

The ultrastructure of fossil ephedroid pollen with gnetalean affinities from the Lower Cretaceous of Brazil

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ABSTRACT

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The micromorphology and fine structure of several dispersed polyplicate, “ephedroid” palynomorphs, originally assigned to *Equisetoporites* spp., are described from the Lower Cretaceous (Aptian–Albian) Santana Formation of northeastern Brazil. Pollen grains are elliptic in shape and have a variable number of longitudinally oriented plicae, but lack a distinct aperture. Plicae do not reach the grain tips and are psilate to faintly scabrate in surface ornamentation. The exine is distinctly two-parted with the sexine typically staining more lightly than the nexine. The sexine is on average 1.5 times thicker than the nexine, and has a tectate granular fine structure with the granular infrastructural layer having a graded organization. Small granules characterize the lower region of the infratectum and are directly contiguous with nexine; these gradually grade into larger granules that appear to fuse with a thick, homogeneous tectum. The nexine is thick and lamellate throughout. Both sexine and nexine sporoderm components are markedly thinner between the plicae (i.e. within the wall furrows), with the granular infratectum laterally thinning until completely absent. An additional wall layer, external to the tectum, is also present within sporoderm furrows; both the tectum and this layer typically occur in a folded, hinge-like fashion in the furrow. This unique feature as well as several other structural characters of the sporoderm are discussed with regard to their possible functional significance. Exine architecture is also compared with that of the only other three fossil polyplicate palynomorphs known at the ultrastructural level (*Ephedripites* sp., *Equisetoporites chinleana*, and *Cornetipollis reticulata*) as well as with that of the pollen of the extant Gnetales (*Ephedra*, *Welwitschia*, and *Gnetum*). By comparison, the Brazilian grains are most similar to *Ephedripites* using fine structural features.

Introduction

The Gnetales consist of three living genera, *Ephedra*, *Gnetum*, and *Welwitschia*, which, although distinctly different in both habit and habitat, share an array of reproductive and vegetative characteristics (e.g. Martens, 1971; Crane, 1988). Recent phylogenetic analyses (Crane, 1985, 1988; Doyle and Donoghue, 1986a,b) suggest that the Gnetales is monophyletic, corroborate early suggestions that many of the group’s unifying features are apomorphic, and indicate that the group, along with Bennettitales, Pentoxylales, and angiosperms, comprises a clade of highly-derived seed plants (= anthophytes).

Although it is clear that all three extant genera are relatively advanced gymnosperms, relatively little is known about morphological evolution in the group over geological time and space. The megafossil record of the Gnetales is extremely sparse, with only one described fossil unequivocally assigned to the group. *Drewria potomacensis* is known from the Early Cretaceous (cf. Aptian) Potomac Group of Virginia (Crane and Upchurch, 1987). Another fossil, *Eoantha zherikhinii* Krassilov (1986) from Barremian–Aptian sediments of Mongolia, has been suggested by Crane (1988) to be a definitive gnetophyte; however, Krassilov (1986) originally advanced the idea that *Eoantha* either represents a “protoangiosperm” or

some type of an intermediate morpho-type between protoangiosperms and gnetophytes. Moreover, Crane and Upchurch (1987) and Crane (1988) also suggested that several other Cretaceous (e.g. *Baisia hirsuta*, *Conospermites hakeaefolius*, *Cyperacites potomacensis*, *C. sp.*, *Casuarina covilli*, *Gurvanella dictyoptera*, and *Montsechia vidali*) and Triassic (e.g. *Dechellyia gormanii*, *Hexagonocaulon minutum*, *Masculostrobos clathratus*, and *Schilderia*) megafossils exhibit some morphological similarities with *Drewria* and *Eoantha*, and thus may also have gnetalean affinities.

The palynological record of the Gnetales is far more extensive than megafossil evidence. Dispersed polyplicate palynomorphs that are morphologically similar to pollen of *Ephedra* and *Welwitschia*, based on observations in transmitted light, occur in sediments ranging from Lower Permian to Recent (e.g. Wilson, 1959; Scott, 1960; Bharadwaj, 1963; Pocock, 1964; Singh, 1964; Stover, 1964; Srivastava, 1968; Azéma and Boltenhagen, 1974; Elsik, 1974; de Lima, 1980; Zavada, 1984, 1990; Crane, 1988; Pocock and Vasanthi, 1988). The most prominent similarities between these *spora dispersae*, "ephedroid" grains and those of *Ephedra* and *Welwitschia* are their elliptic shapes and ribbed, or striate, surfaces. Several genera have been described, differing from one another by such features as number of ribs, orientation of ribs (i.e. longitudinal or spiral), general morphology of grain ends (i.e. more rounded or more pointed, etc.), and attachment of flap-like appendages (i.e. auriculae). The two most commonly reported grains are those assigned to *Ephedripites* Bolkhovitina (1953) and *Equisetosporites* (Daugherty) Pocock (1964). Other polyplicate palynomorphs are recovered less frequently from palynological preparations; examples include grains referred to as *Tornopollenites*, *Hamiapollenites*, *Singhia*, *Gnetaceaepollenites*, *Steevesipollenites*, *Welwitschiaptes*, *Regalipollenites*, and *Multimarginites*. Moreover, relatively young ephedroid palynomorphs (i.e. Tertiary) have often been assigned to species of *Ephedra* (e.g. Wodehouse, 1934, 1935; Scott, 1960). The appressed guard cells on some Pleistocene epidermal remains have also been likened to fossil *Ephedra* pollen (Elsik, 1975).

