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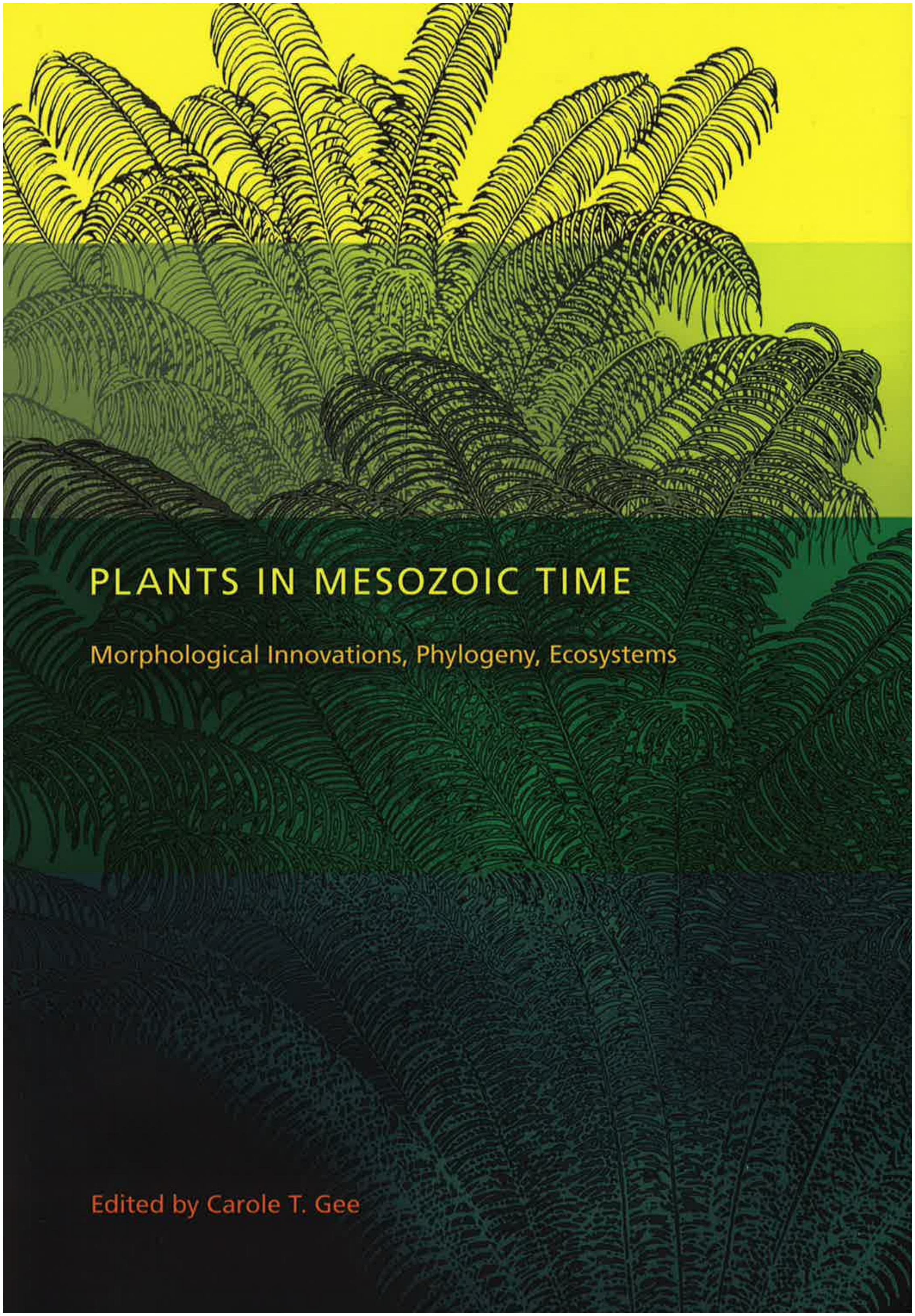


