

## THE POLLEN ULTRASTRUCTURE OF *WILLIAMSONIELLA CORONATA* THOMAS (BENNETTITALES) FROM THE BAJOCIAN OF YORKSHIRE

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The exine ultrastructure of *Williamsoniella coronata* Thomas from the Bajocian of Yorkshire (United Kingdom) was investigated with light, scanning electron, and transmission electron microscopy. The pollen averages 16.5  $\mu\text{m}$  along its short axis and 24.5  $\mu\text{m}$  along its long axis and is monosulcate, and the nonapertural sculpturing is distinctly verrucate. The pollen wall is homogeneous, and the sulcus membrane is composed of thin exine with scattered small granules. The pollen grains differ in exine sculpturing and pollen wall ultrastructure from pollen grains of the bennettitalean taxa *Cycadeoidea dacotensis* (MacBride) Ward and *Leguminanthus siliquosus* (Leuthardt) Kraeusel. They are similar to dispersed pollen grains of *Granamonomolpites luisae* Herbst from the Triassic Chinle Formation of the United States, supporting the bennettitalean affinity of these dispersed pollen grains. The Bennettitales are palynologically characterized by monosulcate “boat-shaped” pollen with a homogeneous or granular pollen wall ultrastructure.

**Keywords:** exine sculpture, exine ultrastructure, Bennettitales, Jurassic.

### Introduction

Pollen morphological and ultrastructural features derived from in situ and dispersed pollen are useful for determining taxonomic position and elucidating, in some groups, evolutionary relationships. The abundance of nonsaccate, monosulcate “boat-shaped” pollen from the Permian and Mesozoic pollen record (Balme 1995) has been of particular interest because of its association with some of the most primitive of flowering plants (Walker and Skvarla 1975; Walker 1976a, 1976b; Walker and Walker 1984, 1986). Pollen wall ultrastructural characteristics have increased the number of useful characters to refine the taxonomic position of various dispersed and in situ pollen produced by a number of gymnosperm groups (sensu lato), e.g., Cycadales, Bennettitales, and Ginkgoales (Zavada 1990, 2004; Meyer-Melikian and Zavalova 1996).

The ultrastructure of in situ pollen has been investigated in two members of the Bennettitales, *Cycadeoidea dacotensis* (MacBride) Ward from the Lower Cretaceous deposits of North America (Taylor 1973; Osborn and Taylor 1995) and *Leguminanthus siliquosus* (Leuthardt) Kraeusel from the Triassic of Switzerland (Ward et al. 1989). In addition, Watson and Sincock (1992) provided photographs from a scanning electron microscope of pollen grains of *Bennettistemon valdensis* (Edwards) Watson et Sincock in their monographic study of the English Wealden (table 1). In this study we investigate the ultrastructure of pollen found in situ in *William-*

*soniella coronata* Thomas from the Bajocian of Yorkshire (see, e.g., Harris 1969; van Konijnenburg-van Cittert 1971).

The hermaphroditic pedunculate flower-like reproductive structures of *W. coronata* bear a series of involucre bracts that subtend a series of eight to 12 microsporophylls, each bearing four pollen sacs. The microsporophylls subtend a receptacle with interseminal scales and seeds and an apical sterile “corona” (Harris 1969; Crane and Herendeen 2009). Remains of *W. coronata* are associated with the foliage of *Nilssoniopteris vittata* (Brongniart) Florin (fig. 1A, 1B, 1G). Leaves of *Nilssoniopteris major* (Lindley et Hutton) Florin also occur at the locality but have been associated with remains of *Williamsoniella papillosa* Cridland (Harris 1969). Our study reports the known suite of characters associated with the details of the pollen morphology, reproductive structures, and vegetative structures of *W. coronata* and the Bennettitales in general. The geographic distribution of this taxon is extended based on similarities between the in situ pollen and known records of similar dispersed pollen.