Ephedroid grains have also been found along with three of the megafossils noted above. Although microsporangiate organs have not been identified for *Drewria potomacensis*, both individual and large masses of polyplicate pollen grains were recovered associated with the vegetative and ovulate organs (Crane and Upchurch, 1987). In fact, the association of these grains was one criterion for assignment of *D. potomacensis* to the Gnetales. The first definitive association of *Ephedripites* grains with any megafossil comes from macerations of in-situ grains within the pollen chambers of *Eoantha zherikhinii* ovules (Krassilov, 1986), while more recently Van Konijnenburg-van Cittert (1992) has documented *Ephedripites* pollen within an enigmatic, synangiate microsporophyll resembling *Piroconites kuespertii*. In addition, in-situ grains assigned to *Equisetosporites chinleana* have been isolated from *Masculostrobos clathratus*, the pollen cones associated with *Dechellyia gormanii*, from the Chinle Formation (Upper Triassic) of Arizona (Ash, 1972). However, pollen wall fine structure of the Arizonan *Equisetosporites* grains is not characteristically gnetalean and suggests other affinities (see below).

The pollen of all three extant gnetalean genera has been studied from a variety of perspectives. Early investigations focused on describing the stages of microsporogenesis and microgametogenesis in *Ephedra*, *Gnetum*, and *Welwitschia* at the gross morphological and anatomical levels (see Martens, 1971, and references therein for the most comprehensive review). Mature pollen of *Ephedra* and *Welwitschia* is similar in its elliptic shape, overall size, and occurrence of longitudinally-oriented, psilate exine ribs. However, pollen of *Welwitschia* has a distinct sulcus, whereas grains of *Ephedra* are generally reported to be inaperturate, although thin areas are present between ribs (i.e. furrows or plicae). Several studies have addressed the nature of grains from these two genera as observed in transmitted light, especially for the purposes of morphological comparisons with fossil polyplicate grains (e.g. Wodehouse, 1935; Steeves and Barghoorn, 1959; Wilson, 1959; Scott, 1960; Bharadwaj, 1963; Huynh, 1974; Kedves, 1987). Pollen wall fine structure (i.e. stratification) is also very similar in the pollen of

Ephedra (Afzelius, 1956; Ueno, 1960; Gullvåg, 1966; Van Campo and Lugardon, 1973; Hesse, 1984; Zavada, 1984; Kurmann, 1992) and *Welwitschia* (Ueno, 1960; Gullvåg, 1966; Hesse, 1984; Kedves, 1987; Zavada and Gabarayeva, 1991). The exine is composed of a lamellated nexine, and a two-zoned sexine consisting of a granular infrastructural layer and a thick tectum. Both sexine components are either very thin or absent in the furrows. *Gnetum* pollen is strikingly different from that of *Ephedra* and *Welwitschia*, in that the former is spherical, inaperturate, and has finely spinulose surface sculpturing. Exine stratification, however, is generally similar. The exine also consists of a lamellate nexine and a two-layered sexine (Gullvåg, 1966; Hesse, 1980; Zavada, 1984; Oryol et al., 1986; Kurmann, 1992). Albeit, both the tectum and granular infrastructural layer are continuous and extremely thin by comparison, only thickening in the spine regions.

Of the numerous genera of fossil ephedroid grains described, only three have been examined at the ultrastructural and micromorphological levels using scanning and transmission electron microscopy (SEM and TEM), and those that have been studied have come from Laurasian localities. Trevisan (1980) sectioned grains of *Ephedripites* sp. from the Lower Cretaceous of Italy. She found the exine of these grains to consist of an inner lamellated component (=nexine) and an outer complex, five-layered component (=sexine) which formed the "ridges and valleys" of the sporoderm. *Equisetosporites chinleana* grains from Arizona were initially investigated by Zavada (1984). The exine was reported to be three-layered, composed of a thin, lamellated inner layer ("foot layer" sensu Zavada), an infrastructural columellar layer, and a thick homogeneous tectum which forms the exine ribs. More recently, however, the Arizona material of *E. chinleana* has been shown to be more diverse regarding both surface sculpturing of ribs and exine ultrastructure (Pocock and Vasanthy, 1988; Zavada, 1990). Pocock and Vasanthy (1988) have emended *E. chinleana* to exclude the new species *Cornetipollis reticulata*. *Cornetipollis reticulata* is principally characterized by foveoreticulate ornamented ribs, larger columellae, and a non-lamellate nexine. *Equisetosporites chinleana*, on the other

hand, is redefined as having psilate ribs and a distinctly lamellate nexine.

