

PALEOBOTANY

A fossil record of land plant origins from charophyte algae

Paul K. Strother^{1*} and Clinton Foster²

Molecular time trees indicating that embryophytes originated around 500 million years ago (Ma) during the Cambrian are at odds with the record of fossil plants, which first appear in the mid-Silurian almost 80 million years later. This time gap has been attributed to a missing fossil plant record, but that attribution belies the case for fossil spores. Here, we describe a Tremadocian (Early Ordovician, about 480 Ma) assemblage with elements of both Cambrian and younger embryophyte spores that provides a new level of evolutionary continuity between embryophytes and their algal ancestors. This finding suggests that the molecular phylogenetic signal retains a latent evolutionary history of the acquisition of the embryophytic developmental genome, a history that perhaps began during Ediacaran-Cambrian time but was not completed until the mid-Silurian (about 430 Ma).

Establishing the timing of land plant (embryophyte) origins provides an important constraint for modeling evolving environmental conditions on the Earth's surface, including many aspects of the global carbon cycle (1), throughout geologic time. However, the origin of the embryophytes did not occur as a singularity in geologic time, it happened over a period of time as preexisting algal genes, and de novo genes were assembled into the genome that specifies embryonic development in the plant sporophyte that we see today (2). The algal-plant transition is also intimately linked to adaptation to living on the land surface. Bower (3) long ago used this fact to propose that it was the serial accumulation of morphological adaptation to the subaerial environment during the evolution of an "interpolated" sporophyte phase in the life cycle that characterized the origin of the land plants. His morphological and developmental model predicted that the plant spore preceded the evolutionary origin of the plant sporophyte, a supposition subsequently borne out by both the spore fossil record (4, 5) and the morphological dynamics of meiosis and spore development in bryophytes today (6).

Refinement of molecular clock time trees (7, 8) continues to approach an Ediacaran to Cambrian maximum age for the origin of crown group land plants. This has led to ad hoc explanations of the much later arrival of the earliest plant mesofossil, *Cooksonia* (9), in the rock record; for example, the incompleteness of the rock record (10), the lack of readily fossilizable plant tissues (7), and the scarcity of lower Paleozoic terrestrial deposits (1). Fossil spores of Middle Ordovician (Darriwilian) age exhibit features that are shared with the

spore-bearing embryophytes, including wall ultrastructure (11), and spores borne in either tetrahedral (12) or dyad (13) configurations. Records of older Cambrian "spore-like" remains (14–16) have been used to inform soft lower boundaries in molecular clocks (7, 17), but their relevance to the question of embryophyte origins has also been dismissed because, it is argued, they lack characters unique to the embryophytes (1, 18).

In fact, Cambrian cryptospores do not have the key synapomorphy that aligns with embryophyte spores: spore bodies borne in tetrahedral tetrads. Instead, they generally occur in spore packets, which include varying numbers of spore bodies that may appear to be in various stages of development. Their interpretation as charophytes, as opposed to other algae (4), is based on the observation that living members of some charophyte algae (e.g., *Coleochaete* Brébisson 1844) undergo irregular forms of meiosis (19) that are consistent with the endosporic development seen in Cambrian spore packets (4, 20). In addition, sporoderm characteristics of Cambrian and Ordovician cryptospores appear to be homologous to the lamellated spore walls of some crown group liverworts (4, 15, 21).

With the exception of a single occurrence in South China (22), Cambrian cryptospores are known only from the Laurentian paleocontinent (14–16). Here, we record an assemblage of cryptospores that occurs in the Tremadocian (Lower Ordovician) of the Canning Basin of northern Western Australia, corresponding to an age of ~480 million years ago (Ma). These populations of cryptospores exhibit morphological variations that bear similarities to both older Cambrian cryptospores from Laurentia and younger Ordovician forms from Gondwana, filling in a temporal gap in the prior cryptospore fossil record and expanding the geographical distribution of the fossil record of the algal-embryophyte transition.

