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PEAT MOSS–LIKE VEGETATIVE REMAINS FROM ORDOVICIAN CARBONATES

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Premise of research. Climatically favorable conditions correspond with fossil evidence for dramatic Ordovician marine biodiversification, but coeval terrestrial biodiversity is less well understood. Although diverse Middle and Late Ordovician microfossils are interpreted as reproductive remains of early bryophyte-like land plants (consistent with molecular data indicating pre-Ordovician embryophyte origin), the vegetative structure of Ordovician plants remains mysterious, as do relationships to modern groups. Because distinctive fungal microfossils indicating land plant presence were previously reported from Ordovician carbonate deposits in Wisconsin, we examined another nearby outcrop for additional evidence of terrestrial biodiversification.

Methodology. Replicate collections were made from well-understood 455–454 Ma Platteville Formation carbonates of relatively low porosity and hydraulic conductivity. We employed measures to avoid contamination, and organic remains extracted by acid maceration were characterized by light and scanning electron microscopy and energy-dispersive X-ray spectroscopy.

Pivotal results. Multicellular organic fragments displayed distinctive cellular features shared with modern vegetative peat mosses but differed from modern materials, e.g., fossil presence of mineral coatings, absence of epibionts. Biometric features of mosslike microfossils isolated from carbonates collected and macerated 12 yr apart by separate investigators did not differ. Putative peat moss remains occurred with foraminifera similar in frequency and thermal maturity to types previously described from the same formation. No diatoms, pollen, or other indicators of post-Ordovician environments were observed.

Conclusions. The peat moss–like fragments described here are the oldest-known vegetative remains of land plants and the oldest fossils having distinctive features linking them to a modern plant group. These findings are consistent with peat moss recalcitrance properties that foster fossilization and molecular evidence that the peat moss lineage is 460–607 Ma of age. The new findings suggest that moss-dominated peatlands—recognized for globally significant roles in modern terrestrial biodiversity and C and N cycling—were present hundreds of millions of years earlier than previously thought.

Keywords: carbonate, Ordovician, peat moss, Sphagnopsida, *Sphagnum*, vegetative microfossil.

The Middle Ordovician through pre-Hirnantian (Katian) Late Ordovician, 470–445 Ma, was a time of mild climate corresponding to fossil evidence for dramatic biodiversification in marine habitats; $\delta^{18}\text{O}$ apatite analyses including both Laurentian and Gondwanan samples indicate that sea surface temperatures of the Middle Ordovician through the Katian Age resembled those of modern equatorial regions (Trotter et al. 2008). Although the pre-Hirnantian Ordovician is associated with high sea levels, periods of marine regression and terrane exposure occurred (Ross and Ross 1995; Nielsen 2004). Ordovician oceanic islands are postulated to have been sites of evolution and dispersal, paths of migration, or centers for evolution of new taxa (Cocks and Torsvik 2004).

These observations suggest that land biota might likewise have diversified during much of the Ordovician, fostered by warm temperatures, high humidity, and atmospheric CO_2 levels estimated to have been 8–18 times present levels (Barnes 2004). Evidence for a widespread flora includes diverse Middle-Late Ordovician microfossils from multiple locations that have been interpreted as the reproductive remains—spores and putative sporangial epidermis—of early bryophyte-like land plants (Strother et al. 1996; Wellman et al. 2003, 2015; Steemans et al. 2009; Rubinstein et al. 2010). An Ordovician terrestrial flora predating the presence of vascular plants is also indicated by carbon isotopic studies (Tomescu et al. 2009). A diversifying Ordovician land flora is consistent with molecular diversification studies (i.e., molecular clock or timescale analyses) indicating pre-Ordovician origin of stem embryophytes (e.g., Clarke et al. 2011) and an age of 460–607 Ma (by at least the Early Ordovician) for ancestors of the early-diverging extant moss genera *Sphagnum* and *Takakia* (Laenen et al. 2014).

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Even so, the vegetative structure of Ordovician plants represented by reproductive microfossils remains mysterious, as do relationships to modern groups. Understanding more about the vegetative structure and relationships of Ordovician land plants would aid comprehension of their biogeochemical impacts, such as potential to sequester carbon, interact with early terrestrial herbivores, and form associations with microorganisms that play important roles in Earth's N, P, and C cycles. Recent metagenomic analyses suggest that earliest land plants might have inherited from ancestral streptophyte green algae tendencies to engage in symbiotic relationships with N-fixing cyanobacteria and rhizobiales, early-diverging fungi, and other biogeochemically significant biota of ancient lineage (Knack et al. 2015).

Previous absence of reports of recognizable vegetative remains of plants from the Ordovician and earlier fossil record is commonly attributed to lack of microbial resistance sufficient to leave fossil remains. Modern bryophytes, commonly used as models of ancient land plants, are widely assumed to lack substantial levels of degradation-resistant materials such as lignin. On the other hand, comparisons of hydrolysis resistance among vegetative (and reproductive) tissues of modern bryophytes revealed that mosses—including the early-diverging genera *Sphagnum*, *Polytrichum*, and *Andreaea*—display diagnostic vegetative structure having high recalcitrance, attributed to degradation-resistant phenolic cell wall polymers inherited from algal ancestors (Kroken et al. 1996; Graham et al. 2004). This concept is consistent with reports that late-diverging streptophyte algae such as *Coleochaete* produce small amounts of lignin or lignin-like polymers (Delwiche et al. 1989; Sørensen et al. 2011). Hydrolysis studies indicate that distinctive bryophyte vegetative structure can be as resistant to hydrofluoric acid extractive treatments as are sporopollenin-walled plant spores and thus in theory as likely to persist in the ancient geological record (Kroken et al. 1996). Such observations—together with molecular evidence for Ordovician (or earlier) age of moss lineages that display distinctive vegetative structure (Laenen et al. 2014)—justify examining Ordovician deposits for vegetative remains of early mosslike land plants.