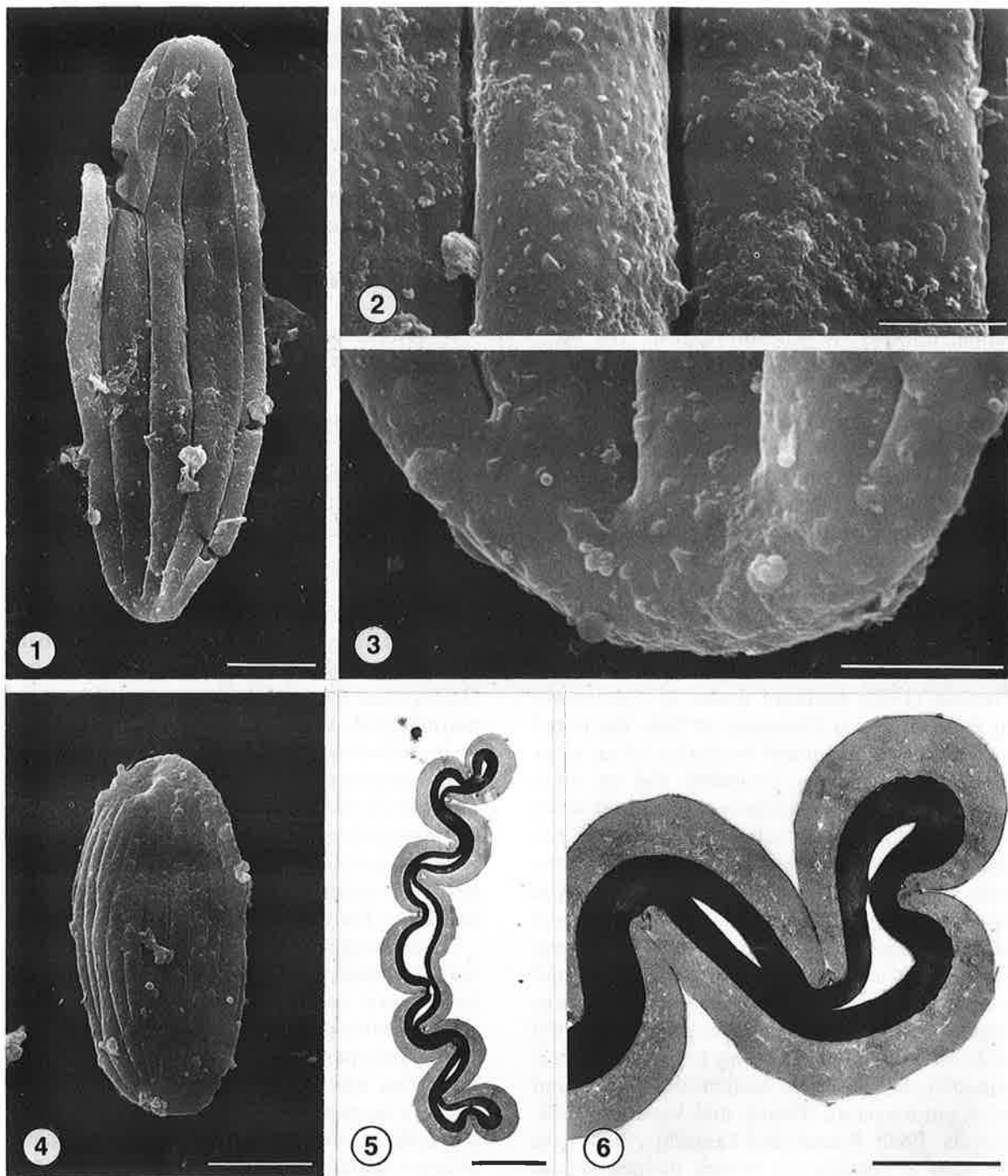
The present investigation, represents the first micromorphological and ultrastructural examination of ephedroid palynomorphs from a Gondwana locality. Data on exine architecture from these grains are addressed with respect to their structural-functional significance as well as their implications regarding systematic and phylogenetic assessments of ephedroid pollen types.

Materials and methods

A diverse flora is known from the Lower Cretaceous (Aptian-Albian) of northeastern Brazil. These fossils occur in the Santana Formation, one of four sedimentary units of the Araripe Group (see de Lima, 1978a,b, for locality details). The Santana Formation is characterized by its exceptionally well preserved fauna, including fish, reptiles, and invertebrates, especially insects (e.g. de Lima, 1978b; Grimaldi, 1990), and its microflora (de Lima, 1976, 1978a,b, 1979, 1980, 1989). The record of megafloral elements is less diverse, mainly due to the fact that these remains have received the least attention in the Santana. Gnetophytes are principally represented by a rich palynological assemblage of polylicate, ephedroid grains including 52 species belonging to six genera (*Equisetosporites*, *Singhia*, *Welwitschiapites*, *Steevesipollenites*, *Regalipollenites*, and *Gnetaceaepollenites*; de Lima, 1978b, 1980). Well-preserved gnetalean leaves and reproductive organs have also recently been recovered from the same sediments (Pons et al., 1992).

Palynomorphs were recovered according to standard techniques (Doher, 1980); however, after hydrofluoric and hydrochloric acid macerations and all washings, it was also necessary to separate the organic fractions of all residues. Palynomorph separation was accomplished by floating grains out in a bromoform-acetone mixture adjusted to a specific gravity of 2.0 (modified Zinc Bromide flotation technique of Doher, 1980). Pollen grains for SEM were pipetted directly from the residues onto polished aluminum stubs, sputter coated with gold-palladium, and then viewed on either a HITACHI S-500 or a JEOL JSM-820 scanning

PLATE I



electron microscope at accelerating voltages of 15–20 kV. For TEM, palynomorphs were pipetted onto cellulose filters under suction, with the filters subsequently coated on both sides with agar. Agar-embedded filters were then dehydrated in a graded ethanol series, transferred to 100% acetone (with at least four acetone changes to completely solubilize the filters), gradually infiltrated with Spurr low viscosity epoxy resin, sectioned into several pieces, and embedded flat in shallow aluminum pans. Individual blocks overlying embedded palynomorphs were then cut out of the large resin disks in order to obtain specific grains. Ultrathin sections were cut with a diamond knife, collected on uncoated 1×2 mm copper slot grids, and dried onto formvar support films (Rowley and Moran, 1975). Grids were triple stained with 1% potassium permanganate (2–12 min), 1% uranyl acetate (2–12 min), and lead citrate (1–6 min; Venable and Coggeshall, 1965), and images were recorded using a ZEISS EM-10 transmission electron microscope at 60–80 kV.

Results

Although six genera of polyplicate pollen grains are known from Santana sediments (Fig. 1; de Lima, 1978b, 1980), specimens of *Equisetosporites* (sensu de Lima) are the most abundant (Fig. 1). Only palynomorphs assigned to this genus are described in the present investigation.

Grains are elliptic, average $41 \mu\text{m}$ in length and $13 \mu\text{m}$ in width, and have a variable number of plicae (typically ranging from 5 to 11) as seen in polar view (Plate I, 1, 4). Plicae extend the majority of the grain lengths, but do not reach the ends (Plate I, 1, 3, 4). Surface ornamentation of plicae ranges from psilate to slightly scabrate (Plate I, 2,

3). Exine surfaces also exhibit some pitting, with pits of varying sizes (Plate II, 4); however, this more than likely represents an artifact of preservation.

