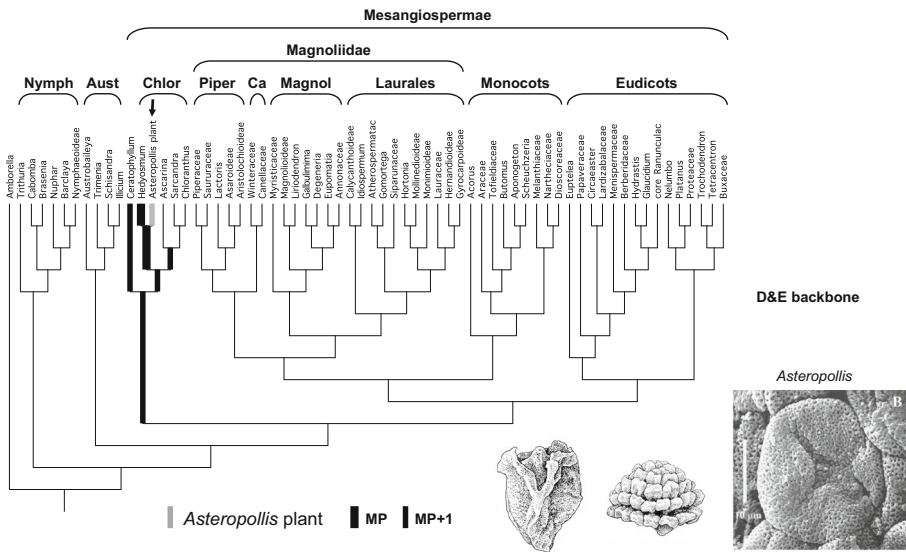
Fig. 3 Relationships of the *Asteropollis* plant obtained after addition to the D&E backbone tree (modified from Doyle & Endress, 2014). SEM photo of *Asteropollis* from Eklund et al. (2004). Conventions and abbreviations as in Fig. 2

Phylogenetic studies of relationships within *Hedyosmum* present a striking example of incongruence between molecular data sets from the nucleus and the plastid (Zhang et al., 2015). In the morphological analysis of Eklund et al. (2004), the basal split was into subgenus *Hedyosmum*, represented by two Antillean species and the Asian species *H. orientale*, and a larger clade including three Antillean-Central American species and all South American species, corresponding to subgenus *Tafalla* of Todzia (1988). A combined analysis of nuclear ITS sequences and two plastid loci by Antonelli and Sanmartín (2011) gave similar relationships, differing most notably in the position of *H. orientale* (nested among Antillean species with morphology, sister to *Tafalla* with the molecular data). However, an analysis of three plastid loci by Zhang et al. (2011) placed *H. orientale* at the base of the genus and nested three species previously assigned to *Tafalla* (*H. arborescens* from the Antilles, *H. gentryi* and *H. neblinae* from South America) in the Antillean-Central American clade that included all other species of subgenus *Hedyosmum*. Zhang et al. (2015) showed that the conflict is due to incongruence between ITS and plastid genes and argued that the ITS tree is closer to the species phylogeny because of its congruence with morphological data, with the conflicting plastid results most likely due to ancient hybridization and chloroplast capture. These uncertainties should not affect the inferred relationship between the *Asteropollis* plant and *Hedyosmum* as a whole.

The Early Cretaceous presence of fossils so similar to modern *Hedyosmum* is of interest in relation to molecular dating analyses, which have given ages for *Hedyosmum* of ca. 29 Ma (Oligocene; Zhang & Renner, 2003), 36–43 Ma (Eocene; Antonelli & Sanmartín, 2011), or 30–50 Ma (Oligocene or Eocene; Zhang et al., 2011). This is not necessarily a conflict, since the molecular dates are crown group ages, whereas the analysis of Eklund et al. (2004) indicated that the fossils could be either near-basal

crown group members or stem relatives of *Hedyosmum*; in the latter case, the crown group could be much younger. In fact, Eklund et al. (2004) took the crown group age of Zhang and Renner (2003) as indirect support for a stem position. As possible morphological support, Eklund et al. (2004) noted that the *Asteropollis* grain in their fig. 3 had verrucate sculpture and a distinct margin, whereas modern *Hedyosmum* has a less distinct sulcus with a "fragmented" reticulum. However, other *Asteropollis* grains have a sulcus of the latter type (Friis et al., 1999, type J4, fig. 105). Two synapomorphies of extant *Hedyosmum* are eucamptodromous leaf venation and stephanocytic stomata (Eklund et al., 2004); association of the *Asteropollis* plant with leaves having the corresponding ancestral states (brochidodromous or semicraspedodromous venation, paracytic stomata) would favor a position on the stem lineage. A Tertiary radiation of the crown group would be consistent with the appearance and expansion of presumed *Hedyosmum* pollen in the Miocene of the Amazon Basin (Martínez et al., 2013). Whether or not molecular age estimates for crown group *Hedyosmum* are accurate, the *Asteropollis* plant provides a remarkable example of morphological stasis since the Early Cretaceous.

Chloranthistemon and *Canrightiopsis*

Flowers with tripartite androecia approaching those of living *Chloranthus* have been described from later in the Cretaceous of Sweden (Crane et al., 1989; Eklund et al., 1997) and New Jersey (Herendeen et al., 1993) as species of the fossil-genus *Chloranthistemon*. The Swedish species (*C. alatus*, *C. endressii*) are known as spikes of bisexual flowers with the androecium attached to the back of a carpel, but the New Jersey species (*C. crossmanensis*) is known only as isolated androecia. *C. crossmanensis* is from a locality that was thought to be late Turonian but may be younger, probably not as young as Santonian (as argued by Clarke et al., 2011), but just as likely Coniacian as late Turonian (Massoni et al., 2015). The Swedish species are younger, namely late Santonian or early Campanian (Eklund et al., 1997; Friis et al., 2011). All three species have one pair of microsporangia on each lateral androecium lobe and two pairs on the central lobe, as in *Chloranthus* (except *C. japonicus*, in which the central lobe is sterile). *C. crossmanensis* is like living species of *Chloranthus* (except *C. erectus*, which is polyporate) in having polycolpoidate pollen (with ca. six short equatorial apertures) and basally fused androecium lobes. By contrast, *C. alatus* has a unique pollen type with a sulcus and a second furrow perpendicular to it on the opposite side, and the three lobes of the androecium are free at the base. *C. endressii* has spiraperturate pollen, with a furrow running around the grain like the seam of a tennis ball. The three lobes of the androecium are connected at one end, which was originally assumed to be basal (Crane et al., 1989) but was reinterpreted by Eklund et al. (1997) as apical, based on immature flowers with the androecium attached to the carpel; if this interpretation is correct, the apical connection was presumably a result of postgenital fusion. The fact that the three androecial lobes in *C. alatus* and *C. endressii* are attached separately to the gynoecium has been considered evidence that the tripartite androecium in *Chloranthus* originated by fusion of three stamens (Eklund et al., 1997; Doyle et al., 2003; Zhang et al., 2011).

The *Chloranthistemon* species were not analyzed by Doyle and Endress (2014), who restricted their attention to Early Cretaceous and Cenomanian fossils, but they were

included in the morphological cladistic study of Eklund et al. (2004). This analysis (Fig. 4) placed the three fossils along the stem lineage of *Chloranthus*, with *C. alatus* most basal and *C. crossmanensis* nearest to the crown group (which is united by papillose pollen sacs). Since *C. crossmanensis* is the oldest of the three fossils, these results imply "ghost lineages" of several million years leading to the two Swedish species, which is not at all implausible considering that all three species are known from single localities.

These results conflict with molecular dating analyses, which indicate a younger divergence of the *Chloranthus* line. These studies assumed an age of 90 Ma (late Turonian; Zhang & Renner, 2003; Zhang et al., 2011) for *C. crossmanensis*, but it could be as young as 86.3 Ma (end Coniacian; Ogg & Hinnov, 2013). Calibrating the tree with a date of 112 Ma for the crown node of Chloranthaceae, based on *Asteropollis*, Zhang et al. (2011) concluded that *Sarcandra* and *Chloranthus* did not diverge until 62–65 Ma (early Paleocene), and they suggested that the *Chloranthistemon* species therefore represent stem relatives of the *Sarcandra-Chloranthus* clade rather than of *Chloranthus* alone. They argued that two morphological characters, stamen number and pollen apertures, might be more consistent with this lower position.

To evaluate these arguments, we used MacClade (Maddison & Maddison, 2003) and the data set of Eklund et al. (2004) to examine the behavior of these two characters on alternative trees. Eklund et al. (2004) scored stamen number (character 110) in
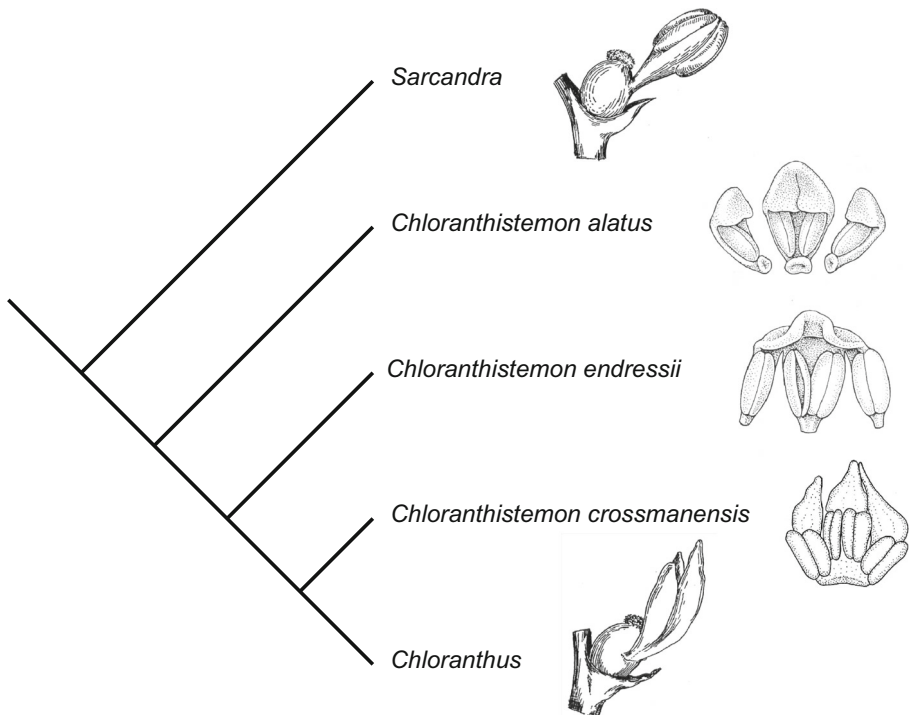

Fig. 4 Most parsimonious relationships of the three species of *Chloranthistemon* found in the morphological phylogenetic analysis of Eklund et al. (2004). Drawings of flowers of *Sarcandra glabra* and *Chloranthus henryi* from Swamy (1953); drawings of fossil androecia courtesy of Helena Eklund

Chloranthistemon and *Chloranthus* as unknown, to avoid assuming whether the tripartite androecium arose by fusion of three stamens or by splitting of one. As noted, Zhang et al. (2011) took the free bases of the androecial lobes in *C. alatus* and *C. endressii* as evidence that they represent three separate stamens, and they argued that this condition went back to the common ancestor of *Sarcandra* and *Chloranthus*. However, even if *Chloranthistemon* and *Chloranthus* are rescored as having more than one stamen, this character undergoes the same number of steps across the tree as before (three), whether the three *Chloranthistemon* species are placed between *Sarcandra* and *Chloranthus* or below their common ancestor. In the aperture character (125), Zhang et al. (2011) incorrectly stated that the spiraperturate pollen of *C. endressii* had been scored as monosulcate (it was actually treated as a separate state), but they correctly noted that placement of the *Chloranthistemon* species below *Sarcandra* and *Chloranthus* would imply that polycolpate pollen was ancestral for the two living genera. In any case, this character undergoes the same total number of steps (six) with the *Chloranthistemon* species in either position. Thus these two characters have no bearing on the relative parsimony of the two positions of *Chloranthistemon*.

Considering the whole data set of Eklund et al. (2004), placing the *Chloranthistemon* species on the stem lineage of *Sarcandra* and *Chloranthus* is four steps less parsimonious than placing them on the stem of *Chloranthus*. For perspective, only two steps are added if the *Asteropollis* plant is moved to the stem of the whole family. If a four-step parsimony debt is accepted in moving *Chloranthistemon* to a morphologically less favored position, one may ask why the *Asteropollis* plant is considered a reliable minimum age constraint for the family. Other possibilities are that divergence of the *Hedyosmum* line occurred long before origin of the distinctive *Asteropollis* pollen type, or that the dating methods used did not deal successfully with rate heterogeneity. In any case, if the trilobed androecium is homologous in *C. crossmanensis* and *Chloranthus*, its persistence since the Turonian-Coniacian is another remarkable example of morphological stasis.

