POLLEN MORPHOLOGY AND ULTRASTRUCTURE OF GYMNOSPERMOUS ANTHOPHYTES

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Abstract

In recent years, phylogenetic analyses of seed plants have suggested a close relationship among angiosperms and three orders of gymnosperms, including Gnetales, Bennettitales, and Pentoxylales. On the basis of several vegetative and reproductive features, these studies have linked the three gymnosperm groups along with angiosperms into a single 'anthophyte' clade. Furthermore, reports of Cretaceous fossils with *in situ Eucommiidites*-type pollen indicate that plants that produced these types of pollen grains (Erdtmanithecales) may also have their affinities nested within the same clade of highly derived seed plants. Two unifying palynological characters are shared among all anthophyte groups; these include a non-saccate condition and a granular sexine infrastructure. In the present paper, pollen morphology and ultrastructure are reviewed for the extant and fossil genera of the four anthophyte orders of gymnosperms that have been studied with transmission electron microscopy. The taxa reviewed include Gnetales (*Ephedra, Welwitschia, Gnetum, Ephedripites, Equisetosporites*), Bennettitales (*Leguminanthus, Cycadeoidea, Monosulcites*), Pentoxylales (*Sahnia*), and Erdtmanithecales (*Eucommiidites, Erdtmanitheca, Eucommiitheca*). Pollen of these taxa is compared with regard to size, shape, aperture type and location, surface ornamentation, and exine infrastructure. These characters are also discussed with regard to their systematic and phylogenetic significance.

Introduction

Many botanists generally consider the term 'anthophyte' as a reference to flowering plants, or members of the Anthophyta (for example, Raven *et al.*, 1999). However, in 1986 the term was also used as an informal designation for a clade encompassing several major groups of highly derived seed plants (Doyle and Donoghue, 1986a). Since the term's initial use in this cladistic context it has been increasingly incorporated into the literature, particularly in other phylogenetic analyses (for example, Donoghue and Doyle, 1989; Crane, 1990; Rothwell and Serbet, 1994).

The anthophyte clade includes angiosperms as well as Gnetales, Bennettitales, and Pentoxylales. The clade, whether specifically referred to as 'anthophyte' or not, is held together by a number of vegetative and reproductive features (for example, Doyle and Donoghue, 1986a, 1992; Crane, 1985, 1990; Rothwell and Serbet, 1994). These characters include syndetocheilic stomata, complex, 'flower-like' reproductive structures (lacking in Pentoxylales according to Rothwell and Serbet, 1994) with aggregated microsporophylls, non-saccate pollen with granular exine infrastructure, and bitegmic ovules. Moreover, reports of Cretaceous fossils with *in situ Eucommiidites*-type pollen suggest that plants that produced these types of grains may also have their

affinities nested within the anthophyte clade (Pedersen *et al.*, 1989a; Friis and Pedersen, 1996). A new order and a new family (Erdtmanithecales, Erdtmanithecaceae) have been established to accommodate dispersed *Eucommiidites* pollen, as well as *Eucommiidites*-producing plants (Friis and Pedersen, 1996).

In the present paper, pollen morphology and ultrastructure are reviewed for the extant and fossil genera of the four anthophyte orders of gymnosperms that have been studied with transmission electron microscopy (Table 1).

Pollen morphology and ultrastructure

Gnetales

The Gnetales are the only group of gymnospermous anthophytes with extant representatives, comprising three living genera: *Ephedra*, *Welwitschia*, and *Gnetum*. Despite marked dissimilarity in habit and habitat among these taxa, as well as one study suggesting that the order is paraphyletic (Nixon *et al.*, 1994), most phylogenetic analyses indicate that the Gnetales are monophyletic. This phylogenetic assessment is based on a suite of shared morphological features (Crane, 1985, 1988; Doyle and Donoghue, 1986a, b; Doyle, 1996), as well as evidence of molecular similarity (*rbc*L data; Hasabe *et al.*, 1992; Price, 1996). *Ephedra* segregates independently, whereas *Welwitschia* and *Gnetum* appear to be more closely related (for example, Doyle, 1996).

Limited information is available about the geologic history of the Gnetales. *Drewria potomacensis* Crane & Upchurch, from the Early Cretaceous Potomac Group of Virginia, U.S.A., is the only described megafossil species with unequivocal gnetalean affinities (Crane and Upchurch, 1987). Additionally, several other Triassic and Cretaceous megafossils have been suggested to have affiliation within the Gnetales (see Crane, 1988, 1996). The majority of data about gnetalean evolution through geologic time comes from palynological evidence. The record of dispersed, 'polyplicate' pollen resembling that of *Ephedra* and *Welwitschia*, at the light microscopical level, extends from Lower Permian to Recent sediments (see Osborn *et al.*, 1993, and references therein). Polyplicate pollen is also produced by several angiosperm groups (for example, Arales, Laurales, Zingiberales). Although some dispersed polyplicate grains are thought to be angiospermous (see below, and Osborn *et al.*, 1993), Hesse *et al.* (2000) have shown that the pollen walls of these pollen types are structurally and chemically different from those of the Gnetales.

EXTANT TAXA
Ephedra

Ephedra consists of approximately 35-45 species (Kubitzki, 1990). No comprehensive palynological survey has been conducted with electron microscopy; micrographs of pollen wall ultrastructure have been published for only eight species (Table 1). However, Steeves and Barghoorn (1959) used transmitted light to systematically examine the pollen of 44 species primarily to generate a database with which to compare fossil pollen.

Pollen of *Ephedra* is elliptic to elongate and ranges from 20-80 μm in length and 16-50 μm in width. Grains are characterised by a series of longitudinal ribs, or plicae, that are typically psilate (Figs. 1-4). Plicae number ranges from 4-19 (Steeves and Barghoorn, 1959). The polyplicate (=striate in many phylogenetic studies) pollen of the genus is typically considered inaperturate, although the exine is considerably thinner between the plicae, or within the furrows (Figs. 5, 7). El-Ghazaly *et al.* (1998) have experimentally shown that the entire exine is completely discarded prior to pollen tube germination. Palynologically, species are principally distinguished from one another by the number of plicae, plica height and degree of slope, and furrow morphology. The furrow may either be straight (Figs. 1-2) or undulated/branched (Figs. 3-4).

TABLE 1. Gymnospermous anthophytes for which pollen ultrastructure has been investigated with transmission electron microscopy.

Taxon	Reference(s)
GNETALES	
EXTANT TAXA	
Ephedra americana	Hesse, 1984; El-Ghazaly *et al.*, 1998
E. californica	Zavada, 1984
E. campylopoda	Hesse, 1984
E. distachya	Van Campo and Lugardon, 1973; Kurmann, 1992; Kurmann and Zavada, 1994; El-Ghazaly *et al.*, 1998
E. foliata	Rowley, 1995; El-Ghazaly and Rowley, 1997
E. intermedia	Bernard and Meyer, 1972
E. monosperma	Afzelius, 1956; Gullvåg, 1966
E. sinica	Ueno, 1960
Gnetum africanum	Oryol *et al.*, 1986
G. gnemon	Gullvåg, 1966; Hesse, 1980; Kurmann, 1992
G. indicum	Bernard and Meyer, 1972
G. montanum	Gullvåg, 1966
G. ula	Gullvåg, 1966
G. sp.	Zavada, 1984
Welwitschia mirabilis	Ueno, 1960; Gullvåg, 1966; Bernard and Meyer, 1972; Hesse, 1984; Kedves, 1987; Zavada and Gabarayeva, 1991; Hesse *et al.*, 2000
FOSSIL TAXA	
Ephedripites sp. 1	Trevisan, 1980
Equisetosporites spp.	Osborn *et al.*, 1993
BENNETTITALES	
Cycadeoidea dacotensis	Taylor, 1973; Osborn and Taylor, 1995
Leguminanthus siliquosus	Ward *et al.*, 1989
Monosulcites sp. 1	Trevisan, 1980
PENTOXYLALES	
Sahnia laxiphora	Osborn *et al.*, 1991
ERDTMANITHECALES (*EUCOMMIIDITES* PLANTS)	
Eucommiidites sp.	Doyle *et al.*, 1975
E. sp. 1	Trevisan, 1980
E. sp. 2	Trevisan, 1980
E. sp. from *Erdtmanispermum balticum*	Pedersen *et al.*, 1989a
E. troedssonii	Scheuring, 1970, 1978; Batten and Dutta, 1997
Erdtmanitheca texensis	Pedersen *et al.*, 1989a
Eucommiitheca hirsuta	Friis and Pedersen, 1996

