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Ultrastructure, morphology, and topology of Cambrian palynomorphs from the Lone Rock Formation, Wisconsin, USA

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ABSTRACT

A combination of white light with scanning and transmission electron microscopic analysis has enabled the detailed characterization of the morphology and topology of problematic spore-like palynomorphs recovered from Upper Cambrian near-shore deposits in Wisconsin, U.S.A. Members of the new taxon, *Agamachates casearius* gen. et sp. nov., are smooth, thick-walled, synoecosporal (within a common wall) packets containing up to four spore dyads. The synoecosporal packets themselves may be aggregated into clusters of two or more packets. The discovery that the smallest purported meiotic units are dyads is supportive of prior hypotheses that attempted to explain the abundance of dyads in the lower Paleozoic fossil record. Their abundance has been especially perplexing given the absence of any modern plants that produce dyads via normal sporogenesis. Dyads appear to precede tetrads as the fundamental resistant-walled propagule in the spore record, indicating a transitional stage in the evolution of sporogenesis in plants prior to the canalization of meiosis into a single coördinated process. The variation in spore number per synoecosporal packet could be due to endoduplication of zygote DNA prior to cytokinesis during sporogenesis–paralleling a process that occurs in *Coleochaete* today.

1. Introduction

Over the past 35 years, our picture of the terrestrialization of the land surface has undergone a series of shifts in perspective. What began as a debate on the taxonomic status of isolated Silurian trilete spores (Gray and Boucot, 1971; Banks, 1975; Gray and Boucot, 1977; Gray, 1985), resulted in the recognition that permanent dyads and tetrads of inaperturate spores were shed as diaspores by Early Silurian land plants (Pratt et al., 1978, Strother and Traverse, 1979). Richardson et al. (1984) soon proposed a new palynomorph class, the cryptospores, to accommodate this pre-trilete spore phase of plant evolution (Richardson, 1996). Paleobotanists have been generally accepting of the cryptospore record acting as a proxy for the existence of embryophytes as far back as the lower Middle Ordovician (Darriwilian) (Wellman and Gray, 2000; Edwards and Wellman, 2001; Steemans and Wellman, 2003). But reports of cryptospores of Middle Cambrian age (Strother, 2000; Strother and Beck, 2000; Strother et al., 2004) have met with some degree of skepticism (Wellman, 2003; Steemans and Wellman, 2003). Chief among the objections to the inclusion of these Cambrian palynomorphs with the cryptospores is their lack of clear tetrahedral symmetry, the variable numbers of spores in some polyads, and the huge time gap (ca. 45 Myr) between the Middle Cambrian material and the accepted cryptospores of Darriwilian age.

In this study, we present a detailed morphologic examination of what initially appeared to be a planar tetrad from a population of palynomorphs extracted from strata of Late Cambrian age. These spore-like palynomorphs possess some morphological and topological qualities that ally them to younger Ordovician cryptospores, but, like their Middle Cambrian counterparts, their morphology and topology is perhaps more varied that seen in younger cryptospores. The current report is part of a larger study that was undertaken to provide detailed structural information on selected, well-preserved Cambrian spore-like microfossils using combined light (LM), scanning electron (SEM), and transmission electron microscopy (TEM). A complete serial set of nearly 250 sections cut from one specimen has provided new information on the number of spores present in each specimen, the nature of the smallest units, and possibly the spore-spore symmetry. We continue to argue that the source of these problematic spore-like palynomorphs is closer to land-derived sporomorphs of plant origin than it is to either freshwater green algae or acritarchs (which carry the implication of a marine origin). These microfossils fit clearly within the palynomorph group, cryptospores, as defined by Strother and Beck (2000), since they appear to represent forms more advanced than the algae, yet less uniformly geometric than spores characteristic of extant tracheophytes.

2. Materials and methods

Sample SBT03-01 was collected from a freshly-worked road cut along Route 131 southwest of LaFarge, Wisconsin (N 43° 33.185', W 90°

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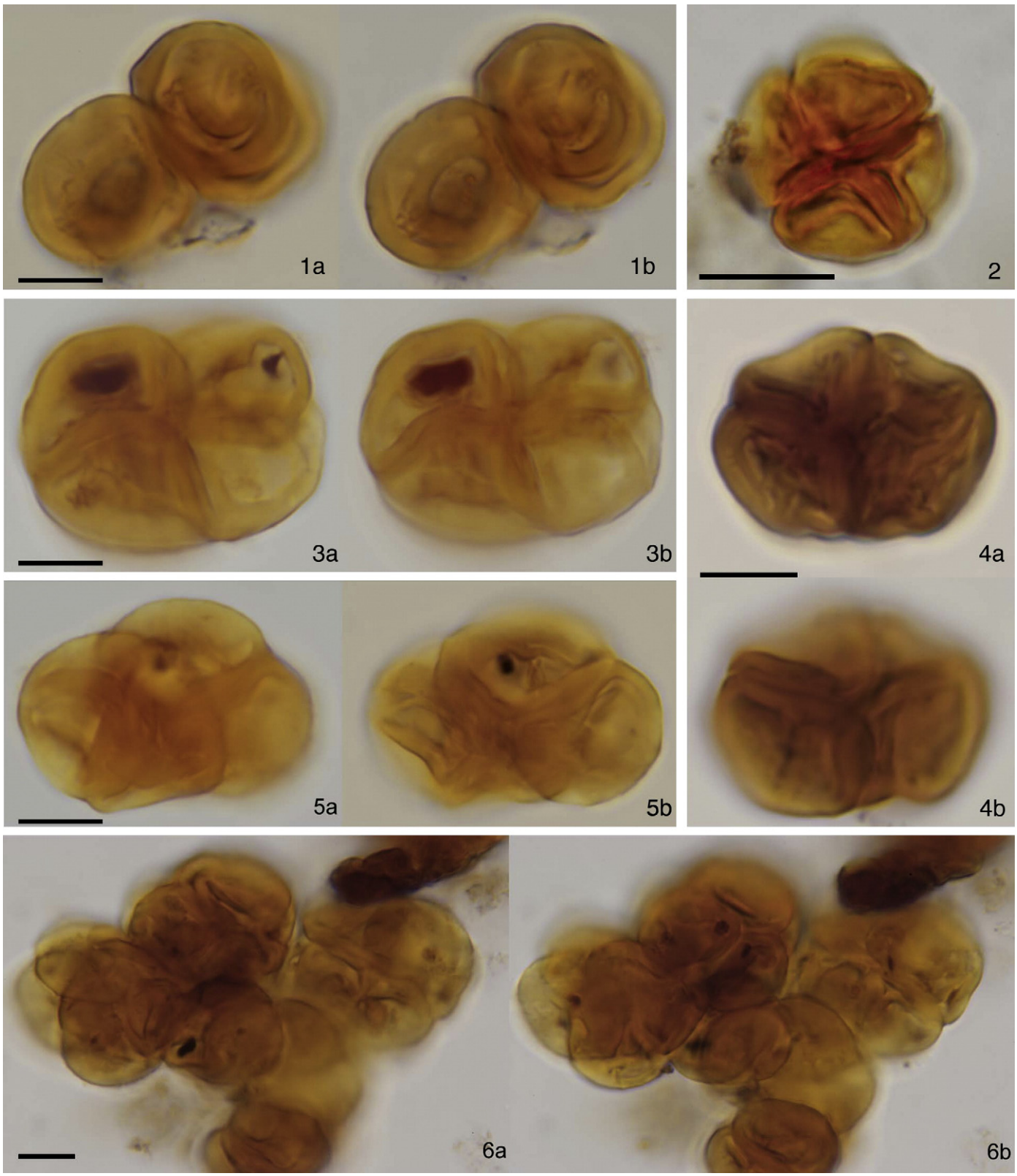
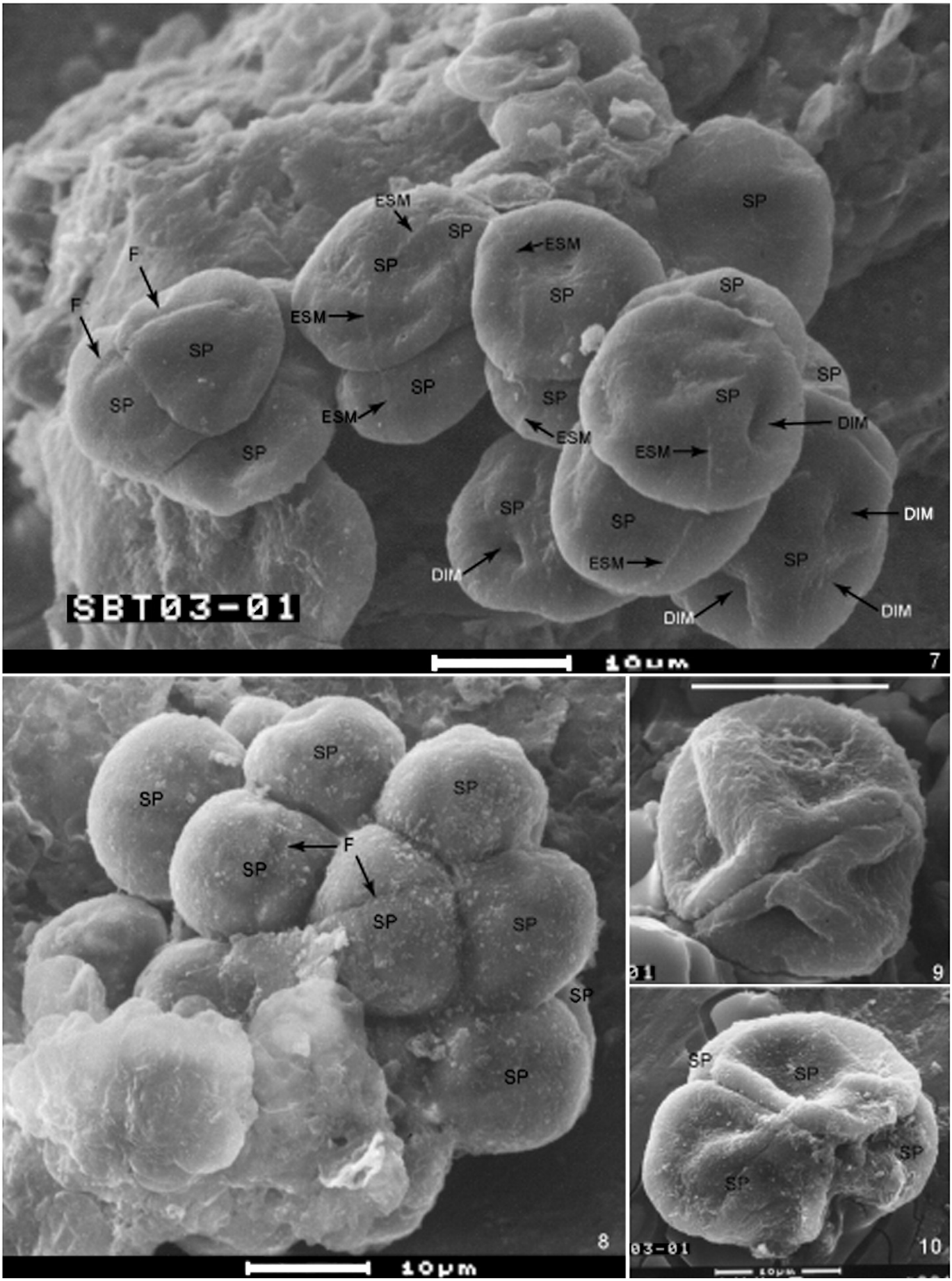