The overall exine averages $1.04 \mu\text{m}$ in thickness and is two-parted, with easily distinguishable sexine and nexine components, based on distinct affinities for heavy metal stains. The sexine generally stains lightly while the nexine stains darkly (Plate I, 5, 6). Interestingly, the nexine of some grains does not stain particularly well and appears significantly less dense in the electron beam in comparison with the sexine of the same specimens. Moreover, the sexine of those grains stains more darkly than the sexine of grains with the typical darkly-stained nexine (Plate II, 2, 3). The sexine averages $0.63 \mu\text{m}$ in thickness and is generally 1.5 times thicker than the nexine. The sexine is stratified into two principal layers, including an inner granular layer and an outer homogeneous layer (=tectum; Plate II, 2, 3; Plate III, 1, 2). The granular infratectum averages $0.35 \mu\text{m}$ in thickness and is directly contiguous with the underlying nexine (Plate II, 2, 3; Plate III, 1, 2). Granules range in size from 0.09 to $0.26 \mu\text{m}$ in diameter, typically with smaller granules located near the nexine and larger granules positioned toward the tectum (Plate II, 3; Plate III, 1). The small granules gradually grade into the larger granules which appear to fuse with the homogeneous tectum (Plate II, 3; Plate III, 1). Some variation with regard to granule density (i.e. density of granules relative to lacunal space) also occurs between pollen grains (Plate II, 1, 3; Plate III, 3). The tectum averages $0.24 \mu\text{m}$ in thickness.

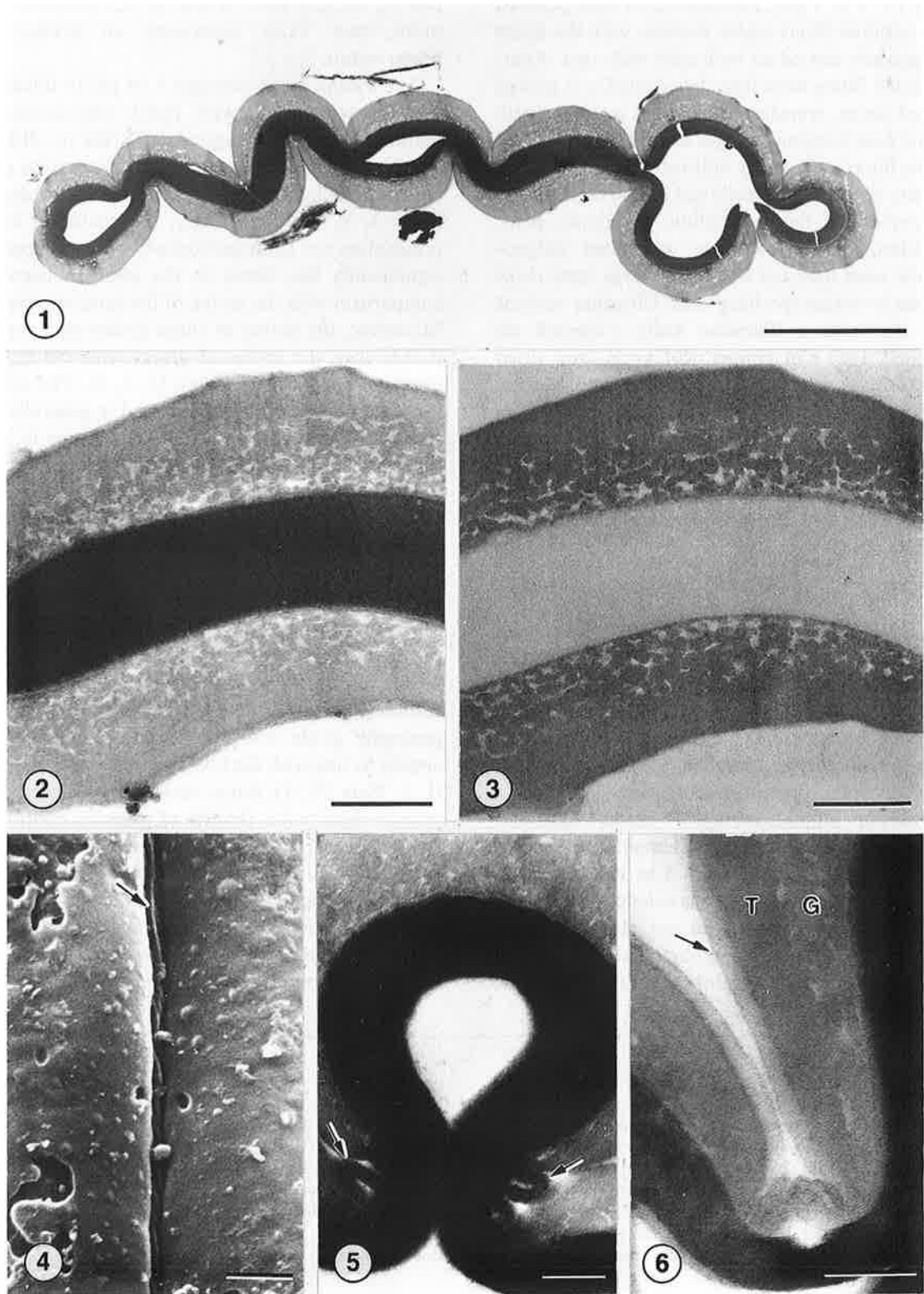
The nexine is relatively thick, averaging $0.30 \mu\text{m}$ in thickness (Plate II, 2, 3; Plate III, 1, 2). In the majority of grains examined lamellae are absent

PLATE I

Ephedroid pollen.