The cryptospore assemblages occur in the Samphire Marsh 1 borehole (Fig. 1A and supplementary text), which was drilled in the southern part of the Canning Basin in 1958 (Fig. 1B). Cryptospore-dominated assemblages occur in the type Nambheet Formation, a 775-m-thick sequence of gray-green glauconitic shales and siltstones interbedded with limestones, which overlies a basal fine- to coarse-grained sandstone unit (23). Paleogeographic reconstruction (fig. S1) places Samphire Marsh 1 in the intertidal zone of a transgressive sequence unconformably overlying Cambrian granitic basement. Nicoll (24) found faunas from overlying cores 4 and 5 belonging to the *Prioniodus elegans*–*Bergstroemognathus extensus* Zone that are Arenig (now, Floian) in age. Assemblages from underlying cores 8 and 9 were assigned to the *Drepanoistodus*–*Paltodus* Zone of late Tremadocian age. On the basis of these conodont biozonations, the age of the cryptospore assemblages reported here from cores 6 and 8 is late Tremadocian (Early Ordovician), corresponding to ~480 Ma (Fig. 1A).

The assemblage is dominated by highly variable clusters of packets of spore-like microfossils (Fig. 2A), along with isolated spore polyads (Fig. 2, B to E), tetrads (Fig. 2, F to M), and dyads (Fig. 3, A to E and G to I). Isolated spore monads such as *Laevolancis divellomedium* (Chibrikova) Burgess and Richardson 1991 (fig. S2A) also occur, and, although their provenance from embryophytes is unclear, it is noteworthy that previous records are from the Late Ordovician (Hirnatian) through the late Silurian (Přídolí) and younger (25). All cryptospore walls in this deposit appear simple, without any evidence of added sculpturing. Surface textures vary from smooth (e.g., Figs. 2, A and B, and 3, C, E, and G) to somewhat blotchy in appearance (e.g., Figs. 2, C and J, and 3, A and H), reflecting variations in wall thickness. Overall, two general kinds of spore walls are present: those with a more or less homogeneous structure (e.g., Figs. 2, C to E, and 3, A and H) and those that show features indicating an underlying laminated sporoderm (e.g., Figs. 2, A, B, H, and L, and 3G). The wall ultrastructure of multilaminate cryptospores is well documented elsewhere (15, 21, 26), and the sporomorphs seen here are similar enough to enable us to interpret their wall structure using light microscopy. Individual laminae in these multilaminate walls may fold back on themselves, generating the appearance of irregular (Fig. 2H) to concentric thickenings or bands (Figs. 2L and 3G, arrows) when viewed under light microscopy. In other cases, linear folds in the spore wall may cross between spore-body pairs, serving to reinforce the fact that these forms are not simply random associations of unrelated cells but rather have a common developmental origin. The first wall