Distinctive fungal microfossils interpreted as glomalean remains had previously been reported from 460–455 Ma Ordovician carbonates (Guttenberg Formation, Wisconsin; Redecker et al. 2000). Such remains are consistent with fungal diversification time analyses indicating that the glomalean lineage originated prior to 500 Ma (Parfrey et al. 2011; Chang et al. 2015). Because modern glomalean fungi are commonly associated with plant partners, including bryophytes (Redecker and Schüssler 2014), and glomalean partnerships have so far not been documented for streptophyte algae (Knack et al. 2015), the Ordovician glomalean remains suggest that early land plants might have co-occurred at this time. Consequently, we examined a nearby carbonate outcrop of similar age for additional evidence of terrestrial biodiversification.

Material and Methods

Replicate collections were made from 455–454 Ma Platteville Formation dolomitized carbonates, Sinnipee Group, Dane County, Wisconsin. Stratigraphy and sedimentology of the

Sinnipee Group carbonates are well understood (Choi 1998). Platteville carbonates are considered to have been deposited in a transgressing sea in a quiet subtidal marine environment of normal salinity on a shallow shelf in deeper water than shoreward facies of the underlying St. Peter Sandstone; the unit displays no exposure indicators or stromatolites; faunal elements include sessile brachiopods, echinoderms, cephalopods, and gastropods (Mossler 1985). Protistan elements of the Platteville Formation of Illinois are reported to include a variety of chambered and globular agglutinated foraminifera having tests with bound siliceous particles (Gutschick 1986).

The time and process of dolomitization have been controversial, with the most recent study arguing the case for a hydrothermal event centered in the Michigan Basin during the late Devonian–Mississippian (Luczaj 2006). On the basis of Platteville carbonates sampled along an 80-mi transect that included the area relevant to this study, Deininger (1964) defined a discrete boundary between fine-grained limestones lying to the west and nearby deposits in which <10% to 100% of the carbonate was patchily dolomitized to the east. According to figure 1 of Deininger (1964), the location sampled in this study lies near that boundary and thus near the boundary of the dolomitization process. Wisconsin Ordovician carbonates display relatively low matrix porosity (1.8%–7.7%) as well as low horizontal hydraulic conductivity (Batten et al. 1999). Total organic carbon percentages in 11 Middle Ordovician Platteville Formation carbonates sampled from Iowa ranged from 0.38% to 1.99% (all samples <2%; Pancost et al. 1998).

Although carbonates (and particularly dolomitic carbonates, in view of potential recrystallization effects on organic microfossils) may not yield concentrated plant remains, important organic microfossils have been reported from carbonates, including dolomites (e.g., Campbell 1980; Blome and Albert 1985; Westphal and Munnecke 1997; Redecker et al. 2000). Yin et al. (2015), for example, reported finding a single fossil interpreted as an early sponge in 600 Ma dolostones, a finding consistent with molecular inference of sponge evolutionary history. This and other examples indicate that taxonomically distinct microfossils can be obtained from carbonates, including dolostones, even though organic remains may be comparatively rare in such matrices.

Sampling Location and Process

Five samples of ~1 kg each were taken in 2000 and 36 of similar size were taken in 2012 from an outcrop on the south side of County Road J near its junction with Country Highway P, northeast of the town of Mt. Horeb, Dane County, Wisconsin (+43°2'44.46", -89°39'25.89"; site 10[PB] of Choi 1998). Collection required a vertical rock climb of ~2 m to reach Platteville carbonates lying above St. Peter sandstones (Byers and Stasko 1978). The surface was forested. Immediately after collection, samples were enclosed within new gallon-size zip-lock plastic bags. No modern peat mosses were observed in the area, which was largely under cultivation.

The outcrop sampled in this study lies ~50 km from the present Baraboo Range, a National Natural Landmark whose weathering-resistant Proterozoic quartzites are an estimated 1.6 Ga of age and thought to have been exposed throughout

the Ordovician (Dott and Attig 2004). During the Ordovician, Laurentia remained in a transequatorial location, with little rotation. Although much of Laurentia was covered by shallow epicontinental seas and surrounded by the Iapetus Ocean and the Panthalassic Ocean during much of the Ordovician (Cocks and Torsvik 2011; Jin et al. 2013), the Baraboo Range represents a potential source of Ordovician terrestrial materials that might have been transported to nearshore sediments by streams and/or wind.

Microfossil Extraction

Six carbonate samples (~1 kg each), none of which had cracks or showed evidence for bioturbation, were macerated in HCl according to protocols described in Redecker et al. (2000). Bulk acid maceration is widely used to extract remains from carbonate and other mineral matrices (e.g., Gutschick 1986; Hübers and Kerp 2012; Yin et al. 2015). Macerations were performed within a modern chemical hood whose working surface was covered with new plastic-backed absorbent paper. Carbonate samples were first rinsed with distilled water and then submerged in concentrated HCl in new plastic containers to remove the outermost layer of carbonate, which was discarded to eliminate surface contamination. The remaining core was macerated in new plastic containers using fresh concentrated HCl, then neutralized, and remains were washed using distilled water until a pH of 5–6 was reached. Following the washing procedure, remains were kept covered to prevent entry of exogenous materials except during the microfossil picking process.

