

IS THE ANTHOPHYTE HYPOTHESIS ALIVE AND WELL? NEW EVIDENCE FROM THE REPRODUCTIVE STRUCTURES OF BENNETTITALES¹

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Bennettitales is an extinct group of seed plants with reproductive structures that are similar in some respects to both Gnetales and angiosperms, but systematic relationships among the three clades remain controversial. This study summarizes characters of bennettitalean plants and presents new evidence for the structure of cones and seeds that help clarify relationships of Bennettitales to flowering plants, Gnetales, and other potential angiosperm sister groups. Bennettitales have simple mono- or bisporangiate cones. Seeds are borne terminally on sporophylls. They have a unique structure that includes a nucellus with a solid apex, no pollen chamber, and a single integument, and they are clearly not enclosed by a cupule or other specialized structures. Such features differ substantially from Gnetales, flowering plants, and the seed fern *Caytonia*, providing no compelling evidence for the origin of the angiospermous carpel. Cladistic tests were performed to assess the strength of the “anthophyte hypothesis” and possible relationships of Bennettitales, Gnetales, and *Caytonia* to flowering plants. Our results do not support the anthophyte hypothesis for the origin of angiosperms by a transformation of fertile organs that were already aggregated into a cone or flower-like structure. However, the anthophyte topology of the seed plant tree continues to be supported by morphological analyses of living and extinct taxa.

Key words: anthophytes; Bennettitales; *Caytonia*; *Cycadeoidea*; Erdtmanithecales; Gnetales; *Williamsonia*.

The Bennettitales is an unusual order of extinct seed plants, fascinating because of its similarities to cycads on the one hand, and on the other because certain very unusual characters are apparently shared with angiosperms and Gnetales. This dichotomy has engendered a long-standing discourse about bennettitalean affinities, and questions persist in attempts to understand their evolutionary significance and relationships, particularly in terms of angiosperms. These questions are more than vexing because they surround a set of taxa that are relatively well represented by fossils, some spectacular in aspect and preservation (e.g., Wieland 1906, 1916; Thomas, 1915; Delevoryas, 1959, 1960, 1963, 1965, 1968a). Frequently referred to as Cycadeoidales in North American literature, bennettitaleans include several extremely well-known plants (Figs. 1, 2) based on excellent fossil material in several modes of preservation (e.g., *Cycadeoidea* Buckland, *Williamsonia* Carruthers, and *Williamsoniella* Thomas). There also are numerous morphogenera assigned to the Bennettitales by some authors that consist of fewer plant parts represented by only a single, sometimes sub-optimal, mode of preservation. Such taxa of uncertain structure and relationships are not included in this study.

There is a long history of debate surrounding the interpretation of bennettitalean fertile structures, with several themes re-

curring from generation to generation of researchers. Whereas some workers have been impressed by similarities of bennettitaleans to cycads, others have favored relationships to gnetales and flowering plants. A lengthy review of this topic is presented in Appendix 1 for readers who wish to revisit historical details of the subject. As exemplified by the paradigm-altering study of Arber and Parkin (1907), hypotheses regarding possible homologies of bennettitalean fertile structures have often involved the search for fossil evidence to document the evolutionary origin of the double integument of the angiosperm seed and the carpel of angiosperm flowers. Phylogenetic analyses have proven to be an additional powerful tool for testing the classical hypotheses that implicated Bennettitales in the origin of flowering plants, and have helped revive the broad views on this subject of Arber and Parkin (1907), Scott (1909), and others (Appendix 1). Crane's (1985) early phylogenetic analysis of seed plants separated Bennettitales from Cycadales and placed the Bennettitales in a clade with Gnetales and angiosperms. It is important to note that virtually all subsequent broad-scale phylogenetic studies that include fossils and employ morphological characters also resolve this “anthophyte” topology, either with (e.g., Fig. 29) or without (e.g., Figs. 30, 31) the seed fern *Caytonia* (Doyle and Donoghue, 1986, 1992; Nixon et al., 1994; Rothwell and Serbet, 1994; Doyle, 1996, 2006; Donoghue and Doyle, 2000; Hilton and Bateman, 2006). In practice, composition of the anthophytes varies among the results of many analyses such that the concept is best regarded as including all the taxa within the least inclusive clade that also contains Bennettitales, Gnetales, and flowering plants (e.g., Figs. 29–31).

In contrast to the results of morphological studies, phylogenetic analyses that employ DNA sequence characters to resolve relationships among living species (i.e., that exclude fossils such as Bennettitales, Caytoniales, or Pentoxylales) yield several conflicting tree topologies (Burleigh and Mathews, 2004; Rydin, 2005; Rai et al., 2008; Mathews, 2009, pp. 228–236 in this issue) that almost never resolve an anthophyte clade (i.e.,

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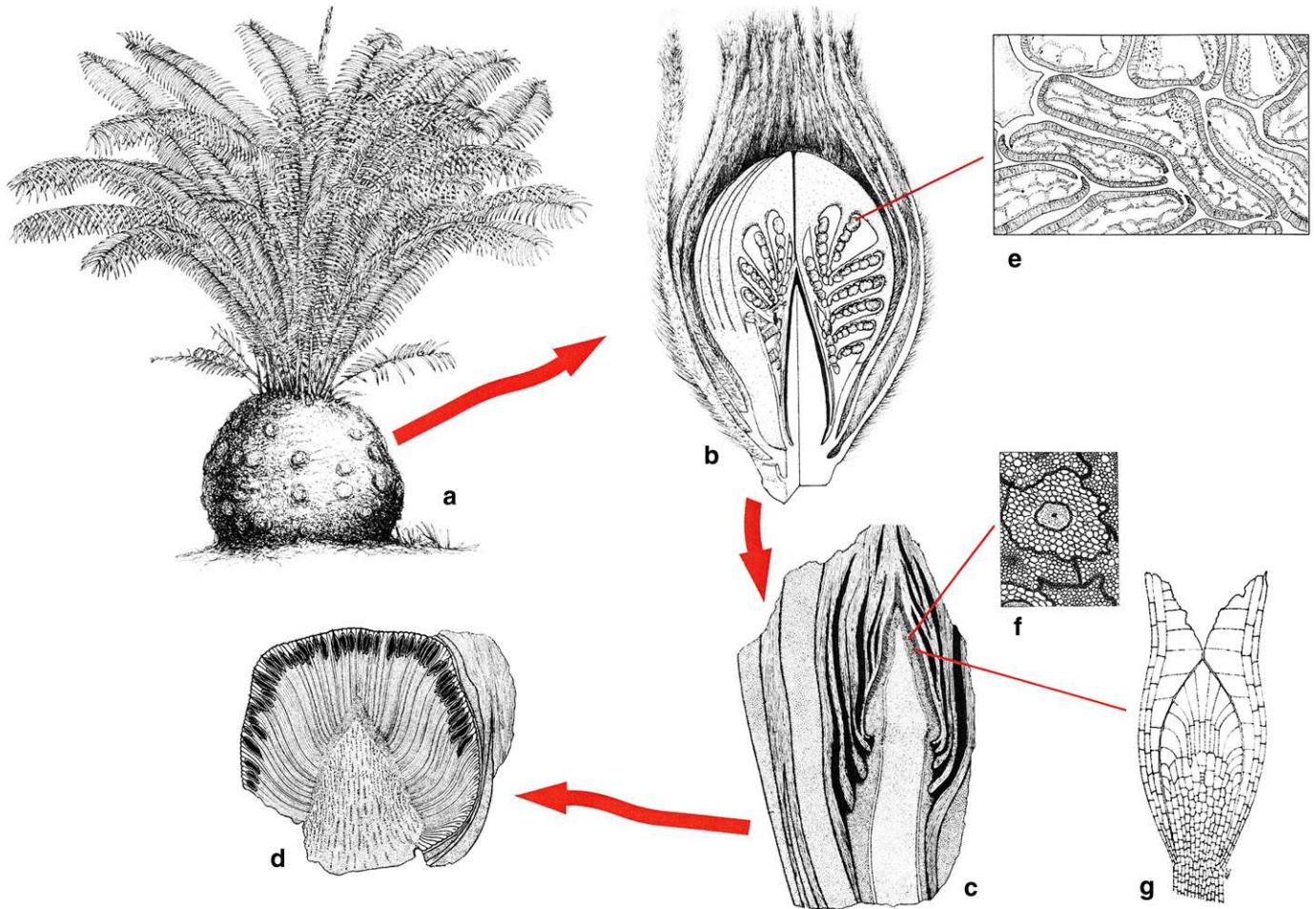


Fig. 1. *Cycadeoidea* spp. Characteristic features of plants. (a) Reconstruction of *Cycadeoidea* plant, with crown of pinnate fronds and cones imbedded among leaf bases (redrawn from Delevoryas, 1971). (b) Cut away reconstruction of bisporangiate cone consisting of bracts surrounding fertile zone. Fertile zone consisting of synangiate ovulate receptacle bearing microsporophylls basally and immature ovulate sporophylls and interseminal scales distally (redrawn from Crepet, 1974). (c) Lower magnification of cone at intermediate stage of maturity; microsporophylls have disintegrated, and ovules are at nucellar stage of development (redrawn from Wieland, 1906). (d) Mature ovulate receptacle of *Cycadeoidea dacotensis* type showing elongated ovulate stalks (i.e., sporophylls) associated with mature seeds among interseminal scales on ovulate receptacle. (e) Cross section of several synangia with included tubular sporangia (from Wieland, 1906). (f) Cross section through stalk of young ovule surrounded by flattened interseminal scales (redrawn from Wieland, 1906). Stalk shows central vascular trace, cortex, and thick-walled epidermis surrounded by broad zone of apparently circular cells that are actually trichomes in cross section. (g) Longitudinal section through young ovule at stage of meiosis illustrating integumentary layers and solid nucellus (from Crepet and Delevoryas, 1972).

Gnetales+angiosperms). The results of nearly all such analyses either move gnetophytes to the base of the seed plant tree or else place flowering plants at the base of that tree, and variously associate gnetophytes in a clade with extant conifers (as their sister group or nested within them; see also Mathews, 2009). This discordance in results lies in stark contrast to the general concordance of results from analyses of living and fossil taxa (or living taxa only; Loconte and Stevenson, 1990; Pryer et al., 2001) based on morphological characters, and argues for the continued viability and possible validity of phylogenies that include the anthophyte topology.

Most recently, descriptions of exceptionally well-preserved bennettitalean seed cones (Rothwell and Stockey, 2002; Stockey and Rothwell, 2003) and of dispersed fossil gnetophyte-like seeds from Cretaceous sediments of Europe and North America (Friis et al., 2007), have offered differing interpretations of bennettitalean seeds and of their putative similarities to gnetophyte

(and dispersed fossil gnetophyte-like) seeds. The fossil seeds of Friis et al. (2007; dispersed in the rock matrix), share with living gnetaleans an outer envelope of fused, integument-enclosing bracteoles, a thin tubular micropyle, and an integument that is fused to the nucellus for much of the length of the megagametophyte (Rydin, 2005; Friis et al., 2007). Friis et al. (2007) emphasize that the outer envelope (= "bracteoles" of living gnetophytes; e.g., Gifford and Foster, 1988) surrounds the integument of the seed and that the envelope has distinctive papillate cells on the inner epidermis. They also stress that the biseriate integument of those seeds is easily identified in cross-sectional views by a diagnostic ring of radially elongated cells that surround and partially occlude the micropylar canal (Rydin, 2005; Friis et al., 2007). Although a similar structure is ascribed to bennettitalean seeds by Friis et al. (2007), that interpretation contradicts numerous previous descriptions (Lignier, 1894, 1911; Wieland, 1906, 1911, 1916; Stopes, 1918; Sharma, 1970;

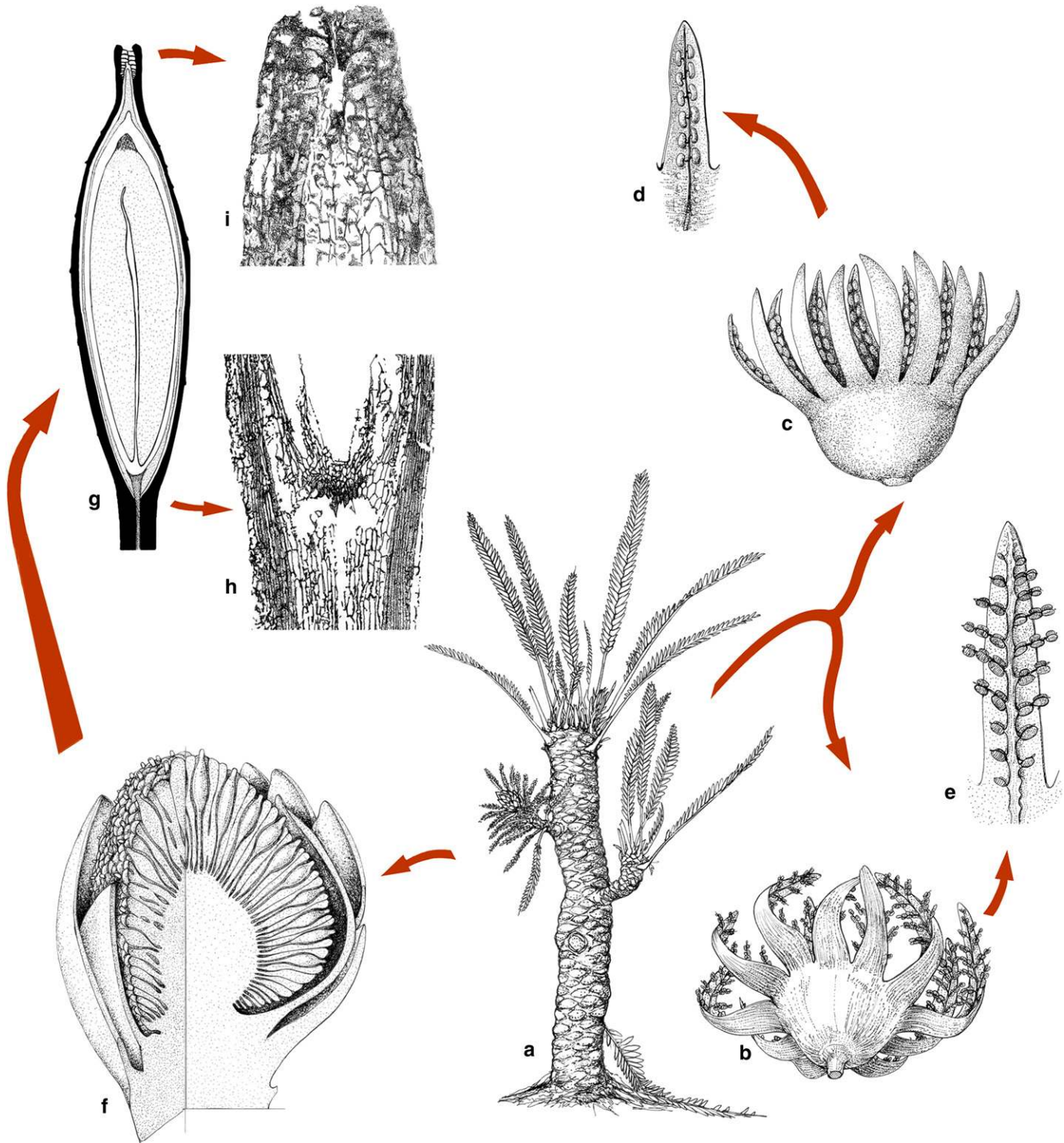


Fig. 2. *Williamsonia* spp. (a) Plant habit of sparsely branched pachycaulis stem with crowns of pinnate fronds and terminal cone (at left). Redrawn after Sahni (1932). (b, c) Two *Weltrichia* pollen cones illustrating variability in this morphotaxon. (b) *Weltrichia spectabilis* pollen cone showing pinnate microsporophylls with synangia (redrawn from Thomas, 1915). (c) *Weltrichia whitbiensis* pollen cone with linear microsporophylls having only two rows of synangia on abaxial surface (redrawn from Nathorst, 1911). (d) Single microsporophyll from cone in Fig. 2c as seen from abaxial surface illustrating pinnate nature of microsporophylls. (e) Enlargement of single microsporophyll from cone in Fig. 2b as seen from abaxial surface illustrating pinnate nature of microsporophylls. (f) Cut away reconstruction of a seed cone (*Williamsonia harrisiana*) showing bracts enclosing ovulate receptacle bearing sporophylls with terminal seeds interspersed with interseminal scales. (g) Longitudinal section of mature seed showing cotyledonary embryo (stippled) within megagametophyte tissue (white), surrounded by nucellus (also stippled) that extends into base of micropylar canal as a plug of tissue, within multicellular integument (black with inner epidermal cells drawn at apex). Note inner epidermis of integument consists of radially elongated cells that partly occlude micropylar canal distal to nucellar plug. (h) Longitudinal section at base of seed showing nucellus separating from multicellular integument at chalaza. (i) Longitudinal section at seed apex showing features of nucellar apex and multicellular integument in immature ovule.

Crepet and Delevoryas, 1972; Crepet, 1974; Nishida, 1994; Rothwell and Stockey, 2002; Stockey and Rothwell, 2003), reviving the century-old debate about the structure of bennettitalean seeds and stimulating a reevaluation of the viability of the anthophyte hypothesis (Friis et al., 2007).

In the current study, we have conducted a detailed review of bennettitalean morphology and anatomy to address the continuing controversy regarding the reality and nature of the anthophytes (Donoghue and Doyle, 2000; Burleigh and Mathews, 2004), to assess the possibly relevant evidence based on recent interpretations of dispersed fossil seeds (Friis et al., 2007), and to provide a more reliable foundation for testing the various hypotheses of bennettitalean relationships. We begin by summarizing well-known and agreed-upon information about the vegetative and reproductive structures of Bennettitales, and then clarify bennettitalean cone and seed structure through a reexamination of classical preparations of anatomically preserved specimens and new information. The latter is derived from closely spaced serial peel preparations of recently discovered specimens from the Apple Bay and other localities on Vancouver Island, British Columbia, Canada that show superb cellular preservation. In the context of the anthophyte debate, we place special emphasis on the morphology and anatomy of seeds and the associated structures borne on bennettitalean ovulate receptacles. We use the resulting information to execute detailed comparisons of bennettitalean cones and seeds to similar organs and tissues of Gnetales and to an array of dispersed charcoalified fossil seeds (hereafter referred to in this paper as “charcoalified seeds”) described by Friis and Pedersen (1996), Rydin (2005), Friis et al. (2007), and Mendes et al. (2008).

The newly developed data have been added to several different morphological character matrices of seed plants for the purpose of conducting comparative numerical cladistic analyses aimed at further resolving relationships among Bennettitales, Gnetales, flowering plants, and related clades. The various results are evaluated in relation to competing hypotheses of seed plant phylogeny to assess the possible role of Bennettitales in the origin of flowering plants and the viability of the classical anthophyte hypothesis (Appendix 1; Arber and Parkin, 1907; Doyle and Donoghue, 1987).

MATERIALS AND METHODS

Material upon which this study is based consists of abundant and excellently preserved specimens of extinct plants that are known from external morphology, cuticular features, and internal anatomical structure (e.g., Figs. 1, 2). Because of additional levels of uncertainty that are introduced by potentially controversial interpretations of less completely preserved and fragmentary fossils of debatable structure and affinities (e.g., some species of permineralized axes assigned to *Bucklandia* Presl, and the compression genera *Bennetticarpus* Harris and *Vardekloeftia* Harris), the latter are not included. Emphasis is placed on anatomically preserved reproductive structures that are represented by large numbers of ground thin sections and cellulose acetate peel preparations of cones that were studied previously by Wieland, Delevoryas, Crepet, Nishida, Rothwell and Stockey, and others. Information from those specimens has been augmented by several recently discovered cones within carbonate marine nodules from Cretaceous deposits of western Canada and by comparisons to previously published fossils from Europe, India, South America, and Japan. Collecting localities, stratigraphy, and preparation techniques for the new material are as presented by Rothwell and Stockey (2002) and Stockey and Rothwell (2003).