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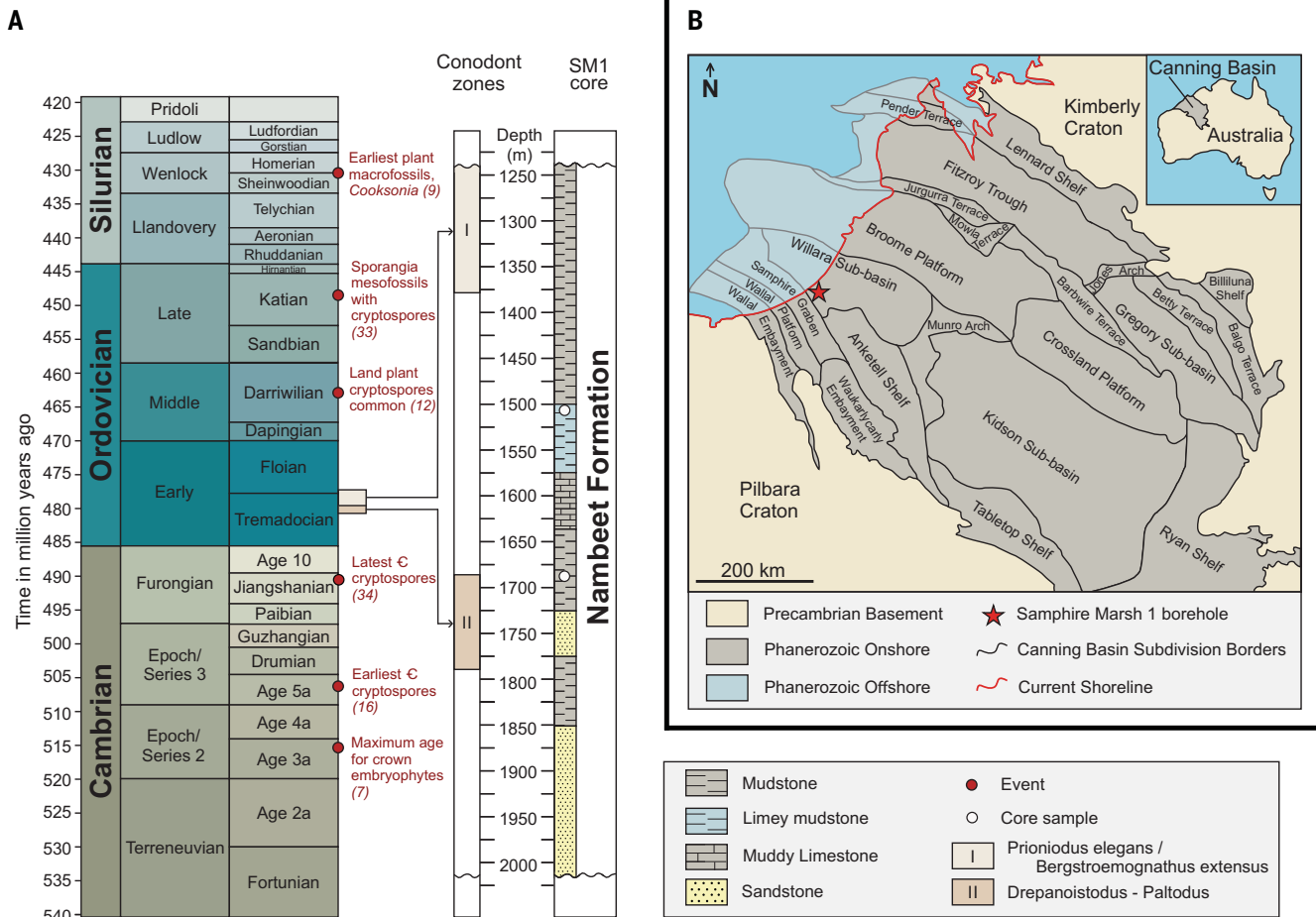


Fig. 1. Stratigraphic placement, evolutionary context, and location of the Samphire Marsh 1 (SM1) borehole. (A) Stratigraphic position of the SM1 assemblages in the context of the major landmarks in lower Paleozoic plant evolution and position within the SM1 core. Important events in plant evolution documented by cryptospores were as follows: “Earliest plant macrofossils,

Cooksonia” (9), “Sporangia mesofossils with cryptospores” (35), “Land plant cryptospores abundant” (12), “Latest Cambrian cryptospores” (20), “Earliest Cambrian cryptospores” (16), and “Maximum age for crown embryophytes” (7). Stratigraphic columns are based on (36). (B) Map showing location of SM1 borehole and geologic structural units of the Canning Basin [according to (37)].

type with thicker homogeneous structure first occurs in the older, Cambrian cryptospore *Spissuspora* Strother 2016, but homogeneous walls are more characteristic of Darriwilian and younger cryptospores (11).

An important feature that is used to discriminate between cryptospores and simple clusters of vegetative algal cells is the intimate nature of cell-cell contact that persists in cryptospores as evidence of developmental continuity during sporogenesis. For example, contact regions between the members of dyads tend to occupy nearly the full diameter of the spore bodies, a feature that is evident in all dyads illustrated in Fig. 3. In simple dyads, the thickened contact surface just corresponds to an upturned fold of the contact face (e.g., Fig. 3A), but in some specimens (Fig. 3C and fig. S2, C and F) a marginal thickening on the proximal spore contact face is quite pronounced, corresponding to a thin equatorial

cingulum in dispersed true spores. Therefore, cell-cell contact in these forms does not appear to be random; these dyads and tetrads were formed together as end products of cytokinesis of a common generative cell, effectively a spore mother cell. Although tetrahedrally aligned spore bodies are not characteristic of the assemblage overall, a few, corresponding to *Rimosotetras* cf. *R. subsphaerica* Strother, Traverse and Vecoli 2015, have been found (Fig. 2F). Similar, although larger, loosely arranged tetrahedral tetrads also occur in the Darriwilian of Saudi Arabia (12, 27). Overall, in this assemblage, most tetrads exhibit either planar (Fig. 2, G to M) or paired dyad (Fig. 3G) configurations. This latter topology is more characteristic of older, Cambrian forms, especially *Adinosporus geminus* Strother 2016. Conversely, planar tetrads composed of isometric spore bodies with simple walls (e.g., Fig. 2, G and I) appear to be comparable to *Tetraplanarsporites*