These dating conflicts have parallels in another early angiosperm group, Nymphaeales (Nixon, 2008; Doyle & Endress, 2014). Yoo et al. (2005) dated the crown group of Nymphaeales (then assumed to consist of only Cabombaceae and Nymphaeaceae) as 44.6 ± 7.9 Ma (Eocene), which led them to suggest that the early Albian flower *Monetianthus*, thought by Friis et al. (2001, 2009) to represent the family Nymphaeaceae, was instead a stem relative of Nymphaeales. However, analyses by Friis et al. (2009) and Doyle and Endress (2014) confirmed that *Monetianthus* is nested within Nymphaeaceae, and in terms of the Doyle and Endress data set it is three steps less parsimonious to attach it to the stem lineage of Nymphaeales.

Other less well understood fossils may be relevant to these problems. Friis et al. (1986) and Crane et al. (1989) described a group of three stamens from the late Albian of the Potomac Group as a tripartite androecium with two pairs of microsporangia on all three parts. In the analysis of Eklund et al. (2004) this fossil was sister to *Sarcandra*. However, its pollen appears to be tricolpate, suggesting that it may be a fragment of a eudicot flower; the fact that the sites of anther dehiscence appear not to be lateral but rather in the median plane also suggests it was part of a larger flower. Other two- and three-lobed androecia from Albian-Cenomanian sediments were briefly reported by Friis et al. (2000b, 2011) and Hartkopf-Fröder et al. (2012). These were interpreted as differing from *Chloranthus* and *Chloranthistemon* in bearing the two microsporangia

of the lateral lobes on the side facing the central lobe rather than on the outer side. Friis et al. (2011) suggested that this might mean the lateral lobes were independently reduced from tetrasporangiate stamens. However, the position of the microsporangia in these fossils is not entirely clear. A problem is that the androecium of *Chloranthus* is highly three-dimensional, with the flanks curved inward. If this is also the case in the fossils, the position of the microsporangia might easily be misinterpreted. Dispersed pollen with four or five colpoid apertures, first described from the middle Albian of Oklahoma by Hedlund and Norris (1968) as *Stephanocolpites fredericksburgensis* and transferred to the new genus *Hammenia* by Ward (1986), was compared with *Chloranthus* using LM and EM by Walker and Walker (1984). However, it is more like *Ascarina*, *Hedyosmum*, and *Asteropollis* in having supratectal spinules, whereas *Chloranthus* has smooth muri (except for *C. japonicus*, which is nested within the genus and presumably derived in this character) (Eklund et al., 2004; Zhang et al., 2011). If any of these Albian-Cenomanian fossils represent stem relatives of *Chloranthus*, they exacerbate the conflict with molecular dates.

Stronger evidence for origin of the *Sarcandra-Chloranthus* clade comes from *Canrightiopsis*, described by Friis et al. (2015) from the early Albian of Portugal. This is a bisexual flower with no perianth but with three separate stamens attached to the dorsal and lateral sides of a uniovulate carpel and pollen of the *Clavatipollenites* type. *Canrightiopsis* resembles *Canrightia* (Friis & Pedersen, 2011), discussed in the next section, in overall shape, the fact that the stamens are attached halfway up the carpel wall, and orthotropous ovule morphology, but *Canrightia* differs in having a reduced perianth, four stamens, and a syncarpous gynoecium.

The relevance of *Canrightiopsis* for understanding *Sarcandra* and *Chloranthus* was supported by Friis et al. (2015) in an analysis using the Doyle and Endress (2010) data set, which indicated that *Canrightiopsis* was sister to the two extant genera. We obtained the same result in our present analysis (Fig. 5; Appendix). With the D&E backbone tree, the *Canrightiopsis-Sarcandra-Chloranthus* clade is supported by the bisexual flower and distinctive intrusive oil cells in the carpel wall, while the two living genera are united by loss of supratectal spinules and an outer integument thickness of four or more cells. With the J/M backbone, bisexuality is equivocal as a synapomorphy of the three genera, since it may be retained from lower in the tree. Other positions are at least two steps less parsimonious with the D&E backbone, at least one step so with the J/M backbone. The same most parsimonious position is found when *Canrightiopsis* is added to both backbone trees along with other fossils, as discussed further below.

Friis et al. (2015) reported *Canrightiopsis* from Famalicão, Catefica, Chicalhão, and other Portuguese localities that they considered late Aptian or early Albian, but which palynological correlations suggest are somewhat above the base of the early Albian (Doyle & Endress, 2014; Tanrikulu et al., 2017). They did not report it from the older Torres Vedras locality, which they considered Barremian-early Aptian but may be as young as earliest Albian, as discussed under the *Asteropollis* plant. These results imply that *Canrightiopsis* provides a minimum age of latest early Albian (ca. 111 Ma: Ogg & Hinnov, 2013) for the split between *Ascarina* and the *Sarcandra-Chloranthus* clade.

Positional relationships of the stamens in *Canrightiopsis* are consistent with a scenario proposed by Friis et al. (2015), in which the three stamens of *Canrightiopsis* were derived by loss of one stamen of a *Canrightia*-like ancestor, after which the three stamens were fused in *Chloranthus* and the two lateral stamens were lost in *Sarcandra*.
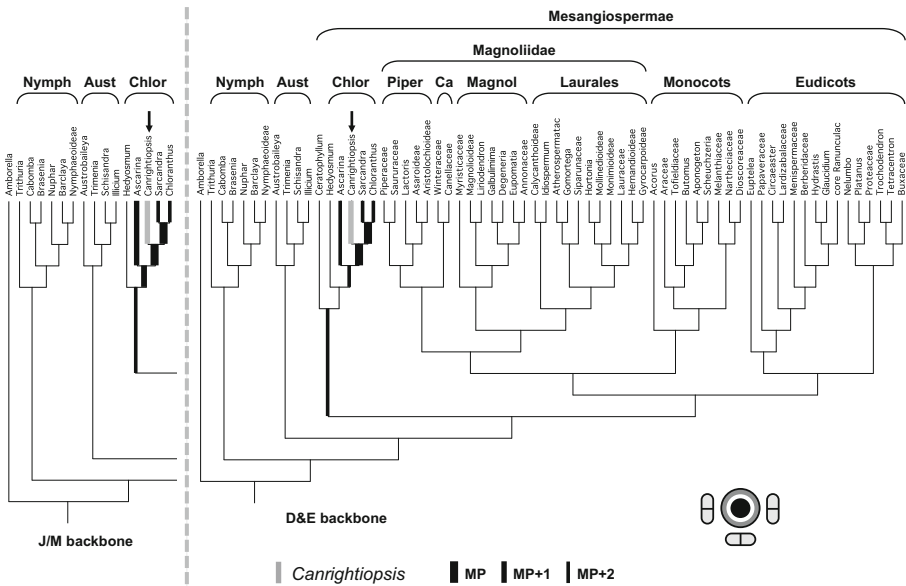
Fig. 5 Relationships of *Canrightiopsis* obtained after addition to the J/M and D&E backbone trees (this study). Conventions and abbreviations as in Fig. 2, but with MP+2 positions for the fossil also shown

With our data set, in which stamen number in *Chloranthus* was scored as unknown because of the problematical morphology of the lobed androecium, the three stamens of *Canrightiopsis* are an autapomorphy and not homologous with the three parts of the androecium of *Chloranthus*. However, if *Chloranthus* is rescored as having more than one stamen, which would be supported by the separate attachment of the androecial lobes in *Chloranthistemon alatus* and *C. endressii*, it is equally parsimonious to assume that its androecium was derived either by lobation of one stamen or by fusion of three stamens homologous with those of *Canrightiopsis*.

Canrightiopsis also has important implications for interpretation of the fossil pollen record. Its pollen is of the type generally assigned to *Clavatipollenites*, with an unbranched, sculptured sulcus and supratectal spinules (nexine characters are unknown). This strengthens the inference (see Fig. 2b) that although this pollen type is restricted to *Ascarina* today, it was previously more widespread and cannot be used as evidence for the *Ascarina* line in the Cretaceous dispersed record.

Canrightia

Flowers from the early Albian of Portugal that Friis and Pedersen (2011) described as *Canrightia* show a level of complexity intermediate between Chloranthaceae and other basal angiosperms and thereby shed light on the origin of the family. They are like female flowers of *Hedyosmum* in having an adnate perianth, which forms a ring with four teeth (presumably reduced tepals), but they are bisexual, with four stamen scars (and in one case an attached stamen) just above the perianth. In contrast to living Chloranthaceae, the gynoecium consists of two to five fused carpels, but the ovules are pendent and orthotropous. The associated pollen, which Friis and Pedersen (2011)

described as of the *Retimonocolpites* type, differs from the *Clavatipollenites* type of *Couperites* and *Canrightiopsis* in lacking supratectal spinules and having a sulcus that extends more than halfway around the grain, approaching the ring furrow of the dispersed pollen genus *Dichastopollenites*. Since Piperales have somewhat similar reduced bisexual flowers, Friis and Pedersen (2011) suggested that *Canrightia* may support a relationship between Chloranthaceae and Piperales, contrary to molecular evidence that Piperales are nested within Magnoliidae (as the sister group of Canellales) and Chloranthaceae are outside that clade.

Phylogenetic analyses of *Canrightia* by Friis and Pedersen (2011) and Doyle and Endress (2014), using different versions of the same data set, gave the same result (Fig. 6): its most parsimonious position was attached to the stem lineage of Chloranthaceae and *Ceratophyllum* with the D&E backbone tree, to the stem of Chloranthaceae alone with the J/M tree. Characters supporting this position clarify the course of floral reduction on the line to Chloranthaceae (and *Ceratophyllum* with the D&E tree). Synapomorphies uniting *Canrightia* with the crown group are sessile flowers, inferior ovary, one perianth whorl, and orthotropous ovule, while the crown group is further united by unisexual flowers, one stamen, and one carpel. Another possible synapomorphy is single-layered endoreticulate endotesta (derived from the inner epidermis of the outer integument), as in *Ascarina*, *Sarcandra*, and *Chloranthus*, but this is equivocal because this layer is unspecialized in *Hedyosmum*; the character is inapplicable in *Ceratophyllum* because it has only one integument. The best position for *Canrightia* in Piperales, sister to Saururaceae and Piperaceae, is two or three steps less parsimonious. The same basal position was found when *Canrightia* was added together with other fossils, as discussed below. Experiments by Doyle and Endress (2014) showed that addition of *Canrightia* had little or no effect on the relative parsimony of a closer relationship between Chloranthaceae and Piperales. Given the
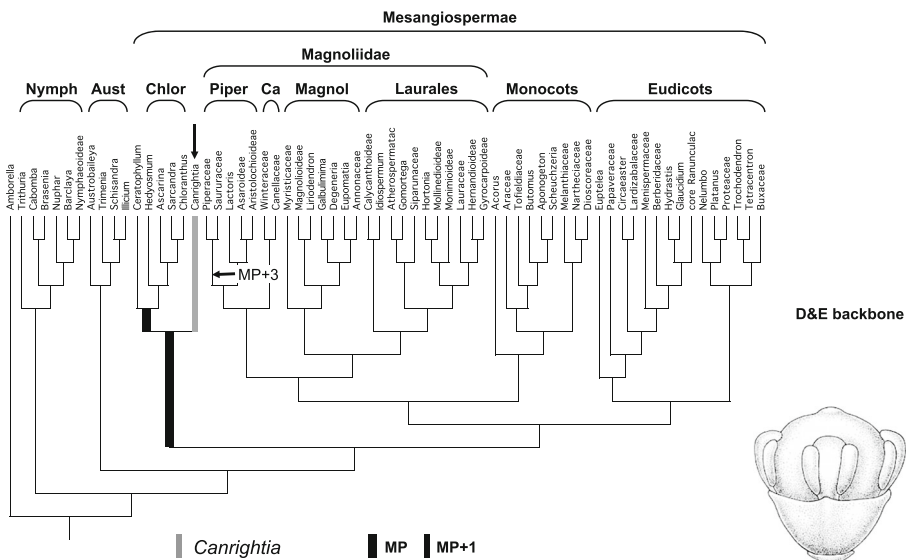

Fig. 6 Relationships of *Canrightia* obtained after addition to the D&E backbone tree (modified from Doyle & Endress, 2014). Conventions and abbreviations as in Fig. 2; no MP+1 positions were found

increasingly strong molecular support for the monophyly of magnoliids, to the exclusion of Chloranthaceae, it is most likely that the similarities between Chloranthaceae and Piperales are the result of parallel trends for floral reduction.

Although *Canrightia* provides a more specific picture of steps in the origin of Chloranthaceae, it should not be taken as a faithful model for their direct ancestor. Its age alone (contemporaneous with the *Asteropollis* plant) implies that it was a persisting side line, and several of its features are probably autapomorphies, such as the tendency for tetramery, extension of the sulcus, and syncarpy.