PLANTS IN MESOZOIC TIME

Morphological Innovations, Phylogeny, Ecosystems

Edited by Carole T. Gee

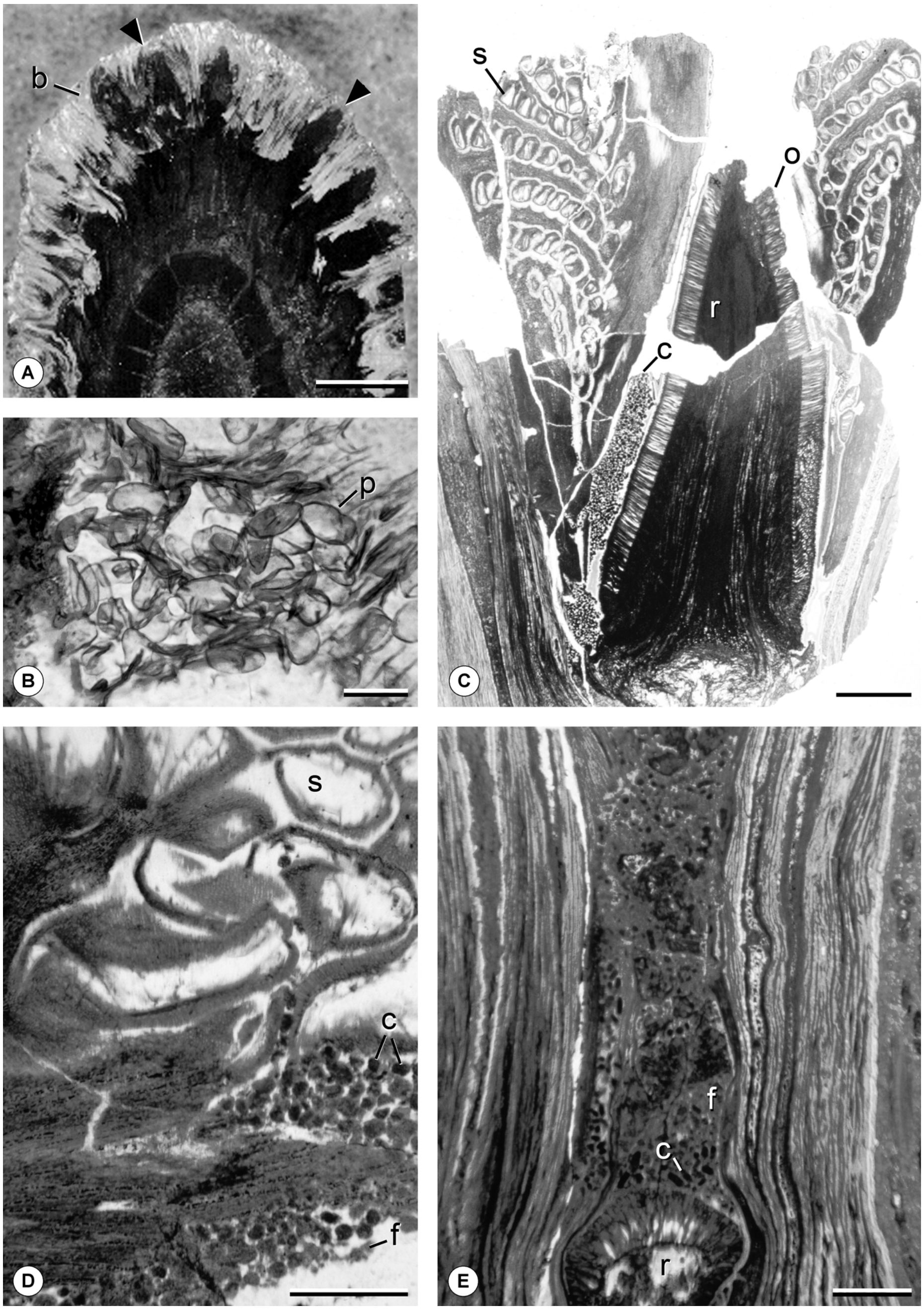


Fig. 3.1. Trunk and cone morphology, *Cycadeoidea.* (A) Transverse section through a trunk showing several cones (arrowheads) surrounded by a thick ramentum of helically arranged bracts (b). Scale bar = 3 cm. (B) Transverse section of a synangium showing a single pollen sac containing in situ pollen grains (p); LM. YPM 5083. Scale bar = 50 mm. (C) Longitudinal section through a bisporangiate cone showing the fleshy microsporangiate region with multiple synangia (s) surrounding a conical ovulate receptacle (r) containing many ovules (o). Note the abundant coprolites (c) in the space between the microsporophyll and the ovulate receptacle, as well as the bore tunnels within the microsporophyll; LM. YPM 5061. Scale bar = 5 mm. (D) Detail of a microsporophyll showing synangia (s) and insect tunnels containing coprolites (c) and frass (f); LM. YPM 5107. Scale bar = 1 mm. (E) Longitudinal section through an immature cone with disarticulated plant material, coprolites (c), and frass (f) surrounding the dome-shaped ovulate receptacle (r); LM. Crepet slide, NS 136 (no YPM number). Scale bar = 5 mm.

POLLEN AND COPROLITE STRUCTURE IN *CYCADEOIDEA* (BENNETTITALES): IMPLICATIONS FOR UNDERSTANDING POLLINATION AND MATING SYSTEMS IN MESOZOIC CYCADEOIDS

3

Jeffrey M. Osborn and Mackenzie L. Taylor

The Mesozoic seed plant group Bennettitales is most comprehensively known from permineralized fossils of *Cycadeoidea* from the Early Cretaceous of North America. Cones were bisporangiate and have been interpreted as remaining closed at maturity. Consequently, self-pollination has been hypothesized in *Cycadeoidea* through developmental disintegration of the fused cones. Additionally, entomophily has been hypothesized as a secondary pollination syndrome because extensive beetle tunnel and gallery systems occur within reproductive tissues. In what is to our knowledge the first study of the structure and contents of coprolites and frass pellets extracted from beetle tunnels within *Cycadeoidea* cones, pollen grains and pollen wall fragments were found to be absent. New data on direct insect interactions, combined with data on pollen ultrastructure, are discussed here as they relate to interpretations of pollination mechanisms and mating system biology in *Cycadeoidea* and the Bennettitales. Because pollination biology determines the frequency and diversity of mating opportunities, the two pollination syndromes would potentially have had different consequences for mating systems in *Cycadeoidea*. Self-pollination alone would have almost certainly resulted in obligate selfing, whereas occasional to predominant insect pollination would have likely resulted in a mixed mating system.

Introduction

The Mesozoic seed plant group Bennettitales is most comprehensively known from permineralized fossils of *Cycadeoidea* from the Lower Cretaceous of North America. Fossils have been collected from a wide range of localities, and many taxonomic species have been described. However, the majority of data on *Cycadeoidea* are derived from investigations of specimens collected in the Black Hills of South Dakota and Wyoming, U.S.A. (Wieland 1906, 1916; Crepet 1974).