1. Pollen grain showing relatively large size and broad ridges. Scale bar = $5 \mu\text{m}$.
2. Detail of exine showing psilate to slightly scabrate surface. Scale bar = $2 \mu\text{m}$.
3. Detail of grain tip showing termination of the plicae and the psilate/scabrate grain surface. Scale bar = $2 \mu\text{m}$.
4. Overall morphology of a smaller grain than the one illustrated in 1 showing narrower ridges. Scale bar = $5 \mu\text{m}$.
5. Ultrathin transverse section through a pollen grain showing overall ultrastructure, distinct ridges and furrows (plicae), and lightly stained sexine and darkly stained nexine. Scale bar = $2 \mu\text{m}$.
6. Detail of lower portion of grain illustrated in 5 showing overall exine stratification. Scale bar = $1 \mu\text{m}$.

PLATE II



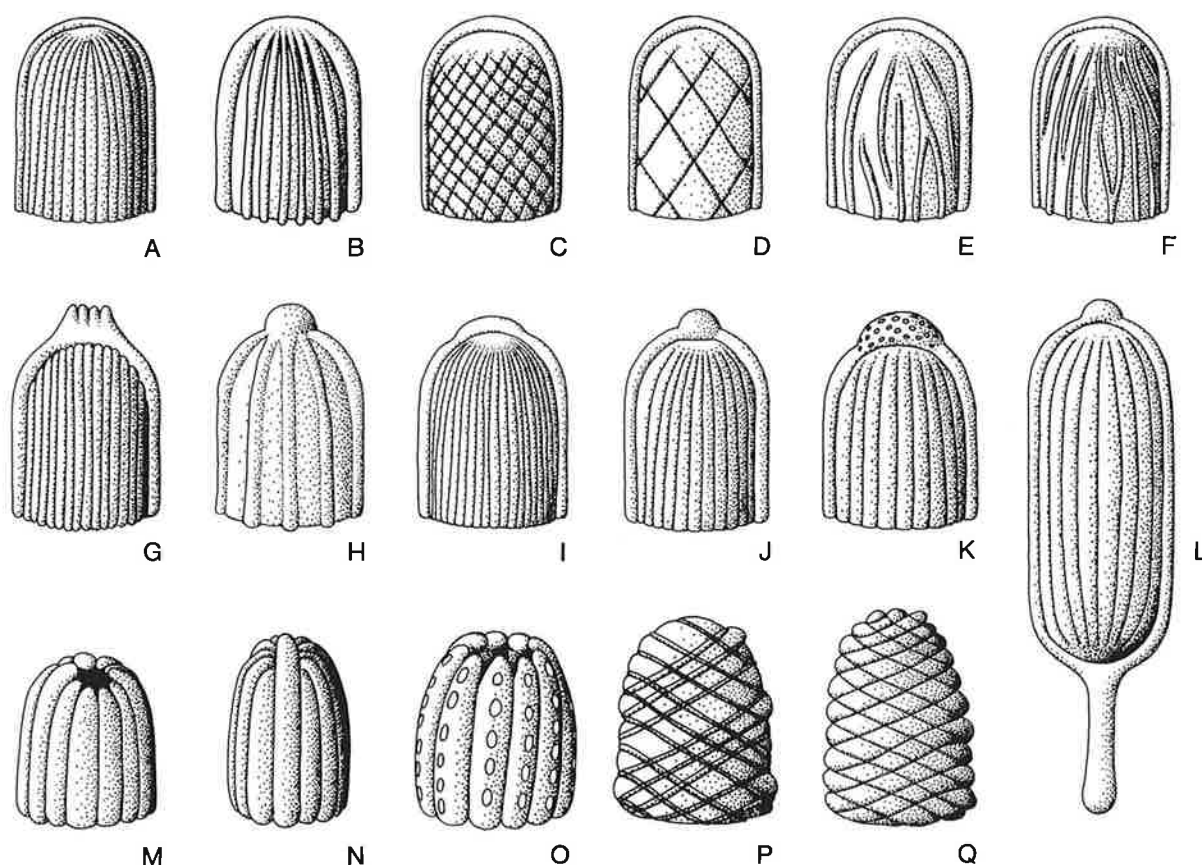


Fig. 1. Morphological diversity of polyplicate, ephedroid palynomorphs recovered from Lower Cretaceous (Aptian–Albian) sediments of the Santana Formation, northeastern Brazil. Genera include (A–D) *Equisetosporites*, (E–F) *Singhia*, (G) *Welwitschiapites*, (H–K) *Steevesipollenites*, (L) *Regalipollenites*, and (M–Q) *Gnetaceapollenites*. Drawings represent partial longitudinal views (redrawn from de Lima, 1980).

from the nexine except in regions near the furrows (Plate III, 2, 5) or in portions of grains exhibiting some minor folding, and then near the sexine interface (Plate III, 5). However, in several other