Zlatkocarpus

Another fossil that appears to be linked to Chloranthaceae but not to any one living genus is *Zlatkocarpus*, from the middle Cenomanian of Bohemia (Kvaček & Eklund, 2003; Kvaček & Friis, 2010), which is known as inflorescences of sessile female flowers and adhering pollen. It is more like *Ascarina* than *Hedyosmum* in having single flowers in the axils of bracts rather than cymes (i.e., spikes rather than thyrses), but more like *Hedyosmum* in having a perianth adnate to the carpel. However, the perianth is more reduced than that of *Hedyosmum*, and the pollen (referred to *Retimonocolpites*) is more like that of *Canrightia* in having a normal unbranched sulcus and lacking the supratectal spinules characteristic of *Hedyosmum*, *Ascarina*, and *Canrightiopsis*. Male structures are not known, and most seed characters are uncertain.

Results of the analyses of *Zlatkocarpus* by Doyle and Endress (2014) differed considerably depending on the backbone tree (Fig. 7a). With the D&E tree, where Chloranthaceae and *Ceratophyllum* form a clade, Doyle and Endress (2014) found that the best position for *Zlatkocarpus* was nested within Chloranthaceae, between *Hedyosmum* and *Ascarina*, based on the fact it has a sessile stigma, like *Ascarina*, *Sarcandra*, and *Chloranthus*, but retains a perianth, which was lost in these three genera. With the J/M tree, where *Ceratophyllum* is well removed from Chloranthaceae, *Zlatkocarpus* can be either attached to the stem lineage of Chloranthaceae or nested in the crown group, sister either to *Hedyosmum* or to the rest of the family.

Inclusion of other fossils appears to favor a more basal position of *Zlatkocarpus*. In our previous study, when we added *Zlatkocarpus* to the D&E tree along with *Canrightia*, the *Asteropollis* plant, and the *Pennipollis* plant, it was attached to the stem lineage of Chloranthaceae and *Ceratophyllum*, immediately above *Canrightia*, in both most parsimonious trees (Doyle & Endress, 2014, fig. 6A, B). In the present study, we obtained the same result when we included *Canrightiopsis* as well (Fig. 7b, c). Apomorphies that place *Zlatkocarpus* closer to living Chloranthaceae are its unisexual flowers and single carpel, but it diverges below the crown group (and the *Pennipollis* plant in Fig. 7b) because its pollen lacks supratectal spinules, which are a synapomorphy of the remaining groups. The smooth muri of *Canrightia* and *Zlatkocarpus* are therefore a symplesiomorphy. When Doyle and Endress (2014, fig. 6C–K) added *Zlatkocarpus*, *Canrightia*, the *Asteropollis* plant, and the *Pennipollis* plant to the J/M backbone, *Zlatkocarpus* was attached to the chloranthaceous stem lineage in five trees but nested in the family in four. However, when *Canrightiopsis* is also included, *Canrightia* and *Zlatkocarpus* are successive basal lines in all six most parsimonious trees (these correspond to Doyle & Endress, 2014, fig. 6C, D, E, J, and K, plus a tree in which *Pennipollis* is linked with *Canrightiopsis*).
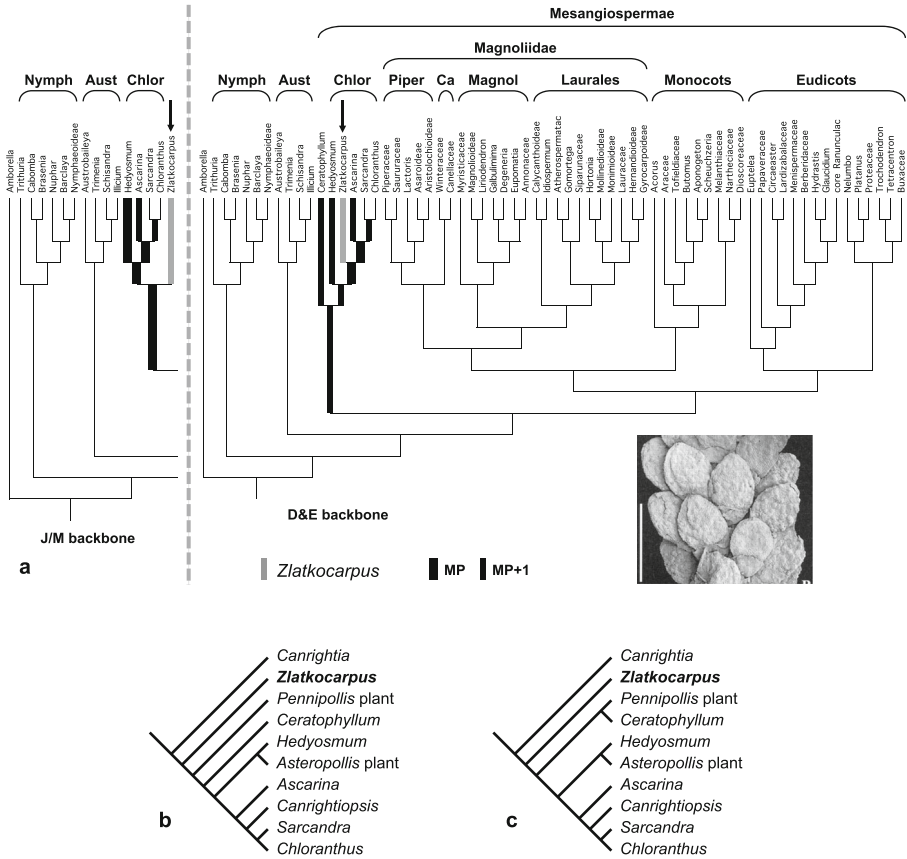
Fig. 7 **a** Relationships of *Zlatkocarpus* obtained after addition to the J/M and D&E backbone trees (modified from Doyle & Endress, 2014). Photo of portion of spike courtesy of Jiří Kvaček. **b**, **c** Most parsimonious arrangements of *Ceratophyllum*, Chloranthaceae, and fossil taxa obtained when *Canrightia*, *Zlatkocarpus*, the *Pennipollis* plant, the *Asteropollis* plant, and *Canrightiopsis* are added to the D&E backbone tree (this study). Conventions and abbreviations as in Fig. 2

Pennipollis Plant

Another major advance in understanding of the early angiosperm record was the association by Friis et al. (2000a) of mesofossils from the early Albian of Portugal with one of the most common Aptian-Albian monosulcate angiosperm pollen types, *Pennipollis*, which is distinctive in having an unusually coarse reticulum that tends to detach from the nexine. Such pollen was first described by Brenner (1963) as two species of *Peromonolites*, a fossil-genus for monolete spores with a perispore, but it was reinterpreted as monosulcate angiosperm pollen by Norris (1967), Doyle (1969), and Singh (1971). Using SEM and TEM, Doyle et al. (1975) and Walker and Walker (1984) showed that it lacked columellae below the reticulum but was otherwise angiosperm-like, with a normal sulcus rather than a proximal monolete scar, supratectal spinules on the reticulum, and a thick nexine made up of foot layer around most of the grain, plus endexine below the sulcus, as in Chloranthaceae and fossil pollen identified

as *Clavatipollenites*. Doyle and Hotton (1991) suggested that it was produced by a relative of Chloranthaceae that had lost its columellae. Friis et al. (2000a) showed that the tectum is underlain by fine granules; this was questioned by Hesse and Zetter (2007), but granules are clearly visible in fig. 4D of Friis et al. (2000a). After Brenner (1963), several authors transferred this pollen to various genera that include columellar species (*Liliacidites*, *Retimonocolpites*, *Brenneripollis*), but Friis et al. (2000a) clarified the situation by placing it in the new genus *Pennipollis*, explicitly restricted to non-columellar pollen. Friis et al. (2000a, 2011) indicated that *Pennipollis* ranges from the Barremian into the Late Cretaceous, but in all cases where its first occurrence is well dated it appears above the Barremian-Aptian boundary, and therefore it is often considered a guide fossil for post-Barremian sediments (Penny, 1988; Doyle, 1992; Hughes, 1994; Heimhofer et al., 2007; Doyle & Endress, 2014).

The carpels associated with *Pennipollis* (*Pennicarpus*) contain one orthotropous seed with no structural layer in the seed coat and an unknown number of integuments. Friis et al. (2000a) interpreted the position of the seed as basal (ascendent) because the micropyle is directed toward the presumed stigmatic end of the carpel, but Doyle and Endress (2014) argued that the off-center position of the chalaza is typical of orthotropous ovules that are apical (pendent). Only isolated carpels are known, so it is not known if the female flowers had any other parts. Of the male structures (*Pennistemon*), the most informative specimen is a short axis with several stamens, which Friis et al. (2000a) interpreted as a fragment of a spike with male flowers that consist of one stamen and have no subtending bract, as in *Hedyosmum* and *Ceratophyllum*.

Friis et al. (2000a, 2011) argued that the *Pennipollis* plant was a monocot, since some Alismatales (including some Araceae) have pollen with a similar loose reticulum and granular infratectal layer, and some have ebracteate male flowers reduced to one stamen. The taxon in Alismatales with the most similar pollen may be *Aponogeton* (Thanikaimoni, 1985), not cited by Friis et al. (2000a, 2011). However, *Aponogeton* has a thin nexine, like many monocots, whereas the thick nexine of *Pennipollis* is more suggestive of Chloranthaceae. A relationship to Araceae was questioned by Wilde et al. (2005) and Hesse and Zetter (2007), who favored a relationship to Chloranthaceae, and Doyle and Endress (2014) argued that although some Araceae have single stamens, these are derived members of the subfamily Aroideae that have very different pollen, with a highly reduced exine, and very different stamen morphology.

The analyses of Doyle et al. (2008) and Doyle and Endress (2014) strongly linked the *Pennipollis* plant with the Chloranthaceae-*Ceratophyllum* clade using the D&E backbone tree and with Chloranthaceae alone using the J/M tree, but they placed it below the crown group because it retains protruding rather than embedded pollen sacs (Fig. 8). With the Doyle and Endress (2014) data set, its best positions in monocots, with *Aponogeton* or *Acorus*, are seven or eight steps worse. With the D&E backbone, it is only one step worse to link the fossil with *Ceratophyllum*. When the *Pennipollis* plant, *Canrightia*, *Zlatkocarpus*, and the *Asteropollis* plant are added to the D&E tree, without (Doyle & Endress, 2014, fig. 6A, B) or with *Canrightiopsis* (Fig. 7b, c), the *Pennipollis* plant attaches either above *Zlatkocarpus* and just below Chloranthaceae and *Ceratophyllum*, or, more remarkably, on the line to *Ceratophyllum*. Both positions are supported by supratectal spinules, which are shared by *Pennipollis*, *Hedyosmum*, *Asteropollis*, *Ascarina*, and *Canrightiopsis*, but not by *Canrightia* and *Zlatkocarpus* (*Ceratophyllum* has no spinules, but its exine is so extremely reduced that all its exine

characters must be treated as unknown). The position with *Ceratophyllum* is supported by a shift from latrorse to introrse or extrorse anthers (in both taxa, the thecae are shifted toward one surface, but whether this is abaxial or adaxial is unknown). With either four or five fossils and the J/M tree, the *Pennipollis* plant is either sister to Chloranthaceae or nested within the crown group (see Doyle & Endress, 2014, fig. 6C–K and discussion of *Zlatkocarpus*); a position with *Ceratophyllum* is four steps worse.

Interestingly, *Pennipollis* pollen is abundant in the organic-rich swamp deposits of the Arundel Clay in the Potomac Group of Maryland, including those at its type locality (United Clay Mine: Brenner, 1963; Doyle et al., 1975), which would be consistent with the hypothesis that it was produced by an aquatic plant related to *Ceratophyllum*.

Appomattoxia, *Tucanopollis*, *Pseudoasterophyllites*, and *Ceratophyllum*

Like the *Pennipollis* plant, the mesofossil genus *Appomattoxia* was not associated with Chloranthaceae when it was first described by Friis et al. (1995) from the middle Albian Puddledock locality in Virginia (for age, see Massoni et al., 2015). It is known as fruits with distinctive hooked hairs that contain one apical orthotropous ovule, as in Chloranthaceae, but the innermost layer of the seed coat around the micropyle has undulate, thickened cell walls recalling the sclerotic endotegmen of Piperaceae and Saururaceae (Piperales). Pollen adhering to the carpels and found in isolated stamens from Portugal (Friis et al., 2006, 2010) has supratectal spinules, a sculptured sulcus, and a thick nexine made up of foot layer, plus endexine under the sulcus, as in *Ascarina*, *Clavatipollenites*, *Hedyosmum*, and *Asteropollis*. However, the tectum is
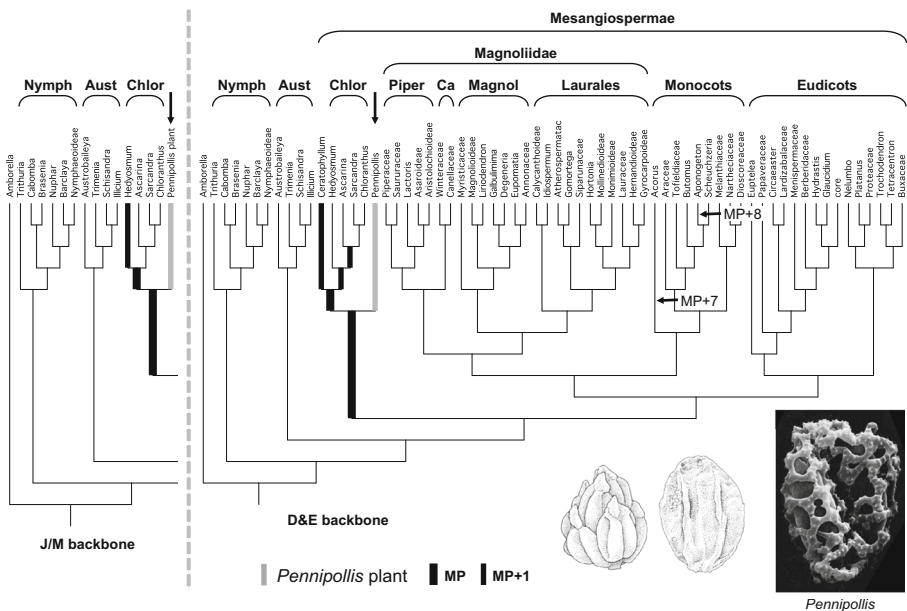

Fig. 8 Relationships of the *Pennipollis* plant obtained after addition to the J/M and D&E backbone trees (modified from Doyle & Endress, 2014). SEM photo of *Pennipollis peroreticulatus* from Doyle et al. (1975). Conventions and abbreviations as in Fig. 2

continuous, as in most Piperales, and a thick nexine occurs in Saururaceae as well as Chloranthaceae (Doyle & Hotton, 1991; Smith & Stockey, 2007).