The reproductive structure and pollination system of *Cycadeoidea* have received much attention over the years. Wieland's (1906) early anatomical and morphological work characterized the reproductive organ of *Cycadeoidea* as flowerlike, and he reconstructed the plant with open “flowers” protruding from the surface of the stem. At maturity, each open “flower” was interpreted to consist of pinnately compound, pollen-bearing microsporophylls surrounding a centrally positioned ovulate receptacle (Wieland 1906). Such an open structure would have permitted wind pollination, as has also been hypothesized for some members of the Williamsoniaceae, the second and more ancient (Late Triassic–Late Cretaceous) family within the Bennettitales (e.g., Watson and Sincock 1992; Labandeira et al. 2007; Taylor et al. 2009).

Wieland’s (1906) interpretation and reconstruction were subsequently shown to be incorrect. Upon further investigation of Wieland’s specimens, as well as of other unstudied fossils, Delevoryas (1968) and Crepet (1974) demonstrated that all *Cycadeoidea* cones were bisporangiate and remained permanently closed at maturity. The cones were shown to have a highly complex ontogenetic structure. The pinnate microsporophylls did not open but rather recurved during development, and the distal tips became ontogenetically fused near the base of the ovulate receptacle (Delevoryas 1968; Crepet 1974). Consequently, self-pollination has been hypothesized in *Cycadeoidea* through developmental disintegration of the fused cones (Crepet 1972, 1974). In addition, entomophily has been hypothesized as a secondary pollination syndrome because extensive beetle tunnel and gallery systems occur within reproductive tissues of *Cycadeoidea* cones (Crepet 1972, 1974).

Labandeira et al. (2007) have recently proposed a generalized plant–insect association between *Cycadeoidea* and beetles (likely belonging to suborder Polyphaga, or its subclade Phytophaga) analogous to the association that characterizes extant cycads (Stevenson et al. 1998) and perhaps extinct cycads as well (Klavins et al. 2005). The model of Labandeira et al. (2007) is based on cone structure and other anatomical characters for *Cycadeoidea*, a range of data on fossil beetles, and the commonality of insect damage to bennettitalean cones described from a range of geographically and geologically disjunct localities (Reymanówna 1960; Bose 1968; Saiki and Yoshida 1999; Stockey and Rothwell 2003). However, there is actually very little direct information about the fossil insects associated with *Cycadeoidea* or other bennettitaleans (see Labandeira et al. 2007 and references therein).

The objective of the present study is to investigate the structure and contents of coprolites and frass pellets extracted from beetle tunnels within *Cycadeoidea* cones, as well as to review and reexamine the pollen structure of *Cycadeoidea*. The new data on insect evidence of phytophagy, combined with the pollen data, are then discussed as they relate to interpretations of pollination mechanisms and mating system biology in *Cycadeoidea* and the Bennettitales.

Materials and Methods

The genus *Cycadeoidea* ranges in age from Late Triassic to Early Cretaceous, and a number of species have been described from a variety of localities (Taylor et al. 2009). Several key Early Cretaceous sites in the Black Hills of South Dakota and Wyoming in North America have yielded the majority of specimens (see Wieland 1906 and Crepet 1974 for historical synopses and locality details). The pollen grains, coprolites, and frass pellets described here were isolated from *Cycadeoidea* fossils collected from these North American sites, and the specimens are housed in the Paleobotanical Collections of the Peabody Museum of Natural History, Yale University, New Haven, Conn. The light micrographs illustrated in Figure 3.1 were photographed from the original specimens and thin sections produced and studied by G. R. Wieland (1906, 1916), T. Delevoryas (1963, 1965, 1968), and W. L. Crepet (1972, 1974). These thin sections, along with the silicified trunk pieces from which the sections were made, are housed in Yale's Peabody Museum (YPM). The pollen grains described and studied here were isolated from cones of *Cycadeoidea dacotensis* (trunk 213) and *Cycadeoidea* sp. (trunk CF 78), and the coprolites and frass pellets were isolated from cones of *Cycadeoidea wielandii* (trunk 77) and *Cycadeoidea* sp. (trunk CF 78). In addition, *Cycadeoidea* pollen from the fossils studied by Taylor (1973) was reexamined; these specimens are housed in the Paleobotanical Collections of the University of Kansas, Lawrence.

For electron microscopy, in situ pollen, coprolites, and frass were isolated directly from synangia and insect tunnels within the cones by building elevated wax wells directly around the areas of interest on the surfaces of the silicified trunk slabs. Pollen grains, coprolites, and frass pellets were then pipette macerated with 48% hydrofluoric acid and washed several times with water. For scanning electron microscopy (SEM), pollen, coprolites, and frass were pipetted directly onto aluminum stubs, sputter-coated with gold–palladium, and imaged with Hitachi S-500 and JEOL JSM-840 scanning electron microscopes at accelerating voltages of 15–20 kV. For transmission electron microscopy (TEM), pollen, coprolites, and frass were pipetted directly onto cellulose filters under suction. The filters were coated on both sides with agar, dehydrated in a graded ethanol series, and then transferred to 100% acetone to dissolve the filters. The agar-embedded specimens were gradually infiltrated with Spurr's epoxy resin and then embedded in flat aluminum pans. Once polymerized, the resin was removed from the aluminum pans and examined with a stereo light microscope to identify specific microfossils of interest. To obtain single-pollen grains, coprolites, and frass pellets in desired orientations for ultramicrotomy, small resin "blocks" surrounding the microfossils were cut from the resin disks with a jeweler's saw. The specimens were then sectioned on a Reichert ultramicrotome with a diamond knife. Thin sections (70–90 nm) were collected and dried on formvar-coated slot grids, stained with 1% potassium permanganate (3–12 minutes), 1% uranyl acetate (6–12 minutes), and lead citrate (3–6 minutes), and then imaged with a Zeiss EM-10 transmission electron microscope at 60–80 kV.