To assess the phylogenetic significance of bennettitalean features in as objective a fashion as possible and to test various hypotheses for relationships among living and fossil seed plants, we analyzed five separate morphology/structure data sets after modifying some of them to include pertinent taxa and recoding some to reflect our findings. The particulars of the modifications for

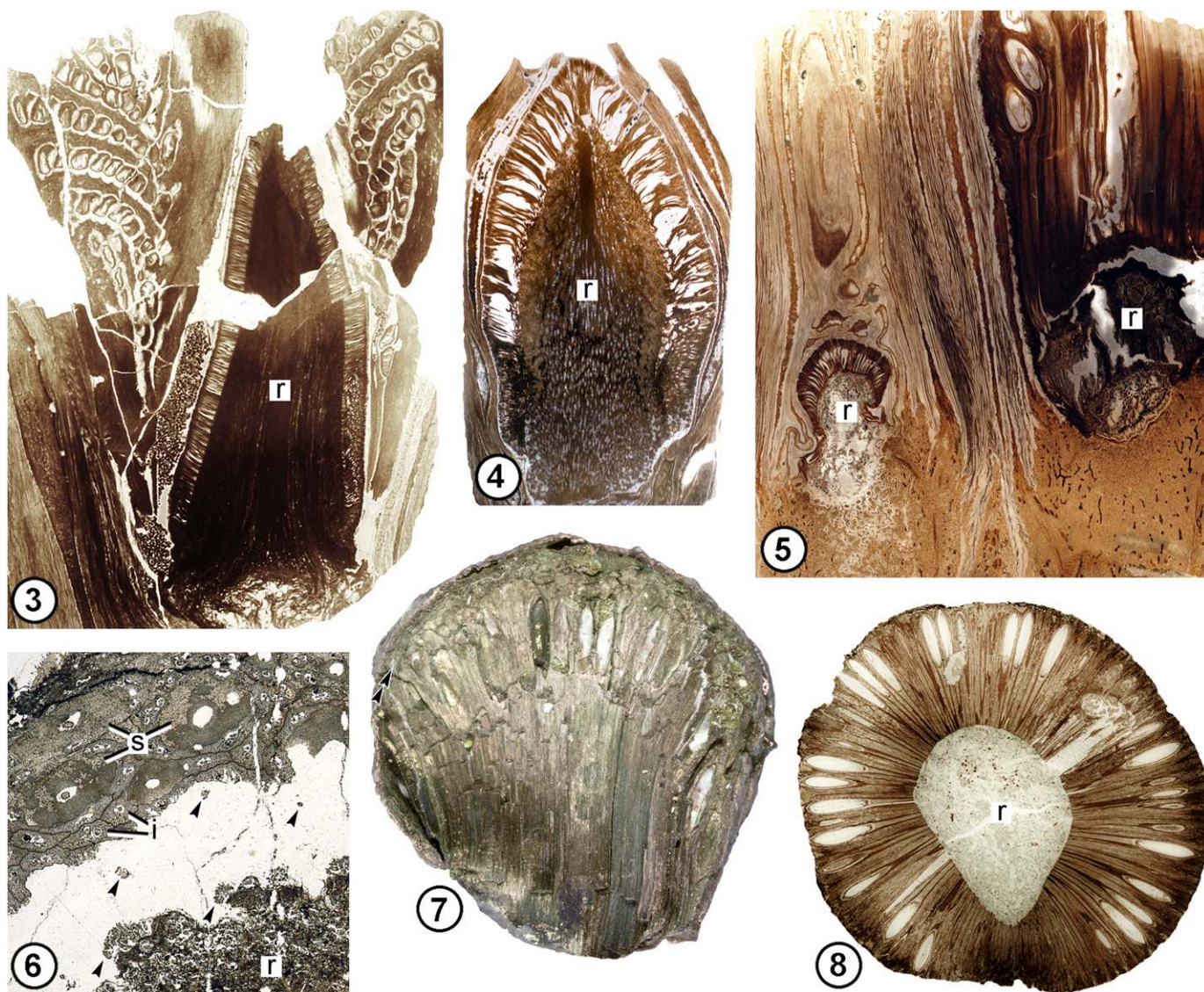
each data set are explained with the results for each analysis. In this study, we used the representative data matrices of Rothwell and Serbet (1994) and Hilton and Bateman (2006). The latter is heavily based upon one matrix of Doyle (i.e., Doyle, 1996). We also combined both matrices and removed evident redundancies in an effort to minimize the effects of subjective character identification and coding that affect all morphological structural data bases by reflecting the biases of their authors (analysis 3). We added the Friis et al. (2007) taxon “charcoalified seeds” to all matrices following the character codings of Friis et al. (2007). The composite taxon Erdtmanithecales (Friis and Pedersen, 1996; Friis et al., 2007) was included in some matrices (i.e., analyses 1 and 5) and excluded from others (i.e., analyses 2–4). Representative genera of the Erdtmanithecales construct were included in one matrix (i.e., analysis 4) to test the effects of introducing uncertainties surrounding composite taxa made up from different organs of different ages from different localities, as explained by Gandolfo et al. (1997). Also, following reasoning put forth by Hilton and Bateman (2006, p. 124; i.e., because of “...critical ambiguities in its reconstruction.”), we did not include the taxon *Piroconites* Gothan in the analyses.

Methods of matrix development are described along with the results of various systematic analyses and in the discussion section of the paper, and follow the rationale presented by Rothwell and Nixon (2006). All maximum parsimony phylogenetic analyses (heuristic search; maximum number of trees to keep = 5 000 000; number of replications = 400) were performed using the program NONA (Goloboff, 1999), spawned through the program Winclada (Asado, version 1.1 beta, by K. Nixon, Cornell University). Characters were all unweighted and unordered. For the reasons explained by Rothwell and Nixon (2006), we have intentionally omitted support values from the nodes of all trees (Figs. 28–32). The character matrices for analysis 1–5 (Figs. 28–32) are included in the appendices in the online Supplemental Data with the online version of this article.

MORPHOLOGY AND ANATOMY OF BENNETTITALES

Species of bennettitalean plants display pachycaulis or slender trunks with a crown of fronds that are either pinnate or transitional to spatulate with pinnate venation (Figs. 1, 2). Some trunks branch most often in an apparent dichotomous fashion, and many species have persistent leaf bases (Wieland, 1906; Sahni, 1932). Branching is infrequent in *Cycadeoidea* (Fig. 1), *Williamsonia* (Fig. 2), and similar genera. Internally, the stems are eustelic with a large pith, broad cortex, and a relatively narrow zone of dense wood (Wieland, 1916; Taylor and Taylor, 1993; Saiki and Yoshida, 1999). Secondary xylem is constructed of slender scalariform (or less often pitted) tracheids and uniseriate or biseriate rays, and the ground tissues contain elongate resin canals (Wieland, 1916; Taylor and Taylor, 1993; Saiki and Yoshida, 1999). Seeds and microsporangia are produced in bisporangiate or monosporangiate simple cones enclosed by bracts (Figs. 1–5). Microsporophylls are either clearly pinnate or apparently pinnately derived (Figs. 2b–2e), or fleshy (Figs. 1b, 3), usually bearing microsporangia in bivalved synangia (Fig. 1e, Appendix 2; Crepet, 1974) that produce monosulcate pollen with granular wall structure (Osborn and Taylor, 1995). Ovules are orthotropous, terminal on narrow stalks that represent sporophylls, and are interspersed with interseminal scales on fleshy receptacles of various shapes and sizes (Figs. 1d, 1g, 2f, 2g, 3–5, 8, 12–14, 23). Such receptacles represent fertile stem tips with determinate growth.

For the purposes of interpreting seed plant systematics and inferring phylogenetic relationships, it is important to focus on key characters that have traditionally defined Bennettitales vs. Cycadales vs. Gnetales in morphological phylogenetic analyses (e.g., Tables 1, 2). There is more variability within certain of these characters revealed in modern data than has traditionally been widely recognized, and there are new relevant fossils that improve our understanding of these characters and their distribution in extinct taxa. Such characters are discussed below.



Figs. 3–8. Anatomically preserved cones of *Cycadeoidea* (Figs. 3–7) and *Williamsonia* in section views. **3.** Longitudinal section (LS) of *C. dacotensis*; microsporophylls are well developed with mature pollen, sporophylls are short with tiny ovules, and interseminal scales remain small and immature. Yale Trunk 214, Wieland Slide 360 $\times 2.6$. **4.** LS of *Cycadeoidea* sp. showing features of somewhat more mature cone than in Fig. 3, in which microsporophylls have matured and disintegrated, conical receptacle is expanding, and there are more elongate seeds and interseminal scales. Yale Trunk 807, Crepet Slide NS 160 $\times 2.5$. **5.** LSs of two cones of *C. wielandi* embedded among leaf bases on trunk surface. Note bisporangiate cones with much smaller, more dome-shaped, ovulate receptacles than *C. dacotensis*. Microsporophylls have matured and disintegrated in both cones and are at significantly different developmental stages. Smaller cone is comparable in developmental stage to conical ovulate receptacle in Fig. 3, indicated by size and maturity of ovules and interseminal scales; larger cone mature, showing very elongated sporophylls with large terminal seeds. Yale Trunk 77, Crepet slide 77.3a. **6.** Cross section of *C. maccafferyi* near base of cone showing traces (at arrowheads) diverging from receptacle (r), and both bases of interseminal scales (i) and sporophylls (s) near their bases. BO 211 Bot #24 $\times 8$. **7.** External view of mature *C. maccafferyi* megasporophylls and interspersed interseminal scales as seen from split surface of cone. VIPM 118 $\times 0.9$. **8.** Oblique section of *Williamsonia bockii* showing relatively mature sporophylls with terminal seeds and interseminal scales radiating from receptacle. VIPM 123 #61 $\times 1.3$.

Secondary xylem tracheary pitting—One of the frequently cited characters separating cycads from Bennettitales is the common presence of scalariform secondary wall thickenings on tracheids in the secondary xylem of the Bennettitales in contrast to the general view that cycads have circular bordered pits in their secondary xylem (e.g., Doyle, 1996; Table 2). To put this character in the perspective of the angiosperms, “scalariform tracheids in secondary xylem” is not considered to be typical of all the so-called angiosperms. Gnetales typically have circular

bordered pits (Table 1), while scalariform thickenings characterize the secondary xylem of many taxa of angiosperms. However, there is more variation in pitting of both angiosperms and gnetophytes than is commonly recognized (Muhammad and Sattler, 1982). Although cycads are generally regarded as having secondary xylem tracheids with circular bordered pits, this is not always the case (Wieland, 1934). Secondary xylem in several taxa of cycads (e.g., *Microcycas* A. DC.; Greguss, 1968) has scalariform thickenings and an accurate coding of secondary

TABLE 1. Contrasting characters of Bennettitales and Gnetales

Character	Bennettitales	Gnetales
Unequivocal axillary branching	?	Present
Pitting of tracheary elements	Circular bordered/ scalariform	Circular bordered pits
Vessels in wood	Absent	Present
Leaf arrangement	Helical	Opposite
Leaf form	Pinnate (simple)	Non-pinnate
Dichotomous leaf venation	Absent	Present
Sheathing leaf bases	Absent	Present
Origin of leaf trace bundles	Variable (1-several)	Two
Rachis vascular configuration	Omega pattern?	Non-omega pattern
Cone complexity	Simple	Compound
Ovules borne on	Sporophyll tip	Stem tip
Ovules enclosed by bracteoles	Absent	Present
Nucellus free from integument	From chalaza	From midregion (variable)
Pollen chamber	Absent	Present
Biseriate tubular micropyle	Absent	Present
Micropyle closure	By nucellar plug	By ingrowth of cells

xylem characters in cycads requires that that condition be scored as such. One can either code cycads as variable for tracheid secondary wall pitting or break the cycads down into groups of taxa (as in Stevenson, 1992) that can be coded accurately.

While Wieland claimed that bennettitalean wood is characterized by scalariform tracheids, he also noted (Wieland, 1906; p. 75) that perhaps “in further” sections pitting might conform with Lignier’s observations of *Cycadeoidea micromyela* Moriere wood in which pitting was “cross-slitted” like “*Araucaria* and *Cordaites newberryi*.” That supposition has been verified in more recent studies of bennettitalean stems (e.g., *Bucklandia kerae* Saiki and Yoshida, 1999).

Cone structure—The so-called bennettitalean “flowers” are compact monosporangiate or bisporangiate cones that consist of a short axis (i.e., a “receptacle”) with determinate growth and that produces helically arranged bracts below the fertile zone (Figs. 1–8). Bisporangiate cones have more-or-less pinnate or variously fleshy microsporophylls attached toward the base of a squat or conical ovulate receptacle. More distally, the receptacle bears erect seeds (i.e., seeds that are terminal on a sporophyll) interspersed among sterile interseminal scales (Figs. 1b, 1d, 1e, 3–5, 7, 8, 17; Appendix 2). The axis has a large pith delimited by a eustele (Fig. 20; Andrews, 1943; Sharma, 1973) from which traces diverge in either a clearly helical arrangement or else are so closely spaced that it is impossible to determine if the traces are helical or whorled (Fig. 9, near top). Several traces enter each bract and microsporophyll. In the ovu-

TABLE 2. Contrasting characters of Bennettitales and Cycadales

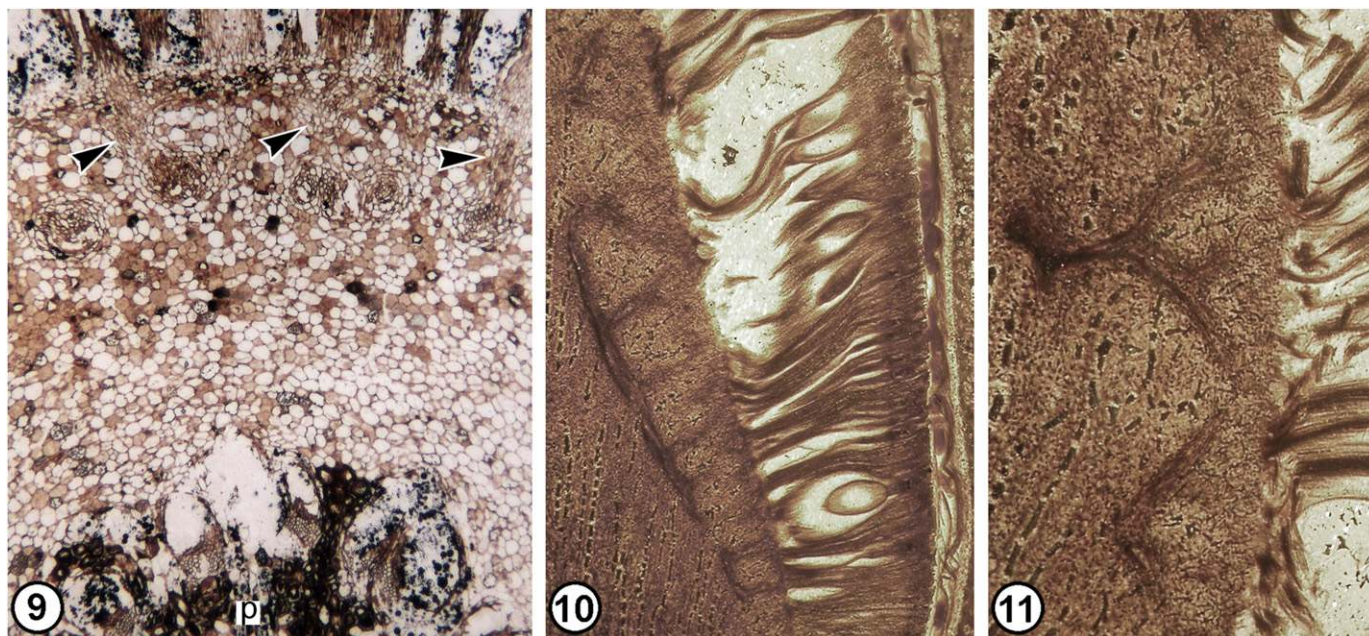
Character	Bennettitales	Cycadales
Girdling leaf traces	Absent	Present
Pitting	Circular bordered/ scalariform	Circular bordered
Stomata	Syndetocheilic	Haplocheilic
Bisporangiate cones in some	Present	Absent
Perianth of bracts	Present	Absent
Microsporangia borne	Adaxial?	Abaxial
Microsporangia	Synangiate	Not synangiate
Nucellus separates from integument	At base	In midregion

late receptacle, eustelar bundles sometimes appear to be reflexed. In such cases, traces to seed stalks and interseminal scales emanate from the reflexed portions of these vascular bundles. Traces to seed stalks and interseminal scales from the stele of the ovulate receptacle typically branch and fuse in the cortex (Figs. 9–11), with a single terete trace entering each ovule-bearing sporophyll and interseminal scale (Figs. 9, 23; Sharma, 1973). We wish to stress that traces to seed stalks (i.e., sporophylls) and interseminal scales are virtually identical, such that the appendage vascularized by any given trace cannot be determined until it actually enters an appendage. Traces that diverge from sympodia of the receptacular eustele (Fig. 20) are collateral bundles that rotate within the ground tissue of the receptacle such that the orientation of xylem and phloem vary from bundle to bundle (Fig. 21).

All of the appendages (i.e., enclosing bracts, microsporophylls, interseminal scales and seed-bearing stalks) diverge directly from the axis or ovulate receptacle (Figs. 10, 17), none arising from another or being located in the axil of another (Figs. 18, 19). Although the seeds are nearly completely surrounded by and variously enclosed by interseminal scales (Figs. 7, 8, 12–19), extremely well-preserved cones (e.g., specimens in Figs. 6, 8, 18) verify that there is no evidence of a specialized “cupule” or other additional enclosing structures (Figs. 15–19). This last point is in agreement with the recent interpretations of Rothwell and Stockey (2002), Stockey and Rothwell (2003), and Friis et al. (2007) and is addressed more completely in the discussion and phylogenetics sections of this paper.

All these characters are consistent with the interpretation that bennettitalean reproductive structures are simple cones wherein both microsporangiate and megasporangiate structures are foliar in nature (i.e., sporophylls). Bracts that enclose the fertile organs (Figs. 1b, 1c, 2f, 5) are linear and display several vascular bundles above the base. Interseminal scales are radial in construction, columnar in shape, and polygonal in cross section (Figs. 18, 19). They display a terete xylem strand (Figs. 15; 16, at arrowheads) that shows no evidence of a maturation pattern. In some interseminal scales, the vascular bundle divides in two (Fig. 15, upper left arrowheads). The xylem is surrounded by a combination of parenchymatous and sclerenchymatous ground tissue (Figs. 15, 16, 18, 19) that sometimes displays resin canals. It is not known whether the interseminal scale bundle is collateral or radial. Except when fused to an adjacent structure, there is a distinct cutinized epidermis at the periphery of each interseminal scale (Figs. 19, 26).

Microsporophylls/synangial/sporangial position—There are a number of undoubted bennettitalean microsporophylls known from direct attachment to reproductive branches, as components of bisporangiate cones, or that have clear synapomorphies of Bennettitales (Wieland, 1906, 1916; Delevoryas, 1965, 1968a). These vary in structure and in themselves raise some interesting questions about sporangial position, microsporophyll homologies, etc. (Figs. 1, 2). There are also a number of compressed morphotaxa that have been assigned to Bennettitales (e.g., *Leguminanthus* Kräusel and Schaarschmidt, 1966) or considered as immediately relevant to their systematic position (e.g., *Piroconites*), that have sometimes been included in phylogenetic analyses of Bennettitales (e.g., Crane, 1988), but their affinities are somewhat problematical. They often are relatively poorly preserved, difficult to interpret and several do not appear to have synapomorphies that allow an unequivocal assignment to Bennettitales (e.g., *Leguminanthus*). These fossils



Figs. 9–11. Vascular architecture of ovulate bennettitalean receptacles illustrated by immature cones. **9.** Cross section of *Williamsonia* sp. showing cauline bundles of eustele surrounding pith (p) at bottom, and ring of traces producing anastomosing bundles (at arrowheads) to seed sporophylls and interseminal scales near top. (UAPC-ALTA) P14653 D, Side #35 $\times 40$. **10.** Longitudinal section of *Cycadeoidea* sp. showing traces near periphery of ovulate receptacle from which anastomosing bundles are diverging to ovulate sporophylls and interseminal scales (note only one trace enters each). Crepet slide 74.2 $\times 27$. **11.** Receptacle of *Cycadeoidea* sp. showing anastomosing traces extending toward bases of ovulate sporophylls and interseminal scales. Crepet slide 74.2 $\times 45$.