Plate I. 1–6 Light micrographs of *Agamachates casearius* gen. et sp. nov. Scale bar in each figure is 10 µm. "a" and "b" designations represent different focal planes of the same specimen.

1. Two attached synoecosporal packets forming an apparent dyad.
2. A quartet of packets that gives the appearance at first of being a simple spore tetrad.
3. Four synoecosporal packets, each with apparent interior spores.
4. A quartet of packets in which the folded walls of the interior spores are more apparent than in Fig. 2.
5. A quartet of adpressed packets that appear as two dyads, but which appear to each contain one or more interior spores.
6. A larger, irregular cluster of packets.



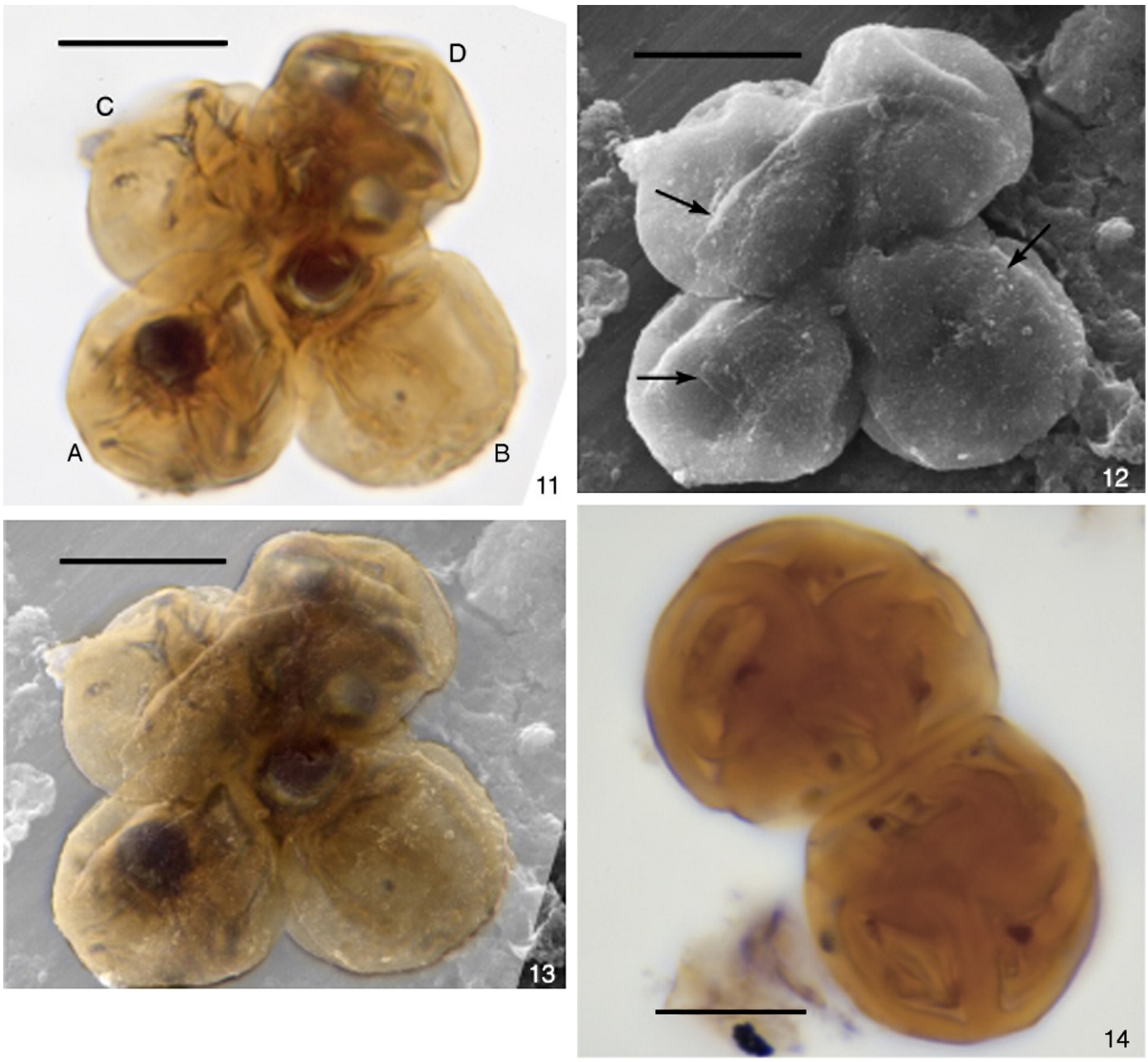


Plate III. 11–14 The sectioned specimen and the holotype. Scale bar in each image is 10 μm.

11. LM of the sectioned specimen with 4 of the attached synoecosporal packets labeled A through D.
12. SEM of the sectioned specimen, arrows point to extra-sporal material attached to all packets.
13. This is a composite image of the sectioned specimen constructed by superimposing the images from Figs. 11 and 12 in Photoshop (see methods.)
14. *Agamachates casearius* holotype. Slide SBT03-01B(SEM), England Finder coordinates, H54/3, Natural History Museum accession number FM2097.

39.747'). In southern Wisconsin, the Lone Rock Formation is one of two glauconite-bearing Upper Cambrian units (Mahoney et al., 1997). The age of the Lone Rock Formation in this region ranges from the *Elvinia* to *Illanurus priscus* trilobite biozones (from Runkel et al., 2007). Since our collection is from the middle part of the formation, we consider the age to be within the lower half of the Sunwaptian Stage (*Conapsis* to *Ptychaspsis-Prosaukia* biozones), corresponding to the middle portion of the Upper Cambrian. The sample, a light grey mudstone, is thoroughly bioturbated at the mm to cm scale. Individual burrowed fabrics are filled with fine-grained quartz sand containing large well-rounded glauconite grains, consistent with their likely origin as fecal pellets. The paleogeographic position of the locality is close to the eastern margin of the

Plate II. 7–10 SEM images of *A. casearius*. Scale bar in each image is 10 μm.

7. Cluster of synoecosporal packets (SP), (F) furrows within packets, (ESM) extra-sporal material, which, in some cases occurs as linear features that appear to represent attachment scars, (DIM) dimple in the synoecosporal wall indicating the presence of an underlying lumen within the spore.
8. Another cluster, (SP) synoecosporal packet.
9. *A. casearius* single packet with infolded contact area and extensive extra-sporal material.
10. A quartet of closely adpressed packets which appear without a common enclosing wall.

sequence in Wisconsin, corresponding to a proximal, very near shore setting on a very shallow, low angle slope.