Overall exine thickness, as well as thicknesses of individual exine strata vary depending on where the pollen wall is measured (i.e., at the crest of a plica vs. grading into a furrow). The sexine consists of a homogeneous tectum and a granular infratectum (Figs. 5-6). Within the plicae, the tectum is typically thinnest at the crest and gradually thickens down the sides (Fig. 5). The tectum typically becomes thinner as it grades into the furrow. Within the furrow, the tectum may either be present as a thin layer (Fig. 7), or may be almost completely absent (Hesse, 1984). The granular infratectum is also absent within the furrow (Figs. 5, 7). The nexine is electron-dense, uniform in thickness in both the plica and furrow regions, and consists of well-defined, sometimes anastomosed lamellae (Figs. 5-7). A thin, electron-lucent "footlayer" has been reported to overlay the lamellated layer in some taxa (for example, Van Campo and Lugardon, 1973; Kurmann, 1992), whereas it is absent in others (Fig. 6).
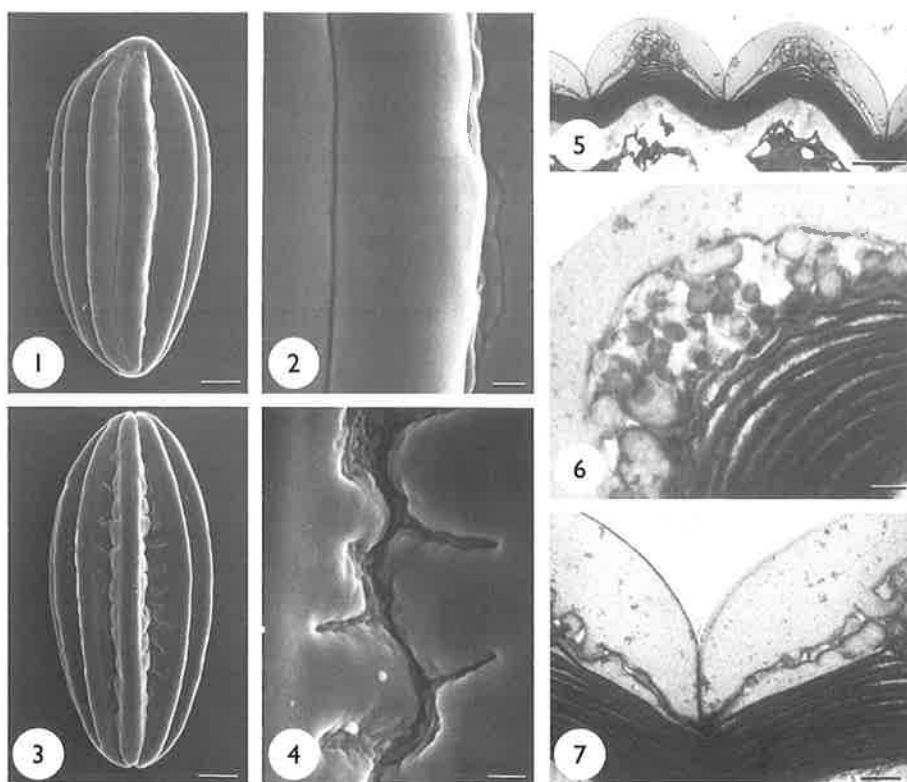
FIGS. 1–7. Gnetales – *Ephedra*. Fig. 1. Pollen of *Ephedra californica*. Bar = 5 μm. Fig. 2. Detail of the pollen surface of *E. californica* showing unbranched furrows. Bar = 1 μm. Fig. 3. Pollen of *Ephedra aspera*. Bar = 5 μm. Fig. 4. Detail of the pollen surface of *E. aspera* showing branched, scabrate furrows. Bar = 1 μm. Fig. 5. Transverse section through the exine of *Ephedra trifurca* showing two complete plicae and three furrows. Note the electron-lucent sexine and the electron-dense nexine. Bar = 1 μm. Fig. 6. Detail of the exine from *E. trifurca* at the crest region of a single plica. Note the homogeneous tectum, large infratectal granules, absence of an electron-lucent 'foot layer' and the white-line-centered, electron-dense lamellae. Bar = 0.1 μm. Fig. 7. Detail of the exine from *E. trifurca* at a furrow region showing thin tectum and lack of infratectal granules. Bar = 0.2 μm.
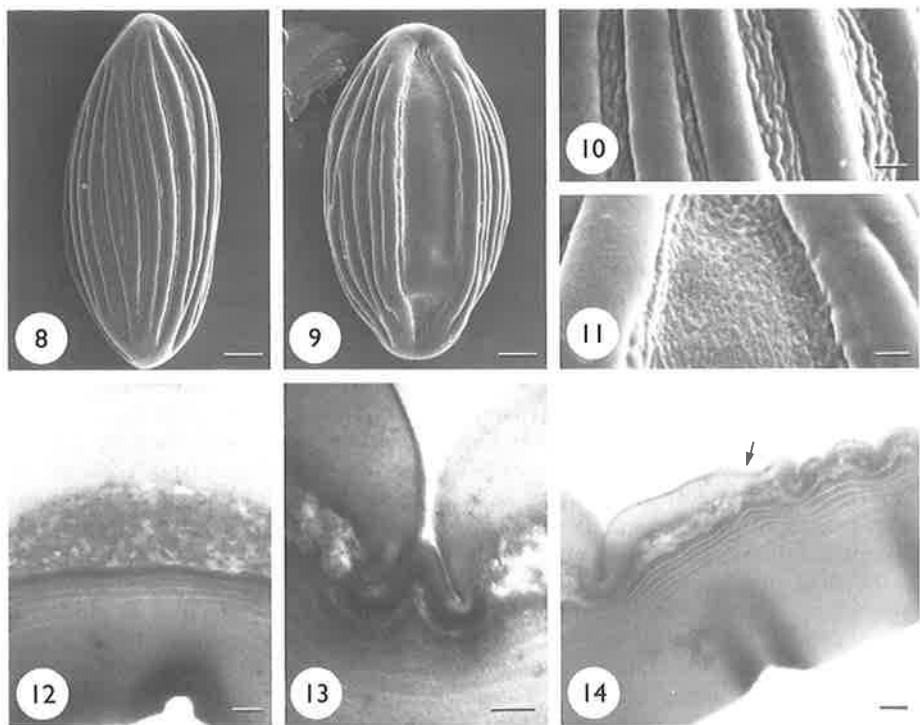
FIGS. 8–14. Gnetales – *Welwitschia mirabilis.* Fig. 8. Proximal view of a pollen grain. Bar = 5 μm. Fig. 9. Distal view of a pollen grain showing a single, broad sulcus. Bar = 5 μm. Fig. 10. Detail of the proximal wall showing five plicae and four furrows. Bar = 1 μm. Fig. 11. Detail of the distal sulcus showing scabrate surface. Bar = 1 μm. Fig. 12. Transverse section through the crest region of a single plica. Note the thick, homogeneous tectum, finely granular infratectum, absence of a well-defined, electron-lucent 'foot layer,' and electron-dense nexine. Lamellae are only detectable in the uppermost region of the nexine. Bar = 0.1 μm. Fig. 13. Detail of a furrow region of the proximal exine showing thin tectum and absence of infratectal granules. Bar = 0.1 μm. Fig. 14. Detail of the distal wall showing the margin of the apertural membrane. Note that the tectum (arrow) gradually thins toward the apertural membrane (at right). Bar = 0.1 μm.

Welwitschia

Pollen of the monotypic *Welwitschia mirabilis* J.D. Hook. (Kubitzki, 1990) is like that of *Ephedra* in being elongate to elliptic in shape and polyplicate (Figs. 8-9). Grains average 51 μm in length and 31 μm in width and have 15-20 psilate plicae (for example, Sahashi *et al.*, 1976; Kedves, 1987). In contrast to *Ephedra*, pollen of *Welwitschia* typically has more rounded plicae and is monoaperturate (Figs. 8-11). The sulcus is relatively broad, extending nearly the entire length of each grain, and the apertural membrane is psilate to slightly scabrate (Figs. 9, 11).