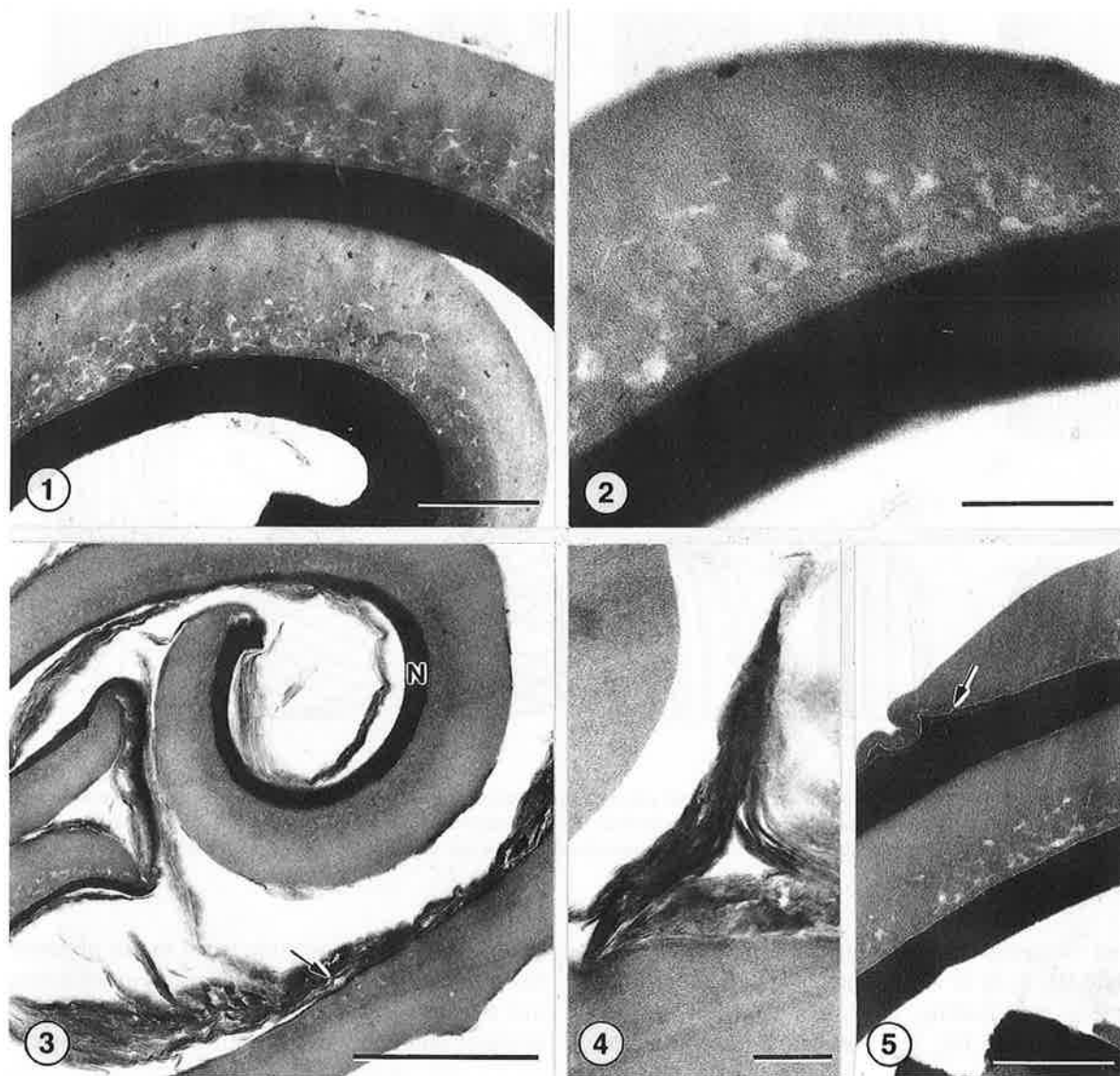
grains that have undergone more severe preservational folding, individual lamellations in the nexine are much more prominent (Plate III, 3, 4). On average, lamellae measure 0.01 μm in thickness.

PLATE II

Ephedroid pollen.

1. Transverse section through a slightly folded grain showing basic ultrastructural organization. Scale bar = 3 μm .
2. Detail of two appressed ridge regions from the same grain illustrated in 1 showing thick homogeneous tectum, granular infratectum, and darkly stained nexine. Scale bar = 0.5 μm .
3. Different section from the same region of the same grain as illustrated in 2 showing opposite staining pattern; note the more distinct appearance of the infratectal granules. Scale bar = 0.5 μm .
4. Exine surface of two contiguous ridges showing slight folding of the wall within the furrow (arrow). Scale bar = 1 μm .
5. Transverse section of two furrows showing hinge-like foldings of the exine (arrows). Scale bar = 0.3 μm .
6. Transverse section of a furrow and its boundary walls showing gradual, lateral thinning of the sexinous granular infratectum (G) and tectum (T) toward the furrow. Note also the presence of an external, homogeneous sexine layer (arrow) within the cavity of the furrow and the basal, hinge-like sexine folding. Scale bar = 0.3 μm .

PLATE III



Ephedroid pollen.

1. Transverse section through a slightly folded grain showing thick, homogeneous tectum and granular infratectum; note that the granules grade into the lower portion of the tectum. Scale bar=0.5 μ m.
2. Detail of sporoderm showing homogeneous tectum, granular infratectum, and darkly stained nexine; note the absence of nexine lamellae. Scale bar=0.2 μ m.
3. Transverse section through a highly folded grain showing portions of the nexine (N) in which individual lamellae (arrow) have separated. Scale bar=1 μ m.
4. Detail of the same grain illustrated in 3 showing well-defined nexine lamellae. Scale bar=0.2 μ m.
5. Transverse section of a non-folded grain showing faint nexine lamellations (arrow) in the region below a furrow. Scale bar=0.5 μ m.

In regions associated with sporoderm furrows, both the sexine and the nexine layers are markedly thinner immediately below the furrows, averaging 0.10 and 0.16 μm in these areas, respectively (Plate I, 6; Plate II, 1, 5, 6). Sexine architecture here exhibits a gradual, lateral thinning of the granular infratectum in marginal regions of the plicae; this zone thins to a point where granules are completely absent over the furrow itself (Plate II, 5, 6). The homogeneous tectum does not thin appreciably over the plicae margins in concurrence with the underlying granular layer, but rather thins only slightly and abruptly just at the furrow (Plate II, 6). This now slightly thinner tectum overlies a thinner nexine layer, which has also gradually thinned, at the furrow itself (Plate II, 5, 6). Over the furrow the tectum is typically folded to a small degree, exhibiting a "hinge-like" appearance (Plate II, 4–6). An additional sexine layer is also present in furrow regions. It is positioned external to the tectum and is also homogeneous, but stains distinctly more lightly (Plate II, 6). Interestingly, this additional layer is thicker over marginal regions of the ribs and gradually thins as it extends toward the crests (Plate II, 6).