Approximately 10 g of crushed sample were dissolved in cold concentrated HF followed by treatment with cold fuming HNO_3 for 5 min and without further oxidation. Gravity separation was performed using $ZnCl_2$ (s.g. 2.0). Isolated specimens were picked, dried on an SEM stub, coated and scanned on an AMRAY 1600 series SEM. Single grains were then picked off the SEM stubs, embedded in glycerin jelly and mounted on standard 1 in.×3 in. microscope slides for examination in LM. The slides were then transferred to WAT. The specimens were removed and embedded in Spurr resin using standard techniques for TEM, and cut with the proper orientation from disks of hardened resin formed in aluminum weighing dishes. All sections from first contact (following initial trimming slightly into the specimen) with the specimen were collected on Formvar coated 1×2 mm copper slot grids. These grids were stained with potassium permanganate (1% aq.), uranyl acetate (1% aq.), and lead citrate (Venable and Coggeshall, 1965) for 5 min, 5 min, and 2 min respectively, and examined and photographed in a JEOL 2010 analytical TEM operating at 80 kV.

Table 1

Packet designation	1st section	Last section (last=230)	Total maximum diam
A	0	131	14 μm
Dyad 1 (bot left)	0	100	
Dyad 2 (up left)	0	60	
Dyad 3 (up right)	0	76	
Dyad 4 (up right)	16	131	
B	0	110	14 μm
Dyad 1	0	110	
Dyad 2	0	110	
C	89	230	14 μm
Dyad 1	89	230	
Dyad 2	89	230	
D	≈112	230	15 μm
Dyad 1 (left)	112	221	
Dyad 2 (right)	≈154	230	
Dyad 3 (top)	≈164	230	
Dyad 4 (bot)	≈162	230	
E	≈26	≈150	14 μm

3. Clarification of terminology

The term *spore*, in our usage, is not meant to refer to a miospore (which implies embryophytic derivation), but rather in its more general biological sense of a single-celled, resistant-walled reproductive unit. In the following description, the term *synoecosporal packet* is used for the spore unit with and including a common enclosing structure, a synoecosporal wall (Taylor and Strother, 2008). This is most clearly seen in SEM images where it is possible to view discrete junctures between individual packets. The included end members that comprise these packets are spore dyads, and, as will be seen below, synoecosporal packets may contain either two or four dyads (4 to 8 cells). This is the reason for employing the neutral term, *packet*, rather than *dyad* or *tetrad*, because the number of spores enclosed within each packet varies. The term *lumen* is used to refer to the space enclosed by each individual spore.

We use the term *cryptospore* in the sense of Strother and Beck (2000) to include spore-like microfossils of probable terrestrial origin and not Steemans (2000) who recommended restricting the cryptospores to a sub-set of miospores. This distinction is important, because we do not wish to insist that what we are calling cryptospores are necessarily of embryophytic derivation. This is needed to keep open the possibility that these spore-like palynomorphs were derived from fresh-water algae that had partially adapted to subaerial habitats. This could have included chlorophytes or charophytes that were on extinct lineages, or those that were ancestral to the embryophytes.

We use the term *land plant* to refer to independent, eukaryotic autotrophs that live on land and incur at least one morphological phase transition (of their life cycle) under subaerial conditions. This definition is only slightly broader than that of Niklas (1997, p. 167), "… any photosynthetic eukaryote that can survive and sexually reproduce on land." Both definitions exclude algae that are desiccation tolerant or resistant, but require fully aquatic conditions to complete their life cycle. Lichens are also distinct from land plants. In today's world, the "land plants" are synonymous with the embryophytes, but, as noted by Niklas (1997), this need not have been the case in the past. Some ancient algae may have adapted to subaerial conditions without possessing the defining characters of the embryophytes — in essence, gametophytes with antheridia and archegonia and sporophytes developing from a polarized embryo. The observation that no living algae are considered in the land plant category would imply that if such organisms did exist in the past, they are now extinct.

4. Assemblage description

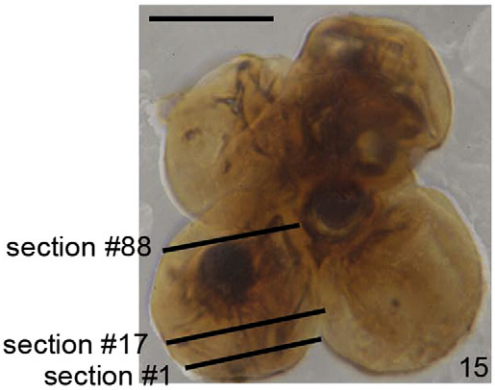
The Lone Rock palynological assemblage contains three major elements. It is dominated by a new cryptospore, *Agamachates casearius* gen. et sp. nov. The second component consists of clusters of thin-walled cells, with characteristic darkened bulbils, or knobs, as sculptural elements. This is a problematic form that remains in open nomenclature, but it has overlapping similarities to some thicker-walled clusters from the Middle Cambrian Bright Angel Shale in Arizona (Strother and Beck, 2000, Fig. 8; Baldwin et al., 2004, Figs. 12c, 14a, 14b, 15n). The third component of the palynological assemblage is a species of *Micrhystridium*, which is closest in form to *M. flexispinosum* Downie.

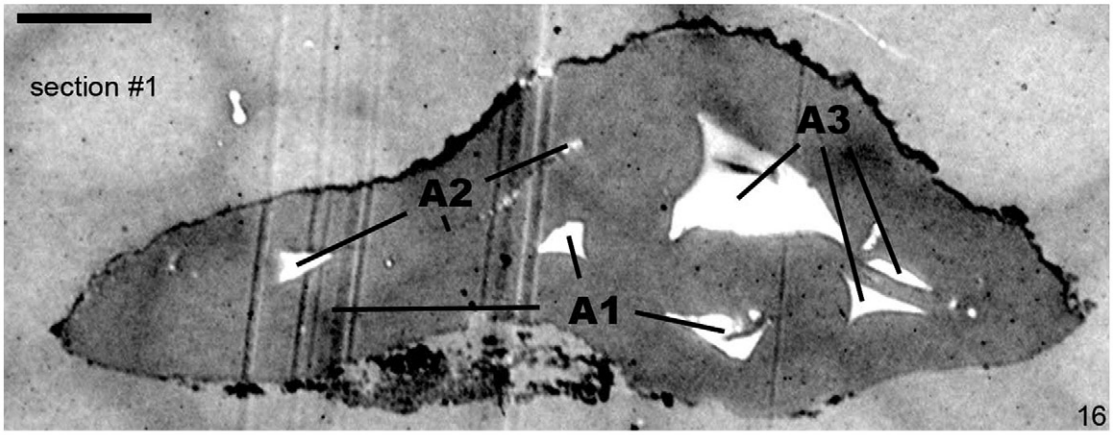
Agamachates appears to encompass a range of topological combinations of two (Plate I, 1a, b) to four cells (Plate I, 2–4) arranged into thick-walled, distinct, enclosed packets. These packets however, can remain adpressed to each other, resulting in larger and irregular aggregates (Plate I, 6a, b, and Plate VII, 27). The packets are recognized most easily with the SEM, because the boundaries between adjacent packets are most often discrete (Plate II, 7). In the l m, these boundaries are much more difficult to recognize, and aggregate clusters can appear to be quite irregular (Plate I, 6a, b). Many of the packets appear to be fundamentally quadripartitioned (Plate I, 2–5, Plate III, 9–13), but TEM analysis now reveals that paired cells are the end-products of sporogenesis. The apparent tetrads in Plate I, 2–5, are probably quartets of synoecosporal packets. (Plate III, 14).

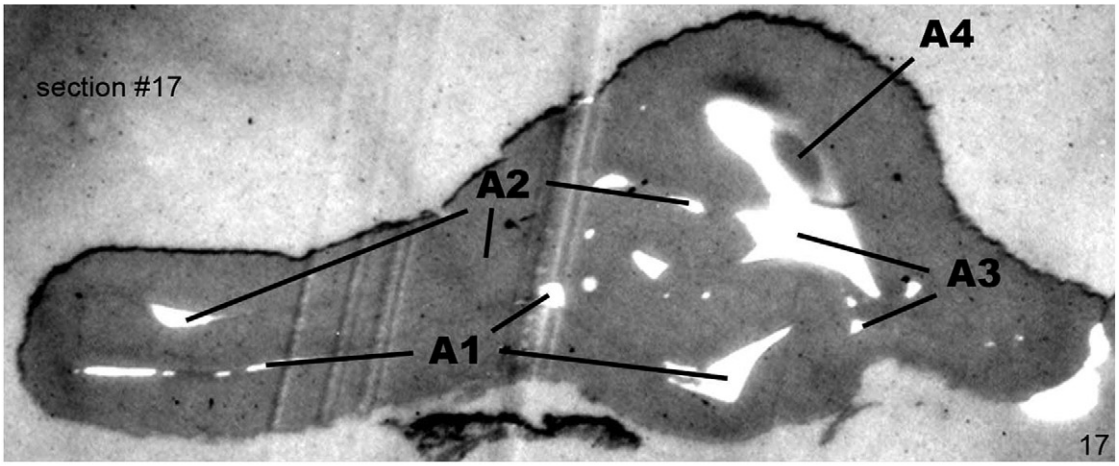
In a slide count of 239 specimens, *A. casearius* comprised 78% of the sample, 14% belonged to the problematic clusters, 5% were referable to *M. flexispinosum* Downie, and 3% were indeterminate acritarchs. Counting palynomorphs such as these, many of which occur in clusters of cells, is far from exact. The tabulation for *A. casearius* records the number of synoecosporal packets, but the packets themselves contain varying numbers of spores. Likewise, in tabulating the problematic sculpted form, cohesive clusters of cells were counted as individuals. The acritarchs, on the other hand, consist of discrete, individual vesicles — each of which is counted as one. This method of counting perhaps