The tectum is generally thicker than that of *Ephedra*, and it is more uniform in thickness within the plicae (i.e., both at the crests and laterally toward the furrows; Figs. 12-13). However, the tectum becomes thinner and is present over both the non-apertural furrows and the apertural membrane (Figs. 13-14). A distinct infratectum is present within the plicae, consisting of relatively small, tightly packed granules (Fig. 12). Zavada and Gabarayeva (1991) have shown that tectum thickness within the plicae of *Welwitschia* is in part due to ontogenetic fusion of infratectal granules.

Distinguishable granules are absent at the non-apertural furrows (Fig. 13) and the apertural membrane (Fig. 14). A well-defined, electron-lucent foot layer is also lacking (Figs. 12-13). The nexine is typically more electron-dense than the sexine, and it is uniform in thickness in both non-apertural and apertural regions (Figs. 12-14). The nexine is lamellate (Figs. 12-14); however, the lamellae are not as easily detectable as they are in the pollen of *Ephedra* and *Gnetum*.

Gnetum

Gnetum consists of approximately 30 species (Kubitzki, 1990), yet pollen wall ultrastructure has been illustrated for only six taxa (Table 1). However, a palynological survey of the genus has been undertaken, and a preliminary summary of the systematic significance of surface morphology has been reported (Gillespie and Nowicke, 1994).

Pollen of *Gnetum* is spheroidal, inaperturate, and relatively small, averaging 12-20 μm in diameter (Figs. 15-16). *Gnetum* is unique among gymnosperms in being the only taxon with a spinose to spinulose ornament, although surface sculpture varies among species (Gillespie and Nowicke, 1994). Asian species are characterised by conical, blunt-tipped spines that are supported by a uniform tectum (Fig. 15). By contrast, the pollen of Neotropical and African taxa have smaller, more rounded, and more numerous spinules. The spinules of these species appear to rest on a discontinuous, irregularly thickened tectum that forms plate-like areas (Fig. 16).

Seen in thin section, it is evident that the surface spines and spinules are not supratectal. Rather, these 'sculptural elements' are sexinous regions in which the tectum folds over conical areas of densely packed infratectal granules (Figs. 17-18). Given this ultrastructure, the spines and spinules appear to be homologous with the longitudinally oriented plicae of *Ephedra* and *Welwitschia* pollen. Interestingly, in *Gnetum*, pollen thickness of the tectum is uniform in these regions, as it is in non-spinose regions (Figs. 17, 19). A distinguishable electron-lucent foot layer is absent. The nexine is more electron-dense than the sexine, and it is evenly thickened in all wall regions. Well-defined and relatively thick nexine lamellae are present (Figs. 17, 19).

FOSSIL TAXA
Equisetosporites

Several dispersed palynomorphs assignable to *Equisetosporites* spp. from the Lower Cretaceous Santana Formation of Brazil have been studied (Osborn *et al.*, 1993). The polyplicate grains are ellipsoidal, average 41 μm in length and 13 μm in width, and have a variable number of plicae (ranging from 5 to 11; Figs. 20-21). Plicae have psilate to slightly scabrate surface sculpture; these grade into straight furrows that do not reach the grain ends (Fig. 20).

The overall exine averages 1.0 μm in thickness in plicae regions (Figs. 21-22). The tectum is uniform in thickness within the plicae (i.e., at the crest and laterally toward the furrows). Infratectal granules range in size from 0.09-0.26 μm in diameter. Small granules are present in the lower portion of the infratectum and are directly adjacent to the underlying, electron-dense nexine; these gradually grade into larger granules in the upper portion and appear to fuse with the tectum (Fig. 22). Within the furrow regions, the tectum thins abruptly at the margins, and it is the only layer of sexine present over the furrow (Fig. 23). The nexine is relatively thick throughout, but thins slightly under the sexine furrows (Figs. 21, 23). Although the nexine is lamellate throughout, this was only detectable in grains that had undergone significant preservational folding (Fig. 24). Most grains studied only exhibited a few lamellae near the nexine/sexine interface or in slightly folded regions near the furrows (Osborn *et al.*, 1993).
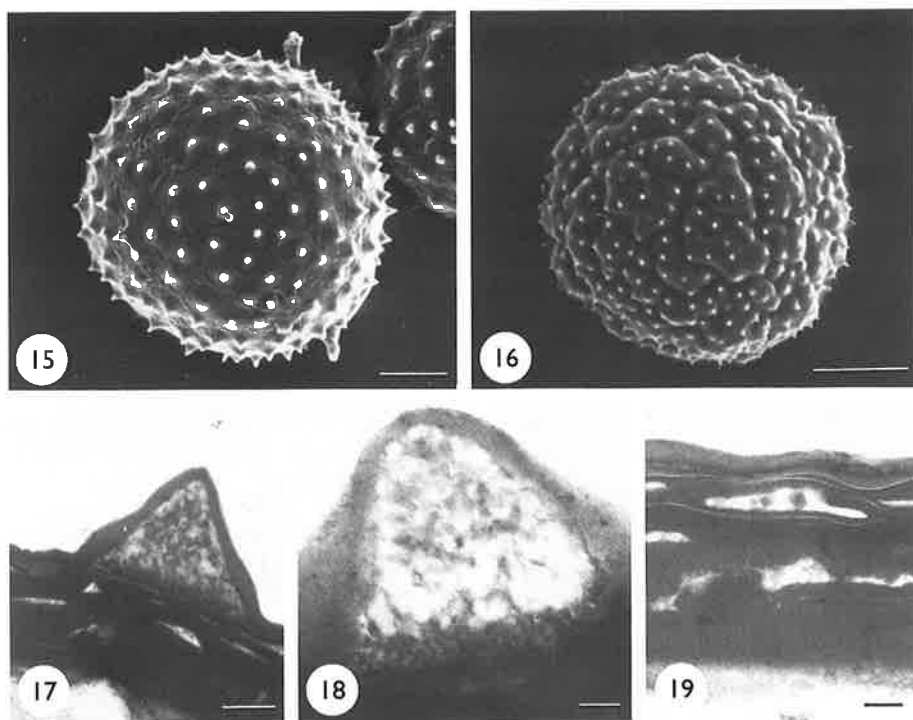
FIGS. 15–19. Gnetales – *Gnetum.* Fig. 15. Pollen of *Gnetum gnemon* showing continuous tectum and surface spines. Bar = 3 μm. Fig. 16. Pollen of *Gnetum nodiflorum* showing plate-like tectum and surface spinules. Bar = 3 μm. Fig. 17. Transverse section of the exine of *G. montanum.* Note the thin, homogeneous tectum, densely packed infratectal granules under the 'spine,' and thick, electron-dense lamellae of the nexine. Bar = 0.3 μm. Fig. 18. Detail of the exine in a spine region of *G. montanum* showing the infratectal granules in direct contact with the electron-dense nexine. Bar = 0.1 μm. Fig. 19. Detail of the exine in a non-spinous region of *G. montanum* showing the tectum in direct contact with the electron-dense nexine. Note also the thick, white-line-centered lamellae of the nexine. Bar = 0.1 μm.

Another species of *Equisetosporites* (*E. chinleana* Daugherty; Zavada, 1984, 1990; Pocock and Vasanthy, 1988), as well as *Cornetipollis reticulata* Pocock & Vasanthy (separated from *E. chinleana*; Pocock and Vasanthy, 1988) have also been studied at the ultrastructural level. Based on surface morphology of the plicae, as well as the presence of infratectal columellae, these two Upper Triassic grains are now thought to be angiospermous (see Osborn *et al.*, 1993) and will not be considered here.