Macerates from samples collected in 2000 were surveyed using a stereomicroscope, and pulled new glass micropipettes were used to extract individual organic remains. During the pulling process, pipette glass is heated to the molten condition, which burns off potential adherent organics. Multicellular irregularly shaped scraps >100 μm in size that resembled vegetative parts of modern peat mosses were found after macerations conducted in 2000, so macerates from samples collected and processed in 2012 were generally first partitioned using a 90- μm brass sieve (that had been washed multiple times with nanopore-filtered water and dried) before discarding flow-through and isolating organic microfossils with the use of a stereomicroscope and micropipettes. The purpose of the sieving process was to concentrate multicellular remains, making them easier to detect. To survey for all remains, some macerates from 2012 were examined without having been sieved.

Some of the 2012 sieved macerations yielded multiple multicellular organic microfossils. In acid macerates that had not been sieved, similar multicellular organic remains were observed together with microfossils similar in size and test structure to foraminifera that had previously been described from Platteville carbonates after HCl maceration (Gutschick 1986). Foraminifera were picked up with micropipettes and transferred to glass slides for imaging with bright-field microscopy to assess color for inference of thermal maturity using the Foraminiferal Colouration Index (McNeil et al. 1996). In imaging putative foraminifera, filters that might have influenced color were not employed.

Acid macerates that were not sieved also contained (in addition to multicellular scraps and foraminifera) spherical or sub-

spherical unicellular remains whose diameters indicated eukaryotic affinity but that lacked definitive structural features (acritarchs). Such remains were imaged with an environmental scanning electron microscope (ESEM; FEI Quanta 200 ESEM, Department of Botany, University of Wisconsin–Madison) without fixation, dehydration, or metal coating of specimens.

Imaging of Multicellular Fragments

Multicellular fragments from unsieved 2000 and sieved 2012 macerates were imaged in bright-field microscopy, and sieved 2012 materials were further imaged in UV and V excitation with a Zeiss Axioplan fluorescence microscope equipped with UV filter G365 FT395 LP420 and violet filter 395-440 FT460 LP470 (Carl Zeiss Microscopy). For comparison, fresh vegetative fragments of a modern peat moss and two herbarium specimens of >150 yr of age were imaged with the same instrument and fluorescence excitation. Fresh materials were harvested in 2014 from *Sphagnum fimbriatum* occurring in a Patagonian bog located on Navarino Island, Chile, and stored in an illuminated chamber at 4°C prior to imaging. The Wisconsin State Herbarium (WIS) provided specimens of *Sphagnum squarrosum* and *Sphagnum wulfianum*, collected by I. A. Lapham from Iron County, Wisconsin, in 1858. Digital bright-field or fluorescence images were recorded with the use of a Nikon D300s digital camera and Camera Control Pro software (Nikon, Melville, NY) using no brightness or other image adjustments. The open-source image analysis program GIMP (GNU Image Manipulator Program; <http://www.gimp.org>) was used to acquire measurements of cell dimensions and counts of cell wall features of multicellular fragments extracted in 2000 and 2012. Student's *t*-test was used to assess the degree to which cellular features of multicellular microfossils extracted in 2000 and 2012 were similar.

Six of the multicellular fragmentary microfossils extracted in 2012 were attached to SEM stubs using carbon tape and then coated with iridium before examination using a Hitachi S-4800 ultra-high-resolution cold cathode field emission SEM operated at 5 kV, at the University of Wisconsin–Milwaukee Electron Microscopy Laboratory. Energy-dispersive X-ray spectroscopy (EDS) was performed at the same facility on the same microfossil materials; C, Si, Ca, O, Cl, Fe, Na, K, and P distributions were mapped.

Control Procedures

To test the possibility that airborne contamination with modern materials might have occurred in the chemical hood during the maceration process (when samples could not be tightly covered), replicate glass slides bearing drops of immersion oil were placed within the chemical hood and left for periods ranging up to 3 mo and then examined with the use of a compound microscope for presence of structures similar to microfossils. Samples from the distilled water supply used to neutralize extracted sedimentary materials were likewise examined using a compound microscope. Although fibers and other microscopic objects were observed, no structures recognizable as organic microfossils were found in immersion oil traps or water samples. No living peat moss or peat was present in the laboratory during macerations, which were performed during the cold season, re-

ducing the likelihood that airborne contamination from natural environments might have occurred. During the processes of microfossil picking and examination of materials by subsequent light or scanning electron microscopy, a search was made for diatoms, pollen, polysporangiate plant remains, or other post-Ordovician indicators; none was observed.

Modern materials were used to assess features expected to distinguish them from multicellular microfossils. Surfaces of fresh *S. fimbriatum* collected from a bog on Navarino Island, Chile, in 2014 that had been stored at 4°C in light until processing for SEM (fixed in glutaraldehyde, dehydrated in an ethanol series, critical-point dried, and metal coated) were examined with the same instrument used to examine multicellular microfossils for surface materials such as Si particles and iron typical of marine sediment samples (but not fresh peat mosses). Samples of commercial peat were used to assess the possibility that the multicellular microfossils might represent peat contamination; commercial peat samples were first treated with concentrated HCl for several days to mimic the maceration process and period and then examined with light microscopy to assess color and morphology.