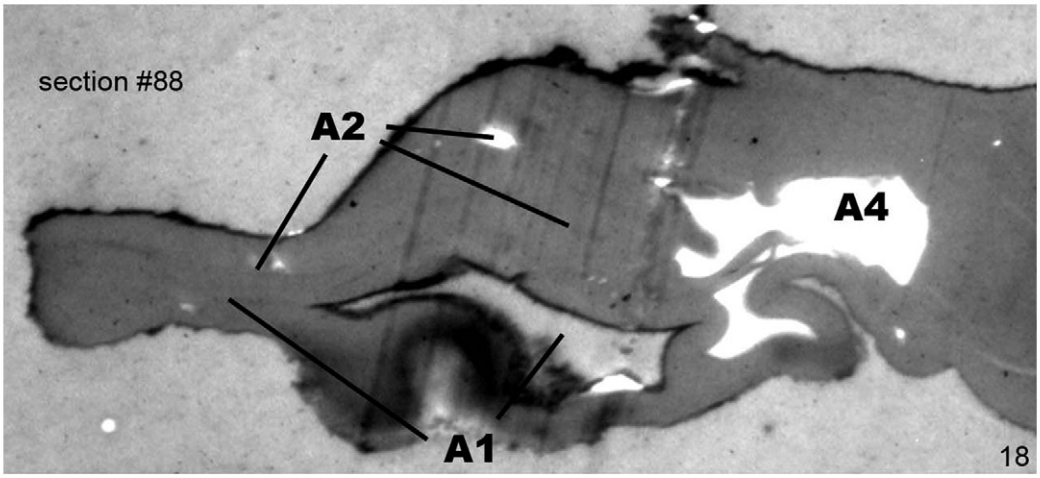
Plate IV. 15–18 Sections through packet A.

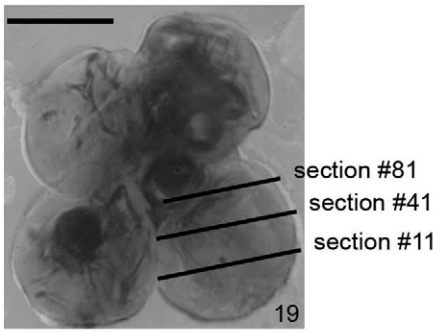
15. Index figure of sectioned specimen. Scale bar=10 μm.
16. Early cross section of packet A (section #1), showing three dyad lumens (A1, A2, and A3). Scale bar=2 μm.
17. Cross section towards the center of packet A (section #17) as compared to that in Fig. 16. The outer wall of dyad A4 is just appearing and will soon replace that of A3. Scale bar same as in Fig. 16.
18. Late cross section of packet A (section #88), showing persistent dyads A1 and A2, and now only A4. Scale bar as in Fig. 16.