Dispersed pollen of the *Appomattoxia* type is known as *Tucanopollis* (originally described as *Inaperturopollenites crisopolensis* by Regali et al., 1974), which is often the most abundant angiosperm pollen in the Barremian-early Aptian of Brazil and Africa (Northern Gondwana; Doyle et al., 1977; Regali, 1989). Especially in the late Barremian, many grains in this complex have a round verrucate aperture about half the diameter of the grain. Sedimentological and paleobotanical data indicate that paleoclimates in most of Northern Gondwana were tropical and semiarid (Brenner, 1976, 1996; Doyle et al., 1982; Heimhofer & Hochuli, 2010). However, similar pollen has also been reported from Southern Laurasia, where the climate was cooler (subtropical?) and more humid, including the Barremian of England, as Barremian-*ring* (Hughes, 1994), and the Albian of Hungary, as *Transitoripollis* (Góczán & Juhász, 1984).

When *Tucanopollis* was known only as dispersed pollen, Doyle and Hotton (1991) suggested that it represented relatives of *Clavatipollenites* and Chloranthaceae that were either more primitive or more derived in having a continuous tectum, but they also recognized its similarities to Saururaceae. Friis et al. (1995, and especially 2011) favored a relationship of *Appomattoxia* to Piperales. Our phylogenetic analyses of *Appomattoxia* (Doyle & Endress, 2014) did not support affinities with Piperales, but they gave ambiguous results on its most likely position.

When we added *Appomattoxia* alone to both backbone trees (Fig. 9a), its four most parsimonious positions were near the very base of the angiosperms: with *Amborella*, on the two adjacent branches, or on the stem lineage of Nymphaeales. Characters that support positions in this part of the tree are the continuous tectum and sessile stigma, as in *Amborella* and Nymphaeales (symplesiomorphies), and the orthotropous ovule, as in *Amborella* (either a symplesiomorphy or a synapomorphy). A pollen character that might link *Appomattoxia* with *Amborella* is a tendency for the tectum to develop low verrucae, which could be homologous with the larger verrucae that are characteristic of *Amborella* and dispersed Hauterivian and Barremian pollen described by Hughes and McDougall (1987) and Hughes (1994) as Hauterivian-*cactisulc* (Doyle, 2001; Hesse, 2001). However, an abundance of ANITA-grade angiosperms in semiarid Northern Gondwana would be surprising ecologically, since *Amborella* and Austrobaileyales are physiologically restricted to wet forest understory habitats today (Feild et al., 2004, 2009). Furthermore, several higher positions for *Appomattoxia* are only one step less parsimonious, including on the stem lineage of Chloranthaceae (J/M) or Chloranthaceae plus *Ceratophyllum* (D&E).

By contrast, when we (Doyle & Endress, 2014) added *Appomattoxia* to the D&E backbone tree along with *Canrightia*, *Zlatkocarpus*, the *Pennipollis* plant, and the *Asteropollis* plant, it attached either to the stem lineage of Nymphaeales (two trees); to the stem lineage of the Chloranthaceae-*Ceratophyllum* clade, between *Zlatkocarpus* and *Ceratophyllum*, with the *Pennipollis* plant nested in Chloranthaceae (Fig. 9b); or to the *Ceratophyllum* line, above the *Pennipollis* plant (Fig. 9c). As with the *Pennipollis* plant, the latter two positions are supported by supratectal spinules. When the *Pennipollis* plant and *Appomattoxia* were both linked with *Ceratophyllum*, the three taxa were united by introrse or extrorse (rather than latrorse) anthers and intermediate infratectal structure (counted as a step toward granular structure in *Pennipollis* because
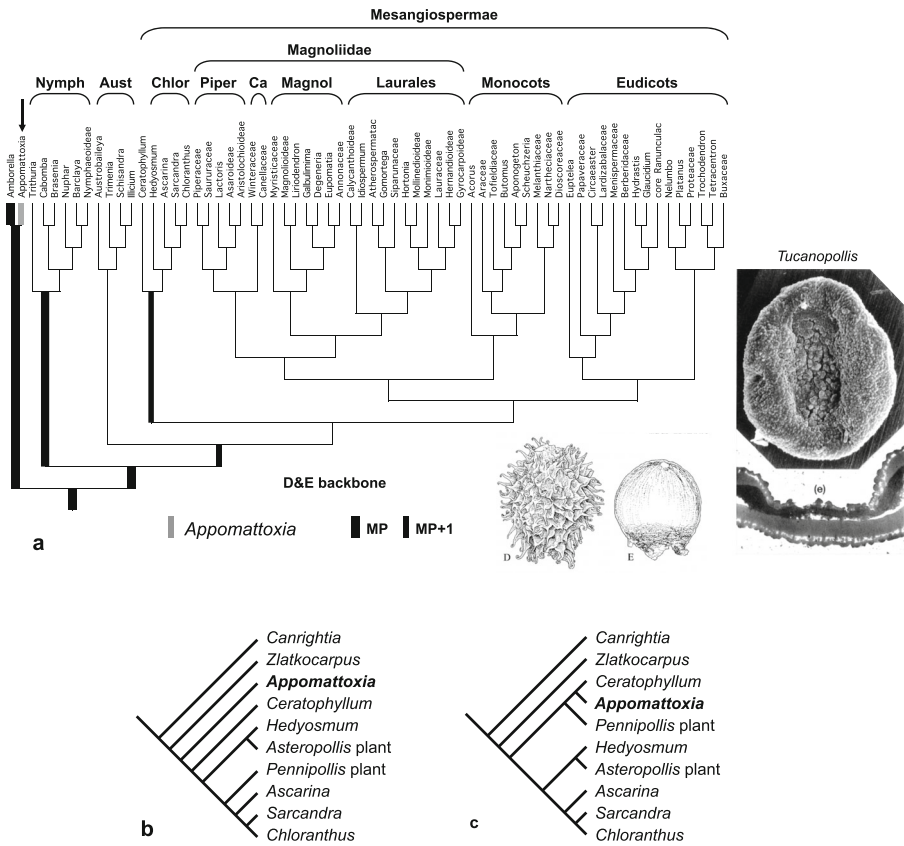
Fig. 9 **a** Relationships of *Appomattoxia* obtained after addition to the D&E backbone tree (modified from Doyle & Endress, 2014). SEM and TEM photos of *Tucanopollis crisopolensis* from Zone C-VI (Barremian) of Congo from Doyle and Hotton (1991). **b–c** Two of four most parsimonious arrangements of Chloranthaceae, *Ceratophyllum*, and fossil taxa obtained after addition of *Appomattoxia*, *Canrightia*, *Zlatkocarpus*, the *Pennipollis* plant, and the *Asteropollis* plant to the D&E backbone tree

this character was ordered). With the J/M backbone tree, *Appomattoxia* had several positions near the base of the angiosperms and below or in crown Chloranthaceae (Doyle & Endress, 2014, fig. 8E–O). These ambiguities might be reduced by information on vegetative morphology or floral organization of the *Appomattoxia* plant; one would expect such characters to be quite different in a plant in the basal angiosperm grade and one linked with Chloranthaceae and/or *Ceratophyllum*.

Another fossil with *Tucanopollis*-type pollen is *Pseudoasterophyllites*, from the latest Albian of France and the middle Cenomanian of Bohemia (Kvaček et al., 2012, 2016), which has reduced (1–11 mm), linear, apparently succulent leaves and occurs in estuarine sediments that suggest it was a halophyte. Using epidermal characters, Kvaček et al. (2012) associated the vegetative remains with dispersed stamens containing *Tucanopollis*-type pollen, and subsequently Kvaček et al. (2016) found both stamens and carpels attached to leafy shoots. The leaves are opposite, as in Chloranthaceae; at first sight they may seem whorled, as in *Ceratophyllum*, but this is because most leaves are borne on short axillary branches in closely spaced pairs, so

that several leaves appear to radiate from the main stem at the same level. The male structures are spikes of flowers that consist of a single stamen, as in *Hedyosmum*, some *Ascarina* species, and *Ceratophyllum*, but they are most like spikes of *Ascarina* in having floral subtending bracts, which are absent in *Hedyosmum* and *Ceratophyllum*. The female flowers consist of a single carpel, which lacks the hooked hairs of *Appomattoxia* but also contains a single pendent, orthotropous seed, which is more elongate than the seed of *Appomattoxia* but similar in having thick-walled cells around the micropyle. However, these flowers are not borne in spikes or thyrses, as in Chloranthaceae, but are instead solitary, as in *Ceratophyllum*.

Phylogenetic analyses by Kvaček et al. (2016) unequivocally associated *Pseudoasterophyllites* with Chloranthaceae, *Ceratophyllum*, or both (Fig. 10a). When *Pseudoasterophyllites* was added to the J/M tree, where Chloranthaceae and *Ceratophyllum* are well separated, its most parsimonious position was sister to Chloranthaceae, supported by opposite or whorled leaves, stephanocytic stomata, sessile, unisexual flowers, one stamen, embedded pollen sacs, supratectal spinules, one carpel, and orthotropous ovule, but a position with *Ceratophyllum* was only one step less parsimonious. By contrast, when *Pseudoasterophyllites* was added to the D&E tree, where Chloranthaceae and *Ceratophyllum* form a clade, it was sister to *Ceratophyllum*. The three groups are united by stephanocytic stomata, sessile flowers, one stamen, embedded pollen sacs, one carpel, and orthotropous ovule, while *Pseudoasterophyllites* is linked with *Ceratophyllum* by the combination of solitary female flower and male flowers in inflorescences. This would imply that *Pseudoasterophyllites* diverged from the line leading to *Ceratophyllum* at an intermediate stage in its evolution from a Chloranthaceae-like terrestrial ancestor, in which the flowers were already strongly reduced.

Kvaček et al. (2016) obtained consistent results when they added *Pseudoasterophyllites* to the two backbone trees along with *Canrightia*, *Zlatkocarpus*, the *Asteropollis* plant, and the *Pennipollis* plant. In two of the three most parsimonious trees found with the D&E backbone, *Pseudoasterophyllites* is attached to the line to *Ceratophyllum*, either alone (Fig. 10b) or with the *Pennipollis* plant (Fig. 10c), while in the third (Fig. 10d) it is sister to *Ceratophyllum* plus the *Pennipollis* plant and crown Chloranthaceae. In the tree in Fig. 10c, the *Pennipollis* plant is linked more closely to *Ceratophyllum* by loss of the bracts subtending the male flowers and introrse or extrorse rather than latrorse anthers. In the one most parsimonious tree found with the J/M backbone (the same as in Fig. 10b but without *Ceratophyllum*), it is sister to crown group Chloranthaceae. When *Appomattoxia* is added as well, *Pseudoasterophyllites* and *Appomattoxia* form a clade united by sclerotic endotegmen, which is related to the *Pennipollis* plant with the J/M backbone tree, but to *Ceratophyllum*, with or without the *Pennipollis* plant, with the D&E backbone, strengthening the conjecture that the two fossils with *Tucanopollis* pollen are related.