Recently, Crane and Herendeen (2009) reported monosulcate psilate pollen recovered from the interseminal scales of *W. coronata* from the same locality as the material investigated by us. The pollen grains range in size from 16.5 to 24.5  $\mu\text{m}$  along the short axis and from 45 to 50  $\mu\text{m}$  along the long axis. Crane and Herendeen (2009) cautiously attributed them to *W. coronata*, recognizing the discrepancy between the reported size of *W. coronata* by Harris (1969) and van Konijnenburg-van Cittert (1971) and their material. The pollen also lacked the characteristic verrucae observed in the pollen extracted from the microsporangia in our study (Crane and Herendeen 2009, figs. 4, 6, 7). This does not rule out, however, the possibility that these pollen grains may belong to

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**Table 1**  
**Comparison of Pollen with Known Bennettitalean Affinity**

Taxon	Average size ( $\mu\text{m}$ )	Sculpturing in nonapertural areas	Sculpturing in apertural area	Wall structure/staining
In situ taxa:				
<i>Cycadeoidea dacotensis</i>	12 × 25	Punctate to scabrate, some specimens psilate	Psilate?	Granular, DBL/KP-UA-Pb
<i>Leguminanthus siliquosus</i>	13 × 23	Scabrate	?	Homogeneous-granular, DBL/UA-Pb
<i>Williamsoniella coronata</i>	16 × 24	Verrucate	Psilate to scabrate	Homogeneous, NDBL/unstained
<i>Bennettistemon valdensis</i>	14 × 23	Psilate	Pitted	No data
Dispersed taxa:				
<i>Granamonocolpites luisae</i> ("small form")	37 × 51	Verrucate	Psilate to slightly scabrate	Distally homogeneous, NDBL/UA-Pb
<i>G. luisae</i> ("large form")	40 × 60	Verrucate	Psilate	Distally homogeneous, NDBL/UA-Pb

Sources. Data derived from Ward et al. 1989; Zavada 1990; Watson and Sincock 1992; Osborn and Taylor 1995; and this study.

Note. All pollen is monosulcate, nonsaccate. DBL = differential electron density of the basal layer, NDBL = nondifferentiated basal layer, UA-Pb = uranyl acetate-lead citrate, KP = potassium permanganate.

a bennettitalean taxon but are probably not derived from *W. coronata*.

### Material and Methods

The reproductive material was collected from the Bajocian of Yorkshire (Grithorpe Bed, Cayton Bay, near Scarborough, specimen 12633) and is deposited in the collections of the Laboratory of Paleobotany and Palynology, Utrecht University. Pollen was extracted from an isolated microsporophyll (~1 cm long) of *Williamsoniella coronata* (fig. 1G). Fragments of a synangium were cleaned with HF followed by maceration in Schulze's solution and KOH. To describe the general morphology of pollen grains, the cleared fragments of cuticles of the synangium with adhering pollen grains were studied in transmitted light with a Zeiss Axioplan 2 and photographed with a Leica DFC-420 digital camera. The pollen grains were photographed under different magnifications; a ×100 oil-immersion objective was used for the greatest magnification. Dozens of pollen grains were screened, but only 16, preserved in more or less longitudinal orientation, were measured. Some pieces of the cuticles with adhering pollen were removed from temporary light-microscopical slides, mounted on SEM stubs, coated with platinum/palladium, and viewed on a CamScan SEM at Lomonosov Moscow State University. The accelerating voltage was 20 kV. Three individual pollen grains have been studied. Some pieces of the cuticles with groups of pollen were removed from temporary light-microscopical slides and embedded for TEM following the method of Meyer-Melikian and Zavialova (1996). Ultrathin sections 50 nm thick were made with an LKB ultramicrotome, viewed unstained on JEOL 100 B and JEOL 400 TEM, and photographed. The accelerating voltage was 80 kV. At least 30 pollen grains have been cut.