Results and Discussion

Cone Morphology

Trunks of *Cycadeoidea* bear cones on short shoots that are embedded within a thick ramentum of leaf bases and bracts; these cones only partially extend beyond the trunk surface (Fig. 3.1A). All cones of *Cycadeoidea* are bisporangiate, producing both ovules and pollen during the course of cone development (Fig. 3.1B, C). Cones have recurved, pinnate microsporophylls, each bearing numerous synangia. Synangia are reniform and multiloculate, typically consisting of 22 elongate locules, and the locules contain abundant in situ pollen grains (Fig. 3.1B–D). After pollen dehiscence from the synangia and disintegration of the microsporangiate organs, the receptacular organs remain intact and continue development (Crepet 1974), and the overall reproductive structure then resembles a monosporangiate ovulate cone (Fig. 3.1A).

Although hundreds of sectioned trunks and cones were examined in the present study to identify specimens with pollen, coprolites, and frass, relatively few *Cycadeoidea* trunks were found with cones bearing microsporangiate organs (i.e., in a bisporangiate developmental stage). The paucity of specimens with microsporangiate organs, also noted by Delevoryas (1968) and Crepet (1974), led early workers to conclude that *Cycadeoidea* bore monosporangiate cones. Critical investigation of the cones with pollen-bearing organs, however, later contributed to the recognition that *Cycadeoidea* actually produced closed, bisporangiate cones with a highly complex ontogenetic structure. The anatomical, ontogenetic, and phylogenetic aspects of cone structure were described in detail by Delevoryas (1968) and Crepet (1974).

Pollen Morphology

Pollen grains of *Cycadeoidea* are typically elliptic to prolate in shape and average 25 µm in length and 12 µm in width (Fig. 3.1B and Fig. 3.2A, C). Spheroidal grains have also been observed (Osborn and Taylor 1995); however, most pollen grains exhibit significant folding of the exine, which contributes to a range of other pollen shapes (Fig. 3.1B). In fact, early reports of the preservation of cellular microgametophyes in *Cycadeoidea* pollen (Wieland 1906) were shown by Taylor (1973) to actually represent exine folds. *Cycadeoidea* pollen is monosulcate, and the relatively broad width of the aperture (seen in section in Fig. 3.2B) causes this region of the pollen wall to become highly folded. This condition has made detailed observation of the external surface of the aperture difficult to achieve (see Osborn and Taylor 1995). The nonapertural pollen surface exhibits a punctate to psilate ornamentation pattern (Fig. 3.2C, E).

The pollen wall is well preserved at the ultrastructural level, averages 0.73 μm in overall thickness, and consists of an electron-translucent sexine and an electron-dense nexine (Fig. 3.2B, D, F). The outer sexine consists of two well-defined layers, a homogenous tectum and a granular infratectum (Fig. 3.2D, F). The tectum has intermittent thin zones corresponding to surface punctuations (Fig. 3.2D, F). In many grains, the tectum and infratectum are not as clearly defined from each other structurally because the granules are so densely packed within the infratectum and appear to evenly grade into the homogeneous tectum (Fig. 3.2D). This structural condition led the first report of pollen wall architecture in *Cycadeoidea* to characterize the exine as homogeneous and lacking ultrastructural detail (Taylor 1973). However, in other pollen grains, individual granules within the infratectum are well preserved, exhibit uniform spacing and diameters, and are clearly delimited from the overlying homogeneous tectum (Fig. 3.2F).