In its morphology *Pseudoasterophyllites* appears intermediate between Chloranthaceae and *Ceratophyllum*, and experiments designed to evaluate its impact on the relative parsimony of competing mesangiosperm trees (Kvaček et al., 2016) indicate that it provides at least a little increased support for a relationship between the two extant taxa. In terms of morphology, the backbone tree in which Chloranthaceae and *Ceratophyllum* form a clade (D&E) is already much more parsimonious than the
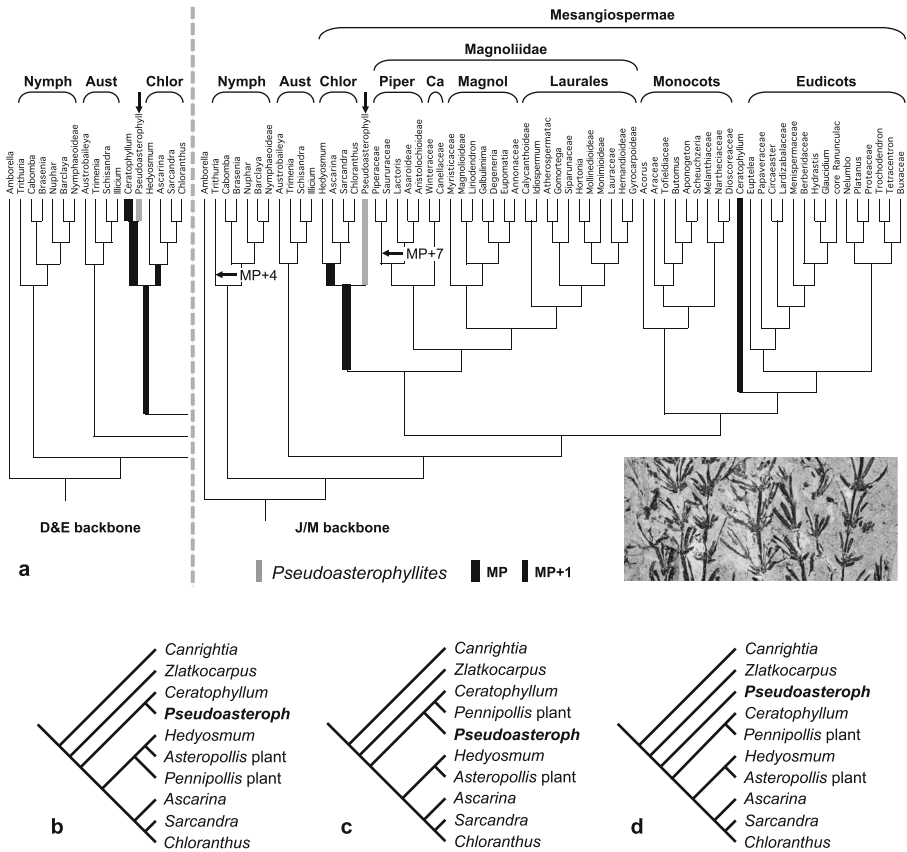
Fig. 10 a Relationships of *Pseudoasterophyllites* obtained after addition to the D&E and J/M backbone trees (modified from Kvaček et al., 2016). Photo of leafy shoots courtesy of Jiří Kvaček. **b–d** Most parsimonious arrangements of *Ceratophyllum*, Chloranthaceae, and fossil taxa obtained after addition of *Pseudoasterophyllites* (*Pseudoasteroph*), *Canrightia*, *Zlatkocarpus*, the *Pennipollis* plant, and the *Asteropollis* plant to the D&E backbone tree. Conventions and abbreviations as in Fig. 2

tree in which they are separated (J/M), by 11 steps. However, if *Pseudoasterophyllites* is added to the two trees at its most parsimonious positions, the relative parsimony of the D&E tree increases by two steps, to 13.

Another probably related fossil is *Montsechia*, a floating aquatic plant with even more reduced leaves from much older Barremian lake deposits in Spain (Martín-Closas, 2003; Krassilov, 2011; Gomez et al., 2015). Historically *Montsechia* was compared with several non-angiospermous groups, but Gomez et al. (2015) associated it with carpels with a single ovule resembling that of *Pseudoasterophyllites* and *Appomattoxia* in being pendent and orthotropous and having an area of thicker-walled cells around the micropyle. Leaves vary between opposite and spiral on different shoots. Unfortunately, male structures and pollen are unknown. Gomez et al. (2015) presented a phylogenetic analysis that linked *Montsechia* with *Ceratophyllum*, with or without Chloranthaceae. Also potentially related are dispersed seeds named *Spermatites* from the Albian-Cenomanian of Greenland (Miner, 1935; Batten & Zavattieri, 1995), which are like seeds of *Appomattoxia*,

Pseudoasterophyllites, and *Montsechia* in being orthotropous and having thick-walled cells around the micropyle.

Plants like *Pseudoasterophyllites* might make sense ecologically as the source of Early Cretaceous *Tucanopollis* pollen in semiarid Africa and Brazil; it is easy to imagine them occupying local wet or saline habitats in such a region. However, even assuming that older *Tucanopollis* pollen was produced by plants related to *Pseudoasterophyllites*, which may be premature based solely on pollen morphology, information on their vegetative morphology is needed to determine whether they were ecologically similar.

General Implications

There is no discernible correlation between the stratigraphic sequence of fossil taxa considered in this review and the pattern of evolution inferred from phylogenetic analysis, except for the younger age of *Chloranthus*-like androecia (*Chloranthistemon*). Even this correlation is problematical because there are Albian-Cenomanian fossils that may also be stem relatives of *Chloranthus* (Hartkopf-Fröder et al., 2012). Of the other fossils treated here, five are early or middle Albian, and our phylogenetic results indicate that the three younger taxa (*Couperites*, *Zlatkocarpus*, *Pseudoasterophyllites*) diverged early in the radiation and must therefore be long-persisting side lines. This lack of a stratigraphic signal is hardly surprising, and it does not contradict the phylogenetic scheme, given the small number of Albian-Cenomanian mesofossil localities, the lack of studied pre-Albian mesofossils, and the diversity of pollen of the *Clavatipollenites* and *Retimonocolpites* types in the Hauterivian, Barremian, and Aptian (e.g., Hughes, 1994), some of which could represent an undeciphered record of earlier events in this line. Mesofossils are known in the lower Potomac Group (Zone I, some of which is presumably Aptian) but have not been studied (Friis et al., 2011); investigation of these and discovery of other mesofossils from older beds elsewhere could reveal temporal trends. Phylogenetic analysis of mid-Cretaceous Chloranthaceae is thus essentially equivalent to inferring relationships among species at the Recent time plane, but without the benefit of DNA data (except in defining backbone trees). However, it has the unique advantage of including extinct taxa with novel character combinations that may provide insights on the course of morphological evolution that could not be gained from living plants alone.

Floral Evolution

Most discussions of the evolutionary implications of chloranthoid fossils have focused on floral evolution. Doyle et al. (2003) found that their trees based primarily on living taxa (Eklund et al., 2004) were consistent with quite divergent scenarios; notably, the most recent common ancestor of Chloranthaceae could be either bisexual or unisexual (Fig. 11a, b, with reconstructed floral diagrams superimposed on nodes). In one scenario (Fig. 11a), this ancestor had bisexual flowers with a perianth. The perianth was lost on the line to *Ascarina*, *Sarcandra*, and *Chloranthus*, but the flowers remained bisexual up to *Chloranthus* and became unisexual independently in *Hedyosmum* and *Ascarina*. However, it was equally parsimonious (Fig. 11b) to assume that the common

ancestor had already become unisexual but still had a perianth in the female flower, as in *Hedyosmum*, and the bisexual flowers of *Sarcandra* and *Chloranthus* were a reversal. Because male flowers in *Ascarina* vary between one and a few stamens, and the unistaminate species appear to form a nested clade (Eklund et al., 2004; Zhang et al., 2011), the first scenario (Fig. 11a) implies one change in stamen number in the *Ascarina* line, whereas the second (Fig. 11b) requires two changes.

Our previous study (Doyle & Endress, 2014) suggested that integration of fossils might help resolve these ambiguities. When we added the four most securely associated fossils to the D&E tree (Fig. 11c), *Canrightia* and *Zlatkocarpus* branched successively from the stem lineage of Chloranthaceae and *Ceratophyllum*, and the *Pennipollis* plant either diverged below *Ceratophyllum* or was linked with it. Both arrangements favor the scenario in which flowers became unisexual on the stem lineage, before divergence of *Zlatkocarpus*, and reversed to bisexual on the line to *Sarcandra* and *Chloranthus*. If the *Pennipollis* plant was linked with *Ceratophyllum*, the perianth was either lost twice, in the *Pennipollis-Ceratophyllum* line and the *Ascarina-Sarcandra-Chloranthus* clade, or lost once and regained in *Hedyosmum*.
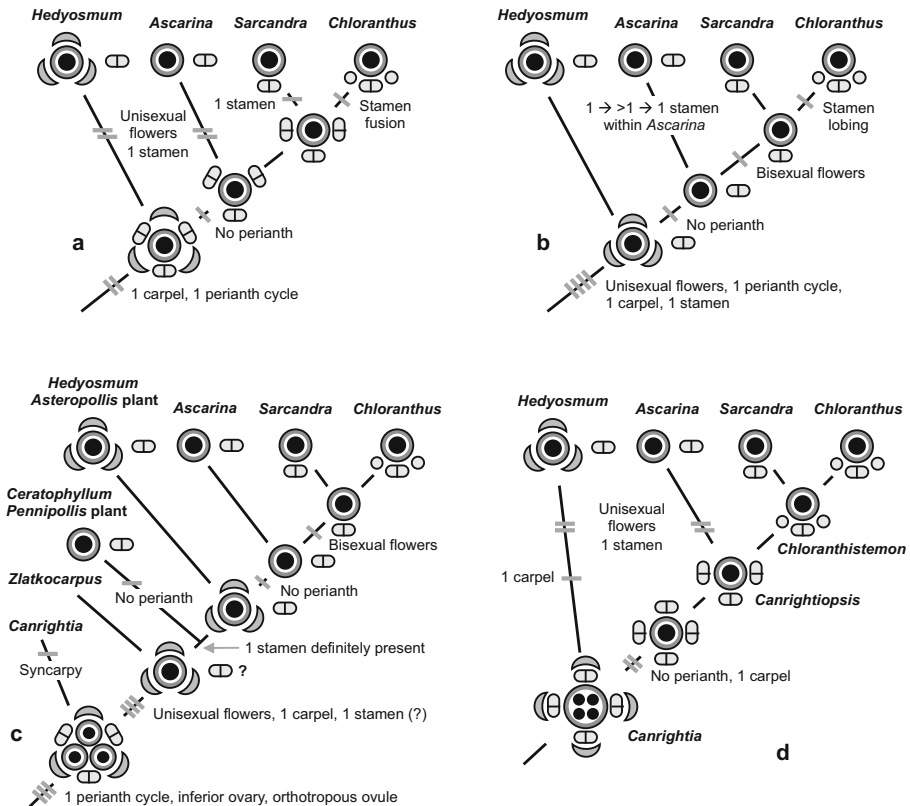
Fig. 11 Scenarios for floral evolution proposed in previous studies, with reconstructed floral diagrams placed at key nodes (redrawn and simplified). **a, b** Two scenarios proposed by Doyle et al. (2003) based on the analysis of living Chloranthaceae by Eklund et al., 2004. **c** Scenario proposed by Doyle and Endress (2014) after addition of *Canrightia*, *Zlatkocarpus*, the *Pennipollis* plant, and the *Asteropollis* plant to the D&E tree. **d** Scenario proposed by Friis et al. (2015). See text for discussion

This scheme was questioned by Friis et al. (2015) based on the discovery of *Canrightiopsis*, which shows similarities to *Canrightia*. They proposed instead (Fig. 11d) that the most recent common ancestor of living Chloranthaceae had a *Canrightia*-like bisexual flower, which gave rise to the *Canrightiopsis* type first by loss of the perianth and reduction to one carpel, then by loss of the stamen on the ventral side of the carpel. However, placing *Canrightia* at the basal node is less parsimonious than attaching it to the stem of the family, since it requires two independent reductions to one carpel, in *Hedyosmum* and the rest of the family. With *Canrightia* in either position, there are two steps in the sexuality character, as was the case when no fossils were considered. This is to be expected, because *Canrightiopsis* by itself does not affect the relative parsimony of the two scenarios, since it is linked with *Sarcandra* and *Chloranthus*, which are also bisexual.

A scenario in which the bisexual flowers of *Canrightiopsis*, *Sarcandra*, and *Chloranthus* are homologous with those of *Canrightia* becomes still less parsimonious if *Zlatocarpus* and the *Pennipollis* plant (with or without *Ceratophyllum*) are also attached to the stem lineage. Both fossils have unisexual flowers, so their inclusion would require additional shifts to unisexuality. Friis et al. (2015) dismissed this result because they did not accept a relationship of *Zlatocarpus* and the *Pennipollis* plant to Chloranthaceae. However, in terms of our data set the best positions of these fossils elsewhere in angiosperms are three or four steps less parsimonious, depending on the backbone tree (Doyle & Endress, 2014). Their relationship to Chloranthaceae and/or *Ceratophyllum* is therefore at least as secure as that of *Canrightia*, which can be placed within Piperales or on the mesangiosperm stem lineage at a cost of only two or three steps (Doyle & Endress, 2014). Friis et al. (2015) also considered floral sexuality in *Zlatkocarpus* as unknown. It is true that only inflorescences with carpels at the fruit stage are known, but if *Zlatkocarpus* had bisexual flowers like those of *Canrightia*, *Canrightiopsis*, *Sarcandra*, or *Chloranthus*, scars where the stamens were attached to the carpel should have been visible. This problem may only be resolved by discovery of male structures; there are unstudied inflorescences in the Czech flora that may be candidates (J. Kvaček, pers. comm.).