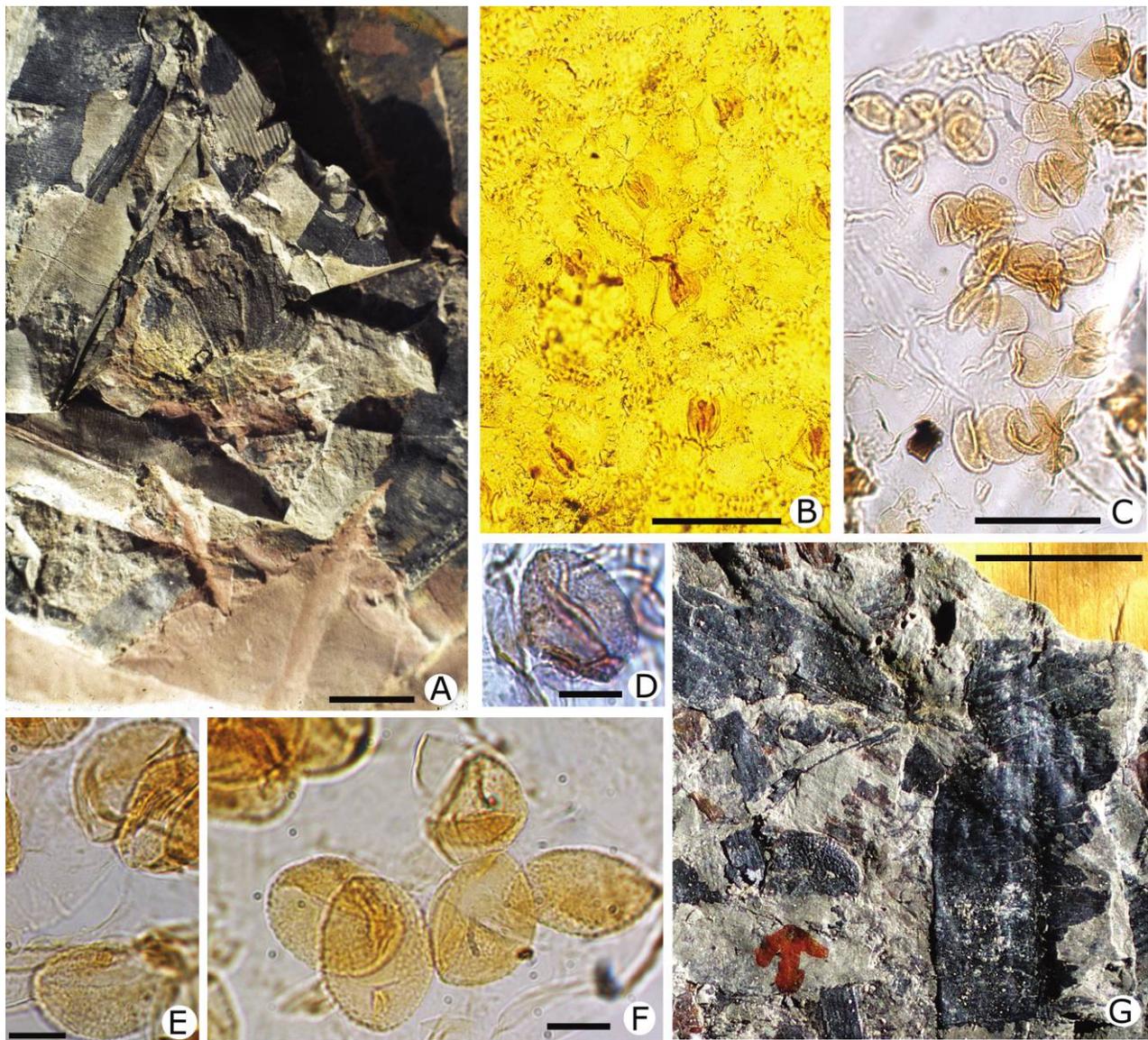
### Morphological Description of the Pollen Grains