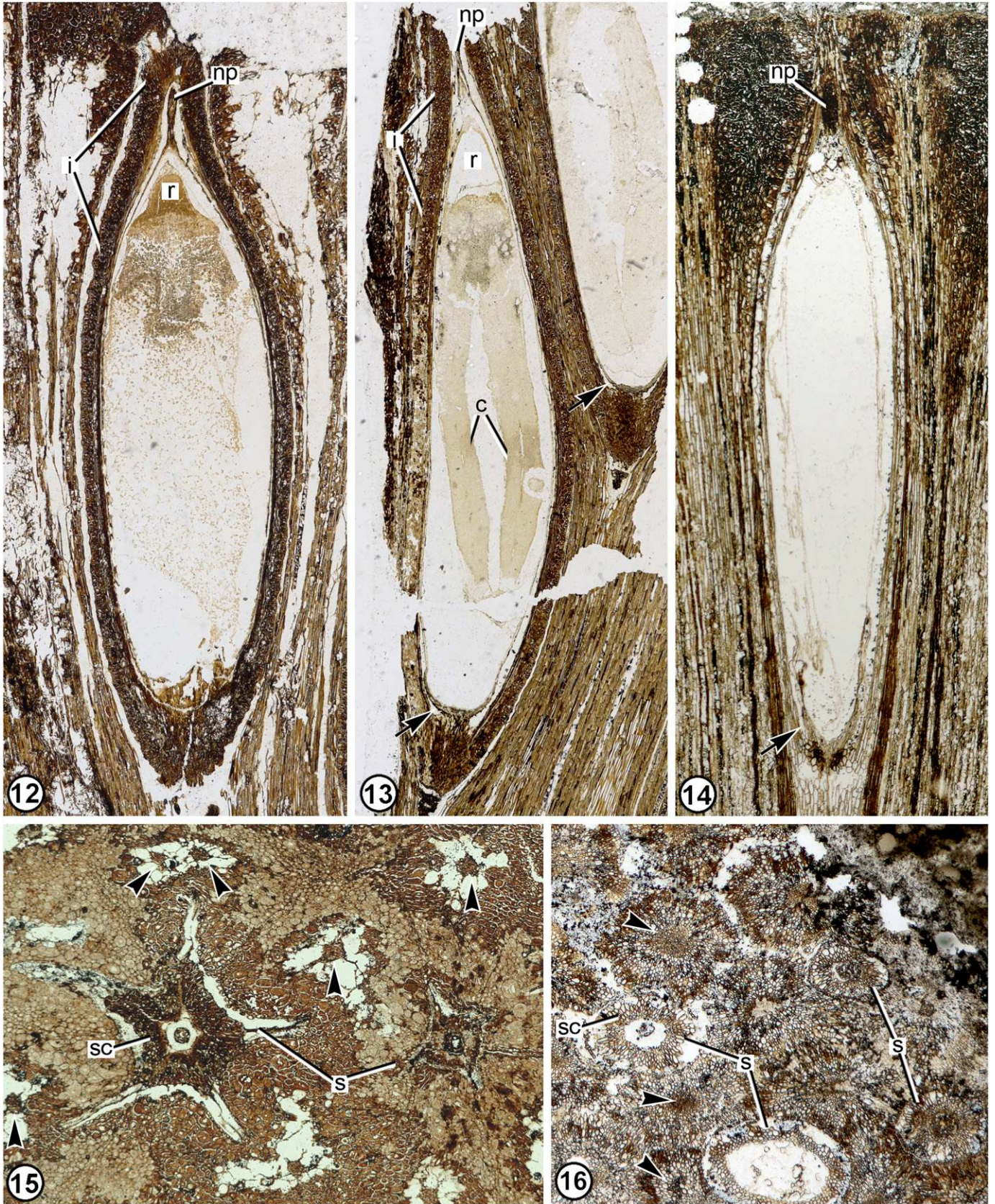
have complicated the understanding of bennettitalean microsporophylls when encoded for phylogenetic analysis in a way that introduces character states presumed present because of assumed affinities with Bennettitales. Some of these morphotaxa comprise genera with remarkably divergent characters. Such fossils are discussed more fully in Appendix 2.

Ovules and seeds—Bennettitalean seeds and their associated structures have been the object of considerable discussion since the time of Lignier (1894), especially in the context of possible links between Bennettitales and Gnetales. Among Bennettitales, the seeds of *Cycadeoidea*, *Williamsonia*, and *Cycadeoidella* vary from one another in several details, but anatomically preserved specimens of excellent preservation reveal that all have a similar basic construction. Seeds are erect and terminate a narrow sporophyll (Figs. 1, 2, 5, 7, 8, 10, 17, 23) that consists of a terete xylem strand that is surrounded by several layers of thin-walled cells (Figs. 2f, 6). In cross sections, the seeds are radial, being more-or-less circular in *Williamsonia* (Figs. 16, 18, 24–26; summarized by Stockey and Rothwell, 2003) and *Cycadeoidella* (Nishida, 1994), and ranging from angular to stellate (Fig. 15) at different levels in *Cycadeoidea* (= *Bennettites* Carruthers of some workers;

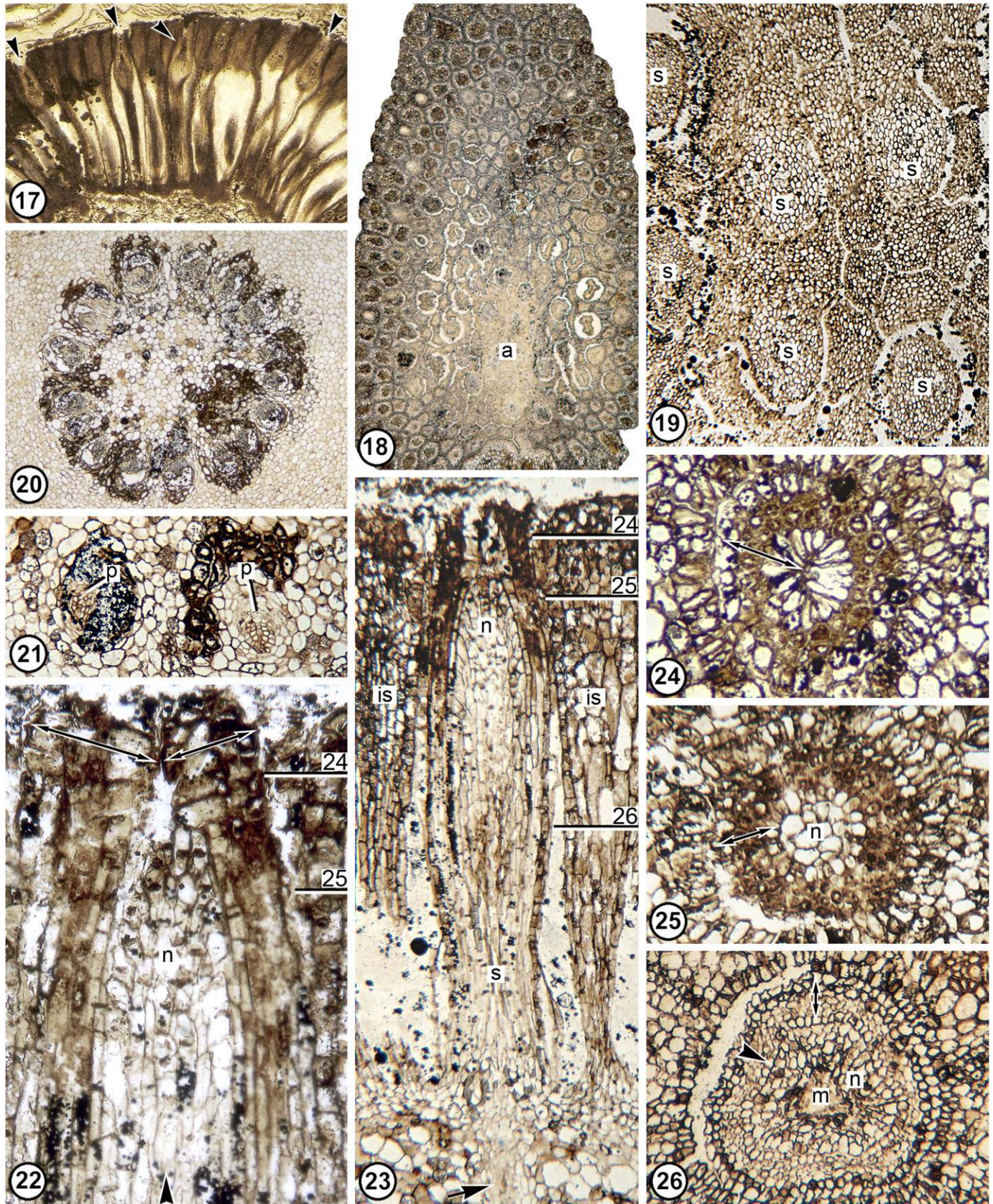
summarized by Rothwell and Stockey, 2002). The seeds of most species taper to a narrow, round micropylar opening (e.g., Fig. 24), which depending on the species, may or may not protrude above the tips of the surrounding interseminal scales (Figs. 2f, 8, 10, 12–14, 17, 23). In contrast to some gnetalean seeds (e.g., Berridge, 1911), excellently preserved permineralized bennettitalean seeds show no evidence of cellular proliferations or secondary cell divisions that occlude the micropyles of mature specimens.

There is a single multilayered integument that is unvascularized and that is differentiated into several types of cells (Figs. 12–16, 22–24). The inner epidermis of the integument typically consists of distinctive radially elongated cells near the tip of the micropylar canal (Fig. 1g, 2g, 2i, 22–24), but cells of that layer are isodiametric at more proximal levels (Figs. 22, 23, 25). The nucellus separates from the integument at the base of the seed (Figs. 1g, 2g, 2h; 13, at arrows; 14, at arrow) and is vascularized by a cup-shaped bundle of tracheids that terminates the vascular bundle of the stalk. Distally, the apex of the nucellus forms a cylindrical- or conical-shaped parenchymatous plug (Figs. 1g, 2i, 22, 23, 25). In both immature and full-sized seeds, the nucellar plug is typically tightly inserted into the base of the micropylar canal (Figs. 12–14, 22, 23). In contrast to Gnetales,

Figs. 12–16. Bennettitalean seeds and interseminal scales. **12.** Longitudinal section (LS) of mature *Cycadeoidea maccafferyi* seed among interseminal scales, showing multicellular integument (i) surrounding nucellus with nucellar plug (np) at apex, and embryo with radicle (r) oriented toward micropyle. BO 211 Side #99 $\times 10$. **13.** LS of mature *C. maccafferyi* seeds among interseminal scales. Note separation of nucellus from integument at chalaza (arrows), multicellular integument (i), nucellar plug in base of micropylar canal (np), and embryo with radicle (r) and cotyledons (c). EMB 052 #22 $\times 10$. **14.** LS of *Williamsonia bockii* seed among interseminal scales, showing integument surrounding nucellus that separates at chalaza (at arrow), and with nucellar plug (np) at apex. VIPM 123 Side #68 $\times 16$. **15.** Cross section of *C. maccafferyi* seeds (s) near apex, and interseminal scales (at arrowheads), showing radiating ribs of multicellular integument surrounding micropyle and sclerotesta (sc). Cellular nucellar plug occupies micropyle of seed at left. 1884 A top #9 $\times 14$.



16. Cross section of *W. bockii* seeds (s), and interseminal scales (at arrowheads), showing circular shape of multicellular integument at midregion (bottom) and closer to apex (at right). Cellular nucellar plug occupies micropyles of seeds at right. Sclerotesta of one seed labeled “sc”. BL 123A #32 ×20.



Figs. 17–26. Bennettitalean cones. **17.** Longitudinal section of receptacle of *Cycadeoidea* sp. showing sporophylls and interseminal scales at surface of receptacle from cone at left in Fig. 5. Because of shrinkage it is easy to see that sporophylls bearing terminal ovules (at arrowheads) and interseminal scales

and nearly all other gymnospermous seed plants, there is no pollen chamber. Within the nucellus, many bennettitalean specimens preserve both tissue of the megagametophyte (e.g., fig. 4D of Stockey and Rothwell, 2003) and an embryo that is dicotyledonous at maturity (Figs. 12, 13). As is also the case for Gnetales and many flowering plants, the megaspore membrane is either extremely thin or not preserved at all in bennettitalean seeds (e.g., Fig. 26).

This relatively simple, but distinctive seed structure is clearly recognized from well preserved permineralized seeds (e.g., Figs. 13, 14), and from immature ovules in which specialized cells of the integument have not yet differentiated (Figs. 17–26) and the nucellus fills the seed cavity below the micropyle (Figs. 1g, 22). In some ovules, meiosis has yet to occur or forms a linear tetrad (Fig. 22, see the possible example at arrowhead; Crepet and Delevoryas, 1972), whereas in others, there is an area at the center of the nucellus that represents the megagametophyte at a stage before cellularization has occurred (Fig. 26, at m).

COMPARISON OF BENNETTITALEAN AND GNETALEAN SEEDS

Seeds and seed characters are included among the features historically thought (by some researchers) to link Bennettitales to Gnetales within an “anthophyte” clade. As described above, however, there are numerous characters that separate seeds of the two clades (Table 1). Recent discoveries of dispersed fossil seeds (Friis et al., 2007) have put an even sharper focus on seed characters and their potential relevance to anthophyte relationships. The extended micropyle is a prominent characteristic of gnetalean seeds, of some species of Bennettitales, and of a few other gymnospermous clades including corystosperms and peltasperms. However, the extended micropylar structures of Gnetales and some Bennettitales occupy different positions with respect to both the outside of seed and internal tissues, and are structurally quite different from each other (Fig. 27). Whereas the micropylar tube of bennettitalean seeds is formed by a multizoned integument, which is the outermost tissue of the seed (Fig. 27a), in Gnetales the thin tubular micropyle of the integument is enclosed by either one, (i.e., *Ephedra*, *Welwitschia* Hook. f., and several fossil seeds recently described by Rydin, 2005; Rydin et al., 2006; and Friis et al., 2007), or two (i.e., *Gnetum* L.) additional (i.e., extra-integumentary) envelopes of heterogeneous cellular construction that are widely regarded as

being formed from fused bracteoles (Bierhorst, 1971; Rydin, 2005; Fig. 27b).

In Bennettitales the multiseriate integument is much thicker than the typically biseriata (i.e., two cell-layered) integument sensu stricto of Gnetales. Moreover, cells of the bennettitalean integument are differentially differentiated (Figs. 12–15, 24, 27a), whereas those of the Gnetalean integument are not (Fig. 27b). Even when the micropyle of gnetalean seeds does consist of more than two cell layers (e.g., *Gnetum* sp., fig. 17 of Thompson, 1916), cells surrounding the inner epidermis are uniformly thin walled in contrast to the heterogeneously differentiated cells of bennettitalean seed integuments (Figs. 12–16, 27). Perhaps the alternative interpretation of bennettitalean seed structure recently offered by Friis et al. (2007) has resulted from those authors consulting old-ground thin sections of incompletely preserved permineralized specimens, rather than the better preserved specimens described by Rothwell and Stockey (2002), Stockey and Rothwell (2003) and figured in this paper.

The integument of Bennettitales often has an outer zone of thin-walled cells that consists of either a continuous epidermis (e.g., *Cycadeoidella* as interpreted herein; fig. 14 of Nishida, 1994), a sheath of tubular cells (e.g., *Cycadeoidea* [= *Bennettites*], Wieland, 1906; Stopes, 1918; figs. 14–16, 22 of Rothwell and Stockey, 2002) or peg-like projections (e.g., *Williamsonia*; figs. 3D, 4A of Stockey and Rothwell, 2003), and a middle zone of thick-walled cells (i.e., sclerotesta = most obvious zone marked as “i” in Figs. 12–13; arrow in Fig. 15). There is also an inner layer of large thin-walled cells that form a distinctive radiating ring in apical cross sections (Figs. 22, 24) and that often fills most of the apex of the micropylar canal distal to the tip of the nucellus (Figs. 1g, 2i, 22–24). In the midregion of bennettitalean ovules, that layer occupies the position of an inner epidermis (Fig. 26, at black arrowhead). In young ovules of *Cycadeoidea* and *Williamsonia*, all the integumentary cell layers are parenchymatous (e.g., Fig. 1g), but in the more mature seeds they often differentiate into sclerenchyma (Figs. 15, 16 at “sc”) and may contain resin canals (e.g., Stopes, 1918). In Bennettitales the integumentary tissue formed by these layers is free from the nucellus above the chalaza (Fig. 27a, at arrow) whereas in Gnetales the nucellus is fused to the integument for varying distances between the chalaza and the pollen chamber (Fig. 27b; Chamberlain, 1935).

The micropyle of Bennettitalean seeds is variable with respect to the degree of elongation. In some taxa it is relatively elongated and narrow at the tip (e.g., *Williamsonia*). In others it

←

all diverge from receptacle at same level. Yale Trunk 77, Crepet slide 77.3a × 10. 18–26. Immature cone of *Williamsonia* sp. (UAPC-ALTA specimen P14653) from Lower Cretaceous of Apple Bay, Vancouver Island, BC. **18.** Tangential section showing axis at bottom center, and progressively more distal levels of ovulate sporophylls, ovules, and interseminal scales in successively more peripheral positions (a = axis or receptacle of cone). D Top #38 × 12. **19.** Tangential section at slightly more peripheral level than area around “a” in Fig. 18, showing incomplete separation of sporophylls (s) and interseminal scales (unlabeled) from cortex and revealing that all diverge at same level. D Top #36 × 60. **20.** Cross section of axis at base of receptacle showing eustele surrounding pith, and parenchymatous cortex. D₁ Side #23 × 35. **21.** Cross section of cone axis showing two traces in cortex, each a collateral bundle, with bundles rotated such that phloem is oriented in different directions. D₁ Side #45 × 80. **22.** Longitudinal section of immature ovule with possible linear tetrad of megaspores near base of nucellus (at arrowhead), nucellus (n) with solid cellular apex, and multicellular integument (double-headed arrows) with radially elongated inner epidermal cells distal to nucellus. Numbered lines correspond to levels at which sections in figures of same number were made. D₁ Side # 53 × 160. **23.** Cross section of cone at margin of axis (at bottom) showing one ovule-bearing sporophyll (s) and two adjacent interseminal scales (is) in longitudinal section. Note multicellular integument surrounding nucellus with solid cellular apex (n). Numbered lines correspond to levels at which sections in figures of same number were made. D₁ Side # 40 × 85. **24.** Cross section of ovule near apex, surrounded by interseminal scales. Note multicellular nature of integument (double-headed arrow) and radially elongated cells of inner integumentary epidermis. Section taken at level numbered 24 in Figs. 22, 23. D Top # 15 × 150. **25.** Cross section of ovule slightly more proximal than Fig. 24 through level where nucellar plug (n) occludes base of micropylar canal. Thickness of multicellular integument indicated by double-headed arrow. Section taken at level numbered 25 in Figs. 22, 23. D Top # 10 × 130. **26.** Cross section of ovule at midlevel, where noncellular central area represents megagametophyte (m), surrounded by multicellular nucellus (n), and multicellular integument (double-headed arrow) with distinct inner epidermis (arrowhead). Section taken at level numbered 26 in Fig. 23. D Top # 20 × 100.

is stellate in cross section to near the tip (e.g., *Cycadeoidea*), while in still others it is relatively short and multilayered to the apex (e.g., *Cycadeoidella japonica*; Figs. 14–16 of Nishida, 1994). In contrast, seeds of living and fossil gnetaleans have a tubular micropyle that is usually constructed of only two layers of integumentary cells that are continuous with the nucellus for variable distances toward the apex of the megagametophyte (Land, 1904; Coulter, 1908; Chamberlain, 1935; Maheshwari, 1935; Eames, 1952; Bierhorst, 1971).

In a recent investigation of Cretaceous charcoalified fossil seeds similar to those of Gnetales, Friis et al. (2007) interpreted bennettitalean seeds to be distinctly different from the structure we describe here. According to those authors, the histologically complex outer tissue that we describe as the integument of bennettitalean seeds is an extra-integumentary outer envelope like that of Gnetales (Friis et al., 2007). Friis et al. (2007) interpret the integument *sensu stricto* of bennettitalean seeds to be a thin structure that is extended into a tubular micropyle distally and that is continuous with the nucellus on the inside. According to that interpretation, the nucellus and integument are so intimately fused throughout their lengths that they appear to be a single tissue. Friis et al. (2007) emphasize that the micropylar canal of their fossil charcoalified seeds and some living Gnetales seeds is lined by large cells of the integument that form a distinctive radiating ring apically and that occlude the micropylar canal. That configuration does look remarkably similar to inner integumentary epidermal cells in a similar position of bennettitalean seeds (cf., Fig. 24 with figs. 1CS2 and S1B of Friis et al., 2007; and *Gnetum* sp., fig. 17 of Thompson, 1916). All show the radiating pattern of large cells at the inner margin of the integument that occlude the apex of the micropylar canal. However, careful examination of other structures reveals that bennettitalean seeds are not as similar to living and fossil gnetalean seeds as that apparently distinctive tissue would suggest.

If one follows the inner epidermis of the gnetalean integument downward to the base of the micropylar canal, it becomes continuous with the nucellus somewhat below the region of the pollen chamber (Fig. 27b, at arrow). In contrast, when cells of the distinctive inner epidermal cells of the bennettitalean micropyle are traced downward, that layer is seen to remain free from the interior tissues all the way to the chalaza (Figs. 12–14; 27a, at arrow; Crepet, 1974).