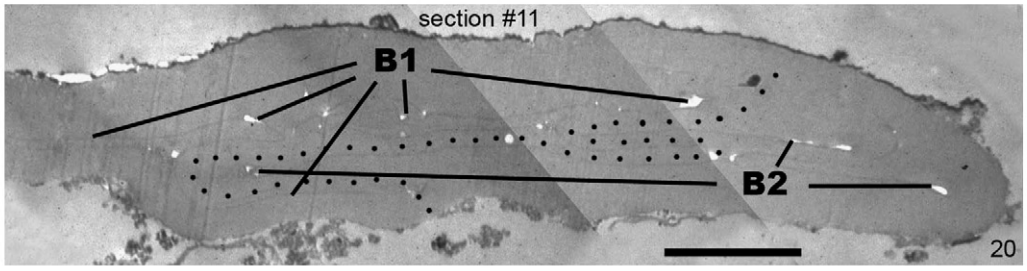


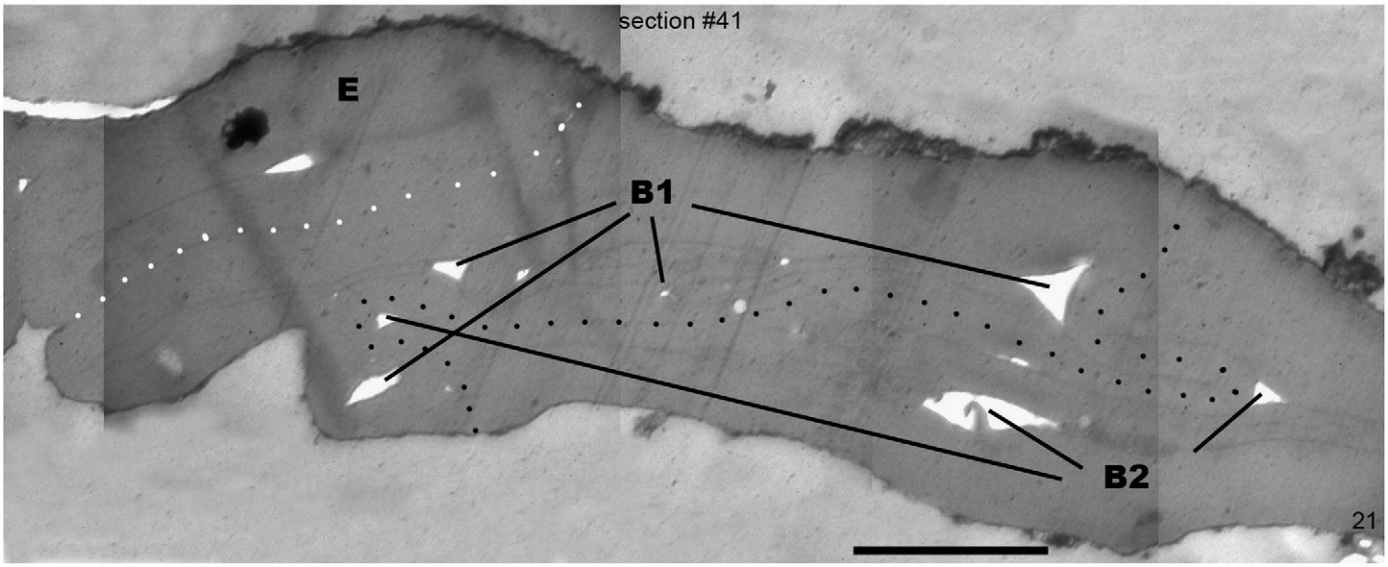












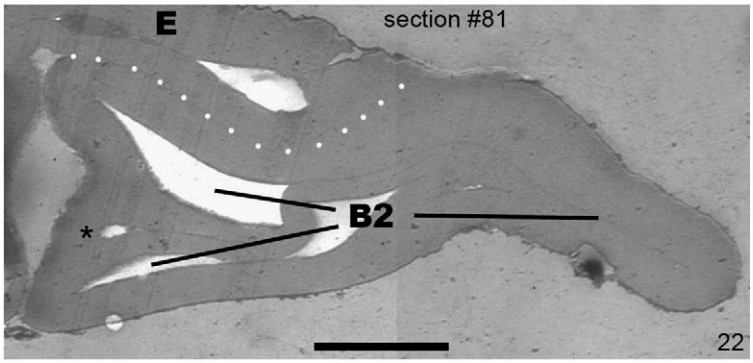
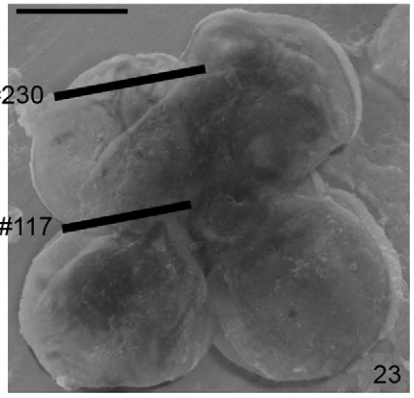
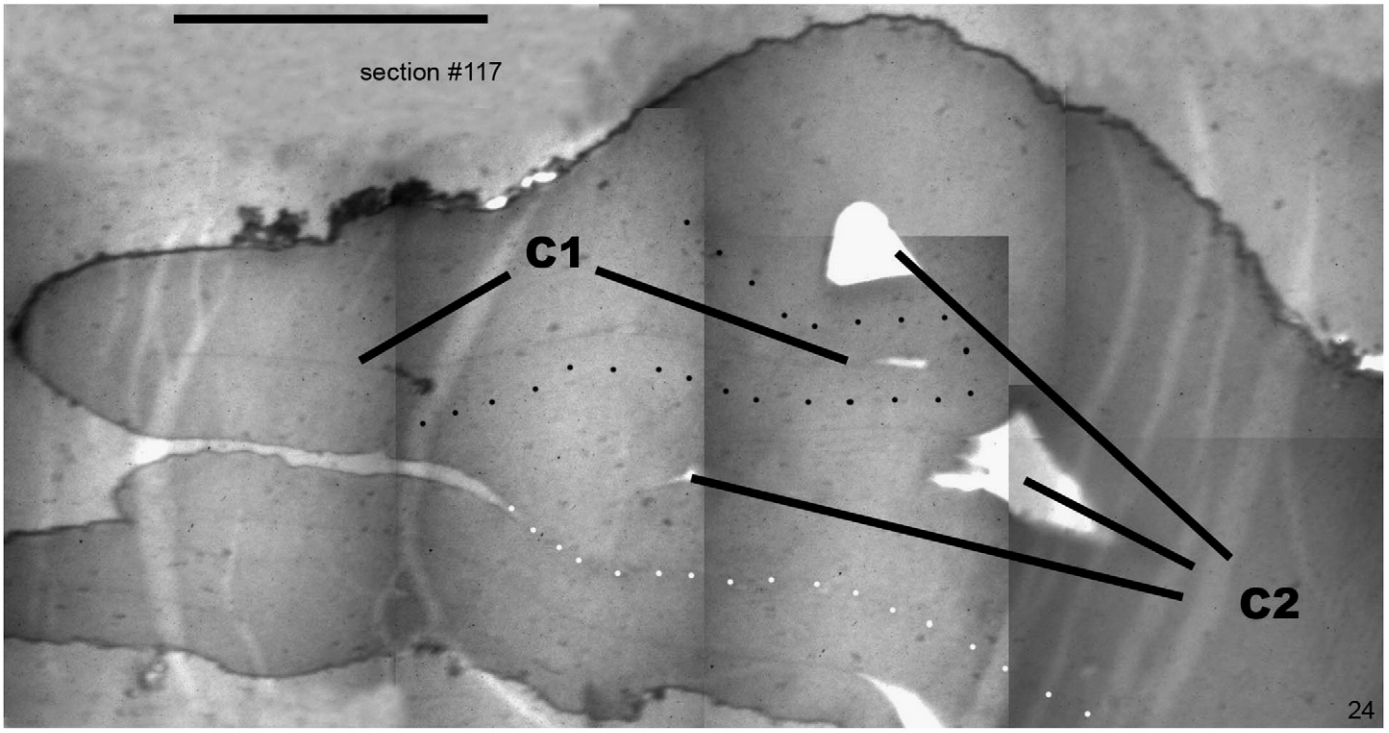


Plate V. 19–22 Sections through packet B.

19. Index figure of sectioned specimen. Scale bar = 10 μm.
20. Early cross section of packet B (section #11) showing two dyads. Black dotted lines indicate the boundary between the two dyads. Scale bar = 2 μm.
21. Cross section near the middle of packet B (section #41). Black dotted line indicates the boundary between the two dyads. Packet E can be seen to upper left. White dotted line shows boundary between packets B and E. Scale bar = 2 μm.
22. Late cross section of packet B (section #81). Only the lumen of B2 remains. The unfused contact face walls of the two dyads are apparent in this section, though their overall thickness is exaggerated due to the glancing orientation of this section. Asterisk marks position where contact face walls of individual dyad members diverge to fuse with synoecosporal wall. Scale bar = 2 μm.





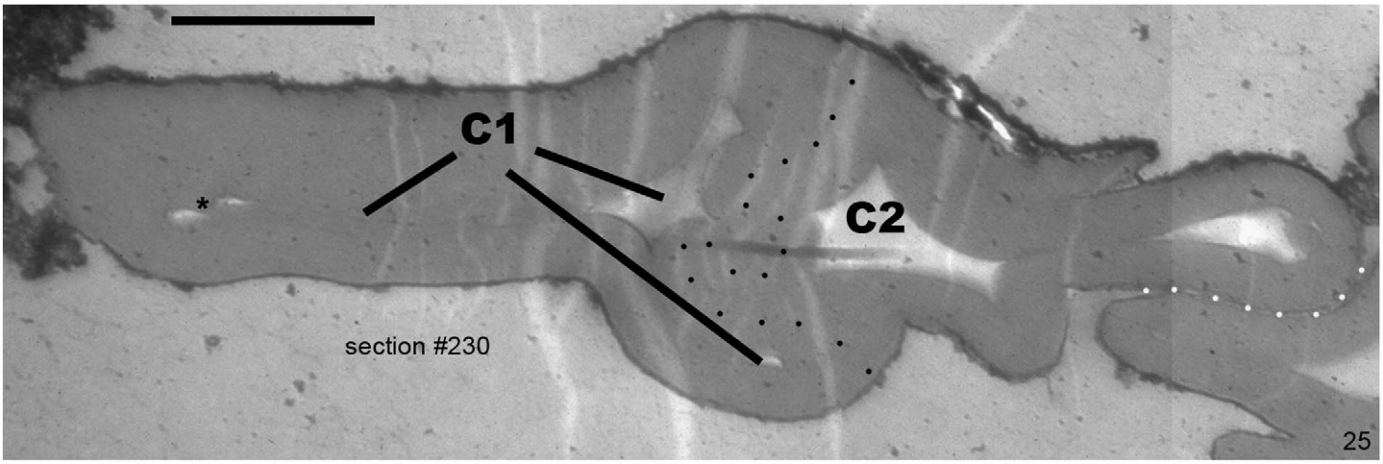


Plate VI. 23–25 Sections through packet C.

23. Index figure of sectioned specimen. Scale bar = 10 μm.
24. Early cross section of packet C (section #117). Two dyads are visible, on either side of the dotted black line. Part of packet A is still visible below the dotted white line. Scale bar = 2 μm.
25. Late cross section of packet C (section #230). The same two dyads are still visible. Scale bar = 2 μm.

exaggerates the relative abundance of acritarchs in the sample assemblage.

The Lone Rock assemblage is not a typical Upper Cambrian palynological assemblage of marine origin. Diversity is very low, with clear dominance by the new cryptospore, *Agamachates casearius*. The acritarch, *Micrhystridium flexispinosum* Downie, belongs to a genus of tiny spiny acritarchs, whose stratigraphic range spans almost the entire Paleozoic and whose ecological provenance is unknown. In short, the Lone Rock assemblage is entirely consistent with an estuarine to near-shore depositional setting. It is highly unlikely to represent an open shelfal to deep-water setting, which would typically present a greater variety of acritarch forms. This proximal assessment matches ongoing regional paleoecological interpretations of Upper Cambrian beach deposits marked by stranded medusae (Hagadorn et al., 2002).

5. TEM analysis of the sectioned specimen

Digital images were taken of every section. In order to trace the exact position of the sections in the specimen, the LM (Plate III, 11) and SEM image (Plate III, 12) were adjusted to the same magnification, using Photoshop, and overlain, with the opacity of the overlying LM image set to be partially transparent. This allowed the opaque crystal inclusion, visible in the LM image but not the SEM image, to serve as points of reference in the blended image (Plate III, 13).

Individual packets are designated with letters: A–D for — front left, front right, back left, back right, respectively (Plate III, 11). A fifth packet was uncovered in the analysis and is designated E.

All packets are attached to a fragment of extra-sporal material (Plate III, 12, arrows) that stains similarly to the walls. This fragment is visible in both SEM and TEM, but in the latter is often difficult to distinguish from the underlying wall. The various measurements of the packets and spores, including where they appear and disappear in the serial sections, are indicated in Table 1.

This study was undertaken specifically because the number of and structural arrangement between the spores in these packets was clearly a central issue when attempting to determine their biological significance. Lacking any information on just how many spores might be present, serial sections afforded the unusual opportunity to track changes in the lumens and the walls that separate them. We assumed at the start that the planar tetrad was just that; four spores. That hypothesis was refuted upon discovery that there were many more lumens within. The second hypothesis was that each unit was a tetrad. When two of the original "spores" were found to contain only two spaces, seemingly bound by a single lamina, through all sections, it became clear that that hypothesis too, was wanting. The discovery that the single lamina that appeared in all the smallest lumens, when sectioned fortuitously, was actually two separate laminae that diverge to fuse with opposite walls led to the conclusion that what was actually being viewed was the unfused central wall of two members of a dyad. In the absence of differential staining of any of these layers, it was necessary to approach the structural analysis in this way as a series of hypotheses.

5.1. Packet A: a quartet of dyads

The maximum diameter of packet A in the plane of sectioning (that is, as measured in a TEM cross section) is 14 μm (Table 1). The maximum diameter perpendicular to the plane of sectioning (traversed by serial sections) is also 14 μm.