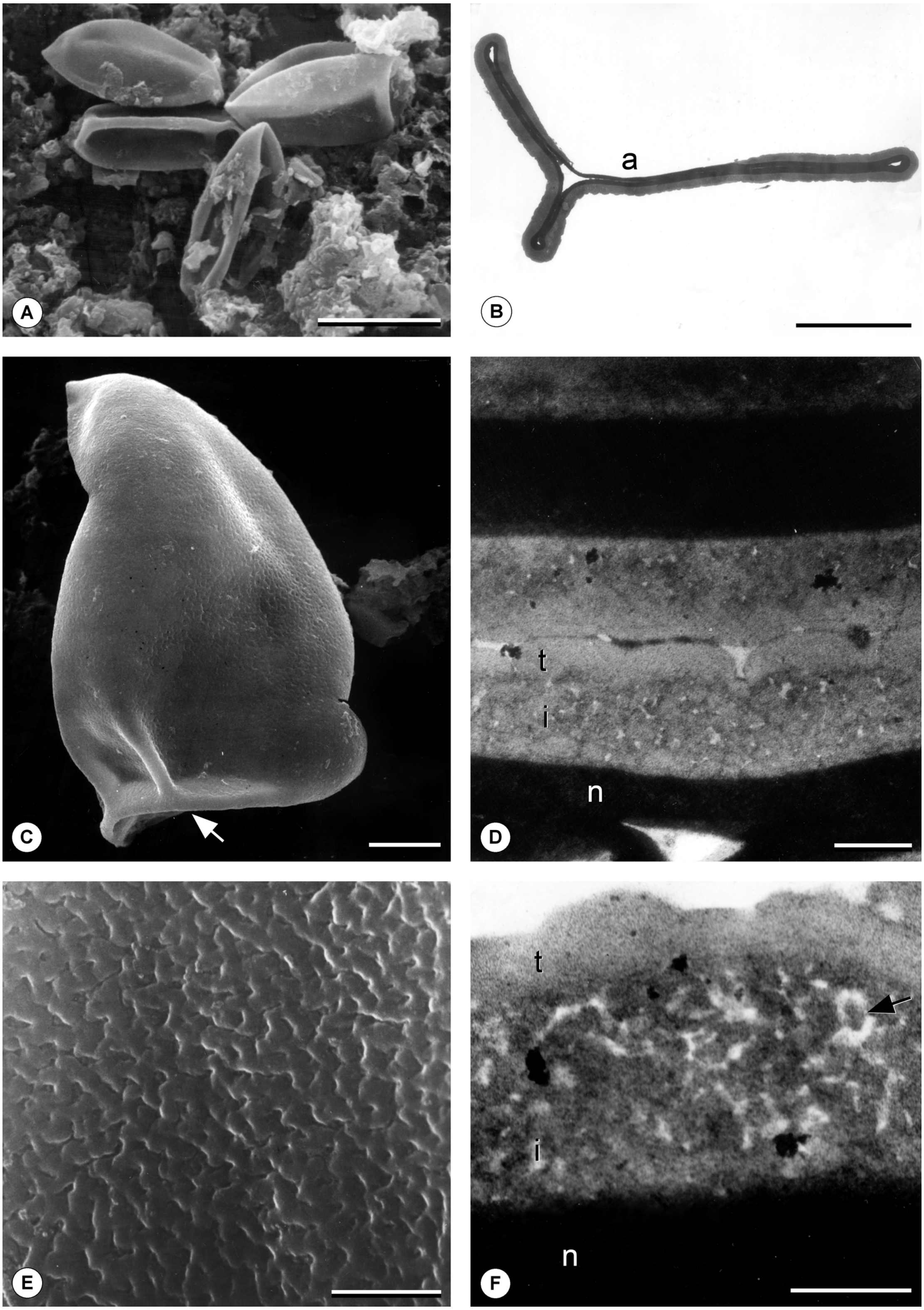
The electron-dense nexine is uniform in thickness throughout the pollen wall, including both apertural and nonapertural regions of the grain (Fig. 3.2B). Endexine lamellations are not discernible in most *Cycadeoidea* pollen grains because the lamellae have become highly compressed during exine ontogeny (Osborn and Taylor 1995; Osborn 2000), a developmental phenomenon also observed in extinct and extant gnetalean pollen (Osborn et al. 1993; Doores et al. 2007). Endexine lamellae may become detectable under certain preservational conditions when fossil pollen grains become stretched or compacted (Osborn and Taylor 1994, 1995; Osborn 2000).

The apertural wall consists of a thin tectal layer, resulting from lateral thinning of both the tectum and infratectum, and a uniformly thick, electron-dense nexine (Fig. 3.2B).

In addition to a range of vegetative and reproductive characters (cf. Rothwell et al. 2009; Crepet and Stevenson 2010; Rothwell and Stockey 2010), several structural characters of the pollen wall from *Cycadeoidea* have been important in evolutionary considerations of the genus, as well as in interpreting the phylogenetic position of the Bennettitales relative to other seed plants. In particular, the granular infratectum and the uniform thickness of the nexine in both nonapertural and apertural regions have played a role in phylogenetic hypotheses about relationships among Bennettitales, Gnetales, and several other gymnospermous taxa, as well as basal lineages of angiosperms (e.g., Osborn 2000; Doores et al. 2007; Taylor et al. 2008).

Coprolite and Frass Morphology

Both coprolites and frass pellets are present in the insect tunnels within *Cycadeoidea* cones. Coprolites are typically oval to spherical in shape and have well-defined outer margins (Fig. 3.3A). The contents of coprolites include a heterogeneous assemblage of plant tissue and cell wall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fragments of varying sizes (Fig. 3.3A–C). The tissue and cell wall fragments are often branched and have an amorphous ultrastructure (Fig. 3.3B, C).

The frass pellets isolated from the same insect boring tunnels are smaller in size, irregular in shape, and have a markedly different ultrastructure (Fig. 3.3D). In comparison to coprolites, the frass pellets consist of plant tissue that is more homogeneous in organization and likely represents chewed plant material that has become disarticulated in chunks (Fig. 3.3D, E).

Labandeira et al. (2007) have proposed a generalized life history pattern of insect herbivory, ovule predation, and pollination in *Cycadeoidea.* Five phases of tissue consumption, associated pollination, and formation of tunnel/gallery systems by small, robust beetles have been hypothesized (Labandeira et al. 2007). The coprolites and frass pellets investigated in the current study were extracted from tunnels within the microsporangiate organs of cones bearing mature pollen, and they therefore represent the fourth and fifth phases of the beetle life history of Labandeira et al. (2007). Consequently, if the beetles were consuming pollen grains, we would expect to find either entire pollen grains or resistant pollen wall layers within the coprolites isolated from the beetle tunnels. In analogous taxa and systems, intact and well-preserved pollen grains have been identified both within the gut contents of several fossil insects (Krassilov et al. 1998; Krassilov and Rasnitsyn 1999) and within the contents of coprolites produced by several fossil beetles (Middle Triassic cycads, Klavins et al. 2005; Late Cretaceous angiosperms, Lupia et al. 2002). However, in the current study, neither entire pollen grains nor pollen walls/exine layers, which are structurally distinctive and exhibit differential staining patterns, were found within the coprolites extracted from *Cycadeoidea* cones (e.g., cf. Fig. 3.2B, D, and F with Fig. 3.3A–E).