In Gnetales, the distinctive inner integumentary epidermis often is continuous with only one additional integumentary cell layer (Fig. 27b; Chamberlain, 1935; Friis et al., 2007), and even when it consists of more layers, the cells are uniformly parenchymatous (Thompson, 1916), rather than differentiated into the several types of cells that characterize bennettitalean integuments (e.g., Figs. 12–16, 24, 25). There is an outer envelope surrounding the integument of gnetalean seeds that is constructed of fused bracteoles (Fig. 27b), that is not attached to the seed *sensu stricto* above the chalaza (Fig. 27b). By contrast, the single integument is the outermost structure of bennettitalean seeds and the only structure that encloses the nucellus (Figs. 12–16, 22–25, 27a). It consists of several layers of differentiated cells that form a histologically continuous tissue (Figs. 12–16, 22–25, 27a). To the outside of the distinctive inner epidermis in immature bennettitalean ovules the several layers are also undifferentiated and consist of exclusively parenchymatous cells (Figs. 1g, 22, 23, 26), but in more mature ovules such cells are differentiated as a middle zone of sclerotic cells and a thin exterior zone of thinner-walled cells (Figs. 15, 16, 24; also see figs. 22 and 26 of Rothwell and Stockey, 2002).

As emphasized earlier, the outermost covering of bennettitalean seeds (i.e., tissue we regard as integument) is interpreted by Friis et al. (2007) to be an outer envelope like that of gnetalean seeds. However, the distinctive radiating cells of bennettitales are at the inner margin of the multicellular outermost covering, which is the only structure (the integument) surrounding the nucellus (Figs. 1g, 24, 27a), rather than at the inner margin of a thinner, tubular micropylar canal that is itself surrounded by the outermost covering (fused bracteoles), as in Gnetales (Fig. 27b; Thompson, 1916; figs. 1, 2 of Friis et al., 2007). That is to say, there is only one structure surrounding the nucellus in bennettitalean seeds, whereas in gnetalean seeds there are at least two (Fig. 27). These data demonstrate that either the distinctive cells lining the micropylar tubes of Bennettitales and Gnetales are not features of homologous tissues (contrary to the interpretation of Friis et al., 2007), or the outermost coverings of gnetalean and bennettitalean seeds are not homologous structures (contrary to the interpretation of Friis et al., 2007).

The nucellus of bennettitalean seeds is free from the integument above the chalaza, whereas the integument and nucellus of gnetophytes are fused at the base of the seed, separating from each other at varying distances ranging from the midregion to the level of the pollen chamber (cf., arrows in Fig. 27). In bennettitalean seeds, the nucellus is vascularized by a pad of tracheids that arise from a terete trace in the structure upon which the seed is borne (i.e., the sporophyll; see detailed explanation of homologies below), and the integument is typically unvascularized. In contrast, seeds of Gnetales are borne at the tip of a secondary shoot (not on a sporophyll; Table 1; Eames, 1952; Bierhorst, 1971; Gifford and Foster, 1988), and two bundles enter the base of gnetalean seeds from the tip of the secondary shoot (i.e., stem tip; Table 1). The bundles extend distally within the fused integument and nucellar tissues up to nearly the level where the two tissue zones separate. These bundles have traditionally been interpreted to be integumentary in position (Eames, 1952; Bierhorst, 1971), but that view is difficult to confirm because the nucellus and integument are adnate. However, if that interpretation is correct, then the integument of Gnetales is vascularized while the nucellus is not. That is just the opposite of Bennettitales seeds, where the integument is not vascularized, but the nucellus is vascularized.

Tissue to the inside of the integument (as interpreted by us) of bennettitalean seeds surrounds the megagametophyte and embryo and provides additional evidence that seeds of the Bennettitales and Gnetales are of dissimilar construction (Fig. 27). Whereas we interpret the internal tissue of bennettitalean seeds to be nucellus that is free from the integument, Friis et al. (2007) interpreted the same tissue as nucellus and integument that are more intimately fused than in living and fossil Gnetales. In gnetophyte seeds, the apex of the nucellus terminates below the base of the micropylar canal, and breaks down to varying degrees during development to form a pollen chamber (Bierhorst, 1971; Gifford and Foster, 1988). In *Ephedra* it forms a large pollen chamber (Fig. 27b; Thoday and Berridge, 1912; Chamberlain, 1935). In *Gnetum* it forms a much smaller pollen chamber (Berridge, 1911; Thompson, 1916), and in *Welwitschia* tissues of the nucellar apex are disrupted by the growth of pollen tubes and archegonial tubes (Gifford and Foster, 1988).

In all bennettitalean seed specimens that show excellent anatomical preservation, the apex of the nucellus (as interpreted by us) extends distally as a solid cone-shaped or finger-like projection

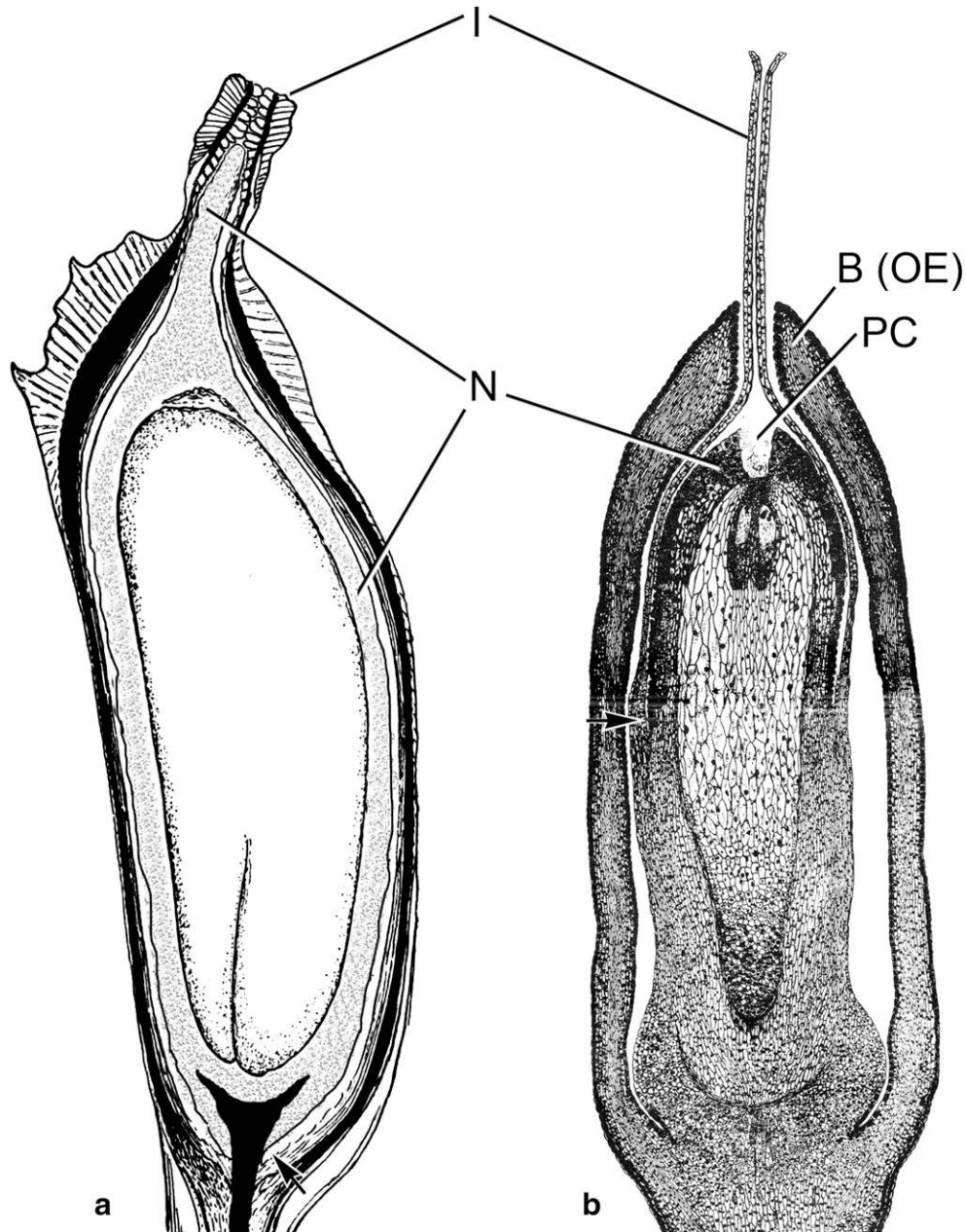


Fig. 27. Diagrammatic representations of (a) bennettitalean and (b) gnetalean seeds in midlongitudinal section to show dramatic structural differences. Fused bracteoles (B = outer envelope [OE] of Friis et al., 2007) surrounding gnetalean seeds are absent from bennettitalean seeds. Integument (I) of bennettitalean seeds is multicellular, while that of gnetalean seeds is biseriate. Nucellus of bennettitalean seeds (N) is free from integument except at chalaza (arrow) and terminates in nucellar plug, while that of gnetaleans is fused to integument up to midregion (arrow), terminates in apical pollen chamber (PC). (a) *Cycadeoidea* seed, modified from Wieland (1906); (b) *Ephedra* seed, modified from fig. 63.2 in Chamberlain (1935).

that plugs the micropylar canal below the level of radiating integumentary cells (Figs. 1g, 2g, 2i, 12–14, 27a; Lignier, 1911; Stopes, 1918; Sharma, 1970; Crepet and Delevoryas, 1972; Crepet, 1974; Nishida, 1994; Rothwell and Stockey, 2002; Stockey and Rothwell, 2003). In contrast to gnetalean seeds, the nucellar apex never breaks down to form a pollen chamber, and there is an apically directed nucellar plug present in bennettitalean full-sized, mature seeds that often shows excellent anatomical preservation (Figs. 12–14, 27a; Rothwell and Stockey, 2002). Although some bennettitalean seeds have been inter-

preted as forming a pollen chamber, those interpretations either are contrary to the structure illustrated (e.g., Fig. 1 of Ohana et al., 1998) or else the interpretation represents an hypothesis for the structure of the nucellus that is based on cuticular envelopes in compression fossils from which internal tissues have been lost and cannot be examined (Harris, 1932; Pedersen et al., 1989b).

The solid rod or cone of tissue that we interpret to be nucellus is tightly inserted into the base of the micropyle in well-preserved, mature bennettitalean seeds (Figs. 1g, 12, 14, 22, 23,

25, 27a). If that tissue does represent nucellus, as interpreted by us, then pollination would have been effected by the growth of pollen tubes (i.e., documented by *Williamsonia bockii*; Stockey and Rothwell, 2003) that extended from pollen grains that were lodged among the radiating cells of the micropyle distal to the nucellar plug (as documented by Sharma, 1970 and others). That mode of pollination compares closely to living conifers of the Araucariaceae, particularly as illustrated by the genus *Agathis* Salisb. (Eames, 1913; Owens et al., 1995a, b; Stockey and Rothwell, 2003). If, on the other hand, the cone of solid tissue in bennettitalean seeds represents intimately fused integument and nucellus, as interpreted by Friis et al. (2007), then there is no micropyle or other integumentary opening to allow for pollination and fertilization.

Ovule enclosing structures—If the Bennettitales is a closely related or sister group to flowering plants, then it is reasonable to expect that bennettitalean cones and seeds may provide evidence for the origin of the double integument and/or carpel or for structures that may have given rise to a second integument and carpel. Early workers (Lignier, 1894; Wieland, 1906; Stopes, 1918) recognized that there is a cup-shaped covering of the seed stalk and integument of *Cycadeoidea* seeds, but disagreed about the nature and significance of that structure. By comparing that structure to the cupule of *Lagenostoma*, Lignier (1894) set the stage for some future workers to interpret Bennettitales as cupulate plants (e.g., Harris, 1932; Crane, 1985, 1986; Doyle and Donoghue, 1986). However, other authors have interpreted the same structures differently. Stopes (1918) provided evidence that the outer covering was originally in organic connection to (or a part of) the seed integument, prompting Rothwell and Stockey (2002) to interpret it as a sarcotesta of tubular cells. It is also possible that these tubular cells of the sarcotesta in *Cycadeoidea* are a dense indument of large trichomes, an interpretation that is consistent with anatomical evidence (e.g., Fig. 1f).

Nishida (1994) recognized a thin, cutinized membrane on both seeds and interseminal scales of *Cycadeoidella japonica* (i.e., figs. 14–17 of Nishida, 1994) and interpreted that structure as a cupule. While that interpretation is plausible, cupules of a similar structure are not known from other bennettitalean fructifications. An alternative interpretation is that the putative cupule represents the cuticle of seeds and interseminal scales in which cells beneath the cuticle are incompletely preserved.

A third category of structure that has been interpreted as a “cupule” in bennettitalean cones is formed by interseminal scales. Many authors have recognized that interseminal scales of bennettitalean fructifications form a ring around each seed (e.g., figs. 16, 17 of Rothwell and Stockey, 2002; fig. 2A of Stockey and Rothwell, 2003). In most examples, the ring consists of what are clearly adjacent interseminal scales (Fig. 19), but in some compressed specimens the surrounding structure appears to be a single entity (i.e., *Bennetticarpus crossospermus* Harris; Crane, 1985). In *Bennetticarpus* Harris, the putative cupule is quite thick, possibly reflecting its origin from several interseminal scales.

Anatomical evidence in support of such an hypothesis possibly could be provided by the cone of *Williamsonia bockii* (Stockey and Rothwell, 2003). In *W. bockii*, adjacent interseminal scales are not separated by a pair of cuticles. Rather, the peripheral cells of adjacent interseminal scales interdigitate, forming the characteristic anatomical fingerprint for postgenital fusion. Although postgenital fusion is a developmental phe-

nomenon widely considered to be a hallmark of angiosperms (e.g., Endress 2001), sections of *W. bockii* reveal that it also occurs in the Bennettitales (Stockey and Rothwell, 2003). While this co-occurrence could be inferred as providing evidence for bennettitalean/flowering plant relationships, the possible distribution of this character in other fossil taxa has not been investigated. Also, there currently is no evidence that the ontogenetic pathway leading to fused interseminal scales in *W. bockii* is the same as for postgenital fusion of angiosperm carpels. Thus, putting too much emphasis on this one character might be premature. In addition, unlike the hypothesized structure of *Bennetticarpus*, all the interseminal scales of *W. bockii* are fused to each other, not just those that surround seeds (fig. 2A, 2B of Stockey and Rothwell, 2003). Despite forming a continuous tissue, in surface views of *W. bockii* the individual interseminal scales and seed micropyles are clearly evident as distinct entities (fig. 8 of Stockey and Rothwell, 2003).

A much thinner cupule has been interpreted as occurring in *Vardekloeftia sulcata* Harris (Harris, 1932; Crane, 1985; Pedersen et al., 1989b), but a more recent interpretation accounts for all of the cuticular layers of *Vardekloeftia* as being derived from the integument and nucellus (Rothwell and Stockey, 2002). More recently still, Friis et al. (2007) also have reinterpreted *Vardekloeftia* as lacking a cupule. At the present time, there is no substantive evidence from bennettitalean fossils for the production of a seed-enclosing structure that is comparable to any of the heterogeneous structures called “cupules” in hydrasperman seed ferns, *Corystospermales*, *Petriellales*, or *Caytoniales* (Rothwell and Serbet, 1994; Hilton and Bateman, 2006; Friis et al., 2007; Stockey and Rothwell, 2009, pp. 323–335 in this issue).

PHYLOGENETIC RELATIONSHIPS OF “ANTHOPHYTES”

Direct examination of classical and recent preparations of anatomically preserved cones has resulted in changes in how we understand certain characters of Bennettitales. A concomitant review of recent and classical literature also reveals more variation in certain homologous characters for the putatively related seed plant taxa Bennettitales and Gnetales than has been reflected in most previous parsimony based analyses (Table 1). To evaluate the potential phylogenetic significance of our findings and to test previous hypotheses of bennettitalean relationships, we conducted a series of phylogenetic analyses.

Analysis 1—We reanalyzed the matrix of Friis et al. (2007) to confirm their results using an alternative program (i.e., NONA, rather than PAUP). Our analysis yielded 1180 most parsimonious trees of 314 steps (CI = 45, RI = 80) with a majority rule consensus topology (not figured) that is roughly equivalent to the 55% majority rule consensus tree figured by Friis et al. (i.e., fig. S2 of Friis et al., 2007). As in the results of the Friis et al. (2007) analysis (strict consensus tree not figured by Friis et al., 2007), the strict consensus tree of our results has a large number of collapsed branches, thus resolving few clades (Fig. 28) and confirming that the general tree topology obtained by Friis et al. (2007) is also obtained using the NONA program. The tree is rooted by the homosporous progymnosperm *Tetrazylopteris* Beck, with successive nodes along the stem including (1) a polytomy of heterosporous progymnosperms; (2) a huge polytomy of seed ferns, cycads, cordaites and conifers,

Pentoxylales, Bennettitales, taxa of the Gnetales, Erdtmanithecales; and (3) *Caytonia* + flowering plants (Fig. 28).

More specifically, in the parsimony-based strict consensus tree based on our analysis, relationships among *Ephedra*, *Welwitschia*, *Gnetum*, the charcoalfied seeds (of Friis et al., 2007), the Erdtmanithecales, and the Bennettitales are all unresolved (arrows in Fig. 28), thereby agreeing with the strict consensus of the most parsimonious trees obtained by Friis et al. (2007) that a clade including Gnetales and Bennettitales is not resolved. Only when parsimony is relaxed by the use of majority-rule consensus is the figured resolution of a clade that includes Bennettitales nested among gnetophytes (i.e., fig. S2 of Friis et al., 2007) approached. Furthermore and as discussed below, it appears that the majority rule consensus may have been misapplied (or over-interpreted) in the Friis et al. (2007) analysis (see explanation below).

Analysis 2—Next, we reanalyzed the matrix of Hilton and Bateman (2006) with the charcoalfied fossil seeds added and coded as in Friis et al. (2007), but with the “Erdtmanithecales” omitted. This allowed us to test what effects the addition of the charcoalfied seeds have on the inferred relationships among Bennettitales and Gnetales without the added variable of an additional new taxon (i.e., Erdtmanithecales).

Our analysis yielded 357 most parsimonious trees of 313 steps (CI = 45, RI = 80) and produced a strict consensus tree that has 23 collapsed branches and better resolution than in the results of analysis 1 (Fig. 29). The topology of this tree is less fully resolved but otherwise roughly equivalent to the results obtained by Hilton and Bateman (2006). Major groups of clades are attached to the stem of the tree in roughly the same order that is found in previous analyses by most authors (Fig. 28; Nixon, et al., 1994; Rothwell and Serbet, 1994; Doyle, 1996; Hilton and Bateman, 2006), and the charcoalfied seeds of Friis et al. (2007) form a polytomy with the extant gnetophytes (Fig. 29, at upper arrow).

The tree is rooted by the homosporous progymnosperm *Tetrazylopteris*, with the polytomies at successive nodes along the stem including (1) heterosporous progymnosperms, (2) a large polytomy containing hydrasperman seed ferns + medullosan seed ferns + higher seed ferns + cycads + cordaites and conifers, and (3) a smaller polytomy containing glossopterids + *Pentoxylon* + gnetophytes (including the charcoalfied seeds of Friis et al., 2007) + a clade consisting of Bennettitales + (*Caytonia* + flowering plants) (Fig. 29). These results contradict the conclusion of Friis et al. (2007) that the addition of the charcoalfied seeds to the matrix of Hilton and Bateman (2006) produces a topology in which Gnetales resolve as a monophyletic group that includes Bennettitales (Fig. 29). In contrast, our results are consistent with the distinct differences between the cones and seeds of Bennettitales and Gnetales that are described earlier in this paper, as is indicated by the attachment of the Gnetales (plus charcoalfied seeds) clade and the Bennettitales clade in separate clades (arrows in Fig. 29). These results suggest that the topology figured by the 55% majority-rule consensus tree of Friis et al. (2007) may derive from addition of the taxon “Erdtmanithecales.”

Analysis 3—To test whether the results of our analyses (and those of Friis et al., 2007) are influenced by the construction of the character matrix being tested, we combined the matrices of Rothwell and Serbet (1994) and Hilton and Bateman (2006) and removed all unambiguously redundant (overlapping) char-

acters, leaving one in each case. Character 63 was modified from Rothwell and Serbet (1994; character 37 “micropyle” modified to “tubular micropyle” with three states: [0] missing, [1] composed of two integumentary layers [2] composed of three integumentary layers). The taxa included in this matrix consist of a compromise between those included in Rothwell and Serbet (1994) and Hilton and Bateman (2006), with the goal of maintaining as high a character state/taxon ratio as possible in the matrix.