Plate IV, 16 shows an early section (#1) of packet A (front left). The exact position of this cross section is shown by the bottom line in Plate IV, 15. At this point, there are three dyad cross sections visible, indicated with numbers in Plate IV, 16. Dyad A1 contains opaque mineral matter that is apparent in Plate III, 11, 13 and Plate IV, 15. This dyad is present until section #100. Dyad A2, located on top of dyad A1, is present in all 131 sections that contain this packet. Dyad A3, which is present in the earliest collected sections, is alone on the right until section #16, where the wall of a fourth dyad, A4, is first encountered (Plate IV, 17). Dyad A3 persists until section #76. Dyad A4 continues through packet A to section #131. Plate IV, 18, which is of section #88, contains three dyad cross sections; A1, A2, and A4.

5.2. Packet B: a pair of dyads

The maximum diameter of packet B is 14 μm.

This packet is present in the first collected sections. At that point (Plate V, 19, section #11), two dyads are evident (B1 and B2, Plate V, 20). By section #31, there is evidence of a third lumen, but later sections will reveal that this is a fifth spore (E) mostly hidden under the extra-sporal material (Plate III, 12, 13). Packet B continues through section #110, but by section #77, only dyad B2 remains (Plate IV, 22). The innermost laminae that line the lumens of the two spores of the dyad are often highly convoluted or fractured, hence difficult to trace. These laminae are often fused to the outer dyad wall that surrounds both spores, but are usually separate in the center of the dyad. Thus, most often two separate thin inner laminae can be seen inside the outer dyad wall. This is well illustrated in Plate IV, 22, which is a glancing section through the wall of dyad B2. At the very left of this figure (asterisk) the paired central laminae diverge to fuse with their common wall.

5.3. Packet C: a pair of dyads

Packet C, at 15 μm in diameter, begins at section #89 and persists through the final section collected (#230). In section #117 (Plate VI, 24) two dyads are present, and the same are still present in section #230 (Plate VI, 25). Though not as apparent as that illustrated in packet B, the point of divergence of the paired laminae is also visible in the very left of Plate VI, 25 (asterisk).

5.4. Packet D: a quartet of dyads

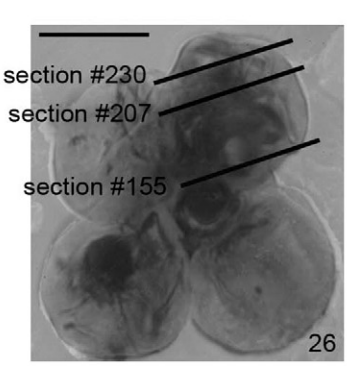
Serial sections of packet D show a single dyad from section #112–154 (Table 1). Thereafter, three additional dyads appear within 10 sections. This pattern is consistent with 4 spore units in a tetrahedral arrangement. A crystal is present in one of the lumens through section #160 (a crystal appears in the other lumen in section #169). In the middle of the first dyad, at section #155, the two lumens can be clearly seen (Plate VII, 27). For a time, all four spores are visible in the cross section (Plate VII, 28), with the inner contact face walls of only one (D3) apparent. In the latest sections that were collected, D1 has disappeared, D2–4 are visible, but the contact face walls of only D2 and D4 are in evidence (Plate VII, 29).

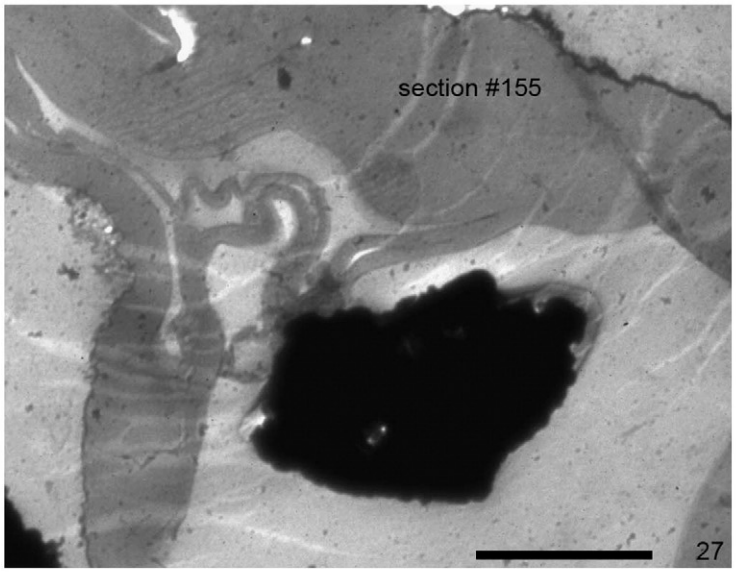
5.5. Packet E: apparently a pair of dyads

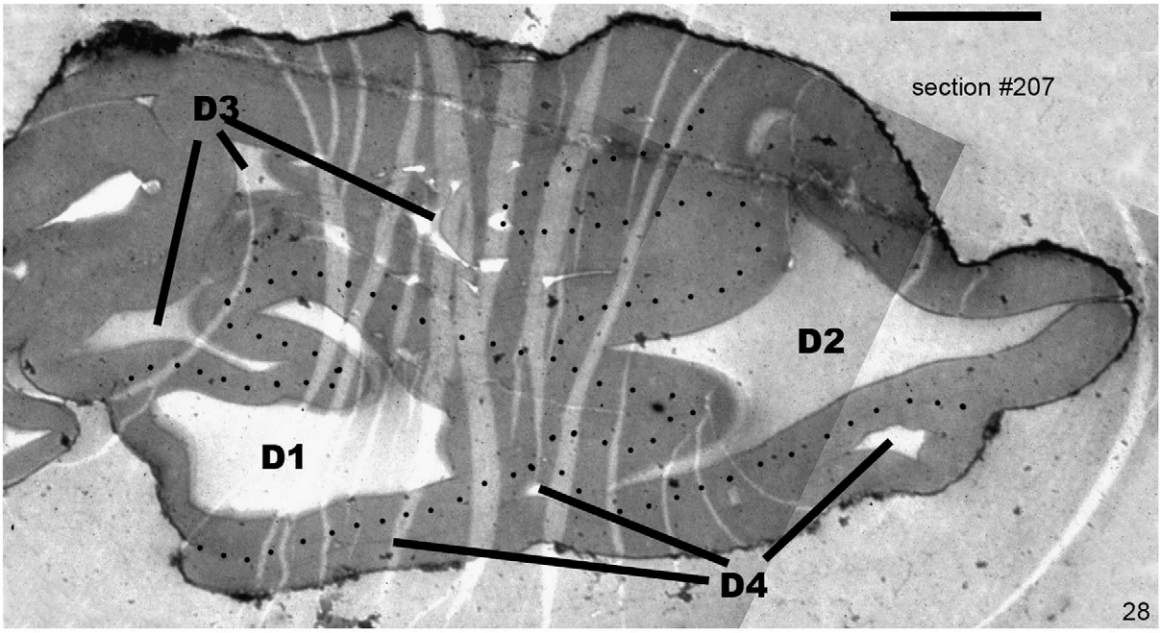
This packet, which is not immediately evident from either LM or SEM, was detected through serial sectioning. It first appears above

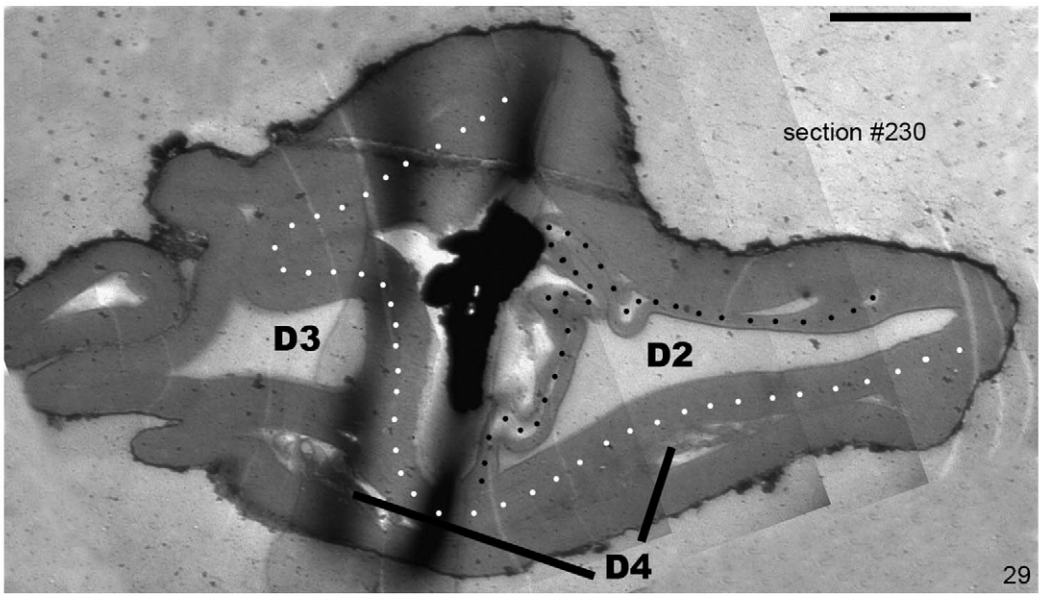
Plate VII. 26–29 Sections through packet D.