Ephedripites

Ultrastructural details for one dispersed taxon of *Ephedripites* have been reported. *Ephedripites* sp. 1, from the Lower Cretaceous of Italy (Trevisan, 1980), is ellipsoidal, averages 44 μm in length and 19 μm in width, and is polyplicate. The palynomorph is also distinctly monoaperturate. The sulcus is relatively broad and extends the entire length of the grain, but does not reach the grain ends (Fig. 25A).
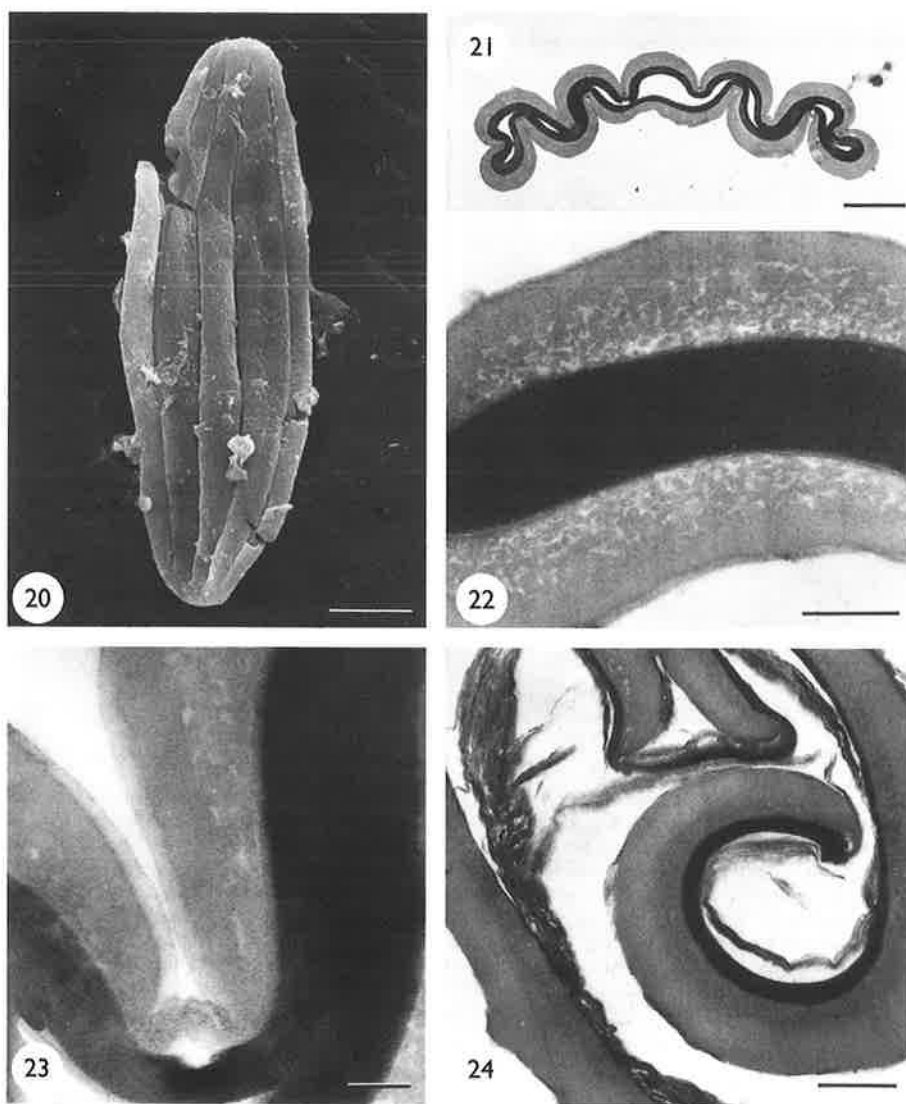
FIGS. 20–24. Gnetales – *Equisetosporites* spp. Fig. 20. Polar view of a pollen grain showing five, broad plicae. Bar = 5 μm. Fig. 21. Transverse section through a grain showing electron-lucent sexine and uniformly thick, electron-dense nexine. Bar = 2 μm. Fig. 22. Detail of two opposing plicae. The pollen lumen is not detectable as the opposing electron-dense nexines are compressed against each other. Note the homogeneous tectum, well-defined infratectal granules, as well as the absence of both a detectable foot layer and nexine lamellae. Bar = 0.5 μm. Fig. 23. Detail of the exine at a furrow showing the absence of a granular infratectal layer and a folded tectum in this region. Bar = 0.2 μm. Fig. 24. Section through a preservationally folded grain showing separated lamellae within regions of the electron-dense nexine. Bar = 0.5 μm.

The exine of *Ephedripites* sp. 1 within the plicae regions consists of an inner lamellated nexine ("layer A" of Trevisan, 1980) and an outer, electron-dense, five-layered sexine ("layers B_1, B_2, B_3, C, D" of Trevisan, 1980). The most prominent sexine layers are the inner three (Fig. 25B). These consist of a thin, homogeneous band (=foot layer?; B_1) appressed to the lamellate nexine, a layer of "anastomosing units" (=granules; B_2), and a homogeneous layer (=tectum; B_3). Layer C is a thin, discontinuous zone just outside the tectum and is intermixed to overlaid by layer D. Layer D consists of variably sized "globulets" (Trevisan, 1980) that have subsequently been suggested to represent debris (Osborn *et al.*, 1993) or tapetal remains (Zavada, 1984). The tectum appears to be uniform in thickness under the plicae and abruptly thins at the margins of the furrows, where it is present as a thin layer (Fig. 25B). The apertural membrane is described as having a similar ultrastructure to that of the non-apertural furrows (Trevisan, 1980).

Bennettitales

The Bennettitales are represented by fossils of Triassic to Cretaceous age. Although the group is now recognised to be somewhat diverse taxonomically, it is best known from the permineralised, cone-bearing trunks of *Cycadeoidea* (see Crepet, 1974, and references therein), as well as a number of compression-impression leaf taxa (for example, Thomas and Bancroft, 1913).

In situ pollen is known from many taxa (Osborn and Taylor, 1995); however, pollen of only two genera has been described at the ultrastructural level, *Leguminanthus* and *Cycadeoidea*. In addition to these taxa, Ward *et al.* (1989) suggested that the dispersed palynomorph *Monosulcites*, for which fine structural data have been published, may have affinities within the Bennettitales. Furthermore, monosulcate pollen identified within micropylar tubes of the Triassic bennettitalean ovules *Vardekloeftia* are reported to be similar to *Monosulcites minimus* Couper (Pedersen *et al.*, 1989b). Given these descriptions, *Monosulcites* will be addressed here, despite previous classification as *Incertae sedis* (Osborn *et al.*, 1991; Osborn and Taylor, 1994).

Leguminanthus

Leguminanthus siliquosus (Leuthardt) Kräusel & Schaarschmidt is a compressed microsporophyll known from the Upper Triassic of Luntz, Austria and Neuewelt, Switzerland (Crane, 1988, and references therein). Ward *et al.* (1989) examined pollen of *L. siliquosus* in a comparative context as part of a study of *Lethosmasites fossulatus* Ward, Doyle, & Hotton, a putative angiosperm monosulcate grain with a granular exine from the Early Cretaceous Potomac Group, U.S.A.