Laminar venation character states (character 8) were modified following Nixon et al. (1994) to more accurately reflect the morphologies of the taxa demonstrating the divergent venation conditions. Instead of one alternative “reticulate,” there are two: “reticulate hierarchical” and “reticulate anastomosing,” the latter reflecting anastomosing venation of similar order veins. *Caytonia* was coded as “reticulate anastomosing” as was “*Glossopteris*.” *Zamiaceae* was coded as multistate for character 10 reflecting actual variation in guard cell configurations (e.g., Thomas and Bancroft, 1913). Cataphylls (character 7) were coded as absent for *Caytonia*. Also, character codings for *Caytonia* were modified to remove embedded hypotheses in character codings as discussed in Nixon et al. (1994) and Rothwell and Serbet (1994). Codings for the characters of *Caytonia* are (1-12-1-1011?0??-210---10--0-----000001100-00-110-02202-0100-10111--1---2106-----111100000---1-----).

Results of this analysis yielded 12 most parsimonious trees of 328 steps (CI = 50, RI = 77), and only five branches collapsed in the strict consensus tree (Fig. 30). This highly resolved tree places *Caytonia* (at arrowhead) among other seed ferns and cycads at a polytomy near the base of the tree. *Ginkgo* occurs within a paraphyletic assemblage of cordaites and conifers at four adjacent nodes on the stem of the tree, but otherwise the tree was free of novel topologies. As in the results of analyses 2 and 4, the charcoalfied seeds nest within the gnetalean clade, and Bennettitales is attached to the stem at a separate node (arrows in Fig. 30).

Analysis 4—For the next analysis, two morphotaxa hypothesized by Friis and Pedersen (1996) and Friis et al. (2007) to be the seed- and pollen-producing organs belonging to the same order of plants (i.e., Erdtmanithecales; Friis and Pedersen, 1996) were added to the matrix for analysis 4 and scored as in Friis et al., (2007), but without pollen characters for *Erdtmanispermum* because, as discussed below, pollen in micropyles does not necessarily belong to the same taxon as the seeds with which they are associated. This analysis was designed to test both the putative assignment of these two morphogenera to the same group of plants and also to assess the impact of their inclusion on the cladistic relationships of Bennettitales and Gnetales.

Results of analysis 4 yielded 151 most parsimonious trees of 330 steps (CI = 50, RI = 77), and 12 internodes collapsed in the strict consensus tree (Fig. 31). Relationships among the taxa were relatively similar to, but somewhat more highly resolved than in the results of analysis 1 (Fig. 28). Notable differences from the results of analysis 1 were the placement of *Caytonia* within the large polytomy that included “higher” seed ferns, Bennettitales, *Glossopteris*, cycads, cordaites and conifers, and Gnetales (Fig. 31). The two taxa of the hypothesized Erdtmanithecales did not nest together in the strict consensus tree of our results. Whereas *Erdtmanispermum* nested within the gnetophyte clade, *Eucommiithea* was not part of that clade (Fig. 31, at arrows), but rather part of a small polytomy at an adjacent

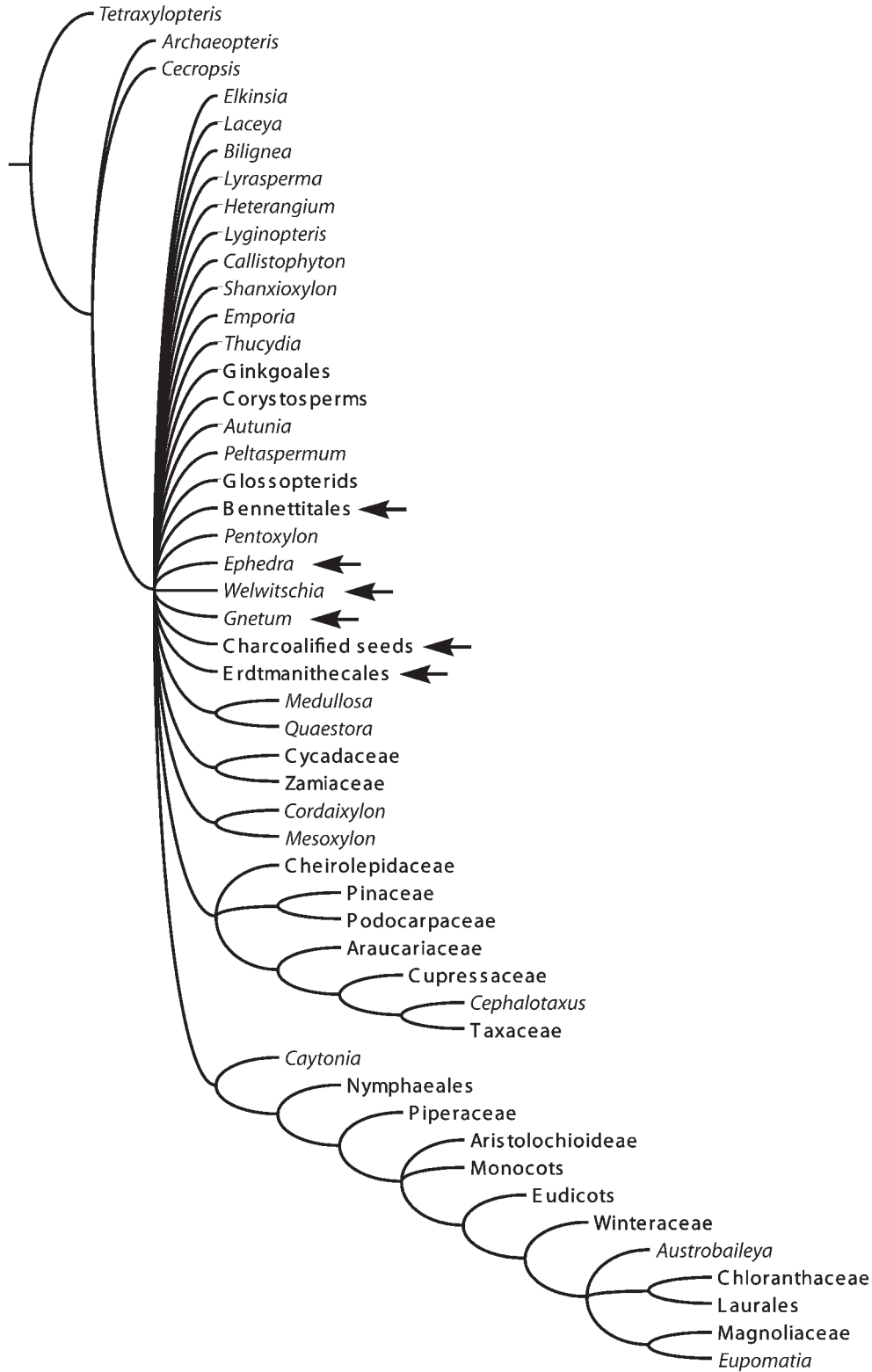


Fig. 28. Strict consensus tree from analysis 1, verifying concordance of results between analysis of Friis et al., (2007) and the same matrix analyzed using an alternative program. Note lack of resolution among taxa traditionally identified as members of "anthophyte" clade. Positions of Bennettitales, gnetophyte taxa, charcoalified seeds, and Erdtmanithecales identified by arrows. See text for details.

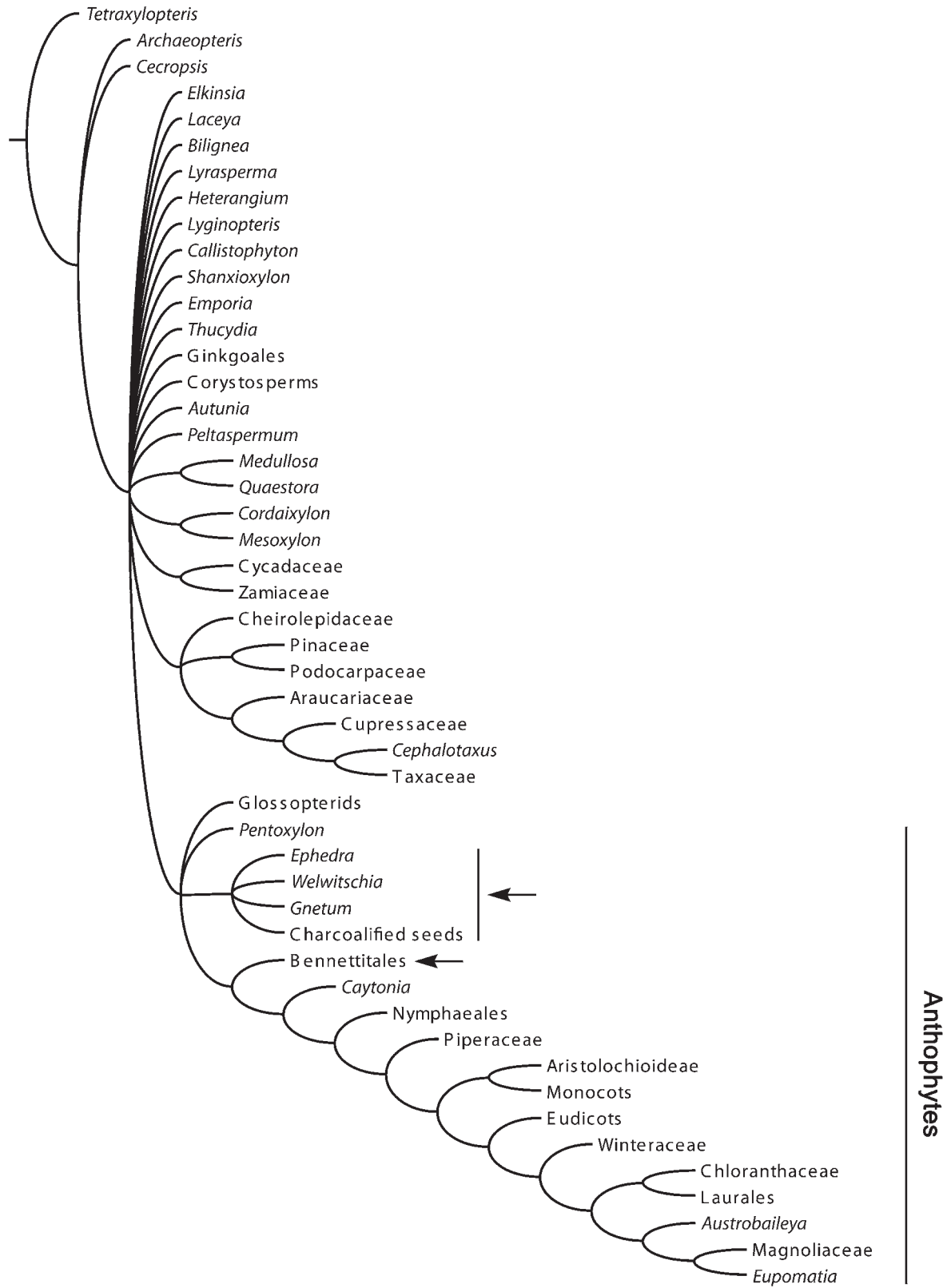


Fig. 29. Strict consensus tree from analysis 2, showing greater resolution than in results of analysis 1 when charcoalified seeds included, but members of “Erdtmanithecales” excluded. Note charcoalified seeds of Friis et al., 2007 occur in a polytomy with gnetophytes, which collectively in turn form a polytomy with glossopterids, *Pentoxylon*, and a clade consisting of Bennettitales + (*Caytonia* + angiosperms). Arrows identify positions of the clade consisting of gnetophytes + charcoalified seeds, and of Bennettitales (see text). Anthophytes include *Caytonia* in these results.

node on the stem. The latter polytomy included Bennettitales, *Pentoxylon*, *Eucommiitheca*, and flowering plants (Fig. 31).

Erdtmanispermum and *Eucommiitheca* were never sister groups in any of the most parsimonious trees unless pollen characters are added to the matrix for *Erdtmanispermum*. Thus, following the methodology explained by Gandolfo et al. (1997; i.e., comparing the results of analyses that treat the different organs as a single taxon with results from analysis that list them as separate taxa) and in the absence of additional information, these data do not support the hypothesis that *Erdtmanispermum* and *Eucommiitheca* are morphotaxa of the same clade.

While the methodology of phylogenetic context (Gandolfo et al., 1997) used to position *Erdtmanispermum* and *Eucommiitheca* in analysis 4 (Fig. 31) may, in speculation, have weaknesses in cases where the combination of organs of a putative fossil taxon represents extreme variation from character complements represented in all known taxa (mosaics), there have been no compelling tests of this concept with fossil taxa. Such speculation depends on the assumption that these separate organs will be defined, principally, by the characters that separate them and that other characters (possible synapomorphies) that may be present in these fossils will fail to nest these morphotaxa even in the context of a more complete matrix. Thus while untested, it is possible that separate organs of the same taxon would not nest together in phylogenetic context under certain circumstances, yet, in the absence of other compelling evidence (see discussion below), there is really no other objective test for the hypothesis that these disparate organs represent the same taxon. Of course, in cases where the relationship between those organs remains in question, as with *Erdtmanispermum* and *Eucommiitheca*, this "phylogenetic context" approach both tests the combined organ taxon concept and identifies the most probable alternative relationship for each organ.

In the results of this analysis, *Erdtmanispermum* nests with the charcoalfied seeds of Friis et al. (2007) and the living genera of Gnetales, with which it shares numerous characters. *Eucommiitheca*, on the other hand, forms a polytomy with Bennettitales, *Pentoxylon*, and flowering plants, which reflects a set of characters that do not conform more closely to any taxon in the polytomy than it does to the others.

Analysis 5—The matrix for analysis 5 was the same as for analysis 4, except that the character codings for *Erdtmanispermum* and *Eucommiitheca* (including pollen characters) were included within a single taxon "Erdtmanithecales." Results of analysis 5 yielded 252 most parsimonious trees of 334 steps (CI = 49, RI = 77), and 19 internodes collapsed in the strict consensus tree (Fig. 32). Relationships among the seed plant taxa were far less completely resolved in this strict consensus tree than in the strict consensus tree of results from analysis 4 (Fig. 31). The monophyletic group reported by Friis et al. (2007) that included Gnetales + Bennettitales + Erdtmanithecales and "Charcoalfied Seeds" (i.e., the "BEG" clade of Friis et al., 2007) did not resolve in the strict consensus of the most parsimonious trees (Fig. 32).

The discrepancy between results obtained here and those reported by Friis et al. (2007) is due in part to over-interpretation of a majority rule consensus tree of phylogenetic results by Friis et al. (2007, see their Supplementary Information). They used this consensus method to support the existence of a BEG clade (Bennettitales + Erdtmanithecales + Gnetales + charcoalfied seeds; see their figs. 3 and S2). However, the BEG clade was not seen in all most parsimonious trees (41.9% of

MPTs; see their Table II), a conclusion supported here by our reanalysis of their data (analysis 1). While the majority rule consensus method is useful, for example, for reporting branch support values from jackknife or bootstrap analysis, it is invalid to present a majority-rule consensus of the MPTs and report it as if it supports either a particular phylogeny or a particular clade (see e.g., Sharkey and Leathers, 2001). Some of their MPTs, which are equally valid interpretations of the data, conflict with the arrangement that they prefer (i.e., the BEG clade). However, in a reanalysis of their data that focused only on characters not missing data for Erdtmanithecales (about 25% of the total), the BEG clade was seen in nearly all MPTs (~99%; their Table II) and also had weak bootstrap support there (60%).

Among most parsimonious trees from analysis 5 were those with Bennettitales and Erdtmanithecales nested within a clade that also included the living gnetophytes and the charcoalfied seeds of Friis et al. 2007 (i.e., the BEG clade, Friis et al., 2007). These, however, were the minority of trees numerically (18 trees had such a monophyletic group) and trees with a clade consisting of Gnetales+charcoalfied seeds+Erdtmanithecales (i.e., with Bennettitales elsewhere) were more common, as were trees with a clade consisting of Gnetales+Erdtmanithecales (i.e., with Bennettitales and charcoalfied seeds elsewhere). While some of the most parsimonious trees (those with the resolved BEG clade, could possibly be interpreted as supporting the hypothesis that the reproductive structures of Bennettitales are comparable to those of Gnetales; Friis et al., 2007), the structural differences between Bennettitales and Gnetales detailed earlier in this paper suggest that an alternative explanation may be more consistent with the loss of resolution of this clade in the strict consensus tree of analysis 5. The appearance of the BEG clade even in relatively few most parsimonious trees (7%) makes analysis 5 unique among the results of our other tests. However, the occasional appearance of a BEG clade is not correlated with the addition of the charcoalfied fossil seeds to the matrix, but rather with the addition of Erdtmanithecales, a taxon of contestable validity.

INTERPRETATION OF SYSTEMATIC RESULTS AND DISCUSSION OF PHYLOGENETIC RELATIONSHIPS

Results of our analyses were surprisingly uniform with respect to the taxa that are most relevant to this study. These results also have interesting implications with respect to the affinities of the "charcoalfied seeds" recently described by Friis et al. (2007), Gnetales vs. Bennettitales, the Erdtmanithecales, *Caytonia*, Cycadales vs. Bennettitales, and therefore the "anthophyte" clade.

"Charcoalfied seeds"—All the results except those from analyses 1 and 5 (where a gnetophyte clade was not resolved) yielded strict consensus trees that included "charcoalfied seeds" in a monophyletic group with Gnetales (Figs. 29–31). Likewise, all of the results except those from analyses 1 and 5 (which show little resolution in much of the tree) resolve Bennettitales and Gnetales as discrete clades that are part of different larger clades (Figs. 29–31). Our results support the interpretation that the charcoalfied seeds are additional representatives of Gnetales. However, because the charcoalfied seeds uniformly nest within the gnetalean clade (when a gnetalean

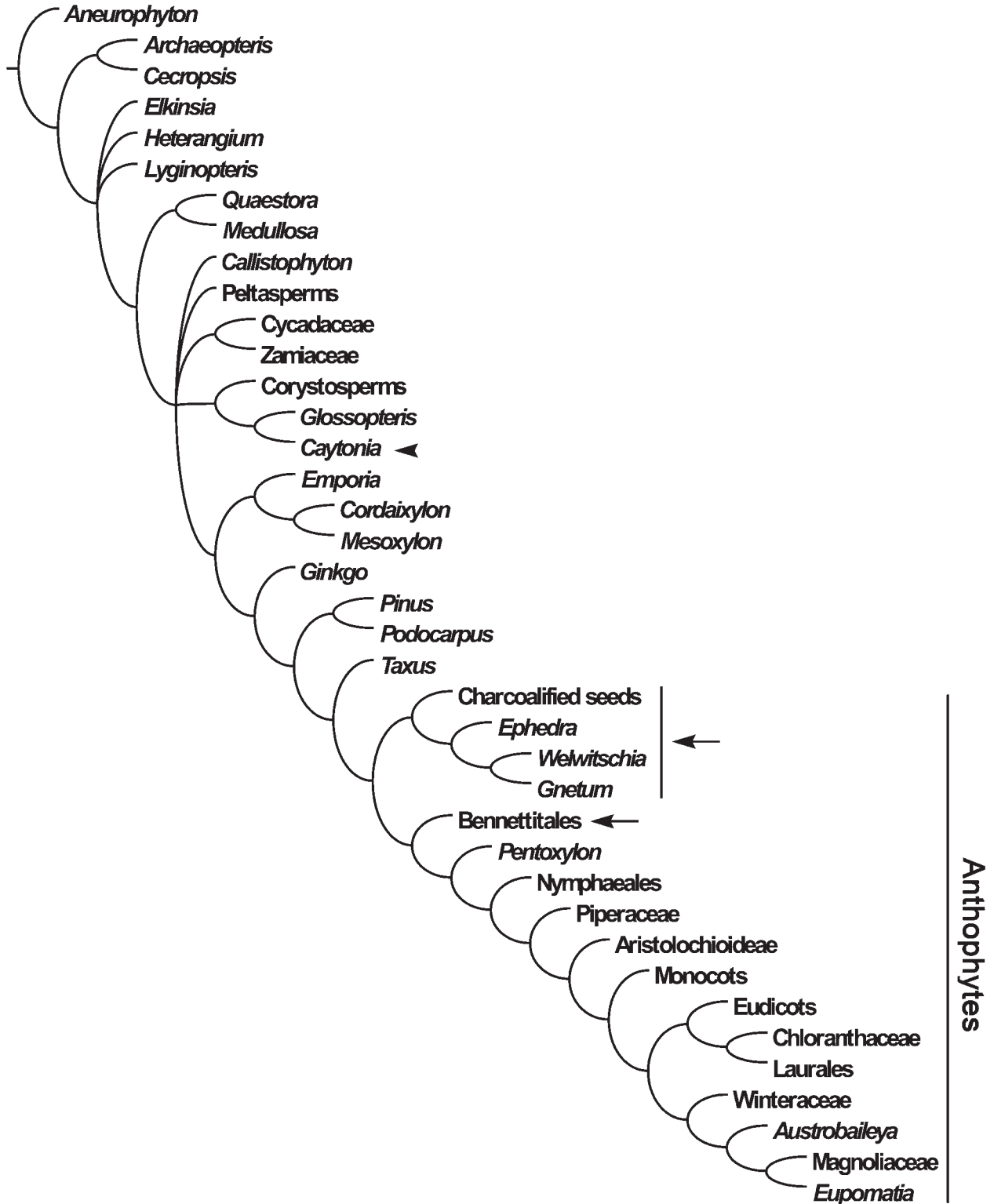


Fig. 30. Strict consensus tree from analysis 3, showing much greater resolution of spermatophyte relationships. Matrix developed by combining and editing matrices of Rothwell and Serbet (1994) and Hilton and Bateman (2006), removing redundant and overlapping characters, recoding characters of *Caytonia*, and adding charcoalified seeds of Friis et al. (2007). Characters of charcoalified seeds scored as in Friis et al. (2007). Note Bennettitales (lower arrow) forms sister group to flowering plants + *Pentoxylon* within anthophyte clade, and Gnetales + the charcoalified seeds of Friis et al. (2007; upper arrow) form a clade that is sister group of Bennettitales + *Pentoxylon* + flowering plants. Note also *Caytonia* (at arrow head) nests with *Glossopteris* and corystosperms at a lower node on stem of tree, rather than nesting with anthophytes in these results.

clade is resolved), they neither provide additional support for a morphologically defined “anthophyte” clade, nor do they help resolve relationships among Gnetales and Bennettitales.