The pollen grains are round to oval (fig. 1C). Sixteen pollen grains were measured, ranging from 13 to 19.1  $\mu\text{m}$  along the short axis, averaging 16.5  $\mu\text{m}$ . The length along the long

axis ranges from 22.2 to 27.5  $\mu\text{m}$ , averaging 24.5  $\mu\text{m}$ . Pollen is monosulcate, and the apertural membrane is smooth with occasional small granules 0.08–0.15  $\mu\text{m}$  in diameter (fig. 2D, 2F). Exine sculpturing is verrucate (fig. 1D–1F; fig. 2A–2C, 2E). The verrucae range in size from 0.3 to 1.5  $\mu\text{m}$  and are spaced ~0.09–0.3  $\mu\text{m}$  apart (fig. 2E). Toward the apertural region the verrucae diminish in size and prominence (fig. 2D, 2F). The verrucae on the proximal face are ~0.6–0.75  $\mu\text{m}$  in diameter, occurring with smaller verrucae of ~0.3–0.4  $\mu\text{m}$  (fig. 2C). Pollen wall structure is homogeneous (fig. 3). In the majority of the unstained sections the outer portion of the pollen wall (verrucae) appears more electron dense than the inner portion of the wall (fig. 3D, 3E, 3G). This is a common occurrence in unstained sections and is attributed to differences in exposure to postdepositional diagenesis between the inner and outer portions of the wall that is in contact with the sedimentary environment. In some sections the outer portion of the wall is less electron dense than the inner portion of the wall (fig. 3F), and in a couple of sections the entire pollen wall is identical in electron density (fig. 3J). In the apertural region the exine thins and the sculpturing elements decrease in prominence; adjacent to the aperture, they are no longer apparent (fig. 3A–3C, 3H, 3I). There appears to be no differentiation of the basal layer into foot layer and endexine based on electron density (fig. 3). The homogeneity of the exine electron density suggests it is entirely made up of ectexine.

### Discussion

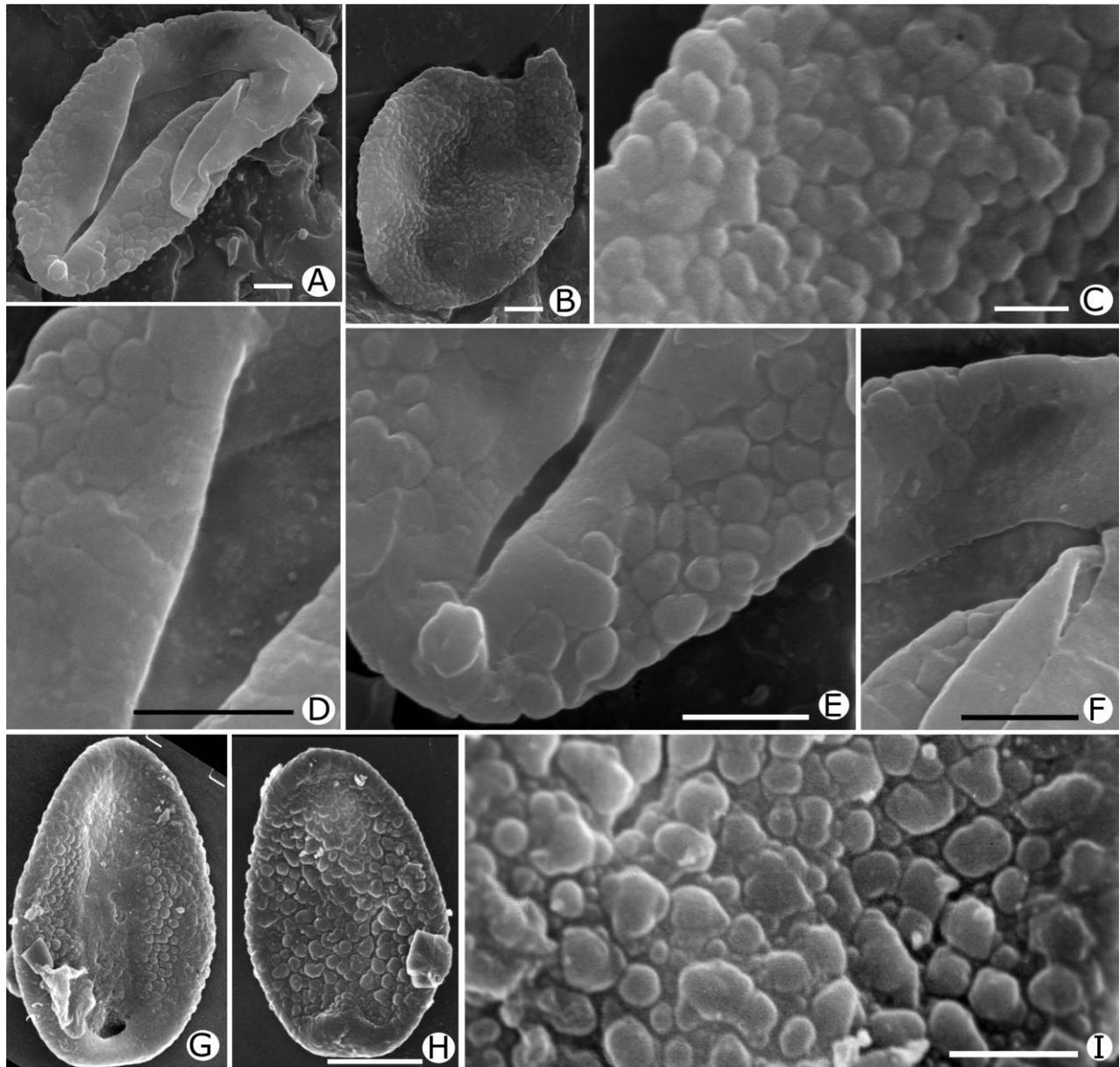
The characterization of the pollen morphology of *Williamsoniella coronata* further broadens our understanding of the Bennettitales. The detailed palynological investigation of *Cycadeoidea dacotensis* (Osborn and Taylor 1995), *Leguminanthus siliquosus* (Ward et al. 1989), and *W. coronata* unequivocally establishes their associated pollen morphologies with these megafossil taxa. The similarity of pollen of *W. coronata* to the Triassic dispersed pollen taxon *Granamonocolpites luisae* Herbst, particularly to its "small form" (Zavada 1990, pl. 3, figs. 1–5), suggests that this dispersed taxon was also bennettitalean (ta-