laevigatus Wellman, Steemans and Miller 2015, which is known from the Upper Ordovician (28, 29). Finally, many smooth-walled dyads illustrated here (Fig. 3, C to E) are not readily distinguishable from the widely distributed taxon, *Dyadospora murusattenuata* Strother & Traverse 1979, a paratype (Silurian, Wenlock) of which is illustrated in Fig. 3F for comparison.

There is a distinct lack of envelopes or enclosing membranes in the assemblage; in fact, we were able to find only two examples of this feature. Figure 3I (arrow) reveals a very thin membrane traversing the marginal gap between the two spore members of a dyad. Such a thin structure is not likely the residual spore wall of a resting cyst, especially given the robust nature of the spores themselves. Figure 3H (arrows) preserves a fragmentary diaphanous membrane that has broken away from a rather robust dyad. Envelopes surrounding

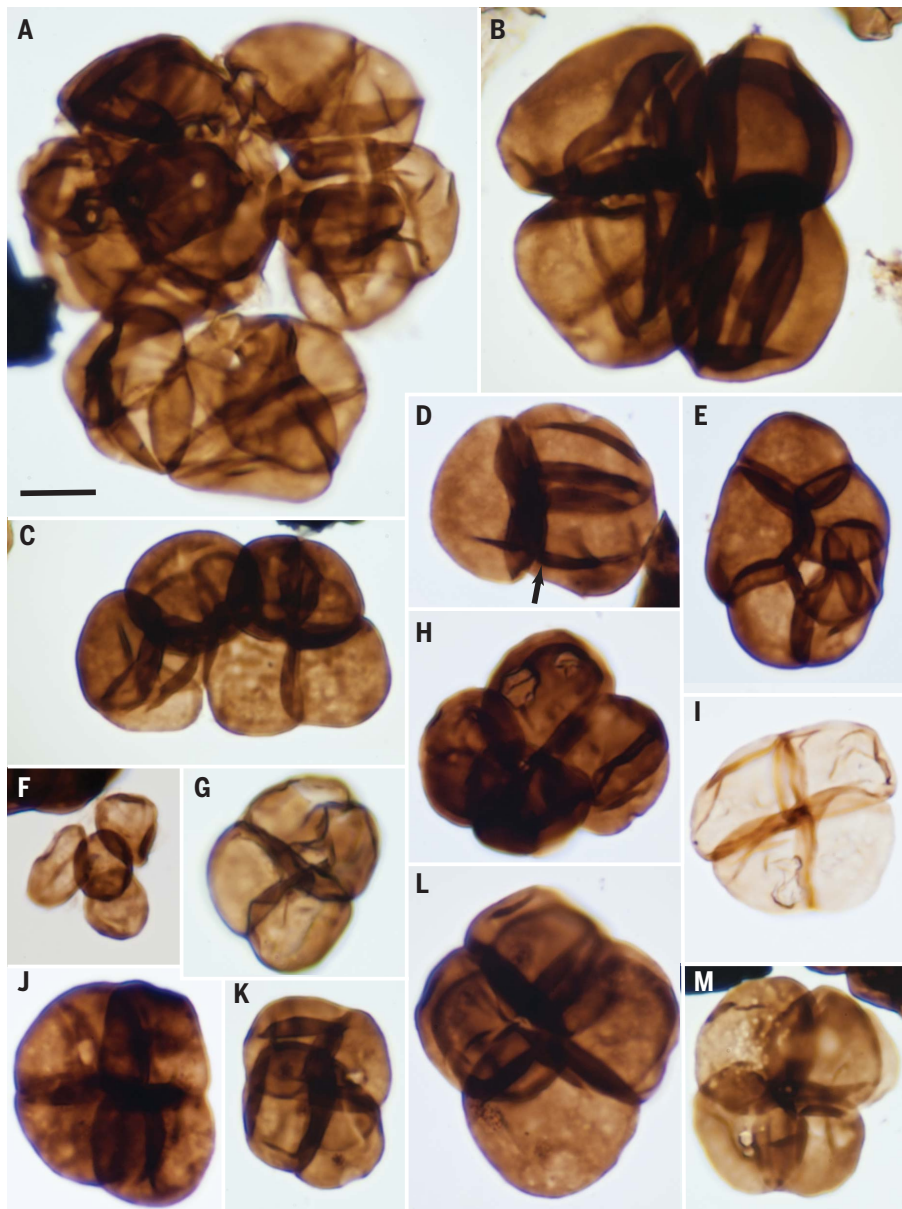


Fig. 2. Cryptospores from the SM1 borehole: spore packets and tetrads. (A) Cluster of six packets of cryptospores typical of preservation in the assemblage. (B) Planar tetrad of smooth-walled cryptospores. (C) Irregular cluster of circular cryptospores, *Spissuspora* cf. *S. laevigata*. (D) Cryptospore triad showing an immature dyad addressed to a larger monad. Note the fold (arrow) that crosses both spore bodies. (E) Quasiplanar cryptospore polyad showing seven spore bodies. (F) *Rimosotetras* cf. *R. subsphaerica*, a tetrahedral tetrad of small, loosely attached isometric spore bodies. (G) Simple thin-walled cryptospore tetrad. (H) Cryptospore tetrad with highly folded wall. (I) Planar tetrad similar to *T. laevigatus* but with a thinner wall and smaller diameter than the type population. (J) Cryptospore tetrad. (K) Cryptospore tetrad with folded walls. (L) Cryptospore tetrad with a singular larger spore body. (M) Cryptospore tetrad with folded walls. Scale bar in (A) is 10 μm and applies to all images; sample depth interval was 5535 to 5547 ft (1687 to 1691 m).