Gnetales vs. Bennettitales—This review, including the analyses described above, detailed descriptive data, and the extensive discussion of comparative seed morphology, suggests that Bennettitales are taxonomically distinct from Gnetales and that there are no unequivocally interpreted fossils to link the groups in a way that would strengthen the “anthophyte” concept in phylogenetic analyses. Moreover, with respect to remaining gnetalean features, there are numerous differences other than contested seed characters (Table 1) and these have been recognized for a long time. For example, Thompson noted in 1912 (p. 1099): “It may be stated at once that on the anatomical side there is very little evidence for connecting the Bennettitales with *Ephedra*, although this genus, being the most primitive of the Gnetales, is the one where the evidence ought to be found...almost every tissue presents grave obstacles to this view.”

Erdtmanithecales—Friis et al. (2007) coded Erdtmanithecales as a composite of the characters found in at least five separate fossil taxa that occur in different localities from beds of different ages spanning the Early to Late Cretaceous interval (Pedersen et al., 1989a; Friis and Pedersen, 1996). However, we wish to caution that associating dispersed organs to create a composite taxon poses a set of challenges (most famously perhaps exemplified by the farrago taxon *Brontosaurus* [vs. *Apatosaurus*], and in paleobotany, by early reconstructions of various Devonian plants, most dramatically the association of different organs in Kidston and Lang’s [1920] reconstruction of *Asteroxylon*). There is always a risk of error in assembling taxa from disparate organs even if, as in this case (Friis et al., 2007), certain plant organs are linked by somewhat similar pollen grains. This is because the actual taxonomic distribution of such grains may not be fully appreciated based on available fossil evidence, and unrelated or distantly related taxa may have been lumped together into a single chimerical entity.

Seeds and pollen producing structures upon which the concept of Erdtmanithecales is based are associated by the occurrence of similar pollen in both. However, there is evidence from both living and fossil plants (e.g., Harris, 1933) that documents that this pollen-based association alone may be misleading. Of special concern in this case is the fact that both wind- and insect-dispersed pollen tend to collect on the stigmas or micropyles of other species, sometimes to the extent of interfering with their reproductive biology (e.g., Sahni, 1915; Neiland and Wilcock, 1999).

Pedersen et al. (1989a) discuss this issue and stress the consistency with which the same kind of pollen is found exclusively within the micropyles of the Danish specimens of *Erdtmanispermum*. However, there are important differences between these pollen grains and those found in the pollen sacs of *Erdtmanitheca texensis*. Those differences leave doubt as to what the pollen-producing organs associated with *Erdtmanispermum* might actually have been like and therefore as to the nature of the taxon that produced them. Such doubt is strengthened further by the distinct possibility that *Eucommiidites*-type pollen may have multiple origins (Tekleva et al., 2006). Inherent reservations concerning the practice of composing taxa based on pollen association rather than on organic connection or on a broader common suite of characters are heightened

in the case of the Erdtmanithecales because the dispersed organs occur at different locations (e.g., in Texas vs. in Denmark, Pedersen et al., 1989a). Specimens assigned to Erdtmanithecales are also of disparate ages, Early Cretaceous vs. Late Cretaceous, in the subset of taxa identified by Pedersen, et al. (1989a) as representing Erdtmanithecales. Moreover, there are notable differences among erdtmanithecalean taxa that even include contrasting morphologies e.g., *Eucommiitheca* vs. *Erdtmanitheca* vs. *Bayeritheca* Kvaček & Pacltová (fig. 6 in Friis and Pedersen, 1996) and phyllotaxy (Kvaček & Pacltová, 2001; Friis et al. 2007).

Finally, there appear to be notable differences between the *Eucommiidites* pollen grains found in the pollen-bearing organs as compared to those found in the micropyles of the seeds assumed to belong to the same taxon, but those differences are not reflected in the character selection or codings of Friis et al. (2007). Pollen grains from *Erdtmanitheca texensis* Pedersen et al. (1989a) have smooth tectal ornamentation with very fine tectal depressions that do not appear to penetrate the tectum (figs. 2A–2F, 3G in Pedersen et al., 1989a). In contrast, pollen grains found in the micropyles of the seed taxon *Erdtmanispermum* are arguably foveolate (fig. 5G in Pedersen et al., 1989a) with tectal perforations (fig. 6 in Pedersen et al., 1989a). In addition, pollen grains in the seed micropyles are dramatically different from grains of *Erdtmanitheca* with respect to certain ultrastructural characters that have been considered taxonomically significant (e.g., Walker and Walker, 1984). Pollen from *Erdtmanitheca* has a poorly defined footlayer, a relatively thin granular layer and an extraordinarily thick tectum (fig. 3D–3G in Pedersen et al., 1989a). In contrast, pollen found in the micropyles of *Erdtmanispermum*, in addition to having tectal perforations (fig. 5G in Pedersen et al., 1989a), has a remarkably thick footlayer, a granular layer with larger granules than those of *Erdtmanitheca*, and a relatively thin tectum (fig. 6 in Pedersen et al., 1989a).

A conservative approach to testing relationships among disparate fossil morphotaxa is to enter the unequivocal characters of individual fossil organs that putatively represent a single taxon into a phylogenetic analysis to gain insights into how the organs are related to other seed plants and to each other (Gandolfo et al., 1997). And as discussed more briefly above, in analysis 4 we conducted such a test of the Erdtmanithecales. That analysis included two representative taxa of putative Erdtmanithecales (i.e., *Erdtmanispermum* Pedersen & Friis without pollen characters, and *Eucommiitheca* Friis and Pedersen with pollen characters) in the combined Hilton and Bateman/Rothwell and Serbet analysis. Results of that analysis do not support the existence of the Erdtmanithecales. The strict consensus tree of those results nests *Erdtmanispermum* with a monophyletic group that also includes Gnetales and the “charcoalified seeds,” while *Eucommiitheca* is part of a polytomy with Bennettitales and *Pentoxylon* that attaches to the stem of the tree at a different node (Fig. 31, at arrows).

Thus, although the strict consensus tree of our results for analyses 3 (Fig. 30) and 4 (Fig. 31) did resolve an anthophyte clade, there were no circumstances among the results of our various experiments (Figs. 29–31) under which including the “charcoalified seeds” and/or the taxon Erdtmanithecales added even minimal support for that topology. (Figs. 28–32). Unless and until future studies verify the validity of the hypothesized Erdtmanithecales, we need to exercise caution in interpreting the results of analyses that include that taxon. Under such circumstances (i.e., no support for the BEG clade in strict consensus

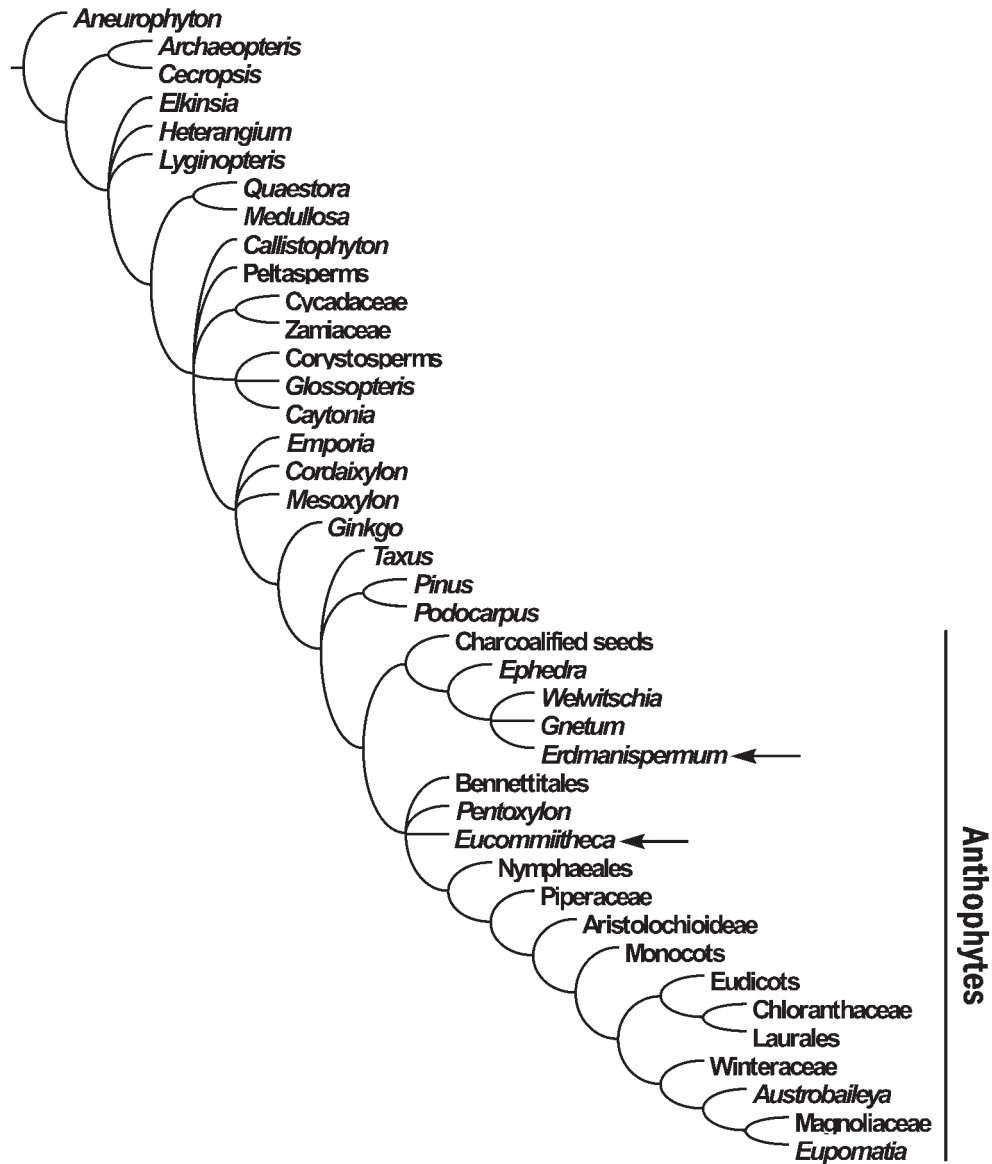


Fig. 31. Strict consensus tree for results from matrix modified from analysis 4 by adding characters for *Erdmanispermum* and *Eucommiitheca* (both identified by arrows) as separate terminals. Note relationships among Bennettitales, flowering plants, and Gnetales + charcoalified seeds clade remain unchanged (although with somewhat less resolution) from results of analysis 3 (Fig. 30); *Erdmanispermum* nests within clade otherwise consisting of gnetophyte taxa + “charcoalified seeds” while *Eucommiitheca* forms polytomy with Bennettitales, *Pentoxylon* and flowering plants at separate node on stem.

trees), inclusion of Bennettitales within a gnetophyte clade remains tenuous at best.

Caytoniales and the origin of angiospermy—The order Caytoniales Thomas (1925) is based on the concept of a *Caytonia* plant, which is derived from an assemblage of compressed organs with excellent cuticular preservation that are associated with each other at the same localities and linked by a number of anatomical characters (Thomas, 1925; Harris, 1933, 1964). Associated organs include dispersed leaves, pinnate pollen organ-bearing structures, and megasporophyll-like branching systems terminating in ovulate cupules (Thomas, 1925; Harris, 1933, 1964; Crane, 1985; Stewart and Rothwell, 1993; Taylor and Taylor, 1993). Larger parts of the plant with interconnected or-

gans and/or internal anatomy have not been found, and on the whole, there are relatively few characters defining the taxon (i.e., available for cladistic analysis).

Caytonia has been associated with angiosperms through various hypotheses since the time of Thomas (1925), who interpreted the cupules to be angiosperm carpels. Carpels of course, along with a second integument are key angiosperm-defining morphological characters and would be compelling evidence of angiospermy if convincingly demonstrated in any fossil. However, *Caytonia* has been removed to the seed ferns following the discovery by Harris (1933) of pollen grains within micropyles of the cupulate seeds (i.e., the absence of angiospermous pollination). Nonetheless, the general similarity between the incurved cupules of *Caytonia* and the anatropous angiosperm

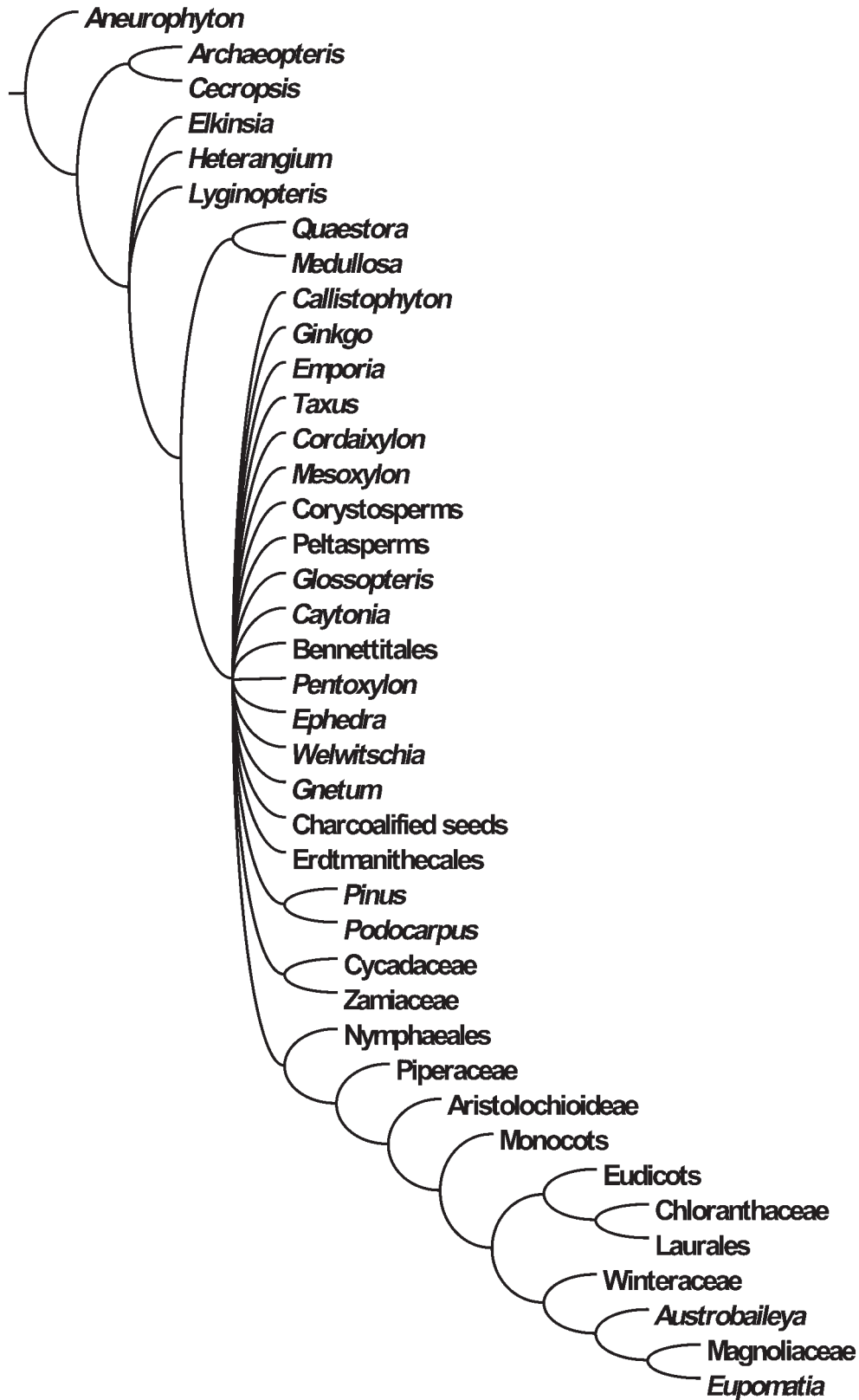


Fig. 32. Strict consensus tree from analysis 5, in which matrix for analysis 4 was altered by combining characters of *Erdtmanispermum* and *Eucomiitheca* as the single terminal *Erdtmanithecales*. Note dramatic loss of resolution from results of analysis 4 (Fig. 31), with no gnetophyte clade resolved and no resolution of sister group for flowering plants.

ovule has inspired a number of successive hypotheses purported to link angiosperms and Caytoniales (Gaussen, 1946; Doyle, 1996, 1998, 2006). Building on this similarity, Doyle has hypothesized a multistep model to explain the origin of the bitegmic ovule plus carpel combination from a *Caytonia*-like ancestor. That model (Doyle, 2006) involves reduction of ovule numbers within the caytonian cupule and the de novo origin of the enclosing carpel from the rachis of the caytonian “megasporephyll”.

Doyle’s hypothesis is well reasoned and an appealing synthesis of structural, evolutionary and developmental data. As engaging as it may be, however, that hypothesis relies on more basic hypotheses about *Caytonia* structures (e.g., abaxial or adaxial identity of the inner cupule surface; Doyle, 2006) that have yet to be verified by anatomical or developmental evidence. In addition, that hypothesis does not sufficiently explain the dramatic differences in some characters between *Caytonia* and angiosperms, especially pollen morphology and ultrastructure (Zavada and Crepet, 1986; Nixon et al., 1994), both of which have well known systematic significance. There is also significant variation in leaf venation that is minimized by codings that offer only one alternative for net-veined (Nixon et al., 1994). Finally, the hypothesis is not supported by any known fossil intermediates. In the context of these observations, the coding of *Caytonia* characters for the systematic analyses performed in this study has been done with care to avoid including possible hypotheses of structure. Results of those analyses (Figs. 30, 31) agree with the results of many earlier phylogenetic studies of seed plants (e.g., Crane, 1985; Nixon, et al., 1994; Rothwell and Serbet, 1994) by removing *Caytonia* from the clade that includes Bennettitales, Gnetales, and flowering plants, and nesting it among other “seed fern” taxa much lower on the stem of the tree (e.g., Fig. 30). This approach emphasizes the need for the development of more compelling evidence for the structure and homologies of *Caytonia* fertile structures and provides a conservative estimate for what we actually know (and what we don’t know) about the origin of the angiospermous outer integument and carpel.

Cycadales vs. Bennettitales—Relationships among cycads and Bennettitales either are unresolved (Figs. 28, 32), or the two taxa remain separated in each of our analyses. In the most highly resolved trees Cycadaceae + Zamiaceae forms a polytomy with the “higher seed ferns” (i.e., *Callistophyton*, *Peltasperms*, *Corystosperms*, *Glossopteris*, *Caytonia*) and Bennettitales are attached to a higher node on the stem of the tree (Figs. 29–31). While certain key homologies of the reproductive structures of cycads vs. Bennettitales remain unclear (ovulate receptacle for example), available and newly available data (Table 2), continue to support the absence of close systematic relationships between these two clades.

Unconfirmed competing hypotheses for flowering plant origins—There are two or three categories of currently competing hypotheses for the origin of angiosperms that are derived from the study of living and fossil plant form. One, as represented by the anthophyte hypothesis, proposes that the outer seed integument and carpel are derived from fertile structures that already were aggregated into a flower-like reproductive organ (i.e., a strobilus or cone). That hypothesis, asserting that a strobilus or flower-like aggregation of fertile organs was inherited from a common ancestor by angiosperms and its sister groups, is most commonly tested by looking for similarities and transforma-

tional series among the fertile organs of angiosperms and hypothesized angiosperm sister groups (e.g., Gnetales, Bennettitales). As elaborated in this paper, that hypothesis has yet to find support from the similar structure of potentially homologous fertile organs among Bennettitales, Gnetales, and flowering plants. Indeed, as elaborated earlier in this paper, the putatively homologous fertile organs of Bennettitales and Gnetales (particularly cone and seed structure) are very dissimilar. Moreover, the paleontological record has yet to yield fossils to help form a transformational series of morphologies between the fertile organs of Bennettitales, Gnetales, and flowering plants.