Pollen of *Leguminanthus siliquosus* is elliptic, relatively small, measuring 22 μm in length and 13 μm in width, and monosulcate (Fig. 25C). The exine is psilate in external ornament, 0.70 μm in overall thickness, and two-parted. The electron-lucent sexine consists of a thick tectum and an infratectal layer of relatively large, tightly packed granules (Fig. 25D). The granular infratectum is reported to be "fused basally into a foot layer" (Ward *et al.*, 1989). The underlying nexine is electron-dense, faintly lamellate (Fig. 25D), and consistent in thickness in both non-apertural and apertural regions. The aperture spans almost the entire length of each grain (Fig. 25C). The apertural membrane also appears to have a thin layer of sexine present.
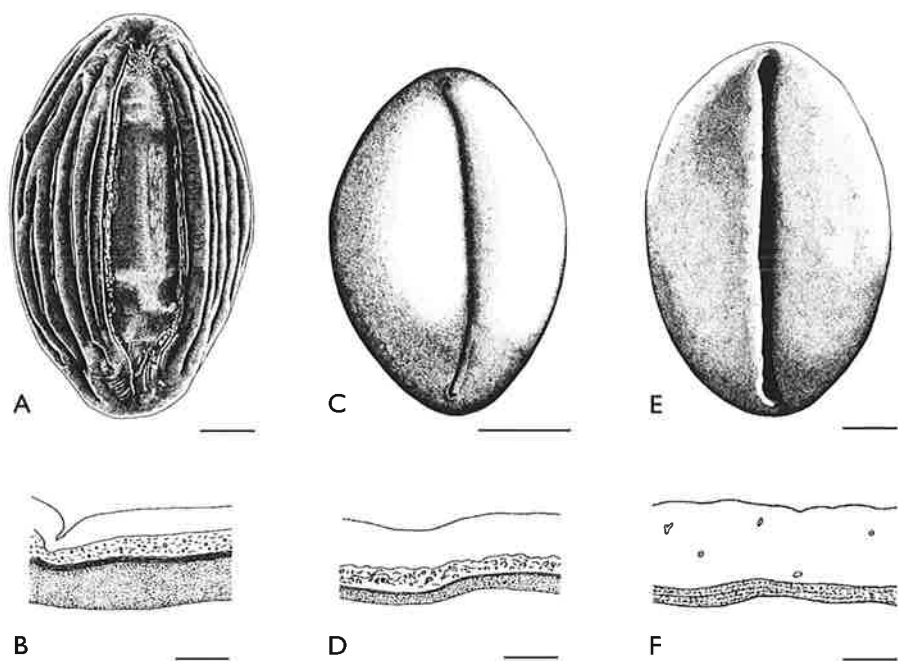
FIG. 25. Gnetales and Bennettitales. A-B. Gnetales - *Ephedripites* sp. 1 (Modified from Trevisan, 1980). A. Distal view of a pollen grain showing elongate sulcus. Bar = 5 μm. B. Section through the non-apertural wall showing thick tectum, granular infratectum, and partially lamellate nexine (stippled). Bar = 0.5 μm. C-D. Bennettitales - *Leguminanthus siliquosus* (Modified from Ward *et al.*, 1989). C. Distal view of a pollen grain showing thin sulcus. Bar = 5 μm. D. Section through the non-apertural wall showing thick tectum, compressed granular infratectum, and partially lamellate nexine (stippled). Bar = 0.5 μm. E-F. Bennettitales - *Monosulcites* sp. 1 (Modified from Trevisan, 1980). E. Distal view of a pollen grain showing broad, elongate sulcus. Bar = 5 μm. F. Section through the non-apertural wall showing relatively homogeneous sexine and lamellate nexine (stippled). Bar = 0.5 μm.

Cycadeoidea

Specimens of *Cycadeoidea* have been recovered from a wide range of Northern Hemisphere localities (Crepet, 1974). However, most information about these plants comes from studies of fossils collected at several Lower Cretaceous sites in the Black Hills of South Dakota and Wyoming, U.S.A. Many species have been described (Wieland, 1906, 1916), but those bearing cones preserved with intact pollen organs and *in situ* pollen grains, or in a bisporangiate condition, are relatively rare (Crepet, 1974). Pollen fine structure has been investigated from a single species, *C. dacotensis* (McBride) Ward (Taylor, 1973; Osborn and Taylor, 1995).

Pollen of *Cycadeoidea dacotensis* is monosulcate and typically elliptic in shape (Fig. 26), although occasional spheroidal grains also occur. Grains average 25 μm in length and 12 μm in width and exhibit punctate to psilate surface ornamentation (Fig. 26). The length and surface sculpture of the sulcus has proven difficult to ascertain,

because the apertural region of most grains is highly folded. The exine averages 0.73 μm in overall thickness. The sexine is two-zoned, consisting of a relatively thin, homogeneous tectum and a granular infratectum (Figs. 27-29). In some grains, the tectum is easily delimited from the underlying infratectum because granule size and spacing is fairly uniform (Fig. 27). However, in many grains granule packing is so dense that the sexine superficially appears homogeneous throughout (Figs. 28-29). The nexine lacks detectable lamellae (Figs. 27-29) and is uniform in thickness all around the grain (Fig. 28). The apertural membrane consists of a thin, homogeneous sexine layer (=tectum), resulting from lateral thinning of both the tectum and infratectum, and an electron-dense nexine (Fig. 29).
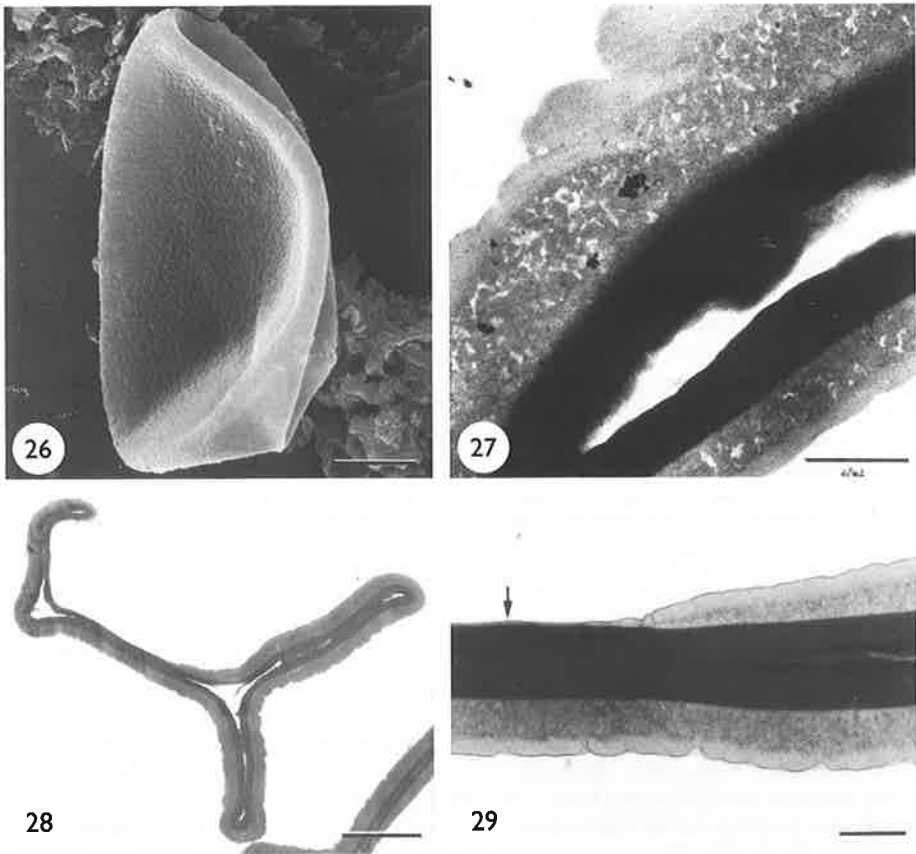
FIGS. 26–29. Bennettitales – *Cycadeoidea dacotensis*. Fig. 26. Partially folded pollen grain showing elongate shape and punctate surface. Bar = 5 μm. Fig. 27. Oblique section through the non-apertural wall showing homogeneous tectum, well-defined granular infratectum, absence of a foot layer, and thick, electron-dense nexine. Note also the absence of nexine lamellae. Bar = 0.5 μm. Fig. 28. Transverse section of a folded grain showing electron-lucent sexine and uniformly thick, electron-dense nexine. Bar = 3 μm. Fig. 29. Transverse section of a compacted grain in the apertural (arrow) and opposing non-apertural regions. The apertural membrane consists of a thin, electron-lucent tectum and thick nexine. Note also that in non-apertural regions the infratectal granules are highly compacted and not individually detectable. Bar = 0.5 μm.

Monosulcites

Two investigations have reported fine structural data for the dispersed taxon *Monosulcites* (Trevisan, 1980; Zavada and Dilcher, 1988). *Monosulcites* sp. 1, from the Lower Cretaceous of Italy (Trevisan, 1980), is ovoid with somewhat pointed ends and averages 47 μm in length and 25 μm in width. The palynomorph is psilate and has a single, slightly folded aperture extending almost the entire length of the grain (Fig. 25E). *Monosulcites* sp. 1 has a thick exine (1.5 - 2.0 μm), consisting of two principal layers (Fig. 25F). The inner layer (nexine) is electron-dense, lamellate, and uniform in thickness in both apertural and non-apertural regions. The outer, electron-lucent layer (sexine) is five to six times thicker than the nexine and appears to consist of a thin tectum and a thick, granular infratectum (Fig. 25F). However, individual granules are not distinct. Granules appear large and highly fused throughout the infratectum, especially in the lower region where they form a basal, homogeneous layer.