**Fig. 1** A, Complete reproductive structure of *Williamsoniella coronata* Thomas co-occurring with leaf remains of *Nilssoniopteris vittata* (Brongniart) Florin in the same bedding plane; specimen 1349, collection of the Laboratory of Paleobotany and Palynology, University of Utrecht, Netherlands; bar = 1 cm. B, Cuticle of *N. vittata* showing characteristically bennettitalean undulated outlines of epidermal cells; bar = 50  $\mu$ m. C–F, Pollen extracted from the isolated microsporophyll of *W. coronata*; specimen 12633, collection of the Laboratory of Paleobotany and Palynology, University of Utrecht, Netherlands. C, Various folded pollen grains adhering to the folded sporangial cuticle under low magnification; bar = 50  $\mu$ m. D–F, Pollen under higher magnification, verrucate exine clearly visible; bar = 10  $\mu$ m. G, Isolated microsporophyll of *W. coronata* (arrow), which contained the pollen grains under study; leaf remains of *N. vittata* are present on the same rock sample; bar = 1 cm.

ble 1; fig. 2G–2I). The megaflora of the Chinle Formation includes *Zamites*, *Williamsonia nizhonia* Ash, and *Nilssoniopteris cinizia* Ash of bennettitalean affinity (Ash 1978, 1980, 1989). The in situ and dispersed pollen of bennettitalean taxa exhibit the range of variation that is considered most primitive among the angiosperms, i.e., monosulcate boat-shaped pollen with a homogeneous or granular pollen wall structure (table 1; Walker and Skvarla 1975; Walker 1976a, 1976b; Walker and Walker 1984, 1986). A distinctive pollen wall structural difference is that *C. dacotensis* (Osborn and Taylor 1995, figs. 6–11, 15, 16) and *L. siliquosus* (Ward et al. 1989, pl. 5, figs. 3,