spore tetrads and dyads from Silurian deposits were once speculated to be the relictual walls of an ancestral algal zygote (30). However, cryptospores from Lower and Middle Ordovician strata do not consistently show envelope-enclosed forms to be more prevalent than in the

Silurian. Neither do Ordovician cryptospore assemblages show evidence of persistent zygospores or aplanospores that might indicate a zygnetacean affinity, adding to a perception that envelopes enclosing cryptospores of late Ordovician and Silurian age were derived from

spore mother cells, not from ancestral zygospore walls. This implies that the robust enveloping walls seen in Silurian *Abditusdyadus* Wellman and Richardson 1996 and *Velatitetras* Burgess 1991 are not plesiomorphic with respect to the algal ancestry of the land plants.

A salient feature of Bower's original antithetic hypothesis was a prediction that mitotic divisions of (diploid) zygotes would first produce multicellular thalli of sporogenous cells, which only later in evolutionary time would lose their totipotency to function as fully differentiated vegetative cells. The fossil record shows that this is likely to be the case, first in the retention of spore-like packets attached in short linear chains (e.g., Fig. 3B), but more substantially in sheets of paired spore-like cells, as seen here in *Grododowon orthogonalis* Strother 2017 (Fig. 4, A and B, and fig. S2J). *G. orthogonalis*, which forms planar sheets generated by successive orthogonal cell divisions, has been described elsewhere from Middle (31) and Upper Ordovician (32) deposits. Rosettes of *G. orthogonalis* show a growth pattern that is similar to thallus growth in extant *Coleochaete* (31), but the basic pattern of orthogonal cell division seen here is certainly not restricted to the charophyte algae. The relation to potential vegetative tissues is unknown for *G. orthogonalis*, but the specimen illustrated in Fig. 4A retains an intriguing, ring-like fragment (arrow) that may represent an attachment feature or a remnant of a vegetative structure or covering.