Discussion

Structural features of the exine

The most striking feature of the Brazilian ephedroid grains examined is the fact that they are exquisitely preserved at the ultrastructural level and exhibit well-stained exine layers. A darkly-stained nexine and lightly-stained sexine generally characterize the sporoderm. This type of differential staining is the most common situation in fossil pollen, especially when grains have been stained with potassium permanganate in association with uranyl acetate and lead citrate. However, some of the sections evaluated here apparently did not readily stain with potassium permanganate, although contiguous sections on the same grid did. These sections show an opposite staining reaction, with the nexine staining lighter than the sexine. Although sections that are reasonably well-stained with potassium permanganate illustrate better overall contrast in the electron beam, the oppo-

sitely stained sections also afford important information. In particular, the infrastructural granules are more easily resolved in the sections with a more darkly-stained sexine.

Nexine lamellae, however, are generally not well-defined, nor easily detectable in either type of stained sections. When lamellae are detectable, they are typically faint, occur at irregular intervals, and are most pronounced in outermost regions of the nexine (i.e. at the nexine/sexine interface). The relative absence of nexine lamellae in these grains is similar to many other Mesozoic grain types (Osborn, 1991); although the fossils are markedly different from the pollen of all three extant gnetalean genera that have very distinct lamellated nexines. However, it is clear that the nexine in the Brazilian grains is unequivocally lamellate throughout. Grains that have undergone a relatively high degree of preservational folding, but have not been altered either diagenetically or thermally, illustrate definitive lamellae that have individually separated from one another.

An especially intriguing structural feature of the Brazilian grains is the nature of the pollen wall in regions of the plicae. In these regions, the sexine has gradually thinned to a single, homogeneous layer (=tectum) that forms a folded, hinge-like structure directly over the furrow. Analogous sexine structures have not been reported in any of the other fossil polyplicate grains that have been sectioned to date (i.e. *Ephedripites*: Trevisan, 1980; *Equisetosporites*: Zavada, 1984, 1990; Pocock and Vasanthy, 1988). However, similar folds are prominent in the sporoderm furrows of extant *Welwitschia* pollen (Kedves, 1987, plate iv, 2, 3). Kedves suggested that these exine folds are the result of crumpling of the tectum, and are manifested further by the concomitant separation of both the tectum and infratectum from the nexine. This "artifactual explanation" for exine folds in the furrows of extant *Welwitschia* pollen originates from similar observations in several additional grains that had been sectioned, especially immature ones. The sexine folds in the Brazilian ephedroid grains, on the other hand, are interpreted as naturally occurring structures, rather than as artifacts of either preservation or preparation. This interpretation is based on the fact that almost all grains

sectioned here are well-preserved throughout, and do not show gross separation of sexine and nexine layers at any position throughout sectional view. This is notably the case in the specific grains that show prominent furrow "hinges".

These furrow "hinges" may in fact have important functional implications, specifically relating to harmomegathy. Pollen grains of extant plants are known to exhibit a variety of harmomegathic modifications in shape and volume in order to accommodate for alterations in their relative degree of hydration. This is especially important for the pollen grains of anemophilous plants, which need to minimize dehydration while being completely immersed in a desiccating medium. Blackmore and Barnes (1986) have identified three principal mechanisms by which pollen grains are harmomegathically altered, including expansion and contraction of the exine, exine folding, and volume modification of exine lacunae. The operative factor with regard to the Brazilian ephedroid grains would appear to be exine folding.

In extant grains, folds in the sporoderm typically occur in apertural regions, although they may also occur in specific non-apertural regions or in non-specific sites altogether (Blackmore and Barnes, 1986). The ephedroid grains sectioned here lack a well-defined aperture; however, the exine is appreciably thinner in the furrows in comparison with the ribs. This is almost identical to pollen of extant *Ephedra* which also lacks a distinct aperture, but is sometimes referred to as polycolpate because of its numerous thin-walled furrows. Although exine folds have not specifically been reported in extant *Ephedra* pollen, two species of this gnetalean genus (*E. trifurca* and *E. nevadensis*) have been shown to be well-adapted for wind pollination (Niklas and Kerchner, 1986; Niklas et al., 1986; Niklas and Buchmann, 1987; Buchmann et al., 1989).

Moreover, insect pollination has also been documented in two species of *Ephedra* (Bino et al., 1984a,b; Meeuse et al., 1990a,b). Although pollen-kitt is not present on the exine surface of *Ephedra* pollen (Hesse, 1984), grains of *E. aphylla* have been suggested to have an anomalous capacity to clump together as well as to adhere to insects (Meeuse et al., 1990b). Documentation of entomophily in the Gnetales may also affect inter-

pretations of fossil ephedroid pollen, and have implications regarding polarity assignments of important grain characters in phylogenetic analyses. For example, as noted above, several ephedroid palynomorphs bear flap-like exine extensions, or auriculae (e.g. *Elateroplicites*, *Elaterosporites*, *Galeacornea*, *Steevesipollenites*, *Regalipollenites*; Elsik, 1974; de Lima, 1980). Crane (1988) has suggested that these types of auriculate grains are more specialized than the non-auriculate ephedroids, and further implicitly suggested that the presence of such "specialized forms" in the fossil record adds to the "evidence of insect pollination in the group."

Whether or not auriculate ephedroid grains are more advanced than non-auriculate ones and how they should be polarized in phylogenetic studies remains a matter of conjecture; however, ultra-structural examinations of these forms may help elucidate this problem. At least two auriculate ephedroid forms occur in the Santana Formation (i.e. *Steevesipollenites*, *Regalipollenites*; de Lima, 1980), and continued fine structural investigations of these palynomorphs are in progress.