Our previous scheme (Doyle & Endress, 2014; Fig. 11c) is generally supported by an analysis of our latest data set (Appendix), including *Canrightia*, *Zlatkocarpus*, the *Asteropollis* plant, the *Pennipollis* plant, *Canrightiopsis*, and *Pseudoasterophyllites*. With the D&E backbone, there are three most parsimonious trees (Fig. 12a–c). In all three trees, *Canrightia* and *Zlatkocarpus* diverge first below the crown clade of *Ceratophyllum* and Chloranthaceae, and *Canrightiopsis* is sister to *Sarcandra* and *Chloranthus*. The *Pennipollis* plant is sister either to *Ceratophyllum* or to *Hedyosmum* and the *Asteropollis* plant. *Pseudoasterophyllites* is either the sister group of the crown *Ceratophyllum*-Chloranthaceae clade or a stem relative of *Ceratophyllum*, with or without the *Pennipollis* plant. With the J/M backbone, there is one most parsimonious tree, which is identical to Fig. 12a except for the absence of *Ceratophyllum*. We use the D&E tree in Fig. 12b as the basis for a more detailed reconstruction of floral evolution (Fig. 12d).

Since phylogenetic analyses indicate that the ancestral flower in mesangiosperms was bisexual and had more numerous free parts, as for angiosperms as a whole (Doyle & Endress, 2011; Sauquet et al., 2017), the first inferred steps in floral evolution on the line to *Ceratophyllum* and Chloranthaceae were departures from this prototype that are
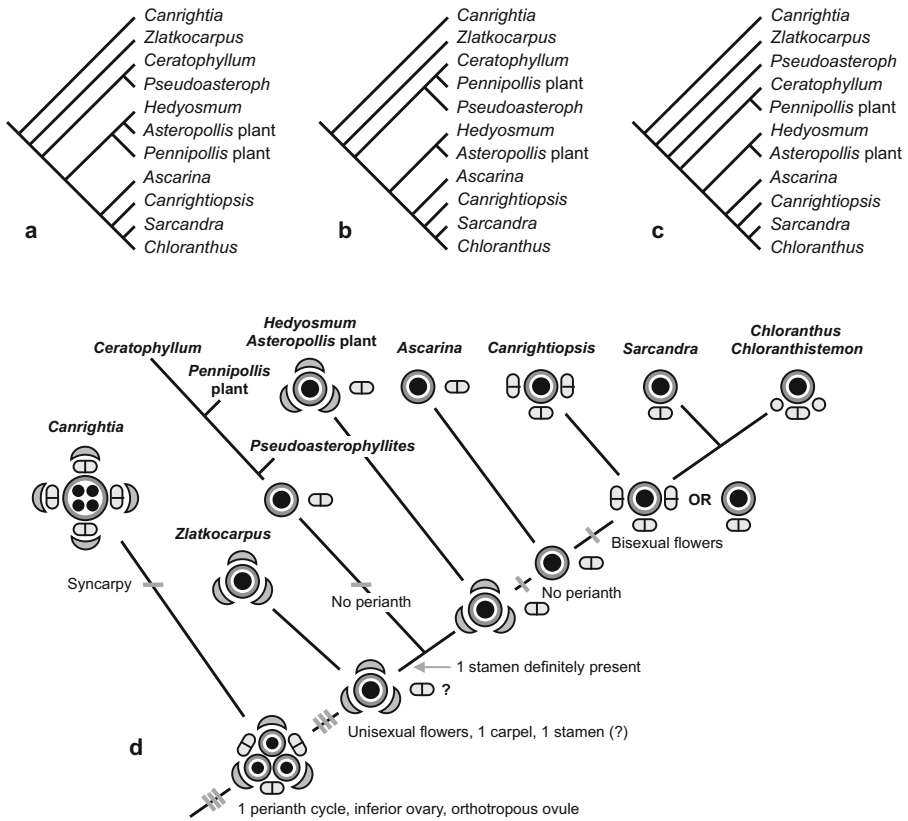
Fig. 12 **a–c** Most parsimonious arrangements of *Ceratophyllum*, Chloranthaceae, and fossil taxa obtained after addition of *Canrightia*, *Zlatkocarpus*, *Pseudoasterophyllites* (*Pseudoasteroph*), the *Pennipollis* plant, the *Asteropollis* plant, and *Canrightiopsis* to the D&E backbone tree. **d** Scenario for floral evolution based on the tree in **b**. See text for discussion

seen in *Canrightia*. These include reduction of the pedicel (sessile flowers), reduction of the perianth to one whorl of tepals, adnation of both the tepals and stamens to a gynoecium consisting of a few carpels, and orthotropous ovule. The androecium was also reduced to one whorl, but whether this occurred on the same line or earlier is equivocal in terms of parsimony, because the state of this character at the mesangiosperm node is uncertain. Because the perianth is trimerous in *Hedyosmum* and the reconstructed common ancestor of mesangiosperms (Doyle & Endress, 2011; Sauquet et al., 2017), the tetramerous organization of *Canrightia* (which it should be noted is somewhat labile) is presumably derived.

The next inferred changes, on the internal branch between *Canrightia* and *Zlatkocarpus*, were a shift to unisexual flowers and reduction of the number of carpels to one. The male flower was also reduced to one stamen, but because male structures in *Zlatkocarpus* are unknown, it is equivocal whether this occurred below *Zlatkocarpus* or the crown node of *Ceratophyllum* and Chloranthaceae. At the latter node, the female flower still had a perianth, as in *Zlatkocarpus* and *Hedyosmum*, but the perianth had been lost in the male flower. This flower (stamen) was still subtended by a bract (as

seen in *Pseudoasterophyllites*), but the bract was lost independently on the line to the *Pennipollis* plant and *Ceratophyllum* and the line to the *Asteropollis* plant and *Hedyosmum*. The perianth in the female flower was lost on the line to *Pseudoasterophyllites*, the *Pennipollis* plant, and *Ceratophyllum* and the line to *Ascarina*, *Canrightiopsis*, *Sarcandra*, and *Chloranthus*; in terms of parsimony, it is equivocal whether it was lost independently in these two lines or lost once in their common ancestor and regained on the line to *Hedyosmum*.

In Fig. 12d we have shown the common ancestor of *Canrightia* and Chloranthaceae as having three free carpels and the syncarpous gynoecium of *Canrightia* as an autapomorphy (in terms of parsimony this is equivocal, since the syncarpy character is undefined in unicarpellate taxa). Parsimony optimization implies that the ovary was at least partly inferior; the combination of free carpels and adnation of other parts to them is rare in living plants, but it does occur (e.g., in *Saruma*, Aristolochiaceae: Dickison, 1992). The unicarpellate gynoecium of the modern genera could thus be derived by a simple reduction in number of carpels. An alternative scenario is that the common ancestor of *Canrightia* and Chloranthaceae was syncarpous, and the gynoecium of living Chloranthaceae was derived from the whole syncarpous gynoecium, with reduction of the number of ovules to one. This seems unlikely, as there are no indications of a syncarpous ancestry in the gynoecium structure of modern Chloranthaceae: the gynoecium appears to be simply unicarpellate. In all four genera, the ascidiate carpel develops from a tilted ring-like primordium and has one dorsal bundle and one ventral bundle supplying the ovule, as in ANITA-grade taxa that are apocarpous or unicarpellate (e.g. *Amborella*, *Cabomba*, *Trimenia*: Endress & Sampson, 1983; Endress & Igersheim, 2000). However, this reasoning does not rule out a third scenario, in which the single chloranthaceous carpel was derived by development of just one of the primordia in an ancestral syncarpous gynoecium.

This analysis also has implications for origin of the bizarre bisexual flowers of *Sarcandra* and *Chloranthus*. As discussed above, *Canrightiopsis* appears to strengthen the hypothesis that the tripartite androecium of *Chloranthus* was derived by fusion of three stamens rather than splitting of one, but this is equivocal in terms of parsimony. In either case, our analysis implies that the flowers of *Sarcandra* and *Chloranthus* are secondarily bisexual, contrary to the predominant trend in angiosperms (cf. Sauquet et al., 2017) and the scheme of Friis et al. (2015). The alternative would imply four shifts from bisexual to unisexual, in *Zlatkocarpus*, the *Ceratophyllum* line, the *Hedyosmum* line, and *Ascarina*, or twice as many steps in this character. The other trees found with the D&E and J/M backbones (Fig. 12a, c) would require as many or more shifts to unisexual flowers.

A hypothesis that might increase the plausibility of the secondary bisexuality scenario is that the supposed bisexual flowers are actually pseudanthia consisting of one female flower and one or more male flowers. Earlier suggestions to this effect were reviewed by Endress (1987) and considered less likely than the flower interpretation. However, cases in *Ascarina* where both stamens and carpels occur in the axil of the same primary bract, such as one stamen and one sterile carpel in *A. lanceolata* and *A. lucida* (Swamy, 1953) and the same plus a lateral bract (bracteole) in *A. diffusa* (Smith, 1976), could represent an analogous situation. In all these cases the stamen is abaxial and the carpel is adaxial relative to the main inflorescence axis, the same configuration as in the bisexual structures of *Sarcandra* and *Chloranthus*. There is

some variation in these features. *A. diffusa* also has unisexual flowers, which are actually more frequent than the bisexual ones (Smith, 1981). In *A. lucida*, Moore (1977) observed that a subtending bract and two lateral bracts are always present, and in some cases the latter subtend additional stamens or carpels, which may have two bracts of their own, indicating further cymose ramification. In general, the primary male flower was abaxial, whereas female flowers and additional male flowers were on the adaxial side. For comparison, it may also be noted that the lateral female units (partial inflorescences) in *Hedyosmum* are cymes, as discussed in Endress (1987) based particularly on *H. mexicanum*. Doria et al. (2012) argued that these units are spikes, but their illustrations are not convincing (Doyle & Endress, 2014). In *H. brenesii* (Todzia, 1988, fig. 15A) each lateral unit has equal numbers of flowers and bracts (including the large subtending bract), confirming that these units are cymes.

Although parsimony favors a shift from bisexual to unisexual followed by a reversal to bisexual in Chloranthaceae, this implicitly assumes that changes in both directions are equally probable. If this assumption is incorrect, and there was a strong intrinsic bias in this character, parallel shifts to unisexual might be favored. However, so far model-based analyses that might detect such bias have not shown a strong asymmetry in evolutionary rates (Goldberg et al., 2017).

Pollen Evolution

Trees including fossils also have implications for the evolution of pollen morphology in Chloranthaceae. Topologies with the D&E backbone (Fig. 13) imply that pollen in the common ancestor of *Canrightia* and Chloranthaceae was globose and monosulcate, with a reticulate-columellar exine, smooth muri, and a sculptured sulcus membrane, as in *Canrightia* and *Zlatkocarpus*, all features inherited from lower in the tree (Doyle, 2005). The sulcus was modified to a several-armed furrow in the *Asteropollis-Hedyosmum* clade, scattered pores in *Sarcandra*, and several colpoid areas in *Chloranthus* plus *Chloranthistemon crossmanensis*. Aperture conditions on the line between *Sarcandra* and *C. crossmanensis* are equivocal (*C. alatus* and *C. endressii* have cross-furrowed and spiraperturate pollen, respectively). The smooth muri seen in *Canrightia* and *Zlatkocarpus* were modified in the common ancestor of the *Pennipollis* plant, *Pseudoasterophyllites*, and the living taxa by origin of supratectal spinules, as in *Hedyosmum* and *Ascarina* (as well as *Clavatipollenites*, *Asteropollis*, *Canrightiopsis*, and *Tucanopollis*), which were later lost on the line leading to *Sarcandra* and *Chloranthus*. The initial inferred change in supratectal sculpture shows an intriguing parallel with an apparent trend in the dispersed pollen record in the Wealden of England (Hughes, 1994), where the proportion of reticulate monosulcate angiosperm pollen types with supratectal spinules increases from relatively rare in the Hauterivian to much more common in the Barremian. However, the relevance of this observation will remain unclear until pollen related to Chloranthaceae can be separated from the reticulate monosulcate pollen of other basal angiosperm lines. Gray shading in Fig. 13 shows the branches of the tree on which the reconstructed pollen type would fit in the dispersed genus *Clavatipollenites* as conventionally defined.