4) are characterized by a differentiated basal layer (based on electron density) in contrast to *W. coronata*, which has entirely homogeneous wall structure (fig. 3) and *G. luisae*, which has predominantly homogeneous wall structure (Zavada 1990, pl. 3, figs. 4, 5; pl. 5, figs. 4–8). Another interesting feature of these taxa is the granular wall structure of *C. dacotensis*, the somewhat structured (“granular”) wall structure of *L. siliquosus*, the somewhat distally structured wall of *G. luisae*, and the entirely homogeneous wall structure of *W. coronata* (table 1). The occurrence of the distinctive granular, homogeneous, and transitional wall structure types in the Bennetti-

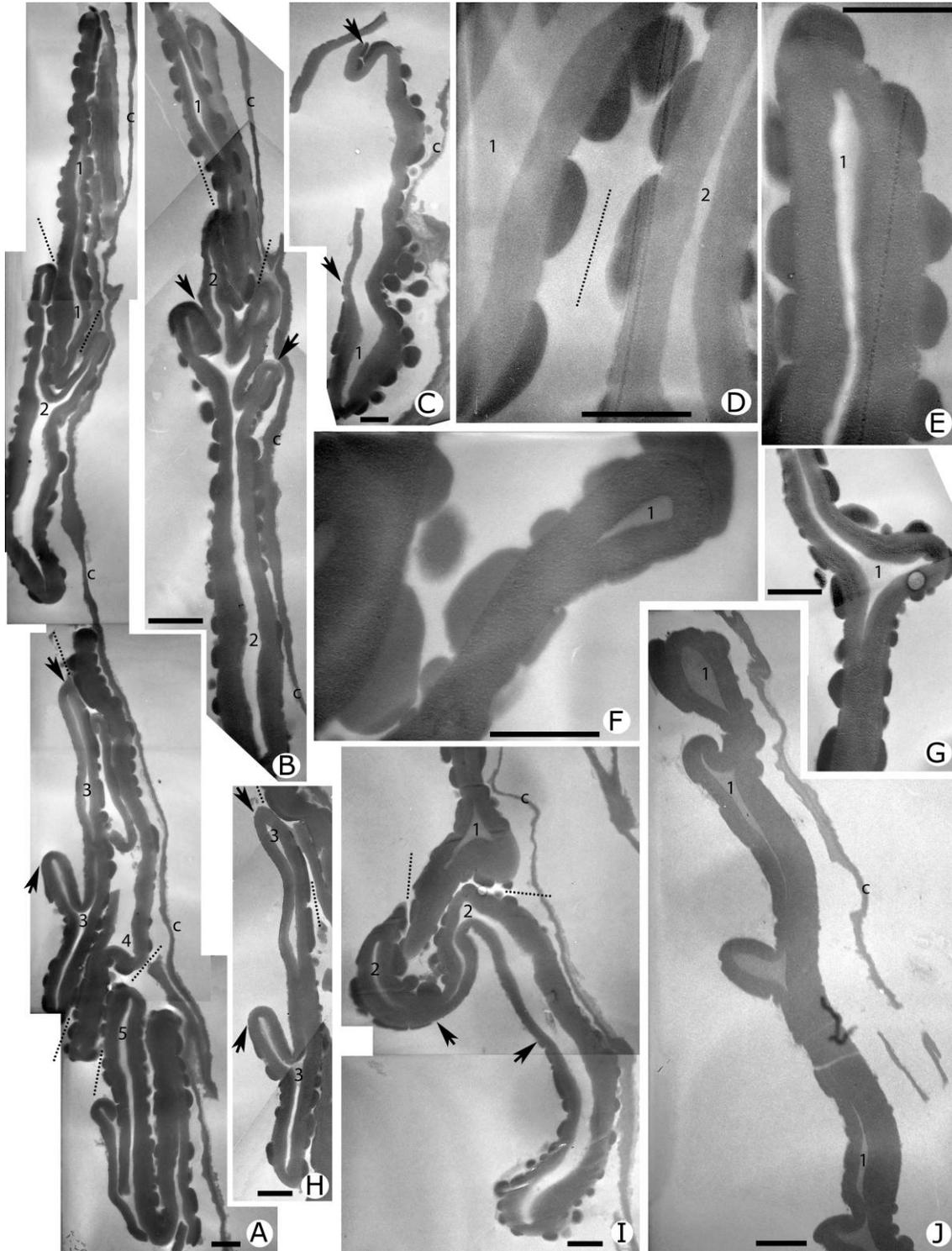


**Fig. 2** In situ pollen grains of *Williamsoniella coronata* Thomas (A–F) and dispersed pollen grains of *Granamonomolpites luisae* Herbst 1970 (“small form”; G–I) under SEM. A, Distal view; the sulcus is distinct; compare with G; bar = 3  $\mu\text{m}$ . B, Proximal view; compare with H; bar = 3  $\mu\text{m}$ . C, Enlargement of B showing proximal verrucae; bar = 1  $\mu\text{m}$ . D–F, Enlargement of A, showing sculpture around or within the sulcus; bar = 3  $\mu\text{m}$ . D, F, Verrucae become flattened toward the sulcus. E, Smaller verrucae are scattered in considerable spaces between larger verrucae (compare with I). G, Distal view of the pollen grain. H, Proximal view of the pollen grain. G, H, Bar = 10  $\mu\text{m}$ . I, Verrucate surface; bar = 4  $\mu\text{m}$ .

tales lends support to their evolutionary relationship (Walker and Skvarla 1975; Walker 1976a, 1976b; Walker and Walker 1984, 1986).

Despite the volatile association of many presumed angiosperm outgroups, the occurrence of bisexual reproductive structures, insect pollination (Crepet 1974), a nucellar plug suggesting the pollen tube may have been transmitted through tissue en route to the archegonium (Friis et al. 2007; see also Rothwell et al. 2009 and references therein), monosulcate boat-shaped pollen, granular to homogeneous wall structure (Walker and Skvarla 1975; Walker 1976a, 1976b; Walker and Walker