Archiving

Permanent slides of representative multicellular microfossils extracted in 2012 and mounted in new Permount Mounting Medium (Fisher Scientific, Fair Lawn, NJ) were archived in the University of Wisconsin–Madison Geology Museum (app. A).

Results

We report here that 455–454 Ma Ordovician carbonates contained multicellular organic fragments that shared distinctive cellular features with modern vegetative peat mosses (Sphagnopsida). Dimensions of cellular features of peat moss–like microfossils isolated 12 yr apart by separate investigators did not statistically differ. The peat moss–like microfossils differed from modern materials in several expected ways (e.g., presence on surfaces of microfossils but not modern fresh peat moss, of silica-rich particles and iron, and lack of evidence in or on fossils for epibionts that are common for modern materials) and occurred with foraminifera of early types that had previously been described from the same formation and in the same concentration. Although additional remains interpreted as acritarchs were found, no diatoms, pollen, polysporangiate plant fragments, or other indicators of post-Ordovician environments were observed, indicating absence of intrusive contamination from later time periods.

Concentration and Structural Features of Multicellular Fragments

Multicellular fragments were found in sparse numbers; fewer than 20 were obtained from two rock samples of approximately 1 kg mass each, and no multicellular fragments were found in four similar rock samples. The overall frequency of multicellular fragments in macerated rock samples was ~2–3/kg. At least 10 of the multicellular fragments were colorless, irregularly shaped, monostromatic sheets that displayed features distinc-

tive for leaves of modern peat mosses (figs. 1A, B1A–B1F, B3A). Two multicellular fragments displayed features distinctive for stems of modern peat mosses (figs. 1C, B1G). (Although moss leaves and stems, lacking specialized lignin-reinforced vascular tissues, are not considered true leaves and stems homologous to those of vascular land plants, such terminology is commonly used to describe moss morphology.)

Most of the peat moss–like multicellular microfossils were flat or partially crumpled monostromatic sheets that displayed cells of two structural types occurring in polygonal arrays. The larger cell type possessed distinctive wall pores and several complete or partial ringlike cell wall thickenings similar in size and number to annular fibrils that occur in the walls of hyaline cells typical of modern peat moss leaves (figs. 1A, 1B, B3, B4). Statistical analysis indicated no significant differences in larger cell length or width, number of ringlike wall thickenings or pores, or pore width in multicellular fragments collected in the years 2000 and 2012 (table 1). The larger cell type lacked evidence for cell contents or epibiotic microorganisms. The smaller cell type occasionally displayed one or a few intracellular spherical structures of unidentified nature.

Multicellular microfossils resembling fragments of modern peat moss stems appeared to be flattened cylinders having a yellowish central region surrounded by colorless cells. Cell walls of the outer colorless cells were ornamented with narrow markings having a spiral pattern (figs. fig. 1C, 1D, B1G). Colorless monostromatic appendages resembling leaf bases extended from flattened cylinders (fig. 1C). Comparative LM observations of commercial dried *Sphagnum* peat samples that had been treated with concentrated HCl retained the original deep brown color and occurred as relatively large crumpled pieces of leaf-bearing stems; pale leaf or stem fragments similar to those obtained from macerates were not observed (fig. B2).

Fluorescence microscopy revealed that cell walls of leaflike and stemlike multicellular fragments extracted from carbonates autofluoresced relatively pale blue-white under UV excitation (fig. B3A) and yellow-green in violet excitation (fig. B3B). Such fluorescence was similar in color but somewhat paler than that displayed by fresh, similarly structured vegetative modern peat mosses imaged with the same equipment (fig. B3C, B3D); fluorescence of similar quality and intensity to fossils was observed for old (>150 yr) herbarium specimens of two *Sphagnum* species (fig. B3E–B3H).

SEM revealed that surfaces of multicellular fragments extracted from carbonates bore many small, irregularly shaped particles that had remained attached through maceration and washing procedures (see fig. 1B). By contrast, particulates were notably sparse on surfaces of natural collections of modern peat mosses similarly processed for and imaged by SEM (fig. B4). Epibiotic microbiota characteristics for modern peat mosses, commonly located within larger diameter porose cells (figs. B3C, B3D, B4), were absent from multicellular microfossils (figs. 1A, 1B, B1B–B1F).

Carbon EDS maps of multicellular fragments extracted from carbonates corresponded with microfossil surfaces and some of the adherent particles (fig. 2A). Silicon mapped with the majority of surface particles observed on microfossil surfaces (fig. 2B). Iron was more or less homogeneously distributed across surfaces of multicellular fragments (fig. 2C). Oxygen and phosphorus maps matched the carbon pattern, while calcium, chlorine, po-

tassium, and sodium distributions were similar to that of iron (data not shown).

Associated Microfossils

Pale chambered structures consistent with classification as foraminifera (figs. 3, B5) were observed in unsieved acid macerates together with multicellular peat moss-like fragments in material collected and processed in 2012. Structural similarities included tests occurring as straight or slightly curved pairs or clusters of three chambers bearing surface particles. Coloration corresponded to Foraminiferal Colouration Index number 0, indicating thermal immaturity. Spherical or subspherical unicellular microfossils that were likewise present in 2012 unsieved acid macerates and imaged with environmental SEM (fig. B6) occurred in sizes ($\sim 100\text{--}200\ \mu\text{m}$) consistent with eukaryotic affinities but lacked distinctive features allowing further classification.