The second category of hypotheses, as represented by interpretations of the ovulate cupules of *Caytonia* and elaborated by Doyle and others (e.g., Doyle, 2006), infers that the angiosperm outer seed integument and carpel are derived from modifications of a seed fern megasporephyll, with aggregation of the fertile parts into a flower presumably having occurred later. As exemplified by the seed-bearing cupules of *Caytonia*, and in the context of associated assumptions, that hypothesis *appears* to be quite plausible. However, crucial structural features of the *Caytonia* cupule and cupule-bearing “rachis” upon which that hypothesis relies have yet to be confirmed by fossils of adequate preservational mode and quality. We reiterate that when the characters of *Caytonia* are coded in a conservative fashion with respect to equivocal structural features (e.g., analysis 3), *Caytonia* relocates from the sister group to flowering plants (e.g., Fig. 28) to a much lower position on the spermatophyte tree (e.g., Fig. 30). Up to the present, the hypothesized transformations of structure leading from the *Caytonia* cupule to an angiosperm carpel have not been confirmed by either developmental transitions or transformational series of mature morphologies.

A third set of hypotheses focuses on the origin of the angiospermous carpel and outer seed integument by development of ovules at positions occupied by pollen-producing structures (i.e., microsporangia) in the putative immediate ancestor of flowering plants. Such hypotheses are reminiscent of Iltis’ famous “catastrophic sexual transmutation” theory for the origin of maize (Iltis, 1983), and include the “gamoheterotopy theory” of Meyen (1987, 1988) and the “mostly male theory” of Frohlich and Parker (2000). The gamoheterotopy theory focuses on transformation of synangiate microsporephylls (Figs. 1b, 1e) to seed-bearing megasporephylls at the apex of the cone of Bennettitales without there being a series of morphological transformations to document the change (Meyen, 1988). Therefore, that hypothesis predicts that it is not testable using the fossil record.

The mostly male theory utilizes the distribution of a specific *LFY* gene paralog in one of the hypothesized topologies of extant seed plant trees to infer that the flowering plant carpel may have originated in the transformation of the apical microsporephylls of indeterminate fructifications to megasporephylls of a fructification with determinate growth (see Frohlich and Parker, 2000 for a detailed explanation). Frohlich and Parker (2000) consider many characters of *Caytonia* to be too dissimilar to those of angiosperms for *Caytonia* to be a plausible ancestor, an opinion with which we agree. Those authors concentrate on the *Corystospermales* as a more plausible angiosperm sistergroup (Sokoloff and Timonin, 2007). Many of the *corystosperm* characters emphasized or hypothesized by Frohlich and Parker (2000) are poorly known or unverified by evidence from the fossils, and other features (e.g., attachment of seeds to the abaxial surface of the cupule in *corystosperms*; Klavins et al., 2002)

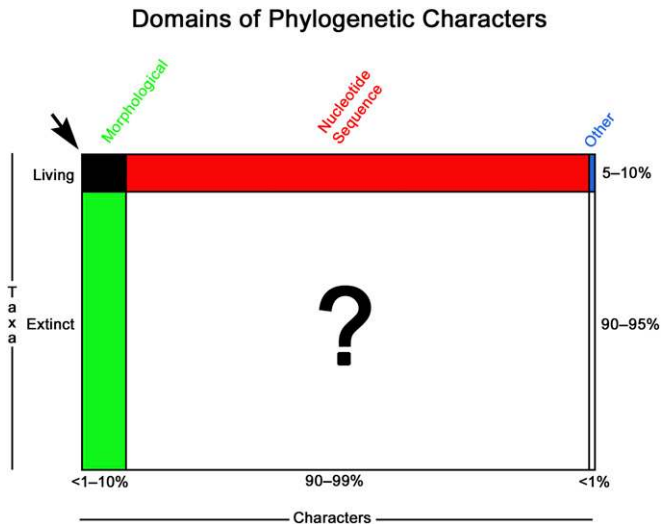


Fig. 33. Diagram depicting subsets of theoretical totality of systematically informative characters currently available for analysis. Estimated sizes of domains of characters available to analyses of differing categories of characters depicted by different colored boxes. Domain of characters available only to nucleotide sequence analyses consists of subset of theoretical character totality depicted in red. Domain of characters available only to morphological analyses that include fossil and living taxa comprises even smaller subset depicted in green. Domains of other types of characters (e.g., biochemical characters, rare genomic changes) constitute tiny percentage of characters depicted in blue. Overlapping domains of nucleotide, morphological, and other characters consist of only a small subset of each available domain of characters; depicted in black (at arrow). Largest domain of potentially informative systematic characters (i.e., nucleotide sequences of extinct organisms; depicted in white with “?”) is currently unavailable for analysis. Percentages depicted here are hypothetical.

are difficult to reconcile with transformation of a crustosperm cupule to an angiosperm carpel (see also Stockey and Rothwell, 2009, pp. 323–335).

The mostly male hypothesis also relies on several nested hypotheses that remain untested. Nevertheless, that hypothesis does predict the presence of ectopic ovules on microsporophylls in apical regions of the fructifications that are transitional to ancestral flowering plants, and therefore at least one aspect of the hypothesis is testable using the fossil record. Up to the present time, no fossil fructifications with the “transitional morphologies” predicted by the mostly male hypothesis have been discovered in the fossil record.

Anthophytes, and alternative sets of characters available for morphological or nucleic acid sequence analyses—The reality of an anthophyte clade has been repeatedly challenged based on analyses of DNA sequence data (e.g., Chaw et al., 2000; Burleigh and Mathews, 2004), and remains as much a question as it did before we conducted the analyses discussed above, and before those analyses that incorporated “charcoalified seeds” (Friis et al., 2007). Because the “charcoalified seeds” described by Friis et al. (2007) are cleanly nested with Gnetales in all analyses to date in which a gnetophyte clade is resolved in the strict consensus tree of most parsimonious trees (i.e., Friis et al., 2007; Figs. 29–31 of this paper), those “charcoalified seeds” do not lend support for the anthophyte hypothesis.

The results of almost all nucleotide sequence based analyses remove Gnetales from the anthophytes (Burleigh and Mathews, 2004), suggesting that many of the characters commonly cited as linking the two groups may be convergent. There is a distinct malleability of all morphology based data sets manifest to anyone who has done such analyses. This malleability is based in part on the inherent subjectivity in selecting and scoring characters. On the other hand, taxon sampling for analyses based on nucleic acid sequences is currently restricted to only the small fraction of clades that are not yet extinct (Crane et al., 2004; Rothwell and Nixon, 2006). This results in domains of available characters that are largely disjunct for morphological and nucleotide sequence based analyses (Fig. 33). These constraints suggest advantages to combining molecular with morphological data sets in analyses aimed at assessing the reality of an anthophyte clade. Such an approach adds a measure of objectivity and broader sampling (save for extinct groups) and allows for the incorporation of valuable information presented by fossil evidence.

Nonetheless, at this time, and based on the data sets we used, our analyses do consistently support an anthophyte topology of the spermatophyte tree (e.g., Fig. 30). This may have been expected because each of the matrices used (or modified) herein to evaluate the significance of bennettitalean characters and the “charcoalified seeds” reported by Friis et al. (2007) already supported a robust anthophyte clade. However it is clear that the constituents of that clade are tightly circumscribed and that connections among them remain as cryptic as ever.

SUMMARY OF CONCLUSIONS

- (1) Bennettiales constitute a clade of fossil vascular plants with distinctive vegetative and fertile structures.
- (2) At this time, there is no compelling evidence that supports classical hypotheses that Bennettiales nest either with Cycadales as a monophyletic group or within Gnetales. Rather, Bennettiales constitute an independent clade on the spermatophyte tree.
- (3) Bennettiales comprise one member of a larger anthophyte clade that also includes flowering plants and Gnetales (and perhaps e.g., *Pentoxylon*; Fig. 30) in the results of phylogenetic analyses of morphological characters that include both living and fossil taxa. However, such relationships are not based on synapomorphies from homologous fertile structures.
- (4) The “charcoalified seeds” of Friis et al. (2007) do not constitute additional morphological evidence that supports the anthophyte clade defined by numerous phylogenetic analyses based on morphological characters alone. It is more probable that those seeds actually represent stem or crown lineages of Gnetales.
- (5) The fossil record has provided a wealth of new information for inferring relationships among major groups of seed plants, but has yet to reveal how the diagnostic reproductive structures of flowering plants (i.e., including outer integument and carpel) arose.
- (6) Although paleontological information is increasingly exposing the pattern of early diversification among flowering plants—the renowned “abominable mystery” (Darwin, 1903; Crepet and Niklas, 2009, 366–381 and Friedman, 2009, pp 5–21) the origin of angiosperms remains as intractable a mystery today as it was to Darwin 130 years ago.

LITERATURE CITED

- ANDREWS, H. N. 1943. On the vascular anatomy of the cycadeoid cone axis. *Annals of the Missouri Botanical Garden* 30: 421–427.
- ARBER, E. A. N., AND J. PARKIN. 1907. On the origin of angiosperms. *Botanical Journal of the Linnean Society* 38: 29–80.
- ARBER, E. A. N., AND J. PARKIN. 1908. Studies on the evolution of the angiosperms. The relationship of the angiosperms to the Gnetales. *Annals of Botany* 22: 489–515.

- ARNOLD, C. A. 1947. An introduction to paleobotany. McGraw-Hill, New York, New York, USA.
- BERRIDGE, E. M. 1911. On some points of resemblance between gnetalean and bennettitean seeds. *New Phytologist* 10: 140–144.
- BIERHORST, D. W. 1971. Morphology of vascular plants. Macmillan, New York, New York, USA.
- BURLEIGH, J. G., AND S. MATHEWS. 2004. Phylogenetic signal in nucleotide data from seed plants: Implications for resolving the seed plant tree of life. *American Journal of Botany* 91: 1599–1613.
- CHAMBERLAIN, C. J. 1920. The living cycads and the phylogeny of seed plants. *American Journal of Botany* 7: 146–153.
- CHAMBERLAIN, C. J. 1935. Gymnosperms: Structure and evolution. Dover, New York, New York, USA.
- CHAW, S. M., C. L. PARKINSON, Y. CHENG, T. M. VINCENT, AND J. D. PALMER. 2000. Seed plant phylogeny inferred from all three plant genomes: Monophyly of extant gymnosperms and origin of Gnetales from conifers. *Proceedings of the National Academy of Sciences, USA* 97: 4086–4091.
- COULTER, J. M. 1908. The embryo sac and embryo of *Gnetum gnemon*. *Botanical Gazette* 46: 43–49.
- CRANE, P. R. 1985. Phylogenetic analysis of seed plants and the origin of angiosperms. *Annals of the Missouri Botanical Garden* 72: 716–793.
- CRANE, P. R. 1986. The morphology and relationship of the Bennettitales. In B. A. Thomas and R. A. Spicer [eds.], Systematic and taxonomic approaches in palaeobotany. Systematics Association Special Volume 31: 163–175.
- CRANE, P. R. 1988. Major clades and relationships in “higher” gymnosperms. In C. B. Beck [ed.], Origin and evolution of gymnosperms. Columbia University Press, New York, New York, USA.
- CRANE, P. R., P. HERENDEEN, AND E. M. FRIIS. 2004. Fossils and plant phylogeny. *American Journal of Botany* 91: 1683–1699.
- CREPET, W. L. 1974. Investigations of North American cycadeoids: The reproductive biology of *Cycadeoidea*. *Palaeontographica* 148B: 144–169.
- CREPET, W. L., AND T. DELEVORYAS. 1972. Investigations of North American cycadeoids: Early ovule ontogeny. *American Journal of Botany* 59: 209–215.
- CREPET, W. L. AND K. J. NIKLAS. 2009. Darwin’s second “abominable mystery”: Why are there so many angiosperm species? *American Journal of Botany* 96: 366–381.
- DARWIN, F. [ed.]. 1903. More letters of Charles Darwin, a record of his work in hitherto unpublished letters, vol. 2. John Murray, London, UK.
- DELEVORYAS, T. 1959. Investigations of North American cycadeoids: *Monanthesia*. *American Journal of Botany* 46: 657–666.
- DELEVORYAS, T. 1960. Investigations of North American cycadeoids: Trunks from Wyoming. *American Journal of Botany* 47: 778–786.
- DELEVORYAS, T. 1963. Investigations of North American cycadeoids: Cones of *Cycadeoidea*. *American Journal of Botany* 50: 45–52.
- DELEVORYAS, T. 1965. Investigations of North American cycadeoids: Microsporangiate structures and phylogenetic implications. *Palaeobotanist* 14: 89–93.
- DELEVORYAS, T. 1968a. Investigations of North American cycadeoids: Structure, ontogeny and phylogenetic considerations of cones of *Cycadeoidea*. *Palaeontographica* 121B: 122–133.
- DELEVORYAS, T. 1968b. Some aspects of cycadeoid evolution. *Botanical Journal of the Linnean Society* 61: 137–146.
- DELEVORYAS, T. 1971. Biotic provinces and the Jurassic-Cretaceous floral transition. Proceedings of the North American Paleontological Convention, part L, 1660–1674.
- DONOGHUE, M. J., AND J. A. DOYLE. 2000. Seed plant phylogeny: Demise of the anthophyte hypothesis? *Current Biology* 10: R106–R109.
- DOYLE, J. A. 1996. Seed plant phylogeny and the relationships of Gnetales. *International Journal of Plant Sciences* 157 (Supplement 6): S3–S39.
- DOYLE, J. A. 1998. Molecules, morphology, fossils and the relationships of angiosperms and Gnetales. *Molecular Phylogenetics and Evolution* 9: 448–462.
- DOYLE, J. A. 2006. Seed ferns and the origin of angiosperms. *Journal of the Torrey Botanical Society* 133: 169–209.
- DOYLE, J. A., AND M. J. DONOGHUE. 1986. Seed plant phylogeny and the origin of angiosperms: An experimental cladistic approach. *Botanical Review* 52: 321–431.
- DOYLE, J. A., AND M. J. DONOGHUE. 1987. The origin of angiosperms: A cladistic approach. In E. M. Friis, W. G. Chaloner, and P. R. Crane [eds.], The origins of angiosperms and their biological consequences, 17–50. Cambridge University Press, Cambridge, UK.
- DOYLE, J. A., AND M. J. DONOGHUE. 1992. Fossils and seed plant phylogeny reanalyzed. *Brittonia* 44: 89–106.
- EAMES, A. J. 1913. The morphology of *Agathis australis*. *Annals of Botany* 27: 1–138.
- EAMES, A. J. 1952. Relationships of Ephedrales. *Phytomorphology* 2: 79–100.
- FRIEDMAN, W. E. 2009. The meaning of Darwin’s “abominable mystery.” *American Journal of Botany* 96: 5–21.
- FRIIS, E. M., P. R. CRANE, K. R. PEDERSEN, S. BENGSTON, P. C. J. DONOGHUE, G. W. GRIMM, AND M. STAMPANONI. 2007. Phase contrast X-ray microtomography links Cretaceous seeds with Gnetales and Bennettitales. *Nature* 450: 549–552.
- FRIIS, E. M., AND K. R. PEDERSEN. 1996. *Eucommiitheca hirsuta*, a new pollen organ with *Eucommiidites* pollen from the Early Cretaceous of Portugal. *Grana* 35: 104–112.
- FROHLICH, M. W., AND D. S. PARKER. 2000. The mostly male theory of flower evolutionary origins: From genes to fossils. *Systematic Botany* 25: 155–170.
- GANDOLFO, M. A., K. C. NIXON, W. L. CREPET, AND G. E. RATCLIFFE. 1997. A new fossil fern assignable to Gleicheniaceae from Late Cretaceous sediments in New Jersey. *American Journal of Botany* 84: 483–493.
- GAUSSEN, H. 1946. Les Gymnospermes, actuelles et fossiles. Travaux du Laboratoire forestier de Toulouse, tome II, Étude dendrologique, sect. 1, vol. 1, fasc. 3. *Chapitre 5*: 1–26.
- GIFFORD, E. M., AND A. S. FOSTER. 1988. Morphology and evolution of vascular plants, 3rd ed. Freeman, New York, New York, USA.
- GOLOBOFF, P. A. 1999. Analyzing large data sets in reasonable times: Solutions for composite optima. *Cladistics* 15: 415–428.
- GREGUSS, P. 1968. Xylotomy of the living cycads with a description of their leaves and epidermis. Akademiai Kiadó, Budapest, Hungary.
- HARRIS, T. M. 1932. The fossil flora of Scoresby Sound East Greenland. Part 3: Caytoniales and Bennettitales. *Meddelelser om Grønland*. 85: 1–130.
- HARRIS, T. M. 1933. A new member of the Caytoniales. *New Phytologist* 32: 97–114.
- HARRIS, T. M. 1964. The Yorkshire Jurassic flora II. Caytoniales, Cycadales & pteridosperms. British Museum (Natural History) London, UK.
- HARRIS, T. M. 1969. The Yorkshire Jurassic flora III. Bennettitales. British Museum (Natural History) London, UK.
- HILTON, J., AND R. M. BATEMAN. 2006. Pteridosperms are the backbone of seed-plant phylogeny. *Journal of the Torrey Botanical Society* 133: 119–168.
- ILTIS, H. H. 1983. From teosinte to maize: The catastrophic sexual transmutation. *Science* 222: 886–894.
- KIDSTON, R., AND W. H. LANG. 1920. On Old Red Sandstone plants showing structure, from the Rhynie Chert Bed, Aberdeenshire. Part III. *Asteroxylon mackiei* Kidston and Lang. *Transactions of the Royal Society of Edinburgh* 52: 643–680.
- KLAVINS, S. D., T. N. TAYLOR, AND E. L. TAYLOR. 2002. Anatomy of *Umkomasia* (Corytospermales) from the Triassic of Antarctica. *American Journal of Botany* 89: 664–676.
- KRÄUSEL, R., AND F. SCHAARSCHMIDT. 1966. Die Keuperflora von Neuwelt bei Basel. IV. Pterophyllen und Taeniopteriden. *Schweizerische paläontologische Abhandlungen* 84: 3–64.
- KVÁČEK, J., AND B. PAČTOVÁ. 2001. *Bayeritheca hughesii* gen. et sp. nov., a new *Eucommiidites*-bearing pollen organ from the Cenomanian of Bohemia. *Cretaceous Research* 22: 695–704.
- LAND, W. J. G. 1904. Spermatogenesis and oogenesis in *Ephedra trifurca*. *Botanical Gazette* 38: 1–18.
- LIGNIER, O. 1894. Végétative fossiles de Normandie. Structure et affinités du *Bennettitites Morieri* (Sap. & Mar.). *Mémoires de la Société linnéenne de Normandie, Caen* 18: 5–78.