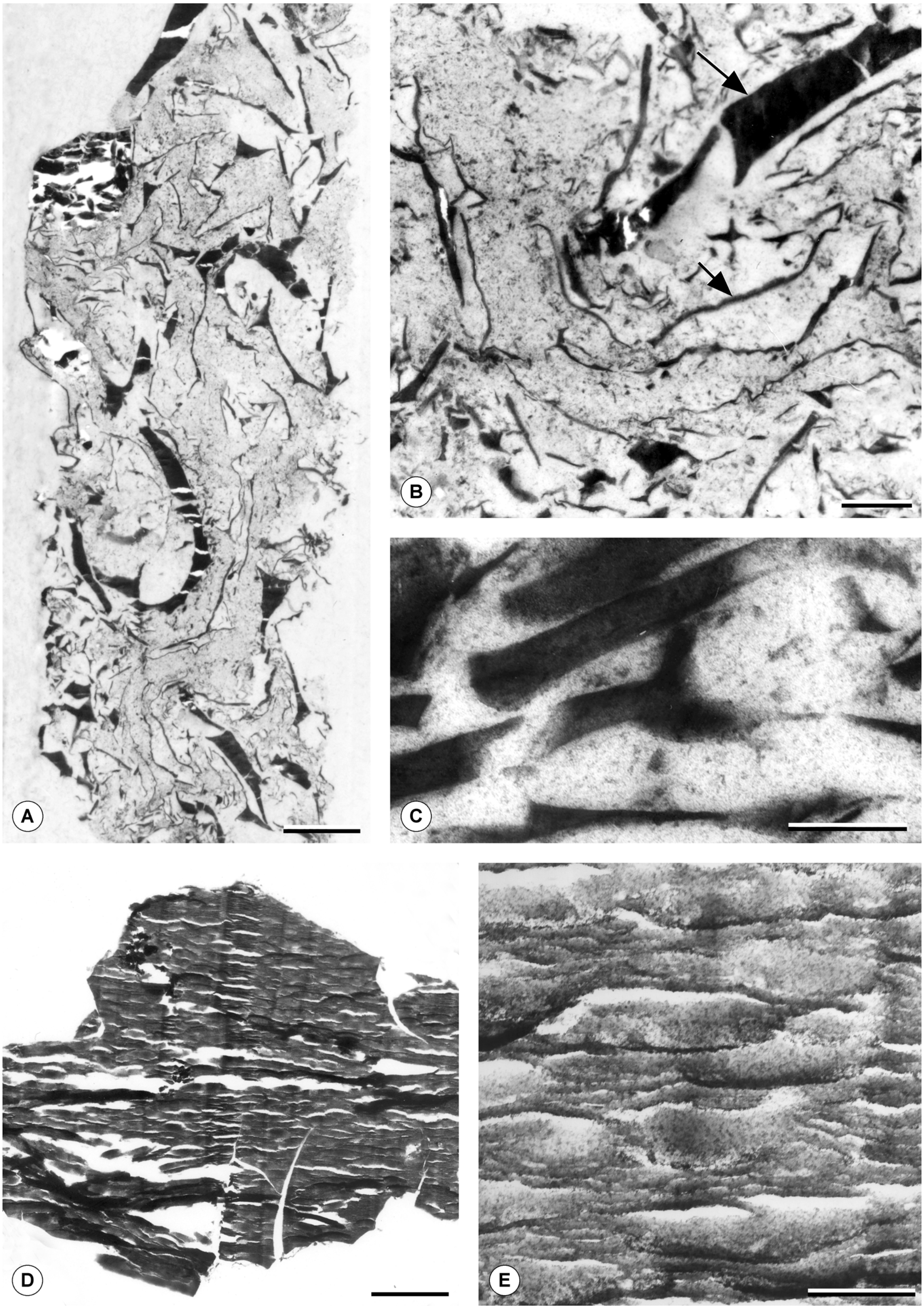
Fig. 3.2. Pollen morphology and ultrastructure, *Cycadeoidea.* (A) Four elongate pollen grains, in which each grain exhibits substantial folding of the exine; SEM. Scale bar = 25 μm. (B) Transverse section through a single pollen grain with a distinctly bilayered exine and thinning in the apertural region (a); TEM. Scale bar = 5 μm. (C) Proximal view of a single pollen grain with slightly folded exine (arrow); SEM. Scale bar = 5 μm. (D) Detail of the pollen walls from two adjacent pollen grains, showing the electron-translucent sexine and the electron-dense nexine (n). The outer sexine consists of two well-defined layers, a homogenous tectum (t) with intermittent thin zones corresponding to surface punctuations, and a granular infratectum (i); TEM. Scale bar = 0.25 mm. (E) Detail of the punctate pollen grain surface; SEM. Scale bar = 2 μm. (F). Detail of pollen wall ultrastructure showing homogeneous tectum (t), infratectum (i) composed of distinct granules (arrow), and electron-dense nexine. Endexine lamellations are not discernible in the nexine (n) of this pollen grain; TEM. Scale bar = 0.25 mm. *All specimens illustrated are from trunk 213.*

Pollination Biology and Mating Systems

Current reconstructions of cycadeoid cones as closed bisporangiate structures indicate that it is highly likely that *Cycadeoidea* exhibited a high rate of selfing. The degree of selfing, which would have depended on the efficiency of insect visitors as pollinators and on post-pollination processes, would potentially have had a profound effect on the ability of cycadeoids to persist and diversify over evolutionary time (Stebbins 1970). On the basis of reconstructions of cone morphology and development, evidence of insect visitors, and comparisons with extant gymnosperms, we consider two major possibilities for mating systems in *Cycadeoidea:* obligate selfing and facultative outcrossing.