In living Chloranthaceae, the supratectal spinules of *Hedyosmum* and *Ascarina* are associated with wind pollination, whereas the smooth muri of *Sarcandra* and *Chloranthus* are associated with insect pollination. As noted by Friis et al. (2011), this
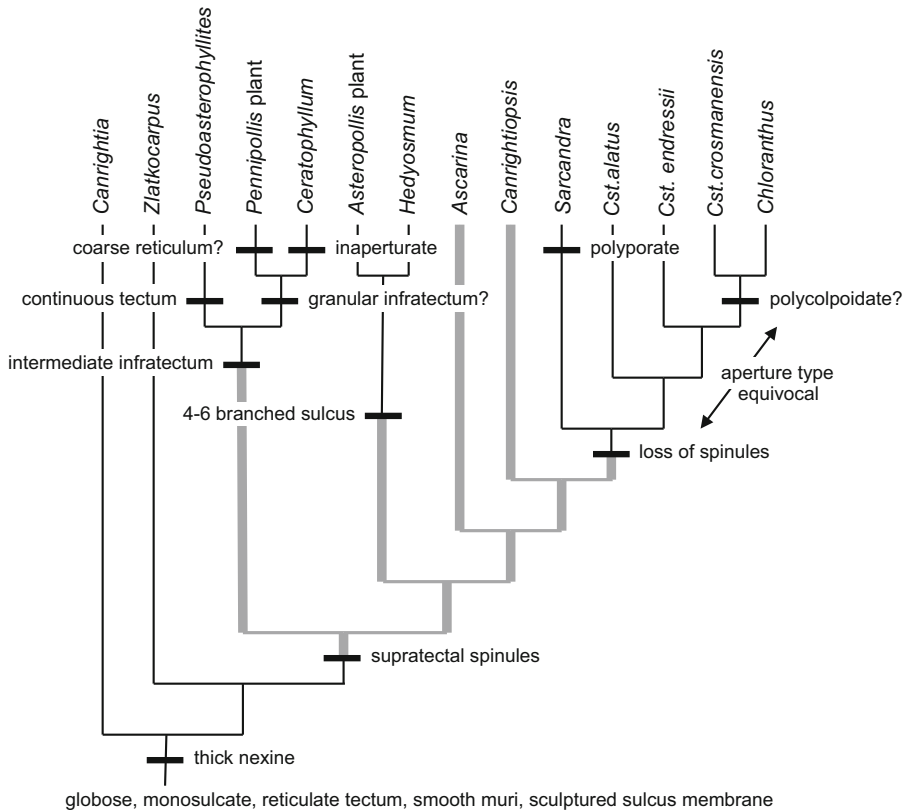
Fig. 13 Inferred events in pollen evolution based on tree (Fig. 12b) found after addition of *Canrightia*, *Zlatkocarpus*, *Pseudoasterophyllites*, the *Pennipollis* plant, the *Asteropollis* plant, and *Canrightiopsis* to the D&E backbone tree, with the three *Chloranthistemon* species (Cst.) placed in the most parsimonious positions found by Eklund et al., 2004. Thick gray lines indicate branches on which the reconstructed pollen corresponds to the *Clavatipollenites* type as defined by many authors

correlation probably also holds in *Canrightia* (which had small, simple bisexual flowers comparable to those of insect-pollinated Saururaceae, Piperaceae, and *Chloranthus*) and in *Chloranthistemon* (Crane et al., 1989). *Canrightiopsis* may appear to contradict this scheme, since it has spinules, suggesting wind pollination, but bisexual flowers, suggesting insect pollination. However, it might represent a stage in which the plant had shifted from wind to insect pollination but its pollen had not yet lost the spinules, consistent with the hypothesis that it was secondarily bisexual.

If *Pseudoasterophyllites* (with *Tucanopollis* pollen) and the *Pennipollis* plant are related to *Ceratophyllum*, this line shows a curious combination of pollen trends. Given that the exine at the node connecting *Ceratophyllum* and Chloranthaceae can be reconstructed as finely reticulate and columellar, *Tucanopollis* and *Pennipollis* represent a trend from columellar to intermediate to granular infratectal structure, plus divergent trends to a continuous tectum in *Tucanopollis* and an unusually coarse, open reticulum in *Pennipollis*. The sulcus and most other pollen characters were lost during extreme exine reduction in *Ceratophyllum*, presumably as a result of a shift to underwater pollination (Takahashi, 1995).

Ecological Evolution

The early record of the chloranthaceous line also has broader implications for under-standing the ecological radiation of angiosperms as a whole. In contrast to their present limited role, members of this line were probably the most common angiosperms in the palynological record until the rise of tricolpate pollen (eudicots), especially if *Pennipollis* and *Tucanopollis* are related as well as types assigned to *Clavatipollenites* and *Retimonocolpites*. It is likely that many of them are over-represented in the pollen record because they were wind pollinated, but this is probably not the whole story, since they are also common as mesofossils and (less definitely) as leaves, and as just noted some (such as *Canrightia*) were probably insect pollinated.

Ecophysiological studies of extant basal angiosperms in a phylogenetic context suggest that Chloranthaceae may have played a special role in the ecological radiation of angiosperms. In the basal ANITA grade, *Amborella* and Austrobaileyales are adapted to disturbed sites in wet forest understory habitats, which Feild et al. (2004, 2009) reconstructed as the ancestral environment for angiosperms (the same analyses implied that the aquatic habit of the remaining ANITA line, Nymphaeales, is a specialization). By contrast, Chloranthaceae are more varied ecologically, ranging from understory to more open disturbed habitats; examples are illustrated in Fig. 14. This variation is associated with monocot-like sympodial establishment growth (Blanc,

Fig. 14 Habitats of extant Chloranthaceae. **a** *Ascarina lucida*, roadside between Harihari and Te Taho, New Zealand; **b** *Hedyosmum brasiliensis*, São Bento do Sul, Santa Catarina, Brazil (photo courtesy of Paulo Schwirkowski); **c** *Sarcandra glabra*, secondary forest understory, Cuc Phuong National Park, Vietnam; **d** *Chloranthus spicatus*, on debris pile in forest behind temple in Dinghushan Biosphere Reserve, Guangdong, China

1986). In *Sarcandra* and *Chloranthus* plants branch repeatedly at ground level in the forest understory and remain more or less herbaceous, but in *Ascarina* and *Hedyosmum* they shift to production of several upright woody stems and sometimes a single trunk in brighter forest gaps and margins (Blanc, 1986; Todzia, 1988; Burrows, 1996; Martin & Ogden, 2002; Feild et al., 2004). Such observations led Feild et al. (2004) to propose that Chloranthaceae were among the first lines with the ability to "break out" of the understory niche as colonizing species, which would have facilitated their spread over the world. In this respect they correspond better to the "riparian weed" model for the first angiosperms (Doyle & Hickey, 1976; Hickey & Doyle, 1977) than do the ANITA lines; this model may be relevant to a second phase in the angiosperm radiation, not the first phase.

If *Ceratophyllum* and the potentially related Cretaceous fossils are part of this radiation, they could represent a line that shifted from disturbed terrestrial sites into saline and aquatic habitats, culminating in the submerged living genus with its dissected leaves and no roots. The chloranthaceous line would then have occupied far more diverse niches in the Early Cretaceous than would be inferred from living Chloranthaceae, but it eventually declined to a minor role, presumably as a result of competition with more derived angiosperms.

Pollination may be another part of this story. Many differences between *Sarcandra* and *Chloranthus* on the one hand and *Hedyosmum* and *Ascarina* on the other correspond to insect- vs. wind-pollination syndromes, respectively (Endress, 1987): flowers bisexual vs. unisexual; reproductive parts of the androecium small (with small amount of pollen) vs. large (with large amount of pollen) relative to non-reproductive parts; androecium colored (yellow or white) vs. not colored (preanthetic anthers may be red, but they become inconspicuously brown when dehiscing); androecium scented vs. not scented; stigma small, often smooth vs. large, papillose. Field observations on four species have shown that *Chloranthus* is specialized for thrips pollination (Ma et al., 1997; Wang et al., 1998; Luo & Li, 1999), although *C. henryi* is reported to be self-pollinated (Wang et al., 1999). A structural expression is the broad three-parted androecium, which covers the pollen sacs and gynoecium with its concave ventral side (von Balthazar & Endress, 1999) and thus provides a shelter for thrips. *Sarcandra*, with its freely exposed single stamen, differs in being pollinated by beetles, bees, Hemiptera, and flies (Tosaki et al., 2001), and not by thrips. By contrast, *Ascarina* and *Hedyosmum* are reported to produce copious wind-borne pollen (van der Hammen & Gonzalez, 1960; Rawlings, 1974; McGlone & Moar, 1977; D'Arcy & Liesner, 1981).

These data from extant plants and the morphology of Cretaceous mesofossils suggest that a shift from insect to wind pollination occurred on the line leading to Chloranthaceae after the divergence of *Canrightia* and was later reversed to insect pollination on the line leading to *Canrightiopsis*, *Sarcandra*, *Chloranthistemon*, and *Chloranthus*. If *Ceratophyllum* is sister to Chloranthaceae, the reduction of its flowers may already have been well advanced as an adaptation for wind pollination before its ancestors entered the water, rather than a consequence of water pollination (Endress & Doyle, 2009). Because wind pollination in temperate plants has been interpreted as an adaptation to the shorter growing season, which favors early flowering, before the emergence of pollinating insects (Whitehead, 1969; Regal, 1982), the inferred shift to wind pollination in the chloranthaceous line might be a result of its spread into higher latitudes. Alternatively, it might be related to occupation of more open sites in both

tropical and temperate areas. In a phylogenetic analysis across angiosperms, Friedman and Barrett (2008) found that evolution of wind pollination is statistically associated with entry into open habitats, but not into temperate regions. However, their analysis did not address the hypothesis that wind-pollinated taxa are more likely to persist and diversify in temperate areas. As pointed out by Friis et al. (2011), it should also be noted that most of the Early Cretaceous pollen types associated with Chloranthaceae are known in presumed insect coprolites, indicating that they were sometimes eaten and therefore potentially transported by insects.

The inferred shift to wind pollination in the chloranthaceous line may be analogous to a somewhat later shift to wind in basal eudicot lines, exemplified by fossils related to *Platanus* (Proteales) in the Albian of Laurasia (Friis et al., 1988, 2011; Crane et al., 1993; Doyle & Endress, 2010), which include some of the first locally dominant angiosperms in leaf floras (*Sapindopsis*, "platanoids": Doyle & Hickey, 1976; Hickey & Doyle, 1977; Doyle & Upchurch, 2014; Sender et al., 2016). The fact that tricolpate pollen was already widespread in the Aptian of Northern Gondwana (Africa-South America) before becoming consistently present in the Albian of Laurasia suggests that eudicots spread from the tropics to higher latitudes (Brenner, 1976; Hickey & Doyle, 1977; Doyle, 1992; Heimhofer & Hochuli, 2010). However, the fact that the perianth parts were larger in *Sapindopsis* and Albian platanoids than in modern *Platanus* suggests that the Albian taxa were still insect pollinated, or transitional between insect and wind pollination (Friis et al., 1988; Crane et al., 1993).

Biogeography

The geographic history of Chloranthaceae is beyond the scope of this paper, but a few remarks may be useful. It is clear that the present restricted occurrences of the living genera are relicts of far wider distributions of their stem relatives in the Cretaceous. Muller (1981) compiled records of dispersed *Clavatipollenites* and *Asteropollis*, as did Krutzsch (1989) for *Clavatipollenites*, and concluded that they document contraction of *Ascarina* and *Hedyosmum* from near-global distributions in the Albian to the present. The use of *Clavatipollenites* pollen for *Ascarina* may be questioned, as can the use of *Asteropollis* for *Hedyosmum* if trichotomosulcate pollen is included, and as emphasized by Friis et al. (2011) there is no recognized dispersed pollen record of *Sarcandra* and *Chloranthus*. They attributed this absence to insect pollination, but it could also be a function of less intensive study of Late Cretaceous palynofloras and the emphasis by palynologists on other stratigraphically useful groups, such as triporate Normapolles (Fagales). However, the presence of both *Asteropollis* flowers and *Chloranthistemon* in the Cretaceous of Europe and North America shows that the *Hedyosmum* and *Chloranthus* lines occurred much more widely than living members of the crown groups; *Hedyosmum* is now restricted to Asia and the Neotropics, *Sarcandra* and *Chloranthus* to Asia.

Conclusions

Studies of chloranthoid mesofossils and their placement in a molecular phylogenetic framework has provided a more detailed picture of the evolution of this now-obscure

early angiosperm group than could be inferred from living plants alone. However, many uncertainties remain that may require discovery of more complete flowers and inflorescences and connections with vegetative organs for their resolution. In addition, better sampling of Chloranthaceae in genome-level phylogenetic analyses of extant angiosperms could confirm or refute the relationship of Chloranthaceae and *Ceratophyllum* and the role of fossils as "links" between these two groups.

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Appendix: Phylogenetic analysis of *Canrightiopsis*

The data set used to evaluate the position of *Canrightiopsis* is that of Kvaček et al. (2016), including all potential fossil relatives of Chloranthaceae (except *Chloranthistemon*) treated in this paper (Table 1). Sources of data on other living and fossil taxa and the rationale for character definitions and scoring of difficult cases are discussed in Doyle and Endress (2000, 2010, 2014), Doyle et al. (2008), Endress and Doyle (2009), and Kvaček et al. (2016). In the following character list we have noted changes made between Doyle and Endress (2014) and Kvaček et al. (2016) that are most likely to affect inferred relationships of Chloranthaceae and potential relatives. We score *Canrightiopsis* as a consensus of the three species *C. intermedia*, *C. crassitesta*, and *C. dinisii* of Friis et al. (2015), whose preserved features do not differ in any of the characters in our data set; the differences among them are largely quantitative. These species are represented by isolated fruits with stamen scars (but no stamens), enclosed seeds, and adhering pollen.