The second *Monosulcites* taxon described, *M.* sp. from the Upper Cretaceous of Minnesota, U.S.A. (Zavada and Dilcher, 1988), will not be considered here. Osborn and Taylor (1995) have suggested that this palynomorph more than likely has its affinities outside of the anthophyte clade, within either Cycadales or Ginkgoales. This suggestion was based on the large grain size and apparent alveolar infratectal elements.
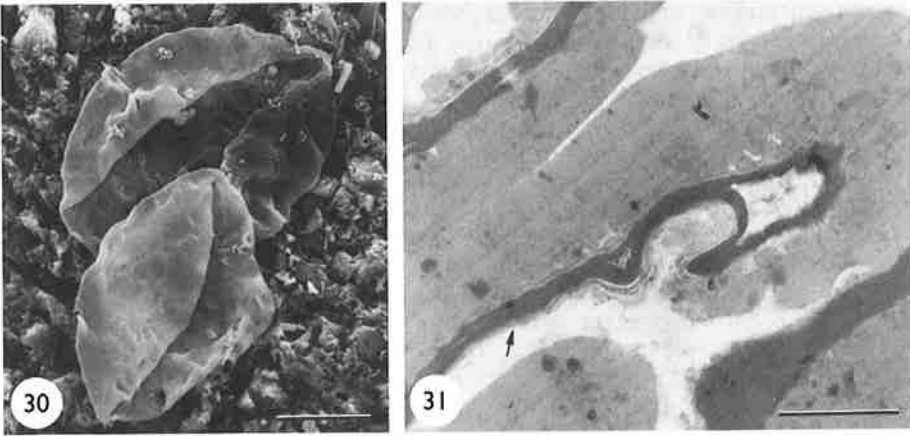
Pentoxylales

The Pentoxylales range from Jurassic to Cretaceous in age, and are known only from Southern Hemisphere localities. The taxon was originally established to classify a number of enigmatic permineralised plants (Sahni, 1948); however, a variety of compression fossils have subsequently been described and assigned to the order (see Osborn *et al.*, 1991, and references therein). Four genera are typically recognised for woody stems (*Pentoxylon*), leaves (*Nipaniophyllum*), ovulate organs (*Carnoconites*), and pollen organs (*Sahnia*). Despite the fact that these organs are not necessarily recovered in organic attachment, many studies, including phylogenetic analyses, often collectively refer to them as the *Pentoxylon* plant (for example, Bose *et al.*, 1985; Crane, 1985; Doyle and Donoghue, 1986a).

Sahnia

Two species of *Sahnia* have been described. *Sahnia nipaniensis* Vishnu-Mittre is based on silicified material of Jurassic age from the Rajmahal Hills, northeastern India (Vishnu-Mittre, 1953), whereas *S. laxiphora* Drinnan & Chambers is known from Lower Cretaceous compression specimens of the Strzelecki Group, southeastern Victoria, Australia (Drinnan and Chambers, 1985, 1986). Although both species contain *in situ* pollen, data on pollen fine structure are only known from *S. laxiphora* (Osborn *et al.*, 1991).

Pollen grains of *Sahnia laxiphora* are ovoid and small, averaging 26 μm in length and 23 μm in width (Fig. 30). Grains are monosulcate, with the relatively broad sulcus extending nearly the entire length of each grain (Fig. 30). The exine is psilate and typically highly folded in the apertural region (Fig. 30). The exine averages 0.95 μm in overall thickness and is two-parted. The sexine consists of a relatively thick, homogeneous tectum and a granular infratectum (Fig. 31). The boundary between the tectum and infratectum is not well-defined, with the infratectal granules grading into the tectum. Granules are most pronounced at the base of the sexine where they are directly contiguous with the underlying nexine (Fig. 31). The nexine is electron-dense and relatively uniform in thickness in both non-apertural and apertural regions (Fig. 31). Faint nexine lamellae are occasionally present and are most prominent at the nexine/sexine interface.

FIGS. 30–31. Pentoxylales – *Sahnia laxiphora*. Fig. 30. Distal view of two compressed pollen grains. Note that both grains are folded in the sulcus regions. Bar = 10 μm. Fig. 31. Transverse section of a grain in the apertural (arrow) and opposing non-apertural region showing electron-lucent sexine and electron-dense nexine. Note that the infratectal granules are compacted and appear to be fused with the thick tectum, as well as that the nexine is uniform in thickness in apertural and non-apertural regions. Bar = 1 μm.

Erdtmanithecales (*Eucommiidites* plants)

The *sporae dispersae* genus *Eucommiidites* was established by Erdtman (1948) to describe 3 'colpate' pollen grains from the Lower Jurassic of Sweden that resembled pollen of the extant angiosperm *Eucommia*. Subsequent studies have shown that *Eucommiidites* is gymnospermous and a variety of dispersed species have been established (see Friis and Pedersen, 1996, and references therein). Furthermore, several intact pollen organs and seeds with *in situ Eucommiidites* pollen have been described. On the basis of these descriptions, Friis and Pedersen (1996) have established Erdtmanithecaceae and Erdtmanithecales to accommodate both dispersed *Eucommiidites* species and *Eucommiidites*-producing plants. The latter includes two pollen organs (*Erdtmanitheca texensis* Pedersen, Crane, & Friis and *Eucommiitheca hirsuta* Friis & Pedersen) and four seeds (*Erdtmanispermum balticum* Pedersen, Crane, & Friis, *Spermatites pettensis* Hughes, *S. patuxensis* Brenner, and *Allicospermum retemirum* Harris). The family is based on a suite of reproductive characters, including three pollen features: elliptic shape having a "distal main colpus" with expanded ends and "flanked by two colpi or a single ring colpus in a proximal to almost equatorial position," granular infratectum, and "thick laminated endexine" (Friis and Pedersen, 1996).

Eucommiidites

Ultrastructural details have been studied from four dispersed taxa of *Eucommiidites*, as well as from *Eucommiidites*-type pollen found within the micropyle of *Erdtmanispermum balticum* seeds (Table 1). All of these taxa are Lower Cretaceous in age, except for *E. troedssonii* Erdtman, which is Lower Jurassic. Most dispersed taxa studied are ovoid to slightly elliptic in outline, have a psilate to slightly scabrate ornament, and range from 22-29 μm in length to 15-26 μm in width (Fig. 32A-C). Furthermore, most palynomorphs have a well-defined sulcus with slightly expanded,

round ends and two lateral furrows with pointed ends (Fig. 32A-C). Variation in surface sculpture is seen in the *Eucommiidites*-type grains recovered within *Erdtmanispermum balticum* micropyles (Pedersen *et al.*, 1989a); these *in situ* grains have a distinctly foveolate surface (Fig. 32D-F).

Exine ultrastructure varies widely among the *Eucommiidites* grains sectioned. Most grains have an electron-lucent, outer layer (sexine) and an electron-dense, inner layer (nexine). However, the entire exine is uniformly electron-dense in *E.* sp. (Doyle *et al.*, 1975), whereas it is uniformly electron-lucent in *E. troedssonii* (Batten and Dutta, 1997). Furthermore, infratectal granule structure and 'foot layer' structure vary. Granules range from being well-defined and easily detectable in section view (Fig. 32G, M) to variably fused and slightly pillar-like (Fig. 32J). The presence of an electron-lucent layer that separates the granules from the electron-dense nexine also varies. Such a 'foot layer' may be absent (Fig. 32L-M), present but thin (Fig. 32H), to present and very thick (Fig. 32G). The presence of distinguishable nexine lamellae is also a variable character (Fig. 32G-H, J, L-M).

Another dispersed species of *Eucommiidites* has also been examined at the fine structural level (*E.* sp.; Zavada, 1984), but is not being considered in the present paper. This Jurassic palynomorph is approximately twice as large, measuring 45 μm in length and 43 μm in width, and does not have as distinctive an aperture and lateral furrows in comparison with other *Eucommiidites* taxa. Because the lateral furrows are not well-illustrated in the light micrograph of *E.* sp. (Zavada, 1984), the identification of this palynomorph to *Eucommiidites* has been questioned (Pedersen *et al.*, 1989a; see also Batten and Dutta, 1997).