Discussion

Overview

This replicated study provides microfossil evidence for the presence of plants having vegetative features distinctive for sphagnopsid peat mosses (Crum and Anderson 1981) at 454–455 Ma. Multicellular microfossils interpreted as leaf fragments displayed features diagnostic for vegetative Sphagnopsida, including dimorphic cellular structure—wider porose cells with annular wall thickenings arranged in a network with narrower cells lacking these wall features. Multicellular remains interpreted as stem fragments displayed spiral ornamentation on outer cell walls and other distinctive peat moss features. The new multicellular microfossils are consistent with well-known peat moss recalcitrance properties that foster fossilization (Kroken et al. 1996) and recent diversification (molecular clock, timescale) analyses that infer an age of 460–607 Ma for the Sphagnopsida

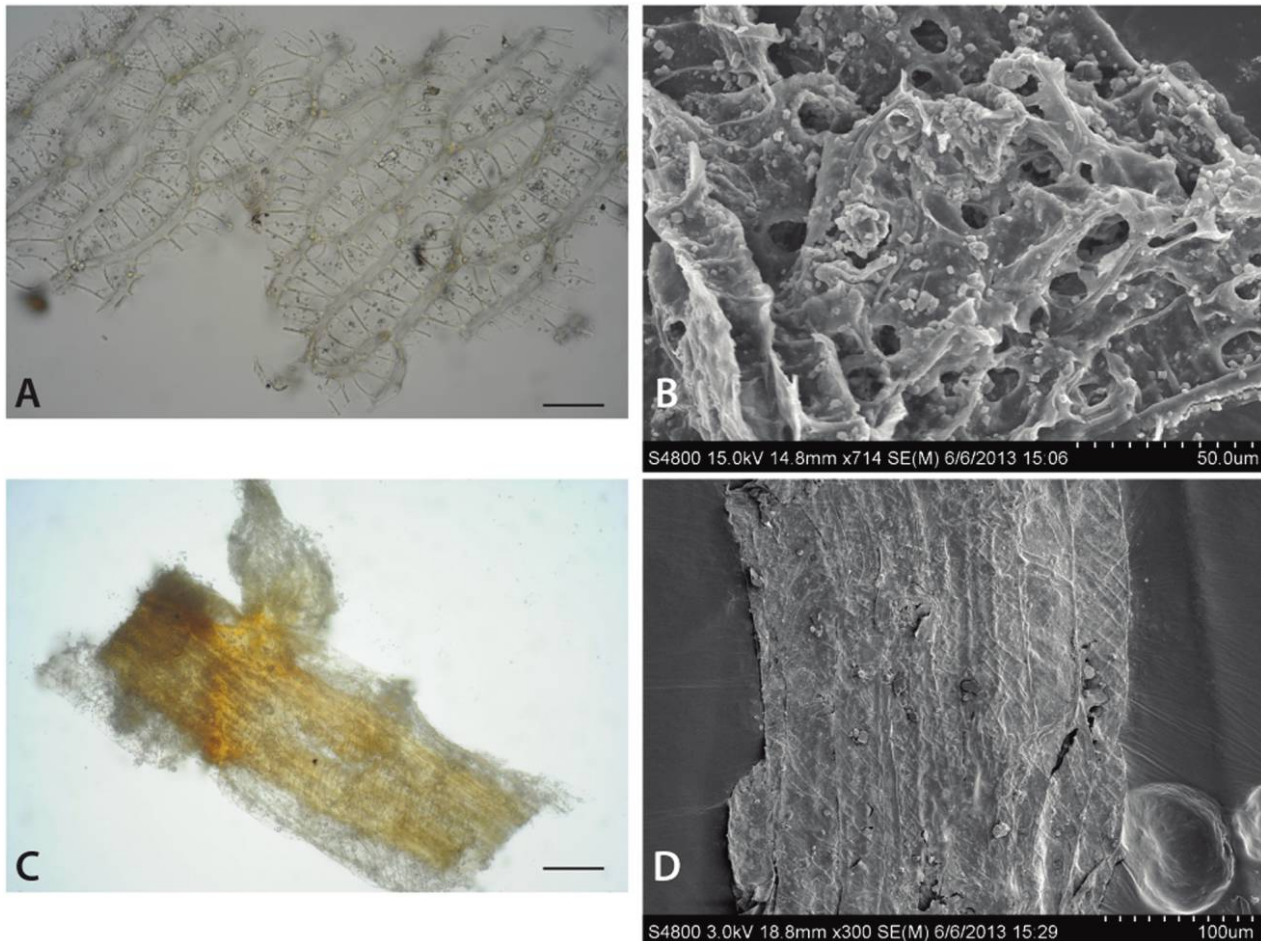


Fig. 1 Representative multicellular organic fragments that resemble leaf or stem pieces of modern peat mosses. *A*, LM of putative leaf fragment showing network of narrow cells lacking wall ornamentation and wider cells with conspicuous annular wall thickenings and wall pores. Surface particles are abundant. Scale bar = $50\ \mu\text{m}$. *B*, SEM of putative leaf microfossil showing conspicuous cell wall pores and annular thickenings. Surface particles are abundant. Scale bar measurement included in SEM data. *C*, LM of putative stem microfossil. Pale central region surrounded by translucent cells with spiral markings. Tissue stubs resemble leaf bases. Scale bar = $100\ \mu\text{m}$. *D*, SEM of putative stem surface cells showing conspicuous spiral markings. Scale bar measurement included in SEM data.