OBLIGATE SELFING

Self-pollination likely occurred in *Cycadeoidea* through autonomous selfing, the transfer of self-pollen without the assistance of an outside vector.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Many fertilized cones have been found that exhibit no evidence of insect boring, providing support for the hypothesis that insect visitation was not necessary for pollination and fertilization (Crepet 1974). Moreover, the morphology of bisporangiate cones, with the synangia positioned above the ovulate receptacle and pollen maturing before the ovules, would have facilitated autonomous selfing. Several stages of bisporangiate cones have been described in which the synangia are in various degrees of disintegration, and the remnants of the synangia have come into contact with the ovulate receptacle (Crepet 1972, 1974). This would have facilitated successful self-pollination if pollen contacted the ovules and remained viable until ovules and female gametophytes became developmentally receptive.

In extant gymnosperms, pollen germination and subsequent pollen tube growth are quite slow, and in extant conifers, there is often a period of dormancy between pollination and fertilization (e.g., Fernando et al. 2005). Preserved pollen tubes in *Williamsonia bockii* have been described as nearly identical to extant araucarian conifers, specifically *Agathis australis* (Stockey and Rothwell 2003). In *A. australis*, pollination and fertilization are separated by a year or more, which results from a 3-month delay in pollen germination, slow pollen tube growth, and pollen tube dormancy during the winter months (Owens et al. 1995). It is probable that the Bennettitales, including *Cycadeoidea*, exhibited similar pollen tube growth patterns that allowed for ovule development between pollination and fertilization.

Autonomous selfing is beneficial in cases where pollinators or mates are limited because such selfing provides assurance of seed set in the absence of these (Lloyd 1992). The ability to autonomously self would also have been beneficial if there were strong selection favoring the evolution of closed cones. Open cones that exhibited cross-pollination via either wind or insects are hypothesized to be plesiomorphic in the Bennettitales (e.g., Crepet 1974; Labandeira et al. 2007; Taylor et al. 2009). Crepet (1974) suggested that the evolution of fused microsporophylls, and possibly the protective position of the cones within the trunk, in *Cycadeoidea* might have been in response to beetle predation. A transition from a predominantly outcrossing mating system to an obligate or predominantly selfing mating system may have accompanied cone closure.

The primary evolutionary consequence of a transition to selfing is a loss of heterozygosity and therefore a reduction in genetic diversity. This reduces the ability of a population to evolve in response to selective pressures, especially in the face of a changing environment. Because of this, obligate selfing is generally considered an evolutionary dead end (Stebbins 1970). Crepet (1974) and Taylor et al. (2009) suggested that a loss of genetic diversity due to self-fertilization may have made the Cycadeoidales less able to evolve in a changing environment in the Late Cretaceous and thus may have contributed to their decline.

In *Cycadeoidea* cones, damage from insect boring, as well as the fact that coprolites and frass are found both near the pollen-containing

Fig. 3.3. Coprolite and frass ultrastructure extracted from *Cycadeoidea* cones. (A) Section through a single coprolite showing heterogeneous contents of plant cell walls and tissue fragments, as well as the well-defined outer margins of the coprolite; TEM. Scale bar = 25 μm. (B) Coprolite contents showing plant tissue fragments of varying sizes (electron-dense cell walls; arrows) and having an amorphous ultrastructure. Note the absence of whole pollen grains, as well as structurally distinctive and/or differentially stained exine layers; TEM. Scale bar = 0.5 mm. (C) Detail of coprolite contents showing amorphous tissue fragments and cell walls. Note that one tissue fragment is branched, and that the fragments lack the structural characters and differential staining of *Cycadeoidea* pollen walls and are also thinner than the exine; TEM. Scale bar = 0.25 μm. (D) Section of disarticulated plant tissue found among frass pellets showing likely chewed plant material. Compare the overall morphology, ultrastructure, and staining pattern of this frass pellet to the sectioned coprolite shown in (A); TEM. Scale bar = 2.5 μm. (E) Detail of frass pellet in (D) showing homogenous nature of the plant tissue; TEM. Scale bar = 0.5 mm. All specimens illustrated are from trunk CF 78.

synangia and near the ovulate receptacle, indicate that insects were often present in cycadeoid cones and may have transferred pollen. Thus, the Cycadeoidaceae may have also exhibited facilitated selfing, in which beetles facilitated the movement of pollen from the synangia to the ovules within the same cone. Facilitated selfing is thought to be much less advantageous than autonomous selfing because it incurs the costs of having a pollen vector—for example, pollen or ovules lost to herbivory, or pollen loss transfer (Lloyd 1992; Harder and Barrett 1996; Holsinger 1996). Facilitated selfing is also dependent on pollinator availability, thus providing less reproductive assurance (Lloyd 1992).