1984, 1986), and the widespread Mesozoic geographic and temporal distribution consistently make the Bennettitales appealing as an angiosperm outgroup. Whether or not the Bennettitales play an important role in our understanding of the origin of angiosperms or favor the anthophyte hypothesis for the origin of angiosperms (Friis et al. 2007; Rothwell et al. 2009), the suite of characters exhibited by the Bennettitales (in addition to a number of other Mesozoic groups, e.g., Pentoxylales) indicates that many Lower Mesozoic taxa were experimenting with features that may have been functionally “angiosperm like,” and the unique juxtaposition of these characters in a number of



**Fig. 3** Pollen grains of *Williamsoniella coronata* Thomas under TEM. Sporangial cuticle is marked as *c*; dotted lines indicate boundaries between exines of individual pollen grains; numbers show the inner hollow of pollen grains; arrows show the position of the apertural area. *A*, Composite image showing exines of several pollen grains on the sporangial cuticle; bar = 1  $\mu\text{m}$ . *B*, Section of two pollen grains, apertural area of the lower one folded; bar = 1.25  $\mu\text{m}$ . *C*, Section of a partially broken pollen exine; bar = 1  $\mu\text{m}$ . *D*, *E*, *G*, Areas of sections showing more electron-dense outer portion of the pollen wall and less electron-dense inner portion of the wall. *D*, *E*, Bar = 0.5  $\mu\text{m}$ . *G*, Bar = 1  $\mu\text{m}$ . *F*, Section showing slightly less electron-dense outer portion of the pollen wall and more electron-dense inner portion of the wall; bar = 0.5  $\mu\text{m}$ . *H*, Another section of the pollen grain shown in *A* and indicated as 3; bar = 1  $\mu\text{m}$ . *I*, Sections of two pollen grains; bar = 1  $\mu\text{m}$ . *J*, Pollen exine without differentiation into two layers differing in electron density; bar = 1  $\mu\text{m}$ .

Early Mesozoic taxa may account for the unresolved nature of the basal portion of the angiosperm phylogenetic tree.