It is tempting to conclude that the spores described here document a Lower Ordovician origin to crown group land plants, thus bringing molecular time trees and fossils into a closer alignment. However, that approach masks the far more intriguing possibility that the fossil record does indeed inform us of the tempo and mode of the evolutionary origins of plant development. This Lower Ordovician assemblage includes some taxa that range into the Late Ordovician and others that are known previously from middle Cambrian deposits (fig. S3). It is the intermediate nature of the assemblage that strengthens our assertion that the earlier cryptospore record is relevant to the question of the origin of land plants. These pre-Darriwilian cryptospores are direct fossil evidence of charophyte adaptation to terrestrial settings. The genomic component of that adaptation that was subsequently incorporated into the embryophyte genome might account for molecular clock dates that precede the arrival of fossilized plant axes in the rock record. This idea, that early cryptospores are a manifestation of spore development that preceded vegetative plant development, is also consistent with sedimentological (33) and other earth system proxies (34) for the effects of land plants indicating a later, Silurian origin to the crown group embryophytes.

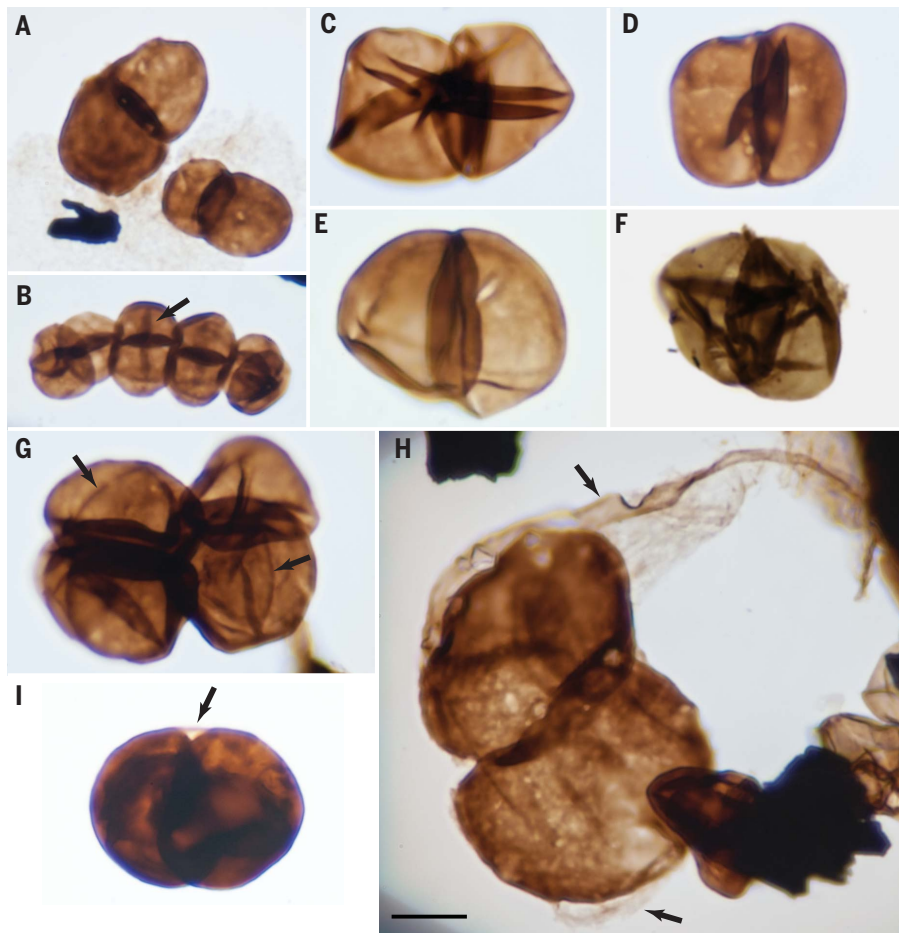


Fig. 3. Cryptospore dyads from the SM1 borehole and the Silurian of Pennsylvania. (A to E and G to I) Cryptospore dyads from the SM1 borehole. (A) Two simple spore dyads that are irregular in size and have blotchy walls. (B) Row of six aligned small dyads. Note the presence of a transverse thickened band (arrow). (C) *D. murusattenuata*. Note the thickened contact ring and folded walls, features that characterize this species. (D) *D. murusattenuata*, a simple smooth-walled dyad pair. (E) *D. murusattenuata*. (F) Cryptospore dyad from the Silurian of Pennsylvania. Shown is a *D. murusattenuata* paratype, which is Silurian (Wenlock) in age. (G) Planar set of two dyads with thin folded walls. This form is similar to the Cambrian species *A. geminus*. (H) *Abditusdyadus laevigatus* with associated partially detached membrane (arrows). (I) *Abditusdyadus* sp. with a very thin enclosing membrane (arrow) and thick, blotchy walls. Scale bar in (H) is 10 μm and applies to all images; sample depth interval was 5535 to 5547 ft (1687 to 1691 m).