- LIGNIER, O. 1911. *Le Bennettites Morieri* (Sap. et Mar.) Lignier se reproduisait probablement par parthénogénèse. *Bulletin Société Botanique de France* 59: 425–428.
- LOCONTE, H., AND D. W. STEVENSON. 1990. Cladistics of the Spermophyta. *Brittonia* 42: 197–211.
- MAHESHWARI, V. 1935. Contributions to the morphology of *Ephedra foliata* Boiss. I. The development of the male and female gametophytes. *Proceedings of the Indiana Academy of Sciences* 1: 586–606.
- MATHEWS, S. 2009. Phylogenetic relationships among seed plants: Persistent questions and the limits of DNA sequence data. *American Journal of Botany* 96: 228–236.
- MENDES, M. M., E. M. FRIIS, AND J. PAIS. 2008. *Erdmanispermum juncaense* sp. nov., a new species of the extinct order Erdtmanithecales from the Early Cretaceous (probably Berriasian) of Portugal. *Review of Palaeobotany and Palynology* 149: 50–56.
- MEYEN, S. V. 1987. *Fundamentals of palaeobotany*. Chapman and Hall, London, UK.
- MEYEN, S. V. 1988. On the origin of the angiosperm gynoecium by gamo-heterotropy. *Botanical Journal of the Linnean Society* 97: 171–178.
- MUHAMMAD, A. F., AND R. SATTLER. 1982. Vessel structure of *Gnetum* and the origin of angiosperms. *American Journal of Botany* 69: 1004–1021.
- NATHORST, A. G. 1911. Paläobotanische Mitteilungen. *Kungliga Svenska Vetenskapsakademiens Handlingar* 46: 1–33.
- NEILAND, M. R., AND C. C. WILCOCK. 1999. The presence of heterospecific pollen on stigmas of nectariferous and nectarless orchids and its consequences for their reproductive success. *Protoplasma* 208: 65–75.
- NISHIDA, H. 1994. Morphology and the evolution of Cycadeoidales. *Journal of Plant Research* 107: 479–492.
- NIXON, K. C., W. L. CREPET, D. W. STEVENSON, AND E. M. FRIIS. 1994. A reevaluation of seed plant phylogeny. *Annals of the Missouri Botanical Garden* 81: 484–533.
- NORSTOG, K. J., AND T. J. NICHOLLS. 1997. *The biology of the cycads*. Cornell University Press, Ithaca, New York, USA.
- OHANA, T., T. KIMURA, AND S. CHITALEY. 1998. *Bennetticarpus yezoites* sp. nov. (Bennettitales) from the Upper Cretaceous of Hokkaido, Japan. *Paleontological Research* 2: 108–119.
- OSBORN, J. M., AND T. N. TAYLOR. 1995. Pollen morphology and ultrastructure of the Bennettitales: In situ pollen of *Cycadeoidea*. *American Journal of Botany* 82: 1074–1081.
- OWENS, J. N., G. L. CATALANO, S. J. MORRIS, AND J. AITKEN-CHRISTIE. 1995a. The reproductive biology of kauri (*Agathis australis*). I. Pollination and prefertilization development. *International Journal of Plant Sciences* 156: 257–269.
- OWENS, J. N., G. L. CATALANO, S. J. MORRIS, AND J. AITKEN-CHRISTIE. 1995b. The reproductive biology of kauri (*Agathis australis*). II. Male gametes, fertilization and cytoplasmic inheritance. *International Journal of Plant Sciences* 156: 404–416.
- PEDERSEN, K. R., P. R. CRANE, AND E. M. FRIIS. 1989a. Pollen organs and seeds with *Eucommiidites* pollen. *Grana* 28: 279–294.
- PEDERSEN, K. R., P. R. CRANE, AND E. M. FRIIS. 1989b. Morphology and phylogenetic significance of *Vardekloeftia* Harris (Bennettitales). *Review of Palaeobotany and Palynology* 60: 7–24.
- PRYER, K. M., H. SCHNEIDER, A. R. SMITH, R. CRANFILL, P. G. WOLF, J. S. HUNT, AND S. D. SIPES. 2001. Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. *Nature* 409: 618–621.
- RAI, H. S., P. A. REEVES, R. PEAKALL, R. G. OLMSTEAD, AND S. W. GRAHAM. 2008. Inference of higher-order conifer relationships from a multi-locus plastid data set. *Botany* 86: 658–669.
- ROTHWELL, G. W., AND K. C. NIXON. 2006. How does the inclusion of fossil data change our conclusions about the phylogenetic history of euphyllophytes? *International Journal of Plant Sciences* 167: 737–749.
- ROTHWELL, G. W., AND R. SERBET. 1994. Lignophyte phylogeny and the evolution of the spermatophytes: A numerical cladistic analysis. *Systematic Botany* 19: 443–482.
- ROTHWELL, G. W., AND R. A. STOCKEY. 2002. Anatomically preserved *Cycadeoidea* (Cycadeoidaceae), with a reevaluation of the systematic characters for the seed cones of Bennettitales. *American Journal of Botany* 89: 1447–1458.
- RYDIN, C. 2005. *The Gnetales: Fossils and phylogenies*. Ph.D. dissertation, Stockholm University, Stockholm, Sweden.
- RYDIN, C., K. R. PEDERSEN, P. R. CRANE, AND E. M. FRIIS. 2006. Former diversity of *Ephedra* (Gnetales): Evidence from Early Cretaceous seeds from Portugal and North America. *Annals of Botany* 98: 123–140.
- SAHNI, B. 1915. Foreign pollen in the ovules of *Ginkgo* and of fossil plants. *New Phytologist* 14: 149–151.
- SAHNI, B. 1932. A petrified *Williamsonia* (*W. sewardiana*, sp. nov.) from the Rajmahal Hills, India. *Palaeontologica Indica* 20 (new series): 1–19.
- SAIKI, K., AND Y. YOSHIDA. 1999. A new bennettitalean trunk with unilacunar five-trace nodal structure from the Upper Cretaceous of Hokkaido, Japan. *American Journal of Botany* 86: 326–332.
- SCOTT, A. C. 1909. *Studies in fossil botany*, 2nd ed., vol. 2. Adam and Charles Black, London, UK.
- SHARKEY, M. J., AND J. W. LEATHERS. 2001. Majority does not rule: The trouble with majority-rule consensus trees. *Cladistics* 17: 282–284.
- SHARMA, B. D. 1970. On the structure of *Williamsonia* cf. *W. scotica* from the Middle Jurassic Rocks of Rajmahal Hills, India. *Annals of Botany* 34: 289–296.
- SHARMA, B. D. 1973. Anatomy of the peduncle of *Williamsonia* collected from the Jurassic of Amarjola in the Rajmahal Hills, India. *Botanique* 4: 93–100.
- SOKOLOFF, D. D., AND A. C. TIMONIN. 2007. Morphological and molecular data on the origin of angiosperms: On a way to a synthesis. *Journal of General Biology* 68: 83–97 (in Russian with English summary).
- STEVENSON, D. W. 1992. A formal classification of the extant cycads. *Brittonia* 44: 220–223.
- STEWART, W. N., AND G. W. ROTHWELL. 1993. *Paleobotany and the evolution of plants*. Cambridge University Press, New York, New York, USA.
- STOCKEY, R. A., AND G. W. ROTHWELL. 2003. Anatomically preserved *Williamsonia* (Williamsoniaceae): Evidence for bennettitalean reproduction in the Late Cretaceous of western North America. *International Journal of Plant Sciences* 164: 251–262.
- STOCKEY, R. A., AND G. W. ROTHWELL. 2009. Distinguishing angiosperms from the earliest angiosperms: A Lower Cretaceous (Valanginian-Hauterivian) fruit-like reproductive structure. *American Journal of Botany* 96: 323–335.
- STOPES, M. C. 1918. New bennettitalean cones from the British Cretaceous. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 208: 389–440.
- TAYLOR, T. N., AND E. L. TAYLOR. 1993. *The biology and evolution of fossil plants*. Prentice-Hall, Inglewood Cliffs, New Jersey, USA.
- TEKLEVA, M., V. A. KRASSILOV, J. KVAČEK, AND J. H. A. VAN KONIJENBURG-VAN CITTERT. 2006. Pollen genus *Eucommiidites*: Ultrastructure and affinities. *Acta Palaeobotanica* 46: 137–155.
- THODAY, M. G. 1911. The female inflorescence and ovule of *Gnetum africanum*, with notes on *Gnetum scandens*. *Annals of Botany* 25: 1101–1135.
- THODAY, M. G., AND E. M. BERRIDGE. 1912. The anatomy and morphology of the inflorescence and flower of *Ephedra*. *Annals of Botany* 26: 953–985.
- THOMAS, H. H. 1915. On *Williamsoniella*, a new type of bennettitalean flower. *Philosophical Transactions of the Royal Society of London B, Biological Sciences* 207: 113–148.
- THOMAS, H. H. 1925. The Caytoniales. A new group of angiospermous plants from the Jurassic rocks of Yorkshire. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 213: 299–363.
- THOMAS, H. H., AND N. BANCROFT. 1913. On the cuticles of some Recent and fossil cycadean fronds. *Transactions of the Linnean Society, London* 8: 155–204, pls. 61–77.
- THOMPSON, W. P. 1912. Anatomy and relationships of the Gnetales. I. The genus *Ephedra*. *Annals of Botany* 27: 1077–1104.
- THOMPSON, W. P. 1916. The morphology and affinities of *Gnetum*. *American Journal of Botany* 3: 135–184.

- WALKER, J. W., AND A. G. WALKER. 1984. Ultrastructure of Lower Cretaceous angiosperm pollen and the origin and early evolution of angiosperms. *Annals of the Missouri Botanical Garden* 71: 464–521.
- WATSON, J., AND C. A. SINCOCK. 1992. Bennettitales of the English Wealden. Paleontographical Society Monographs, London, UK.
- WETTSTEIN, R. R. VON. 1907. *Handbuch der systematischen Botanik*, II. Band. Franz Deuticke, Leipzig, Germany.
- WIELAND, G. R. 1899. A study of some American fossil cycads. Parts I–III. *American Journal of Science* 7: 219–391.
- WIELAND, G. R. 1901. A study of some American fossil cycads. Part IV. On the microsporangiate fructifications of *Cycadeoidea*. *American Journal of Science* 11: 423–436.
- WIELAND, G. R. 1906. American fossil cycads. Carnegie Institution, Publication no. 34. Washington, D.C., USA.
- WIELAND, G. R. 1911. On the *Williamsonia* tribe. *American Journal of Science* (series 4) 32: 433–476.
- WIELAND, G. R. 1916. American fossil cycads. Carnegie Institution, Publication no. 34, vol. II, Washington, D.C., USA.
- WIELAND, G. R. 1934. Fossil cycads, with special reference to *Raumeria reichenbachiana* Göppert sp. of the Zwinger of Dresden. *Palaeontographica B* 79: 83–130, pls. 9–20.
- ZAVADA, M. S., AND W. L. CREPET. 1986. Pollen grain wall structure of *Caytonanthus arberi* (Caytoniales). *Plant Systematics and Evolution* 153: 259–264.

APPENDIX 1. Historical summary of systematic hypotheses, interpretation of fertile structures, and development of systematic concepts

Affinities of the Bennettitales have been the subject of intense interest and controversy for over a hundred years (e.g., Lignier, 1894, 1911; Wieland, 1906, 1916; Arber and Parkin, 1907; Berridge, 1911; Thoday, 1911; Stopes, 1918; Chamberlain, 1920, Arnold, 1947; Delevoryas, 1968b; Norstog and Nicholls, 1997; Friis et al., 2007). In the late 19th and early 20th centuries the combination of frond structure, sparsely branched main axes, pachycaul with persistent leaf bases, and stem anatomy found in the genus *Cycadeoidea* was considered so strikingly similar to corresponding characters of some extant cycads (e.g., *Macrozamia*) that affiliations with that modern group were often inferred (e.g., Wieland, 1906). Even after the discovery of the bisporangiate *Cycadeoidea* cone by Wieland, around 1900, the notion that cycads and cycadeoids were closely related endured (e.g., Wieland, 1899, 1901, 1906).

At about the same time, Arber and Parkin (1907) developed a different interpretation of bennettitalean relationships that, in essence, has more recently been named the anthophyte hypothesis by Doyle and Donoghue (1987) and is based to some extent on perception of similarity between the bisporangiate cone and the angiosperm flower (fig. 1 of Arber and Parkin, 1907). Originally viewed as an alternative to a hypothesis of von Wettstein (1907), Arber and Parkin (1907) suggested that Bennettitales were transitional to Gnetales + Angiospermae through a hypothetical group, the “Hemiangiospermae” (Arber and Parkin, 1907, 1908). That point of view, especially as expressed in 1907, required a rather complex hypothetical intermediate to explain the transformation between a bennettitalean cone, a common ancestor, and the angiosperm flower. That hypothesis also involved highly interpretive assessments of homology (Arber and Parkin, 1907).

In the same vein and bolder in interpretation, D. H. Scott in his book *Plant Evolution* (1909), suggested that the orthotropous ovules of cycadeoids were in fact borne in reduced carpels and that the interseminal scales among them were sterile carpels. Such an interpretation required a significant reduction of several structures (including the microsporangiate synangia), for which there was (and still is) no fossil evidence in the form of either putatively ancestral morphologies or transformational series. Nevertheless, the general view that Bennettitales were roughly transitional to angiosperms and separate from cycads gained ground as a result of an influential study by Thomas and Bancroft (1913), who also provided criteria for discriminating between cycad and cycadeoid foliage based on epidermal characters, particularly those of the stomatal apparatus.

A second and related line of controversy developed around the possibility that Bennettitales were related to Gnetales, as was suggested by some unusual similarities and recently reemphasized by Friis et al. (2007). These included the bisporangiate cone of *Cycadeoidea* as compared to the “flower” of *Welwitschia* and controversial interpretations of similarities between the seeds of bennettitaleans and gnetaleans. Following the

description of well-preserved bennettitalean seeds by Lignier (1894; i.e., *Bennettites morieri*), Wieland (1906; i.e., *Cycadeoidea* spp.), Thoday (1911) and Berridge (1911) interpreted the seeds of *Gnetum* as similar to those of Bennettitales based on the number of integuments, a proliferation of the micropylar tissue into a sealing “plug,” and the presence (according to Thoday, 1911) of a micropylar flange that in *Gnetum* envelopes the distal rim of the outer integumentary layer. Additional similarities cited by Thoday (1911) included an expanded (at least in some planes of section) shoulder area in the integument that has radially aligned palisade cells as well as a thickened sclerotesta.

Stopes (1918), however, vigorously objected to the assertion that seeds of Bennettitales and Gnetales displayed significant novel characters not also shared by Paleozoic pteridosperm seeds. Stopes (1918) noted that Thoday (1911) ascribed the identification of certain bennettitalean seed characters, particularly the double integument, to Lignier (1894) when Lignier had actually and explicitly described the seeds as “unitegumentées” (Stopes, 1918). Lignier (1911) himself joined the fray to express strong disagreement with some of the seed interpretations advanced by Berridge (1911) and Thoday (1911) that suggested Gnetalean-Bennettitalean affinities, particularly the mode of micropyle closure (Lignier, 1911). Notably, in reference to Berridge’s (1911, p. 144) assertion that the micropyles were closed in similar fashion in both Bennettitales and Gnetales (i.e., by an ingrowth of cells lining the micropyle). Lignier stated: “Je conteste absolument la possibilité de cette interprétation du ‘bec nucellaire’ dont l’état massif est absolument primaire et qui, du reste, est totalement indépendant du tube micropylaire” (viz., “I absolutely dispute the possibility for such an interpretation of the ‘nucellar beak,’ which in contrast to the remainder of the fused nucellus, is totally independent from the micropylar tube”).

After 1920, conventional botanical thought seemed to turn ambivalent regarding a close relationship between the Bennettitales and angiosperms + Gnetales (Chamberlain, 1920), possibly due to attacks on purported (seed) homologies as discussed and to the difficulties comparing reproductive structures of the two groups. Against a backdrop of apparently compelling morphological and anatomical similarities between cycadeoids, and some “lower” pteridosperms (e.g., Chamberlain, 1935) enthusiasm for close relationships among Bennettitales, Gnetales, and flowering plants waned. While some botanists continued to favor a separation between Bennettitales and Cycadales (e.g., Arnold [1947, p. 267], even though he noted that with respect to their internal vegetative structures cycads and cycadeoids [Bennettitales] demonstrate “few fundamental differences and many points of genuine resemblance”), others continued to explicitly favor a closer relationship between the Bennettitales and Cycadales (e.g. Norstog and Nicholls, [1997, p. 200]; who note that among other similarities, “girdling leaf traces are reputedly absent in a few living cycads”).

APPENDIX 2. Microsporangiate structures of uncertain structure and putative bennettitalean affinities.

A wide array of microsporangiate structures have been assigned to the Bennettitales (Crane, 1985, 1988), but they are incompletely understood with respect to both structure and systematic relationships. The compression genus *Bennettistemon* Harris includes a species with synangia (an apparent bennettitalean synapomorphy) and one without. The latter, while questionable with respect to affinities, has been identified as a possible

link between the Bennettitales and the Gnetales through another poorly understood fossil *Piroconites* as mentioned, but has been omitted from recent analyses due to its ambiguous nature (Hilton and Bateman, 2006). One species of equivocal relationships, *Bennettistemon ovatum* Harris, has monosulcate pollen and adaxial sporangia that are not aggregated in enclosed synangia. In other species of that genus, non-synangially

aggregated sporangia are arranged pinnately on "plates" (*Bennettistemon amblum* Harris; plates 11, 12 of Harris, 1932). Harris notes that stomatal type and monosulcate pollen suggest affinities with Bennettitales and that "It is of interest because no type of bennettitalean microsporophyll has yet been described in which the sporangia are borne this way" (p. 99).

There are numerous additional examples of poorly understood putative bennettitaleans (e.g., Watson and Sincock, 1992; text figure 106B). These compressed morphotaxa are difficult to interpret because, while some may well represent species that are relevant to understanding bennettitalean origin and relationships, others may not be. So while further studies of these fossils have undoubted potential, for the moment, in our opinion, there are too many presumptions necessary to include such fossils in any sort of rigorous morphological analysis, and attempts to do so introduce errors into a field of characters that have already been too subject to conflicting interpretation. However, because we are interested in higher relationships of anthophytes, already controversial given the contrast between DNA sequence-based analyses and those grounded in morphology, we have restricted our matrix to including the generalized taxon "Bennettitales" coded appropriately to reflect character variation among the major taxa. We decided to do so partly because the resulting tree topology remained the same as trees in which we included three of the best understood genera of Bennettitales as separate terminals: *Cycadeoidea*, *Williamsonia*, and *Williamsoniella*. These genera consistently come out in a monophyletic group in the same position as the umbrella taxon "Bennettitales" (W. Crepet et al., Cornell University, unpublished data).

Of the unequivocally identified Bennettitales, many have microsporophylls that appear to be pinnate, pinnately derived or fleshy. Some synangiate bennettitalean microsporophylls are entire-margined, while other entire-margined morphotaxa lack synangia. However, all unequivocal bennettitalean microsporophylls produce synangia either borne on short, sometimes thick, stalks, or are almost sessilely adherent to the microsporophylls (that Harris [1969] sometimes calls "segments"). Sporangial position is a character often used in phylogenetic analyses of seed plants. Thus, it is a character of some interest among bennettitalean taxa. There are several ways to place the position of bennettitalean synangia in perspective. The pinnate microsporophylls of *Weltrichia spectabilis* Nathorst, *Williamsoniella coronata* Thomas, and *Williamsoniella papillosa* Cridland could be construed as homologous with the incurved pinnate microsporophylls of *Cycadeoidea*. In other taxa, e.g., *Weltrichia sol* Harris, the microsporangiate apparatus is so different from a pinnate archetype that similarities to pinnate fronds are only defensible in the context of a hypothesized morphocline. Of course, such an interpretation best follows from a phylogenetic analysis rather than being the basis

(through character coding) of such an analysis. Thus, the morphocline ranging from the overtly pinnate microsporophylls of *Cycadeoidea* spp. and *Weltrichia spectabilis* Thomas, to the more modified ones of *Williamsoniella* Thomas, to the even more modified ones of *Weltrichia santalensis* Sitholey and Bose may or may not be reflective of evolutionary changes within the clade, and are in any case not congruent with the timing of the appearances of these microsporangiate organs in the fossil record. Moreover, such a transformational series does not logically include what appear to be overtly abaxial sporangia in the Triassic taxon *Wielandiella* Thomas, although there is considerable uncertainty as to structural details of *Wielandiella* microsporangiate organs (Harris, 1969).

Another interpretive difficulty arises because the positions of synangia vary according to how pinnate such structures are and because sporangial position (which is fixed within bivalved synangia) varies according to the position of the synangia, whose orientation can best be determined from their point of attachment and the disposition of the suture separating the two valves. This variation introduces some uncertainty in applying the character "sporangia adaxial or abaxial." At one extreme of the morphocline of bennettitalean microsporophylls that begins with the pinnate microsporophyll, "pinnae" are so reduced that the synangia are almost directly borne on the "rachis" in which case the synangia are adaxial (disposed with sutures facing the cone axis). This same phenomenon can be observed even in overtly pinnate microsporophylls where the distal pinnae are so shortened that the synangia are borne directly on the rachis (see Crepet, 1974, pl. 61, fig. 21).

In pinnate microsporophylls such as those of *Weltrichia spectabilis* and *Cycadeoidea* spp., synangia borne along the pinnae are pendant with the suture facing away from the pinnae, which can be interpreted as facing abaxially (or, depending on the flexibility of the synangial attachment and position in life, marginal at most; Fig. 1b). In such species, the synangia themselves consist of an outer two-valved capsule that includes tubular sporangia. The outer synangial wall may be homologous to the lamina of the pinnule, an interpretation supported by position and by the facts that the synangial wall in Bennettitales is not formed by the fusion of sporangial walls and because there are trichomes and stomata on the outer synangial walls of some taxa (e.g. *Weltrichia setosa*; Harris, 1969). Thus, there is variability in synangial position according to the specific taxon of bennettitaleans, and our coding reflects this variability when appropriate. Insights into basic synangial position in Bennettitales may be forthcoming from phylogenetic context and could be confirmed in future studies by incorporating more bennettitalean taxa. In this study, pending further analyses, we code sporangial position as "?" rather than identifying a single condition that encompasses the entire order.