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### Literature Cited

- Ash SR 1978 Plant megafossils. *In* SR Ash, ed. Geology, paleontology and paleoecology of a Late Triassic lake in western New Mexico. Brigham Young Univ Geol Stud 25:23–43.
- 1980 Upper Triassic floral zones of North America. Pages 153–170 *in* DL Dilcher, TN Taylor, eds. Biostratigraphy of fossil plants. Dowden, Hutchinson & Ross, Stroudsburg, PA.
- 1989 The Upper Triassic Chinle flora of the Zuni Mountains, New Mexico. Pages 225–230 *in* New Mexico Geological Society Guidebook, 40th Field Conference, Southeastern Colorado Plateau.
- Balme BE 1995 Fossil in situ spores and pollen grains: an annotated catalogue. *Rev Palaeobot Palynol* 87:81–323.
- Crane PR, PS Herendeen 2009 Bennettiales from the Gristhorpe Bed (Middle Jurassic) at Cayton Bay, Yorkshire, UK. *Am J Bot* 96:284–295.
- Crepet WL 1974 Investigations of North American cycadeoids: the reproductive biology of *Cycadeoidea*. *Palaeontographica* 148B:144–169.
- Friis EM, PR Crane, KR Pedersen, S Bengston, PCJ Donoghue, GW Grimm, M Stampanoni 2007 Phase contrast x-ray microtomography links Cretaceous seeds with Gnetales and Bennettiales. *Nature* 450:549–552.
- Harris TM 1969 The Yorkshire Jurassic flora. III. Bennettiales. Trustees of the British Museum (Natural History), London.
- Meyer-Melikian NR, NE Zavalova 1996 Dispersnye distal'no-borozdnye pyl'tsevye zerna iz nizhneyurskikh otlozhenii Zapadnoi Sibiri [Dispersed distal-sulcate pollen grains from the Lower Jurassic of Western Siberia]. *Bot Zh* 81:10–22.
- Osborn JM, TN Taylor 1995 Pollen morphology and ultrastructure of the Bennettiales: in situ pollen of *Cycadeoidea*. *Am J Bot* 82: 1074–1081.
- Rothwell GW, WL Crepet, RA Stockey 2009 Is the anthophyte hypothesis alive and well? new evidence from the reproductive structures of Bennettiales. *Am J Bot* 96:296–322.
- Taylor TN 1973 A consideration of the morphology, ultrastructure, and multicellular microgametophyte of *Cycadeoidea dacotensis* pollen. *Rev Palaeobot Palynol* 16:157–164.
- van Konijnenburg-van Cittert JHA 1971 In situ gymnosperm pollen from the Middle Jurassic of Yorkshire. *Acta Bot Neerl* 20:1–97.
- Walker JW 1976a Comparative pollen morphology and phylogeny of the Ranalean Complex. Pages 241–299 *in* CB Beck, ed. Origin and evolution of angiosperms. Columbia University Press, New York.
- 1976b Evolutionary significance of the exine in pollen of primitive angiosperms. Pages 1112–1137 *in* IK Ferguson, J Muller, eds. The evolutionary significance of the exine. Academic Press, London.
- Walker JW, JJ Skvarla 1975 Primitive columellaless pollen: a new concept in the evolutionary morphology of angiosperms. *Science* 187:445–447.
- Walker JW, AG Walker 1984 Ultrastructure of Lower Cretaceous angiosperm pollen and the origin and early evolution of flowering plants. *Ann Mo Bot Gard* 71:464–521.
- 1986 Ultrastructure of Early Cretaceous angiosperm pollen and its evolutionary implications. Pages 203–217 *in* S Blackmore, IK Ferguson, eds. Pollen and spores: form and function. Academic Press, London.
- Ward JV, JA Doyle, CL Hotton 1989 Probable granular angiosperm magnoliid pollen from the Early Cretaceous. *Pollen Spores* 31:113–132.
- Watson J, CA Sincock 1992 Bennettiales of the English Wealden. *Monogr Palaeontol Soc Lond* 588:1–228.
- Zavada MS 1990 The ultrastructure of three monosulcate pollen grains from the Triassic Chinle Formation, western United States. *Palynology* 14:41–51.
- 2004 Ultrastructure of Upper Paleozoic and Mesozoic monosulcate pollen from southern Africa and Asia. *Palaeontol Afr* 40: 59–68.