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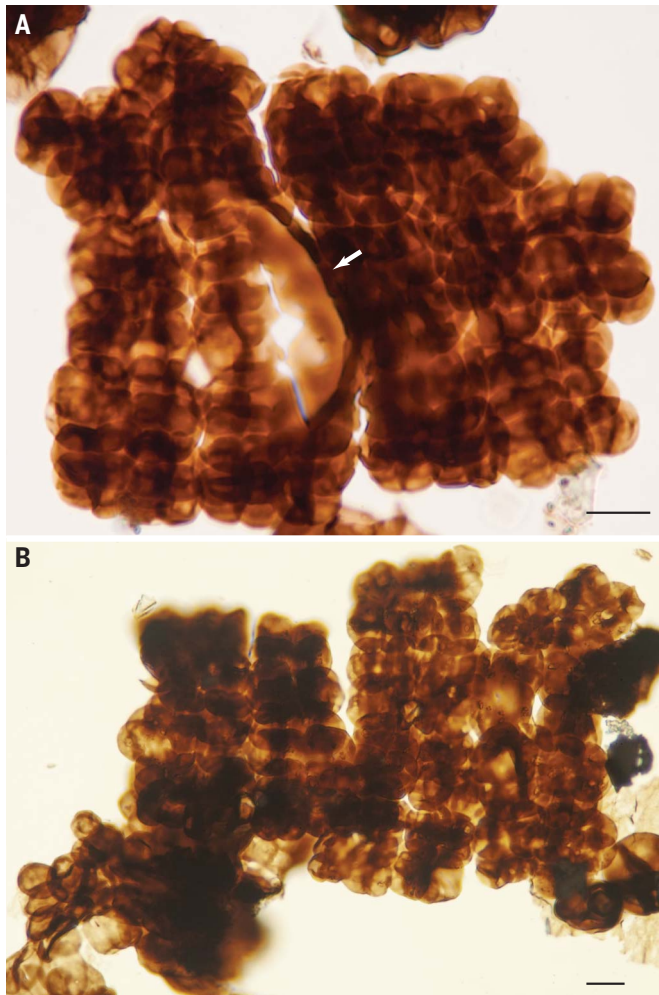
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Fig. 4. *G. orthogonalis* from the SM1 borehole.

(A) *G. orthogonalis* comprises planar sheets of geometrically aligned spore packets, here with an attached arcuate structure (arrow).

(B) This larger specimen shows varying degrees of preservational quality but retains the orthogonal alignment of small spore dyads that characterize the genus. Scale bars are 10 μm ; sample depth interval was 5535 to 5547 ft (1687 to 1691 m).



Gordon Wood, who first brought to our attention the potential existence of a lowermost Paleozoic spore record. **Funding:** None. **Author contributions:** C.F. made the initial discovery, including recognition and specimen acquisition, and prepared all figures. P.K.S. wrote the initial draft and was responsible for all photomicrography. Both authors were responsible for conception, review, and editing. **Competing interests:** The authors declare no competing interests. **Data and materials availability:** This report is based on material housed and curated by the WA Department of Mines, Industry Regulation and Safety, which may be accessed through the WAPIMS database <https://wapims.dmp.wa.gov.au/wapims>. One sample, slide S-05-77/2, is housed at the Paleobotany Laboratory at Weston Observatory of Boston College; contact P.K.S. for access. All data are available in the manuscript or the supplementary materials.

SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S3
Tables S1 and S2
References (38–46)

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A fossil record of land plant origins from charophyte algae

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The timing of land plant origins

Until now, the first fossil evidence of land plants was from the Devonian era 420 million years ago. However, molecular phylogenetic evidence has suggested an earlier origin in the Cambrian. Strother and Foster describe an assemblage of fossil spores from Ordovician deposits in Australia dating to approximately 480 million years ago (see the Perspective by Gensel). These spores are of intermediate morphology between confirmed land plant spores and earlier forms of uncertain relationship. This finding may help to resolve discrepancies between molecular and fossil data for the timing of land plant origins.

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