FACULTATIVE OUTCROSSING (MIXED MATING SYSTEM)

The occurrence of insect visitors in cones of *Cycadeoidea* indicates that these plants may have also been capable of both selfing (either autonomous or facilitated selfing, as described above) and outcrossing, a condition referred to as a mixed mating system or facultative outcrossing (see Goodwillie et al. 2005). This is a strategy common in extant gymnosperms (Williams 2007) and can be considered a "best of both worlds" situation (Becerra and Lloyd 1992). Occasional cross-pollination maintains heterozygosity and preserves genetic diversity, while self-pollination provides reproductive assurance in the absence of pollinators (Lloyd 1992; Goodwillie et al. 2005). The balance between self- and cross-pollination in gymnosperms has been best studied in extant conifers, which often exhibit moderate to high rates of self-pollination (Franklin 1969; Williams 2007). However, pollination alone does not determine the proportion of self-fertilization, but instead determines the frequency and diversity of mating opportunities that post-pollination processes then filter (Harder and Barrett 1996).

Most gymnosperms exhibit mechanisms to promote cross-pollination, including the separation of male and female function spatially into different cones (moneocy) or different plants (dioeocy), and developmentally (i.e., protandry). The bisporangiate cone in *Cycadeoidea* does not provide spatial separation of male and female function, but the delay in ovule development until after disintegration of the synangia may have increased the probability that cross-pollination by insects could have occurred, especially if insects were drawn to cones with mature pollen, as suggested by Crepet (1979).

After pollination, there may be additional processes that promote cross-fertilization despite high rates of self-pollination. In gymnosperms, these are thought to primarily act postzygotically and to include embryo competition and low viability of selfed embryos due to lethal alleles (see Fernando et al. 2005). Reduced seed set and fewer self-progeny than expected are well-documented phenomena in conifers (Franklin 1969; Owens et al. 1991, 2005; Williams 2007). If cycadeoids did experience both self- and cross-pollination, it is possible that these plants also had mechanisms that promoted outcrossing, such as those in extant gymnosperms, increasing the ratio of cross-to-self-fertilization when cross-pollination did occur. It is also possible that *Cycadeoidea* exhibited pre-zygotic mechanisms to promote cross-fertilization, which are common in angiosperms, but are thought to occur much less frequently in gymnosperms (Willson and Burley 1983; Mulcahy and Mulcahy 1987; but see Runions and Owens 1996).

The evolutionary stability of mixed mating systems is highly controversial (see Goodwillie et al. 2005). Lande and Shemske (1985) provided compelling theoretical evidence that selection will move mating systems toward one of two extremes, either outcrossing with high inbreeding depression or selfing with low inbreeding depression. This is because the level of inbreeding depression, the major cost to selfing, should evolve with the selfing rate. As populations self, recessive deleterious alleles are exposed to selection and purged. Thus, over time, the cost of selfing due to inbreeding is reduced and the rate of selfing should increase (Lande and Shemske 1985). However, recent analyses indicate that the selective pressures on mating systems are dependent on many factors and that mixed mating systems are more common than previously thought (Goodwillie et al. 2005). Therefore, the lack of evolutionary stability is far from certain, and it is possible that *Cycadeoidea* was able to maintain a mixed mating system. If *Cycadeoidea* was dependent on a mixed mating strategy, the changing environment of the Late Cretaceous may have contributed to the disruption of this system and to the subsequent decline of the lineage.

POLLINIVORY AND INSECT POLLINATION

It is often suggested that insect pollination evolved from plant–insect interactions involving herbivory on pollen (pollinivory) or ovules. Subsequent specialization of pollinator rewards and flower morphology may have then led to increasingly consistent and specialized relationships between plants and pollinators (e.g., Faegri and van der Pijl 1979; Crepet 1979, 1983).

Crepet (1979) suggested that pollinivory, which was widespread by the Permian (e.g., Krassilov et al. 1998; Krassilov and Rasnitsyn 1999), occurred in *Cycadeoidea* on the basis of his observations that cones with mature pollen exhibited the most insect damage. However, our study indicates that pollen was not present within coprolites or frass pellets isolated from *Cycadeoidea* cones, and there is no other direct evidence that insect visitors were foraging on *Cycadeoidea* pollen. The lack of pollinivory would have made pollen transfer much less efficient because beetles would have had to fortuitously come into contact with pollen (Crepet 1979). This situation, in which insects feed on ovules but not pollen, is thought to be less likely to result in successful pollination than the reverse (Crepet 1979).

It is possible that even in the absence of pollinivory, the beetle–plant interaction in *Cycadeoidea* represents an intermediate step in the evolution of insect pollination. Fertilization could occur successfully without pollen vectors, but the presence of beetles in the cone allowed for fortuitous, incidental insect pollination that was the precursor to more specialized relationships between plants and pollinators. It certainly shows that insect predation on cones was prevalent. If we assume Crepet's (1974) hypothesis that closed cones evolved in response to beetle predation, then it is likely that some insect pollination was occurring before the evolution of the closed cone, such as in the bennettitalean genus *Williamsoniella* (Williamsoniaceae), a bisexual, chasmogamous taxon. It is possible that *Cycadeoidea* evolved a closed cone and a high degree of selfing, whereas members of the Williamsoniaceae maintained an open cone and evolved more efficient insect pollination. However, there is also indirect evidence that selective pressure from insect predation was pushing williamsonians to evolve in the direction of sporophyll closure (Taylor et al. 2009). Thus, plant–beetle interactions in Mesozoic cycadeoids demonstrate the complexity of early plant–insect associations and their potential ramifications on morphology, reproductive biology, and ultimately evolutionary success.

Acknowledgments

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