26. Index figure of sectioned specimen. Scale bar = 10 μm.
27. Partial early cross section of packet D (section #155). Though convoluted, the thin, unfused contact face walls of dyad D1 can be seen. Scale bar = 2 μm.
28. Late cross section of packet D (section #207). Four dyads are visible, separated by dotted black lines. Scale bar = 2 μm.
29. Slightly later cross section of packet D (section #230). In this image, the dotted white line marks the boundary of dyad D2. The dotted black line marks the contact face between the two lumens of dyad D2. Scale bar = 2 μm.









packet B, between sections #21 and #31. By section #51 (Plate VIII, 31), two lumens of a dyad can be seen. At section #81 (Plate VIII, 32), it is apparent that the growth of mineral inclusions "inflated" the second lumen encountered. Unfortunately, this inflated area was poorly infiltrated, rendering interpretation difficult. E appears below D (Plate VI, 24), and persists until about section #150. Based on the overall distance traversed by the sections, it seems likely that there is a second dyad, though the poor infiltration makes this hard to confirm.

6. Discussion

It is apparent from the TEM analysis that each of the spore end-packets, that is, the smallest recovered cellular unit, is always a member of a dyad (Fig. 1). These end-member dyads are packaged either in pairs or in quartets. The packets are defined by their common, synoecosporal wall, which, in this instance, effectively corresponds to a spore mother cell (smc) wall. Paired sets of cells show an apparent genetic relation by immediate prior division from a common generative cell, and, in the absence of other topological information, this relation points toward mitotic, rather than meiotic division. However, the notion that these cells are simply random vegetative cells that were fortuitously preserved seems less likely than reduction division resulting in resistant-walled cells acting as diaspores, i.e. spores.

It might be argued that preservation of terminal dyads should be viewed simply as evidence of normal vegetative cell division, which has nothing to do with reduction division. In studies of younger cryptospores the occurrence of tetrads has long been used as proof of reduction division, paralleling that seen in all extant plants that produce four haploid spores per diploid sporocyte through meiosis. Our argument against vegetative (mitotic) division is that the spore-like end-products described here are serially encased within enclosing resistant walls. In order to form these synoecosporal walls, cell division must occur within a generative cell with a pre-existing, resistant wall. (The resistant-wall cannot be formed after its contents have divided, because this would require that the two recently divided cells jointly contribute to the production of an extra-cellular product that was applied centrifugally to the inside wall of the pre-existing generative cell.) This method of cell division is very different from vegetative division in the algae, which produces discrete cells, each of which forms its own cell wall (Fritsch, 1948; Fig. 2).

Extant chlorococcalean algae that form cell aggregations, coenobia, or colonies, typically do so through the production of extra-cellular mucilage or cellulose, which acts to bind vegetative cells together. The production of aggregated forms is usually cited as an adaptation to a planktonic mode of life in freshwater habitats (Smith, 1938; Fritsch, 1948). The resultant aggregated topologies are very different from cells that form through the endogenous division within a generative or smc wall to produce aplanospores, zoospores, or meiospores. However, there are a few green algae, for example *Nephrocytium* (Chlorococcales/Oöcystaceae), that divide and yet retain the smc wall. In *Nephrocytium ecdysiscepanum* West, the smc wall continues to produce mucilage after the spores have formed, which leads to irregular clumps of related cells (Fritsch, 1948, Fig. 45A). These aggregates bear a superficial resemblance to the aggregated packets of *Agamachates casearius*, although the cell morphology and topology in *Nephrocytium* does not match the fossil. None of the algae that divide endogenously to produce resistant spores within a retained smc wall is known to produce algaenan or a similar sporopollenin-like substance in association with this combination of morphology and developmental topology.

The combination of dyads as the end products of sporogenesis with the differing number of spores produced per smc, seems to indicate that meiosis in this fossil was messy — somehow, not precisely coordinated to produce four meiospores per smc. This condition, the production of more than four haploid meiospores from a single (initially diploid) generative cell, occurs today, interestingly enough, during meiospore generation in *Coleochaete* (Graham, 1993). Nuclear DNA in the *Coleochaete* zygote replicates endogenously multiple times, with meiosis resulting in 4 to 32 meiospores per zygote. In this example, the duplication of DNA is separated in time from later cytokinesis and cell wall formation. *Coleochaete* does not produce resistant-walled spores in packets, so it is unlikely that *Agamachates* represent the remains of this alga. However, endogenous duplication of the genome, prior to the onset of cytokinesis during sporogenesis, would explain the irregular number of spore dyad pairs seen in adjacent packets of the fossil form. So, even though the meiospores of *Coleochaete* do not provide an exact homologue, the *process* of meiospore production (meiosporogenesis) in *Coleochaete* is consistent with preserved spore topology in *Agamachates*.

Dispersed dyads are a major component of assemblages of cryptospores in the Siluro-Devonian record. The asynchronous development of cytokinesis and cell wall formation during sporogenesis has been invoked as a mechanism to explain the production of cryptospore dyads (Strother, 1991; Strother and Beck, 2000; Wellman et al., 1998a,b) and is discussed perhaps most thoroughly by Fanning et al. (1991) and Hemsley (1994a). Cryptospore dyads are well documented in Upper Silurian and Lower Devonian (Fanning et al., 1991; Wellman et al., 1998a,b) rhyniophytoid sporangia, where they are found *in situ*. Dyads appear to be restricted to this early phase in plant evolutionary history. The geological persistence of the dyad form back to these problematic Cambrian palynomorphs (Strother et al., 2004; Taylor and Strother, 2008) could be an important clue in reconstructing the algal-plant transition in evolution.

The common occurrence of packets adpressed in pairs (Plate I, 1, Plate III, 14) and in quartets (Plate I, 3–5) that share a common size and wall structure, strongly suggests that these aggregates each have a common source. In the case of the sectioned specimen, the degree of fusion of the extra-sporal fragment to the packet walls further suggests a developmental association; i.e., the fragment and walls were in contact when one or both were forming. So why are these synoecosporal packets aggregated together? One possibility is that packets represent endosporic development that occurred immediately after reduction division (meiosis). Since aggregated packets do not retain a common resistant-wall, this implies that the (2n) smc did not form a resistant-wall, and that all resistant-wall production occurred within a gametophytic (1n) phase. In this case, then, a smc divides meiotically to produce tetrads and paired dyads of meiospores. Then each meiospore produces a resistant wall (and becomes a synoecosporal packet) and proceeds to divide internally into different sets of dyads, each with its own resistant wall. If this is the case, and Hemsley's (1994a,b) hypothesis of sporopollenin "transfer" from the diploid to the haploid phase is correct, then *Agamachates* represents spore formation in organisms living after such an evolutionary transition.

Could the wall fragment seen in the sectioned specimen be part of a tapetum or sporangial wall? Perhaps, but it is equally likely that this tissue was torn from the wall of an adjacent associated packet, so its presence does not promote an association with any particular macroscopic tissue or structure. Furthermore, the dispersed forms of *Agamachates casearius* examined in LM are not found with coverings or cuticles that would indicate a prior intimate association with a sporangium.

In either case, having spore dyads as the end-products of sporogenesis is direct evidence of the postulated heterochronic process of sequential (mitotic) divisions during meiosis giving rise to dyads of spores by serializing the timing of cell wall formation. At this point in evolutionary time, perhaps the process of (meio)sporogenesis was not yet canalized, resulting in variable numbers of spores produced from each sporocyte. In all cases, however, the final product – the last mitotic division – produced dyads. During the Ordovician, the increasing dominance of tetrads of spores, and, ultimately, in tetrahedral tetrads of spores, seems to indicate the progressive canalization of meiosporogenesis as a progressive feature of land plant evolution. *Agamachates casearius* represents diaspores adapted to subaerial dispersal, but it is