Erdtmanitheca

Pedersen *et al.* (1989a) described *Erdtmanitheca texensis* from the Upper Cretaceous of Arthur City, Texas, U.S.A., the first pollen organ unequivocally known to produce *Eucommiidites*-type pollen. *In situ* pollen of *E. texensis* is ellipsoidal to ovoid and averages 24 μm in length and 18 μm in width. The pollen surface is psilate to slightly scabrate. Pollen grains have a well-defined sulcus with somewhat rounded ends and two, more slit-like lateral furrows with pointed ends. All three are approximately the same length and do not reach the grain ends. The exine ranges from 0.7-1.3 μm in overall thickness. The electron-lucent sexine consists of a thick, homogeneous tectum and thinner granular infratectum (Fig. 32K). Granules are relatively small, tightly packed, and appear to be directly adjacent to the underlying, electron-dense nexine. Although the

FIG. 32. Erdtmanithecales. A-C. Views of the psilate surface of most *Eucommiidites*-type pollen grains. Bars = 5 μm. A. Distal view showing the sulcus with rounded ends. B. Equatorial view showing sulcus (top) and two, lateral furrows. C. Proximal view showing two lateral furrows with pointed ends. D-F. Views of the foveolate surface of *Eucommiidites*-type pollen found within the micropyles of *Erdtmanispermum balticum* seeds. Bars = 5 μm. D. Distal view. E. Equatorial view. F. Proximal view. G-M. Sections of *Eucommiidites*-type grains shown at the same scale. Bars = 0.5 μm. G-I. Sections of grains with a detectable, sexinous foot layer present above the nexine (stippled). G. Non-apertural exine of *E.* sp. from *Erdtmanispermum balticum* seeds. (Modified from Pedersen *et al.*, 1989a). H. Non-apertural exine of *Eucommiidites* sp. 2. (Modified from Trevisan, 1980). I. Non-apertural exine of *Eucommiitheca hirsuta*. (Modified from Friis and Pedersen, 1996). J. Non-apertural exine of *Eucommiidites* sp. 1 showing infratecum with ill-defined granules fused into pillar-like units. (Modified from Trevisan, 1980). K-M. Sections of grains with infratectal granules in direct contact with the underlying nexine (stippled). K. Non-apertural exine of *Erdtmanitheca texensis*. (Modified from Pedersen *et al.*, 1989a). L. Non-apertural exine of *Eucommiidites troedssonii*. (Modified from Batten and Dutta, 1997). M. Non-apertural exine of *Eucommiidites* sp. (Modified from Doyle *et al.*, 1975).
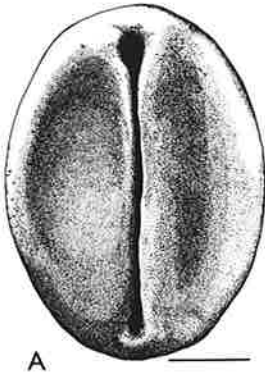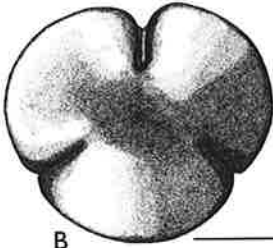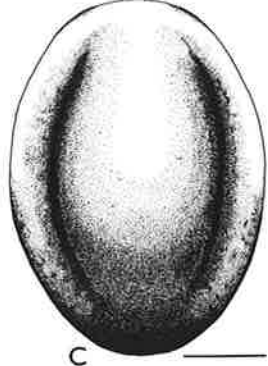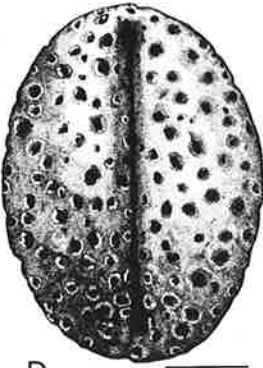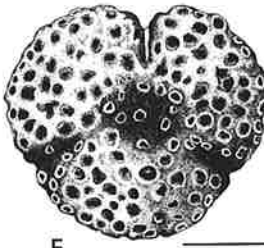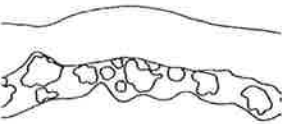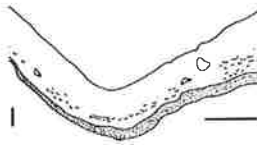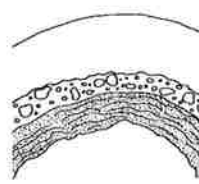
nexine is not particularly well-preserved, lamellae are clearly detectable (Fig. 32K). The nexine appears to be uniform in thickness in all grain regions, thickening slightly below the apertural membrane and the two furrows.

Eucommiitheca

In situ pollen of *Eucommiitheca hirsuta*, from the Early Cretaceous of Portugal, has been described ultrastructurally (F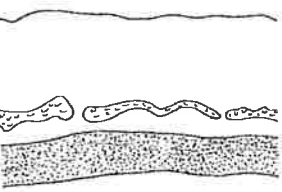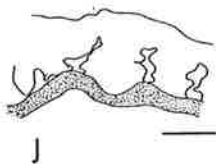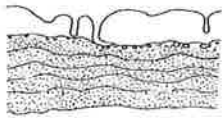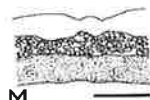riis and Pedersen, 1996). Grains are elliptic with pointed to truncate ends and range from 15-20 μm in length and 10-12 μm in width. Grains have a psilate to slightly foveolate surface and are "3 colpate" (Friis and Pedersen, 1996). The colpi are parallel, approximately the same length, and extend nearly to the grain ends. The exine is distinctly two-parted, consisting of an electron-lucent "ektexine" and an electron-dense "endexine" (Friis and Pedersen, 1996). In non-apertural regions the ektexine averages 0.35 μm in thickness, and it gradually thins towards the margins of the colpi. Although the grains are not well-preserved at the ultrastructural level, a thick, homogeneous tectum and a granular infratectum have been described (Fig. 32I). Furthermore, although an electron-dense basal layer is present, preservation has affected characterisation of this layer in apertural regions. Lamellae are also not detectable in the basal layer (Fig. 32I).

Discussion

Pollen of the four groups of gymnospermous anthophytes exhibits several common character trends. These include the absence of sacci, a relatively small size (12-50 μm), an elliptic shape (except for *Gnetum*), a monosulcate aperture type (except for the inaperturate gnetalean taxa: *Ephedra*, *Gnetum*, and *Equisetosporites*), a granular infratectum, and a thick basal layer (nexine) that is uniform in thickness in non-apertural and apertural regions. In addition to reviewing structural data for the gymnosperm taxa addressed above, an initial goal of this paper was to provide a comparative table that included a variety of ultrastructural and morphological characters (for example, sexine and nexine thicknesses, tectum architecture, granule size and ultrastructure, lamellae thickness, etc.). It was hoped that such a table would be useful in standardising the descriptions of salient characters and thereby provide for more accurate and consistent character scoring in phylogenetic analyses. However, the summary table was omitted because direct comparisons of many palynological characters are not possible, primarily due to the significant variation that was identified among the taxa.

Character Variation

Clearly, natural variation exists among pollen types, and this variability is critically important in a phylogenetic context. However, palynological characters can also reflect a wide range of other biological and physical processes that are often phylogenetically uninformative and ambiguous. Osborn and Taylor (1994) have discussed the phylogenetic utility of several ultrastructural characters from an array of fossil gymnosperms. Four palynological features are relevant to the present paper with regard to gymnospermous anthophytes. These include staining properties of ultrathin sections, nexine structure, infratectal granules, and foot layer.

Overall exine structure, as well as particular layers, may exhibit a range of electron densities after staining for transmission electron microscopy. For example, different sections of the same pollen grain of *Equisetosporites* have been shown to exhibit both

typical staining (i.e., electron-lucent sexine and electron-dense nexine), as well as an opposite staining pattern (i.e., electron-dense sexine and electron-lucent nexine) (Osborn *et al.*, 1993). The latter pattern is also evident in sections of *Ephedripites* (Trevisan, 1980). Similar examples of stain variation have been reported in *Ephedra foliata* Boiss. ex K.C.A. Mey, in which El-Ghazaly and Rowley (1997) have suggested that differential leaching of pollen wall materials may contribute to the electron-lucent endexine in this species. The relative staining pattern in turn plays an important role in perceived ultrastructure, especially the ability to resolve whether or not particular characters are present (for example, nexine lamellations and foot layer).