Characters

Character states scored for *Canrightia* are indicated in bold font. Uncertain scorings (e.g., 0/1) are shown by putting both states in bold font. When no state is in bold font, the character is scored as unknown (including inapplicable).

1–41 Vegetative characters: unknown; see Kvaček et al. (2016).

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. Although Kvaček and Friis (2010) assumed that *Zlatkocarpus* had unisexual flowers, and we scored it accordingly in Doyle and Endress (2014), Friis et al. (2015) scored *Zlatkocarpus* as unknown.

However, we assume that if *Zlatkocarpus* was bisexual there would be visible remnants or scars of stamens below or on the fruits, as there are in *Canrightia*, *Canrightiopsis*, *Sarcandra*, and *Chloranthus*.

48. Inflorescences of unisexual flowers (0) both sexes with more than one flower, (1) male with more than one flower, female with one flower (uniflorous, solitary). Bisexual taxa scored as unknown.

49 Floral base (0) no hypanthium, superior ovary, (1) hypanthium, superior, **(2) inferior ovary**. See Kvaček et al. (2016) for rescoring of *Sarcandra* and *Chloranthus* as inferior. Friis et al. (2015) used a character that distinguished partly epigynous from epigynous, but this is not likely to be informative for placement of *Canrightiopsis* among Chloranthaceae and related fossils, since only *Hedyosmum* and the *Asteropollis* plant are fully epigynous.

50 Floral receptacle (female portion) **(0) short**, (1) elongate.

51 Pits in receptacle bearing individual carpels **(0) absent**, (1) present.

52 Cortical vascular system (0) absent or supplying perianth only, (1) supplying androecium, (2) supplying androecium plus gynoecium.

53 Floral apex (0) used up after production of carpels, (1) protruding in mature flower. Not applicable in taxa with one carpel.

54 Perianth (0) present, **(1) absent**.

55–61 Perianth organization, features of perianth parts: inapplicable; see Kvaček et al. (2016).

62 Calyptra derived from the last one or two bracteate organs below the flower **(0) absent**, (1) present.

63 Stamen number **(0) more than one**, (1) one.

64 Androecium phyllotaxis (0) spiral, (1) whorled. Because of the uneven, one-sided position of the three stamens, we consider characters 64 and 65 inapplicable in *Canrightiopsis*.

65 Androecium merism (0) trimerous, (1) dimerous, (2) polymerous.

66 Stamen whorls (series when phyllotaxis is spiral; includes inner staminodes) **(0) one**, (1) two, (2) more than two. See Kvaček et al. (2016) for rescoring of *Ascarina* as unknown for characters 66 and 67.

67 Stamen positions (0) single, (1) double (at least in outer whorl). Inapplicable with one whorl of stamens.

68 Stamen fusion **(0) free**, (1) connate.

69 Inner staminodes (0) absent, (1) present. Inapplicable with one whorl of stamens; see Kvaček et al. (2016) for rescoring of *Canrightia* as unknown.

70–81 Stamen morphology, pollen development: unknown; see Kvaček et al. (2016).

82 Pollen unit **(0) monads**, (1) tetrads.

83 Pollen size (average) (0) large (> 50 μm), (1) medium (20–50 μm), **(2) small** (< 20 μm).

84 Pollen shape (0) boat-shaped, **(1) globose**, (2) triangular, angulaperturate.

85 Aperture type **(0) single** (presumably polar, including monosulcate and monoporate) **or disulcate** (one furrow at each pole), (1) inaperturate, (2) sulculate, (3) (syn)tricolpate with colpi arranged according to Garside's law (with or without alternating colpi), (4) tricolpate.

86 Single aperture shape **(0) elongate**, (1) round.

87 Single aperture branching **(0) unbranched**, (1) with several branches.

88 Infratectum (0) granular (including "atectate"), (1) intermediate, **(2) columellar**; ordered.

89 Tectum (0) continuous or microperforate, **(1) perforate** (foveolate) **to semitectate** (e.g., reticulate), (2) reduced (not distinguishable from underlying granules).

90 Grading of reticulum **(0) uniform**, (1) finer at ends of sulcus (liliaceous), (2) finer at poles (rouseoid). Applicable only in taxa with state 1 in character 89.

91 Striate muri **(0) absent**, (1) present.

92 Supratectal spinules (smaller than the width of tectal muri in perforate and semitectate taxa; includes rounded as well as pointed elements) (0) absent, **(1) present**.

93 Prominent spines (larger than spinules, easily visible with light microscopy) **(0) absent**, (1) present.

94 Aperture membrane (0) smooth, **(1) sculptured**.

95 Extra-apertural nexine (0) foot layer, not consistently foliated, distinctly staining endexine absent or only discontinous traces, (1) foot layer and distinctly staining, continous endexine, or endexine only, (2) all or in part foliated, not distinctly staining.

96 Nexine thickness (0) absent or discontinuous traces, (1) thin but continuous, (2) thick (1/3 or more of total exine); ordered.

97 Carpel number **(0) one**, (1) two–five in one whorl or series (when spiral), (2) more than five in one whorl or series, (3) more than one whorl or series.

98 Carpel form (0) ascidiate up to stigma, (1) intermediate (both plicate and ascidiate zones 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. Because developmental or anatomical evidence is often needed to distinguish these states, we have scored this character as unknown in fossils, except when they have a clear ventral slit.

99 Postgenital sealing of carpel (0) none, (1) partial, (2) complete.

100 Secretion in area of carpel sealing (0) present, (1) absent.

101 Pollen tube transmitting tissue (0) not prominently differentiated, (1) one cell layer prominently differentiated, (2) more than one cell layer prominently differentiated.

102 Style **(0) absent** (stigma sessile or capitate), (1) present (elongated, distinctly constricted apical portion of carpel).

103 Stigma **(0) extended** (half or more of style-stigma zone), (1) restricted (above slit or around its upper part). In contrast to many fossils, the stigmatic zone in *Canrightiopsis* is distinct and similar to that in *Sarcandra* and *Chloranthus*.

104 Multicellular stigmatic protuberances or undulations (0) absent, (1) present. Characters 104 and 105 are not visible from the surface because of the abundant stigmatic secretion.

105 Stigmatic papillae (most elaborate type) (0) absent, (1) unicellular or with single emergent cell and one or more small basal cells, (2) uniseriate pluricellular with emergent portion consisting of two or more cells.

106 Extragynoecial compitum (0) absent, (1) present. Characters 106 and 107 are not applicable in unicarpellate taxa.

107 Carpel fusion (0) apocarpous, (1) parasyncarpous, (2) eusyncarpous (at least basally).

108 Oil cells in carpels (0) absent or internal, **(1) intrusive**. Inapplicable in taxa with no oil cells anywhere in the plant. Compared by Friis et al. (2015) with the intrusive oil cells of *Chloranthus spicatus*.

109 Long unicellular hairs on and/or between carpels (0) absent, (1) present. Characters 109–112 are usually not scored in fossils.

110 Short curved appressed unlignified hairs with up to two short basal cells and one long apical cell on carpels (0) absent, (1) present.

111 Nectary on dorsal or lateral sides of carpel or pistillode (0) absent, (1) present.

112 Septal nectaries or potentially homologous basal intercarpellary nectaries (0) absent, (1) present.

113 Number of ovules per carpel **(0) one**, (1) two or varying between one and two, (2) more than two.

114 Placentation (0) ventral, (1) laminar-diffuse or "dorsal." Described as ventral by Friis et al. (2015) based on the relation of the ovule to the bract, but the bract shows the orientation of the carpel relative to the inflorescence axis, not to the floral axis, which is unknown. We scored living Chloranthaceae and *Ceratophyllum* as ventral, based on development (Endress & Doyle, 2009), but potentially related fossils as unknown.

115 Ovule direction **(0) pendent**, (1) horizontal, (2) ascendent.

116 Ovule curvature (0) anatropous (or nearly so), **(1) orthotropous** (including hemitropous).

117 Integuments **(0) two**, (1) one.

118 Outer integument shape (0) semiannular, (1) annular. Orthotropous taxa are scored as unknown.

119 Outer integument lobation (0) unlobed, (1) lobed.

120 Outer integument thickness (at middle of integument length) (0) two cells, (1) **two and three to four**, (2) four and five, or more; ordered. Friis et al. (2015, p. 199) stated that there may be only two cell layers, but that this is uncertain because it is difficult to distinguish the exotesta from the fruit wall and because there may be additional cells above holes in the endotesta (see Friis et al., 2015, fig. 14). Based on the latter, we score *Canrightiopsis* as 1.

121 Inner integument thickness (0) two cells, **(1) two and three, or three**, (2) three **and more**. Friis et al. (2015) described *Canrightiopsis* as having "several" layers of thin-walled cells, which are often collapsed, but their fig. 14 shows three cell layers; because we consider the state uncertain, we score *Canrightiopsis* as 1/2.

122 Chalaza (0) unextended, (1) pachychalazal, (2) perichalazal. Orthotropous taxa scored as unknown.

123 Nucellus (0) crassinucellar (including weakly so), (1) tenuinucellar or pseudocrassinucellar.

124 Fruit wall **(0) wholly or partly fleshy**, (1) dry.

125 Lignified endocarp **(0) absent**, (1) present. Scored only in fleshy fruits.

126 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.

127 Hooked hairs on fruit **(0) absent**, (1) present.

128 Testa (0) slightly or non-multiplicative, (1) multiplicative. Because this character is defined by comparison with the number of cell layers in the ovule stage, it is not applicable in fossils.

129 Exotesta **(0) unspecialized**, (1) palisade or shorter sclerotic cells, (2) tabular, (3) longitudinally elongated, more or less lignified cells.

130 Mesotesta lignification **(0) unlignified**, (1) with sclerotic layer, (2) with fibrous layer. Characters 130 and 131 are scored as unknown (inapplicable) in taxa with a two-layered outer integument.

131 Mesotesta fleshiness **(0) not juicy**, (1) wholly or partly modified into a juicy sarcotesta.

132 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.

133 Tegmen **(0) unspecialized**, (1) thick-walled exo- and endotegmen, (2) fibrous to sclerotic exotegmen.

134 Ruminations **(0) absent**, (1) testal, (2) tegminal and/or chalazal.

135 Operculum **(0) absent**, (1) present.

136 Aril **(0) absent**, (1) present.

137 Female gametophyte (0) four-nucleate, (1) eight- or nine-nucleate.

138 Endosperm development (0) cellular, (1) nuclear, (2) helobial.

139 Endosperm in mature seed **(0) present**, (1) absent. Characters 139 and 141 are clearly visible with synchrotron radiation X-ray tomographic micrography.

140 Perisperm (0) absent, (1) from nucellar ground tissue, (2) from nucellar epidermis.

141 Embryo **(0) minute** (less than 1/2 length of seed interior), (1) large.

142 Cotyledons (0) two, (1) one.

143 Germination (0) epigeal, (1) hypogeal.

Table 1 Data matrix used for analysis of the positions of *Canrightiopsis* and other fossil taxa

A = 0/1, B = 0/2, C = 0/3, D = 0/4, E = 1/2, F = 1/3, G = 2/3, H = 0/1/2

Taxa included in the matrix:

- Amborella
- Cabomba
- Brasenia
- Nuphar
- Barclaya
- Nymphaeoideae
- Trithuria
- Austrobaileya
- Trimenia
- Illicium
- Schisandra
- Liriodendron
- Magnolioideae
- Degeneria
- Galbulimima
- Eupomatia
- Annonaceae
- Myristicaceae
- Calycanthoideae
- Idiospermum
- Atherospermatac
- Siparunaceae
- Hortonia
- Monimioideae
- Mollinedioideae
- Gomortega
- Lauraceae
- Hernandioideae
- Gyrocarpoideae
- Winteraceae
- Canellaceae
- Saururaceae
- Piperaceae
- Lactoris
- Asaroideae
- Aristolochioideae

Table 1 (continued)

The data matrix (a morphological character-state table with column position rulers numbered 1–4 across the top) lists the following taxa in the left column, each followed by a long string of coded character states (digits, letters, and ? for missing data):

- Euptelea
- Papaveraceae
- Lardizabalaceae
- Circaeaster
- Menispermaceae
- Berberidaceae
- Glaucidium
- Hydrastis
- Core Ranunculac
- Nelumbo
- Platanus
- Proteaceae
- Tetracentron
- Trochodendron
- Buxaceae
- Acorus
- Tofieldiaceae
- Butomus
- Aponogeton
- Scheuchzeria
- Araceae
- Nartheciaceae
- Dioscoreaceae
- Melanthiaceae
- Hedyosmum
- Ascarina
- Sarcandra
- Chloranthus
- Ceratophyllum
- Pseudoasterophyll
- Pennipollis plant
- Couperites
- Canrightia
- Canrightiopsis
- Zlatkocarpus
- Asteropollis plant
- Appomattoxia