Table 1
Comparative Biometrics of the Wider Cell Type in Leaflike Microfossil Fragments Extracted from Ordovician Platteville Formation Carbonates in 2000 versus 2012

	2000 (mean \pm 1 SE)	2012 (mean \pm 1 SE)	<i>t</i>	df	<i>P</i>
Cell length (μm)	212 \pm 5.36	220 \pm 8.50	-1.09	5	.391
Cell width (μm)	45.5 \pm 2.43	52.5 \pm 2.33	-1.49	5	.275
No. annular wall fibrils per cell	9.59 \pm 1.54	11.9 \pm .90	-1.46	5	.281
No. wall pores per cell	4.50 \pm .53	3.27 \pm .64	1.82	5	.210

lineage (Laenen et al. 2014). Because meso- and microfossils of polysporangiophytes that possessed degradation-resistant tissues had become well established by the late Silurian (Gensel 2008), the absence of degradation-resistant plant materials other than peat moss-like remains argues against the possibility that Platteville carbonates had been infiltrated by Silurian-Holocene materials.

The multicellular remains described here represent the oldest vegetative remains of land plants reported to date and the oldest fossils yet known that can confidently be linked with any modern plant group. The new microfossils also indicate that peat moss-dominated peatlands, recognized for globally significant roles in modern terrestrial biodiversity and C and N cycling (Gorham 1991; Kip et al. 2010), were likely present hundreds of millions of years earlier than previously thought.

Evidence for Antiquity of Peat Moss-Like Remains

Several lines of geological, biological, and chemical evidence argue for antiquity of the peat moss-like multicellular remains found in macerates of Ordovician carbonate.

1. The Platteville carbonate matrix, which is not a microbialite (Mossler 1985), displays relatively low porosity and horizontal hydraulic conductivity (Batten et al. 1999), features indicating resistance to infiltration. Absence of materials characteristic of post-Ordovician environments (e.g., pollen, diatoms, polysporangiate plant remains) in macerates is consistent with these matrix properties.

2. The multicellular peat moss-like remains co-occurred with spherical objects whose dimensions were similar to those of acritarchs (presumed eukaryotes whose affinities are otherwise unclear) known to occur in Ordovician limestones (Servais et al. 2004). Co-occurring remains that were similar in size and test structure to foraminifera previously described from the Platteville Formation likewise provide expected extractives. Gutschick (1986) reported that only about 25 whole or fragmentary specimens of the agglutinated foraminiferan *Reophax* were recovered from 10 kg or more of carbonate sampled from the Platteville Formation. This frequency ($\sim 2.5/\text{kg}$) is similar to that of the peat moss-like microfossils we found ($\sim 2\text{--}3/\text{kg}$). Similar early types of foraminifera have been found in other Ordovician deposits (e.g., Moreman 1930; Nestell et al. 2009).

3. The pale color of the Platteville foraminifera and multicellular mosslike remains is consistent with an interpretation of thermal immaturity, indicating that these materials had experienced similar low thermal environments. The sampled locale occurs at the extreme western edge (Deininger 1964) of a region of carbonates reportedly influenced by hydrothermal dolomiti-

zation centered in the Michigan Basin (Luczaj 2006) and therefore less likely to have been heated.

4. Color (pale vs. dark brown) and structural (small monostromatic fragments vs. larger crumpled conglomerates) differences indicate that the multicellular remains we extracted from carbonates are unlikely to represent contamination by modern commercial or natural peat. The comparison also suggests that the Platteville peat moss-like microfossils did not originate from long-buried peat but rather from living antecedents.

5. Absence of cellular contents, absence of evidence for epibionts, and presence of coatings of silica-rich particulates were features of the multicellular remains inconsistent with origin from modern peat mosses. The latter display cellular contents, epibiontic microorganisms within porose hyaline cells (Hingley 1993), and smooth outermost surfaces when imaged by SEM.

6. Reduced levels of autofluorescence of peat moss-like microfossils by comparison to fresh peat moss and similarity of fossil autofluorescence to that of old (>150 yr) peat moss herbarium specimens indicate origin from ancient materials that retained some degree of autofluorescence over long time periods. Organic cell walls of thermally immature Neoproterozoic and Early Cambrian acritarchs (such as *Tasmanites*) are likewise known to retain autofluorescence in UV excitation (Talyzina 1998; Arouri et al. 2000). If *Tasmanites* and similar autofluorescent acritarchs represent degradation-resistant phycoma stages of early-divergent green algae, as is commonly thought (Graham et al. 2015), it is possible that similar chemical features are responsible for the persistent autofluorescence properties of such acritarchs and the pale peat moss-like microfossils described here. *Gloeocapsomorpha prisca* (aka *Gloeocapsomorpha prisca*), of widespread occurrence in Ordovician deposits (including the Platteville Formation; Pancost et al. 1998) and an important source of Ordovician oil deposits (Kukersite-type source rock; YongGe et al. 2013), though of uncertain taxonomic affinity, is likewise commonly autofluorescent (Obermajer et al. 1999), a property attributed to presence of phenolic polymer (Derenne et al. 1991; Versteegh and Blokker 2004). Retention of some degree of autofluorescence by thermally immature Ordovician peat moss-like multicellular fragments is therefore not surprising, particularly in light of our evidence that herbarium specimens of two peat moss species collected >150 yr ago display similar autofluorescence.