Systematic comparisons

Ephedroid palynomorphs are especially abundant in the Cretaceous when several groups of gymnosperms flourished, presumably including Gnetales, and angiosperms were undergoing massive radiations (see Crane, 1988, and references therein). Even upon a cursory examination, it is evident that the diverse assemblage of fossil ephedroid grains currently known represents a distinctly artificial group. In fact, several palynostratigraphic studies have emphasized that different, dispersed ephedroid grains may share affinities with either Gnetales, Coniferales, Monocotyledoneae, or Araceae in particular (e.g. Mchedlishvili and Shakhmoundes, 1973; Elsik, 1974). Moreover, although only two genera of dispersed ephedroid grains have previously been studied at the fine structural level (i.e. *Ephedripites* and *Equisetosporites*), exine architecture of these grains clearly demonstrates disparate taxonomic affinities.

Only one species of *Ephedripites* has been sec-

tioned to date (Trevisan, 1980). These grains are monosulcate with longitudinally oriented ribs. Both rib morphology and exine stratification of this ephedroid species suggests gnetalean affinities, based on its tectate-granular sexine that gradually thins toward the furrows, and a nexine that is apparently lamellate throughout. The recently emended palynomorph *Equisetosporites chinleana* and the newly separated species *Cornetipollis reticulata* are both strikingly different in surface morphology and exine fine structure from one another, as well as from *Ephedripites* (Pocock and Vasanthy, 1988). Both *Equisetosporites* and *Cornetipollis* are inaperturate and have a discontinuous tectum (= semi-tectate) that is underlaid by infratectal columellae. *Cornetipollis* has more or less longitudinally oriented ribs with foveoreticulate ornamentation, stouter columellae than those of *Equisetosporites*, and a non-lamellate nexine. Based on these features, *Cornetipollis* is thought to be angiospermous (Pocock and Vasanthy, 1988). In *Equisetosporites*, exine ribs are psilate and more spiral in their orientation, columellae are more variable in distribution as well as narrower in diameter, and the nexine is three-layered with distinct lamellae in an intermediate position (Pocock and Vasanthy, 1988). In addition to Pocock and Vasanthy's work on these two grains, the fine structure of *E. chinleana* pollen was also initially realized to be "non-gnetalean", principally because of its columellar vs. granular infratectum (Zavada, 1984). Furthermore, despite the presence of nexine lamellae in *E. chinleana*, Zavada (1990) has subsequently suggested that these grains are definitively more angiospermous than gymnospermous and, in particular, most similar to the pollen of *Dahlgrenodendron natalensis* (Lauraceae) and *Spathiphyllum* (Araceae).

Of the three ultrastructurally known fossil polyplicate palynomorphs, the Brazilian grains sectioned in this study most closely resemble *Ephedripites* both in surface morphology and exine stratification. The exine of *Ephedripites* consists of an inner lamellated nexine (layer *A*) and an outer complex, five-layered sexine (layers *B*₁, *B*₂, *B*₃, *C*, *D*; Trevisan, 1980). The most prominent layers of the sexine are the three inner zones. These consist of a very thin, homogeneous band (*B*₁) appressed

to the lamellate nexine, successively overlaid by a layer of "anastomosing units" (= granules; *B*₂) and a homogeneous layer (= tectum; *B*₃). Layer *C* is a thin and discontinuous layer just outside of the tectum and is intermixed to overlaid by layer *D*, that consists of variably sized "globulets." The comparison between the Brazilian grains and *Ephedripites* is based principally on a similar tectate-granular sexine organization and faintly lamellate nexine. Although Trevisan (1980) did not specifically describe the infratectum of *Ephedripites* as granular, but rather referred to it as consisting of "anastomosing units", it is clear from her transmission electron micrographs that these "units" are indeed granules.

Another interesting comparison with *Ephedripites* is the possible occurrence of a sexine layer in the Brazilian grains that is analogous to Trevisan's layer *C*. In the ephedroid grains sectioned here, a thin, homogeneous sexine layer is present external to the tectum in the plicae and absent over the crest regions of the sporoderm ribs. This additional sexine layer may also have functional significance relating to harmomegathy, as it is restricted to furrow regions, and occurs concomitantly with sexine hinges (see above). A layer analogous to the layer *D* observed in *Ephedripites* grains has not been detected in the Brazilian grains. Zavada (1984) suggested that the globulets making up *D* layer in *Ephedripites* may represent tapetal remains; however, we interpret these as fragments of adhering palynodebris.

When the Brazilian grains are systematically identified, using Pocock and Vasanthy's (1988) "key to polyplicate or ridged and furrowed pollen", they key-out very close to *Ephedripites*; however, they also show some features of *Ephedra*. The key only employs micromorphological and ultrastructural data, is based in part on information acquired from the literature, and includes five taxa. These include three dispersed—*Cornetipollis*, *Equisetosporites*, and *Ephedripites*—and two extant—*Ephedra* and *Spathiphyllum* (Araceae)—forms. However, we view the key as slightly flawed concerning two characters that delimit *Ephedripites*, including "tectum almost disappearing in furrows" and "infratectum non-granular." Data on this genus were gained from Trevisan's

(1980) descriptions and published micrographs. We believe, on the other hand, that a tectum is indeed present in the furrows of *Ephedripites*. Although it is markedly thinner here, it does not "almost disappear" (see Trevisan, 1980, plate II, 1c) as Pocock and Vasanthi (1988) suggest, and, in this regard, it is similar to the tectum of extant *Ephedra* pollen. Regarding the fine structural assignment of "non-granular" to *Ephedripites*, as has been stated above, we interpret the infratectum of *Ephedripites* as granular.

Using fine structural features, the Brazilian grains are clearly most similar to *Ephedripites* in comparison to *Equisetosporites*, although originally assigned to the latter genus by de Lima (1980). In addition, when the Brazilian ephedroids are compared with the pollen of extant gnetophytes, both in surface morphology and exine fine structure, they are in fact more similar to the pollen of *Ephedra* than to that of *Welwitschia*, based principally on the lack of a distinct aperture in the former. Exine stratification of the pollen of both extant genera is almost identical. Based on these comparisons with extant and fossil taxa, the Brazilian grains sectioned in this investigation appear to have their affinities unequivocally rooted in the Gnetales.

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