Nexine structure is a particularly variable character among the pollen of gymnospermous anthophytes, especially with regard to the presence of lamellations. Preservational aspects clearly influence the ability to detect nexine lamellae. For example, lamellations were only identifiable in *Equisetosporites* pollen after ultrathin-sectioning numerous palynomorphs; this sample size provided the opportunity to identify grains that had undergone significant preservational folding thereby separating the highly compressed, individual lamellae (Osborn *et al.*, 1993). In other cases, although lamellae-like structures may be distinguished in ultrathin sections, these may represent poorly preserved intine remains that become tightly appressed against the nexine as observed in *Cycadeoidea* pollen (Osborn and Taylor, 1994, 1995). Furthermore, a significant portion of the basal layer may be absent altogether in fossil pollen, thereby precluding the identification of lamellae that may have in fact been present before fossilisation. Under analogous conditions, the endexine of many extant taxa may be completely lost following acetolysis. This occurs because the endexine is often structurally supported by the underlying intine (Blackmore and Crane, 1988), which is not acetolysis-resistant (Erdtman, 1960).

Although a granular infratectum is a shared character among gymnospermous anthophytes, considerable variation occurs in granule size, shape, and packing. The wide range of granular infratecta observed in the pollen of both *Sahnia* (Osborn *et al.*, 1991) and *Cycadeoidea* (Osborn and Taylor, 1995) is due primarily to preservational influences. In fact, the sexine appears entirely homogeneous in most grains of *Cycadeoidea* that have been ultrathin-sectioned (Taylor, 1973; Osborn and Taylor, 1995); infratectal granules were only detected in this fossil after sectioning multiple grains (Osborn and Taylor, 1995). Exine development also plays a role in the ultrastructural detection of infratectal granules. For example, during sexine ontogeny in *Welwitschia*, the outermost granules fuse with, and contribute to, the developing tectum (Zavada and Gabarayeva, 1991). Infratectal granules also undergo ontogenetic aggregation and fusion to form columellae-like elements in *Ephedra foliata* (El-Ghazaly and Rowley, 1997).

The pollen of many gymnospermous anthophytes lacks an ultrastructurally detectable foot layer. In most taxa, the electron-lucent infratectal granules are directly contiguous with the underlying, electron-dense basal layer. It is possible that an ectexinous foot layer is present in some pollen grains, but is not identifiable because of variation in staining. Foot layer and endexine commonly have similar electron densities following conventional staining in both gymnosperms and angiosperms (for example, Osborn and Taylor, 1994; Weber, 1998; Kreunen and Osborn, 1999).

Future Research: Identifying Phylogenetically Relevant Characters

Determining which palynological characters are phylogenetically valuable while taking into account the external factors (for example, preservation, specimen preparation, ontogeny, pollination) that can introduce significant variation in pollen ultrastructure and morphology is particularly challenging. Osborn and Taylor (1994)

have emphasised a conservative approach with regard to 'homologising' characters when a limited number of ultrathin sections have been prepared from a single or a small number of pollen grains. Clearly, it is evident from the review above that important structural data are available for the pollen of gymnospermous anthophytes; however, greater phylogenetic resolution will come from additional research that focuses on a greater diversity of taxa, larger sample sizes, pollen ontogeny, and megafossils with *in situ* pollen.

Pollen morphology and ultrastructure from relatively few taxa of gymnospermous anthophytes have been studied. New insight will come from investigation of additional species clearly nested within anthophyte groups, as well as other putatively allied taxa. For instance, phylogenetic analyses by Doyle (1996) have linked Gnetales with *Piroconites*, an enigmatic microsporophyll with *in situ Ephedripites*-type pollen from the Lower Jurassic of Germany (van Konijnenburg-van Cittert, 1992). Although the pollen of *Piroconites kuespertii* Gothan appears polyplicate in surface view, when ultrathin-sectioned the plicae appear to be major folds in the whole exine (Osborn and van Konijnenburg-van Cittert, unpublished data) rather than the alternating thick and thin regions that characterise gnetalean pollen. Furthermore, three additional dispersed polyplicate taxa from the Lower Cretaceous of Brazil and Ecuador have recently been described at the fine structural level (Dino *et al.*, 1999). Although ultrastructural data on these unusual 'elater-bearing' palynomorphs were not available at the time the current paper was prepared, *Elateroplicites africaensis* Herngreen, *Elaterosporites klaszii* (Jardiné & Magloire) Jardiné, and *Sofrepites legouxiae* Jardiné appear to have gnetalean affinities (Dino *et al.*, 1999).

In addition to the need for new species of gymnospermous anthophytes to be studied, larger sample sizes of pollen grains from individual taxa need to be investigated. This is particularly important for fossils. As discussed above, preservational influences can alter phylogenetically informative data and introduce dubious characters. The degree of preservational alteration can be determined and overcome in many cases by studying multiple pollen grains. Increased sample sizes can also be achieved by examining entire reproductive organs with *in situ* pollen.

Ultrastructural details about pollen development in gymnospermous anthophytes, or related taxa, are also not well-understood. As discussed above, infratectal granules undergo various degrees of fusion during pollen ontogeny in *Welwitschia* (Zavada and Gabarayeva, 1991) and *Ephedra foliata* (El-Ghazaly and Rowley, 1997). Therefore, the developmental stage at which a pollen grain is prepared for study will have significant bearing on the observed ultrastructure and potential character scoring. Ontogenetic data may also help resolve questions regarding nexine structure. Mature gymnosperm pollen typically has a uniformly thick and lamellated basal layer, whereas mature angiosperm pollen has a thinner basal layer with fewer lamellae that are typically restricted to apertural regions (see Osborn and Taylor, 1994). Developmental data may indicate that the thinner basal layer in angiosperms is due to loss of the innermost endexine lamellae. In some basal angiosperms a layer of electron-dense granules develops below a layer of well-defined, endexine lamellae during ontogeny (for example, *Nymphaea*, Nymphaeaceae, Gabarayeva and El-Ghazaly, 1997; and *Nelumbo*, Nelumbonaceae, Kreunen and Osborn, 1999). It is possible that these granules represent flocculents of later-formed sporopollenin of the endexine that were unable to aggregate onto phylogenetically lost sub-structural, white-line units. Hemsley *et al.* (2000) have modelled flocculation patterns using polystyrene latex and suggested that white-line units may be sites for sporopollenin deposition. Furthermore, the endexines of gymnosperm and angiosperm pollen are considered by Rowley (1995) to be structurally and functionally equivalent. If correct, this provides support for the white-line loss hypothesis presented in the current paper.

Ultrastructural details about ancient developmental patterns can be ascertained by studying whole pollen organs with *in situ* grains (see Taylor *et al.*, 1996). Limited information is known about pollen ontogeny in fossils (Taylor, 1990), but investigations of *in situ* pollen have the potential to yield phylogenetically valuable data. For example, although mature pollen of the Jurassic fossil *Caytonanthus arberi* (Thomas) Harris is saccate and has an alveolar infratectum (Pedersen and Friis, 1986; Zavada and Crepet, 1986; Osborn, 1994), identification of early ontogenetic stages indicates that granular sexine units are present and later aggregate to form the plate-like alveolae observed in mature pollen (Osborn, 1994). Although the Caytoniales has been viewed as more distantly related to anthophytes (for example, Crane, 1990; Rothwell and Serbet, 1994), Doyle (1996) has suggested that this group of 'Mesozoic seed ferns' may be linked to angiosperms.

It is likely that new data on pollen development from both fossil and extant taxa will require a re-evaluation of how traditional ultrastructural characters such as 'granular infratectum' are interpreted in a phylogenetic context. Although pollen characters alone will not definitively answer all of the systematic questions about anthophyte relationships and angiosperm origins, when enough palynological information becomes available these data can add considerable resolution to phylogenetic analyses.

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