7. Silica-rich particle coatings on mosslike microfossils and coeval arenaceous, aggregated foraminifera (e.g., Gutschick 1986) are consistent with relatively high silica concentrations (at least 110 mg/L SiO_2 characteristic of Cambrian–Early Cretaceous oceans); by contrast, modern oceans tend toward Si depletion in consequence of the emergence of diatoms in the Cretaceous (Grenne and Slack 2003). The surfaces of modern

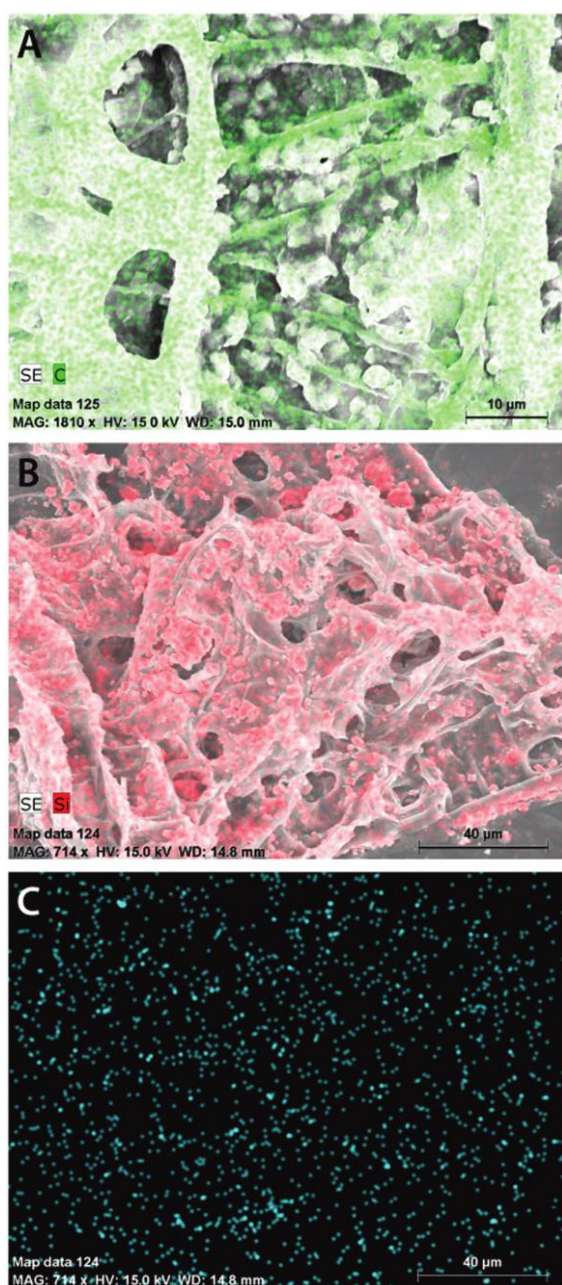


Fig. 2 Energy-dispersive X-ray spectroscopy. *A*, Carbon map of typical multicellular microfossil of the leaf fragment type showing correspondence with cell walls, including annular wall projections present on wider cells. *B*, Silicon map showing correspondence with many of the particles associated with microfossil surfaces. *C*, Iron map showing comparatively homogenous distribution. Scale bar measurements included in SEM data.

peat mosses prepared and imaged similarly lacked equivalent adherent particulates, consistent with low Si concentrations known for peat moss-dominated wetlands (Bendell-Young 2003).

8. Carbon, oxygen, and phosphorus mapping with EDS indicated the organic nature of the peat moss-like fragments, and EDS mapping also demonstrated association of the peat

moss-like multicellular microfossils with iron, which is known to promote the preservation of organic matter in sediments, shielding it from microbial degradation (Lalonde et al. 2012). Surfaces of microfossils resembling peat moss leaf and stem fragments also displayed ions (Ca, Na, K, and Cl) that would be expected to occur in association with marine fossil remains, but not modern peat mosses, which occur in terrestrial wetland systems of low Fe and other mineral content (Bendell-Young 2003). Together, these observations indicate that the peat moss-like Ordovician remains are unlikely to represent contamination by younger materials.

Evolutionary Significance of Ordovician Peat Moss-Like Microfossils

The peat moss-like fossils described here add new depth to Pre-Wenlock (Late Silurian) plant history that until now largely consisted of reproductive structures: spores or spore-like objects known as cryptospores and putative sporangial epidermis (Gray et al. 1982; Strother et al. 1996; Wellman et al. 2003, 2015; Steemans et al. 2009; Rubinstein et al. 2010). Vegetative aspects of the plants that produced these reproductive structures and their relationships to modern lineages have been unclear, though a trilete mark on certain Late Ordovician spores has been regarded as evidence for vascular plant provenance, since bryophytes generally lack such spore ornamentation (Steemans et al. 2009; Wellman et al. 2015). In this regard, the peat mosses are interesting exceptions because their spores display a true trilete region where the spore wall is thinner than elsewhere and other distinctive spore ornamentation (Brown et al. 1982, 2015; Cao and Vitt 1986). These considerations indicate that the surface structure of trilete Ordovician spores might be compared in more detail with those of modern peat mosses, whose spore surface variability is well understood. For example, spore ornamentation has been surveyed in 87 peat moss populations representing 33 *Sphagnum* species (Cao and Vitt 1986). The new fragmentary but distinctive vegetative remains described here are consistent with an earlier interpretation (Kroken et al. 1996) of a Caradocian (ending in the mid-Katian) cellular sheet (Gray et al. 1982) as a fragment of peat moss sporangial epidermis because these materials displayed similar color, cell patterning, and recalcitrance attributes. Additional remains of this type might ultimately be found in Ordovician deposits that are rich in spore remains.