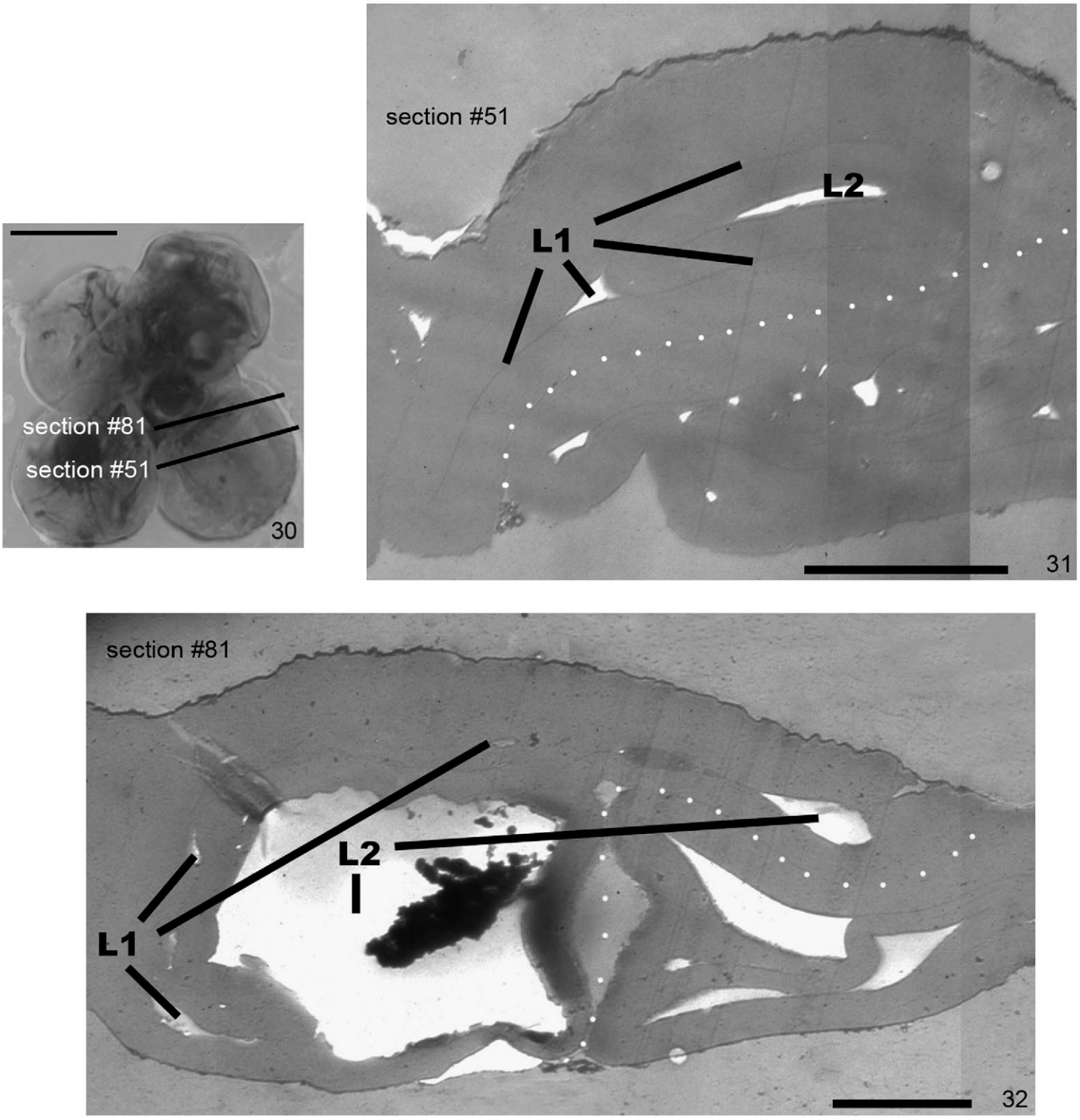


Plate VIII. 30–32 Sections through packet E.

30. Index figure of sectioned specimen. Scale bar = 10 μm.
31. Early section of packet E (section #51), up and to the left of the white dotted line. This section is soon after the appearance of the second lumen of the first dyad (L2). Scale bar = 2 μm.
32. Later section of packet E (section #81), up and to the left of the white dotted line. The increase in size of lumen 2 (L2), mostly as a result of crystal growth in the space of the lumen, has compressed the space of lumen 1. Scale bar = 2 μm.

not possible to assert whether its parent plant evolved before or after the origin of the embryophyte condition.

7. Conclusions

Agamachates casearius retains a topology that parallels the predictions of Hemsley (1994a,b) in the conceptual evolutionary transfer of sporopollenin deposition from the sporophyte to the gametophyte phase. However, the analogy is even more powerful here, because the topology of 2 to 8 enclosed spores more closely matches meiosporogenesis in *Coleochaete*, which is dependent upon endogenous DNA duplication, than it does extant sporogenesis in extant embryophytes. There is an extremely important difference with respect to any *Coleochaete* analogy, however, in that the smallest units

in the fossil form are completely encased in resistant polymeric substances (algaenan or sporopollenin-like substances) which is not the case in any extant *Coleochaete* species. Given the position of *Coleochaete* as a model for the ancestral alga from which the embryophytes evolved (Graham, 1993 and references therein), it is important to note that the fossils described here fit a model that posits an intermediate condition between the algae and the embryophytes (and in this respect it is notable that zygotes of *Coleochaete* have been reported with a resistant wall around them; Graham, 1990). And, in our view, a subaerial, *Coleochaete*-like alga that possessed thick-walled meiospores would have functioned as a "land plant" if such spores were used as diaspores in subaerial habitats. However, no such organism exists today.

The preservation of spore dyads reflects the asynchronous timing of cell wall formation during meiosporogenesis, which effectively stretches out reduction division into a series of discrete, sequential cell divisions. This has the effect of making the terminal spore-generating cell division appear mitotic (forming a division pair) rather than forming a tetrad of meiospores. The terminal formation of dyads in a spore packet seems to demonstrate that the dyad form is a primitive character state in the evolutionary transition processes. Only later did plants evolve a more compact and synchronized form of meiosporogenesis in which only two mitotic divisions became coördinated into the single process of meiosis. Thus, this example appears to link charophyte ancestors to future land plant derivatives.

The tedium of TEM analysis has been invaluable in discerning topological arrangements of spore bodies that was not detected in either the LM or SEM. This adds to the worthiness of TEM in providing far more than wall ultrastructure to the debate about the algal–land plant transition. This report expands the range of the Middle Cambrian cryptospores reported on earlier (Strother et al., 2004; Taylor and Strother, 2008) and is beginning to close the temporal gap between the oldest accepted Ordovician cryptospores and the more problematic Cambrian forms.

Systematic Paleontology.

Incertae Sedis.

turma Cryptosporites Richardson, Ford & Parker 1984.

The turma Cryptosporites accommodates spore-like, spherical to sub-spherical to discoidal palynomorphs that occur in populations that typically include attached dyads and tetrads. We use the term in the sense of Strother and Beck (2000), and do not follow the recommendation of Steemans (2000) who restricted the term cryptospore to be a subset of miospores, which are necessarily of embryophytic derivation.

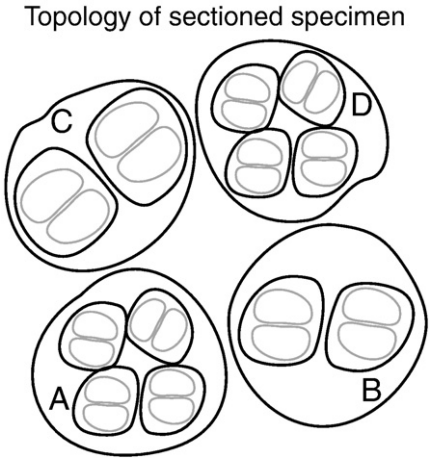


Fig. 1. Diagrammatic representation of the spore topology in the sectioned specimen (packets A–D). The spore end-products are represented by gray walls, resistant walls, presumably also impregnated with sporopollenin or a similar compound, are in black.

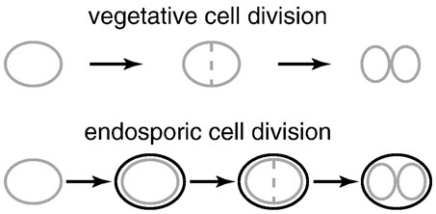


Fig. 2. Comparison of vegetative cell wall formation and endosporic cell division which occurs within an existing resistant wall (black). Cellulosic plant walls represented in gray, resistant (spore-forming) `walls represented in black.

genus *Agamachates* new genus

type species *Agamachates casearius* sp nov.

Diagnosis: Subspherical to discoidal simple, thick-walled spores enclosed within a packet characterized by a homogeneous, common (synoecosporal) wall; each packet contains 1 to 8 cells, most typically 1 or 2 paired cells (dyads); packets may be solitary or occur in closely adpressed pairs, quartets, or larger aggregated clusters.

Etymology: From the Latin *agamus* (alone, solitary) and *achates* (stone, rock).

Agamachates casearius new species.

Diagnosis: As per genus.

Description: Packet with smooth synoecosporal wall; packet quartets most typically planar or nearly so, with thickened contact margins and walls often collapsed toward cell interiors. Packets are around 14 μm in diameter; individual interior cells typically about 9 μm in diameter (n=45).

Holotype: The specimen pair in Plate III, 14 is designated as the holotype. Holotype slide is housed in the Micropalaeontological Collections at the Natural History Museum, London, accession number, FM2097.

Stratigraphic Distribution: The Lone Rock Formation, lower Sunwaptian Stage (*Conapsis* to *Ptychaspsis-Prosaukia* biozones).

Etymology: From the Latin *casearius*, pertaining to cheese.

Discussion: The individual spores within each packet appear to be organized into either 2 or 4 sets of dyads as deduced from the TEM analysis presented here; however, within the population observed using the LM, there appear to be packets that contain only one cell (e.g. Plate I, 2). In general, it is quite difficult to discern dyad pairs embedded in the packets, and the packets themselves seem to serve as the spore-body end members. Thus, the specimen in Plate I, 2, which has the appearance of a planar tetrad of four discrete bodies, is interpreted as four packets, each containing only one spore. The internal content of the packets consists of a varying number of spore end members whose walls appear to be of similar composition and structure to the synoecosporal walls, which makes it difficult in the LM to distinguish the actual spores. But it is clear in the holotype that each packet contains multiple spore end-members (Plate III, 14).

The specimens from the Lone Rock assemblage are somewhat yellowish to reddish brown in color, corresponding to a very low TAI of around 2.

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