Figure 1 of Jin et al. (2013) and figure 10 of Cocks and Torsvik (2011) suggest that the site from which the newly discovered peat moss-like remains were obtained was located approximately 10° south of the equator. Warm, humid climatic conditions at this latitude (and significantly higher than present atmospheric CO₂ levels) would seem optimal for the growth and spread of early land plants. The wide physiological tolerances displayed by the modern peat mosses, including occurrence in modern tropical, subpolar, and other environments (Rydin and Jeglum 2006; Yao et al. 2009), may explain how peat mosses persisted through periods of widespread glaciation occurring in the Hirnantian and other times of extreme environmental conditions into milder modern conditions. Early adaptation to tropical conditions might also help to explain why modern peat moss productivity and persistence seem relatively unaffected by environmental warming (Lang et al. 2012).

*Comparison of Ordovician Microfossils to
Later Peat Moss–Like Remains*

Triassic *Sphagnophyllites* from India—described as having acostate oblong leaves with darker cells forming a meshwork around hyaline regions—was classified by these workers into the order Sphagnales (Pant and Basu 1976). The order Protosphagnales was erected for fossils from Permian deposits in Russia that display leaf cell dimorphism as well as a leaf midvein (costa; Neuberg 1956, 1960). Protosphagnales currently includes the genera *Protosphagnum*, *Intia*, *Kosjunia*, *Vorcutannularia*, and *Jungagia*, differentiated by leaf developmental patterns (Maslova et al. 2012). Some remains found in bulk macerations of Mississippian (Carboniferous) shales displayed features in common with peat mosses, though investigators deemed the plant fragments too small for detailed classification (Hübers and Kerp 2012).

The Ordovician remains reported here were too fragmentary to determine whether a midvein (costa) might have been present. Even so, the remains seem appropriately classified with Sphagnopsida. Such a classification is consistent with recent diversification time analyses involving hundreds of *Sphagnum* accessions and multiple parameter assumptions, which indicated that the ancestors of the modern Sphagnopsida were significantly older (460–607 Ma; Laenen et al. 2014) than the Ordovician microfossils described here (455–454 Ma). The molecular data suggest that Sphagnopsida existed at least 5 Ma before the formation of the deposits in which peat moss–like remains were found and probably longer. These time periods seem long enough to allow populations to spread and become abundant enough to leave remains in the fossil record.

Together, the microfossil and molecular diversification data indicate that microanatomical and morphological characteristics of the peat moss vegetative body may have been conserved over hundreds of millions of years. Long-term vegetative structure conservation has been noted for a number of other plant genera, notably, the archaic pteridophyte genus *Equisetum*, whose distinctive features have been conserved for more than 150 Ma (Channing et al. 2009; Stanich et al. 2009; Husby 2013). Peat mosses may likewise represent a structurally archaic lineage.



Fig. 3 Representative early-type multichambered foraminifera-like microfossil found with peat moss–like fragments. Pale color provides evidence for thermal immaturity of organic materials in the sampled deposit. Scale bar = 25 μ m.

*How a Long-Lived Peat Moss Genus Might
Influence Ecosystems over Time*

Features of the Ordovician peat moss–like microfossils reported here suggest strong structural similarity to the modern genus *Sphagnum* and close relatives (Shaw et al. 2010a), which display efficient N uptake (Fritz et al. 2014), mixotrophic capacity (Graham et al. 2010), structural adaptation to changes in temperature and UV conditions (Cardona-Correa et al. 2015b), light-directed gametophytic growth response enabling reorientation after disturbance (Cardona-Correa et al. 2015a), beneficial interactions with microorganisms (Bragina et al. 2014), capacity for rapid asexual propagation leading to the ability to dominate large landscape areas, and effective sexual dispersal (Whitaker and Edwards 2010) occurring on a continental to intercontinental scale (Sundberg 2013). If ancestral peat mosses likewise possessed these characteristics, persistence for hundreds of millions of years to the present day seems understandable and effects on past ecosystems inferable.

One major ecosystem service that modern peat mosses provide is fostering the biodiversity of several groups of organisms whose lineages almost certainly predate the Ordovician: bacteria (including cyanobacteria; e.g., Bragina et al. 2014), photosynthetic protists such as the green streptophyte algae Zygnematales and Desmidiaceae that today are most diverse in *Sphagnum*-dominated peatlands (Graham et al. 2015), and arcellinid amoebozoans (Fiz-Palacios et al. 2014). Although the latter authors assumed that *Sphagnum*-dominated peatlands arose in the Miocene during the radiation of current peat moss species diversity (Shaw et al. 2010b), peat mosses might actually have been abundant much earlier, indicated by the evidence presented here as well as Triassic, Permian, and Carboniferous remains that display some features similar to modern Sphagnopsida. If so, ancient peat moss–dominated environments might have fostered biodiversity of bacteria and protists since the Ordovician and later pteridophyte, gymnosperm, angiosperm, and invertebrate groups that today favor peatlands dominated by peat mosses. Likewise, if peat mosses are as old as molecular diversification (Laenen et al. 2014) and the present fossil evidence indicate, peatlands might have influenced Earth’s atmospheric chemistry and climate since the mid-Ordovician, as suggested by modeling studies based on physiological features of modern mosses (Graham et al. 2004; Lenton et al. 2012).

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Appendix A

Voucher Deposition

Permanent slide mounts of representative peat moss–like microfossils are deposited at the University of Wisconsin–Madison Geology Museum, 1215 West Dayton Street, Madison, Wisconsin 53706.

Appendix B

Supplemental Figures

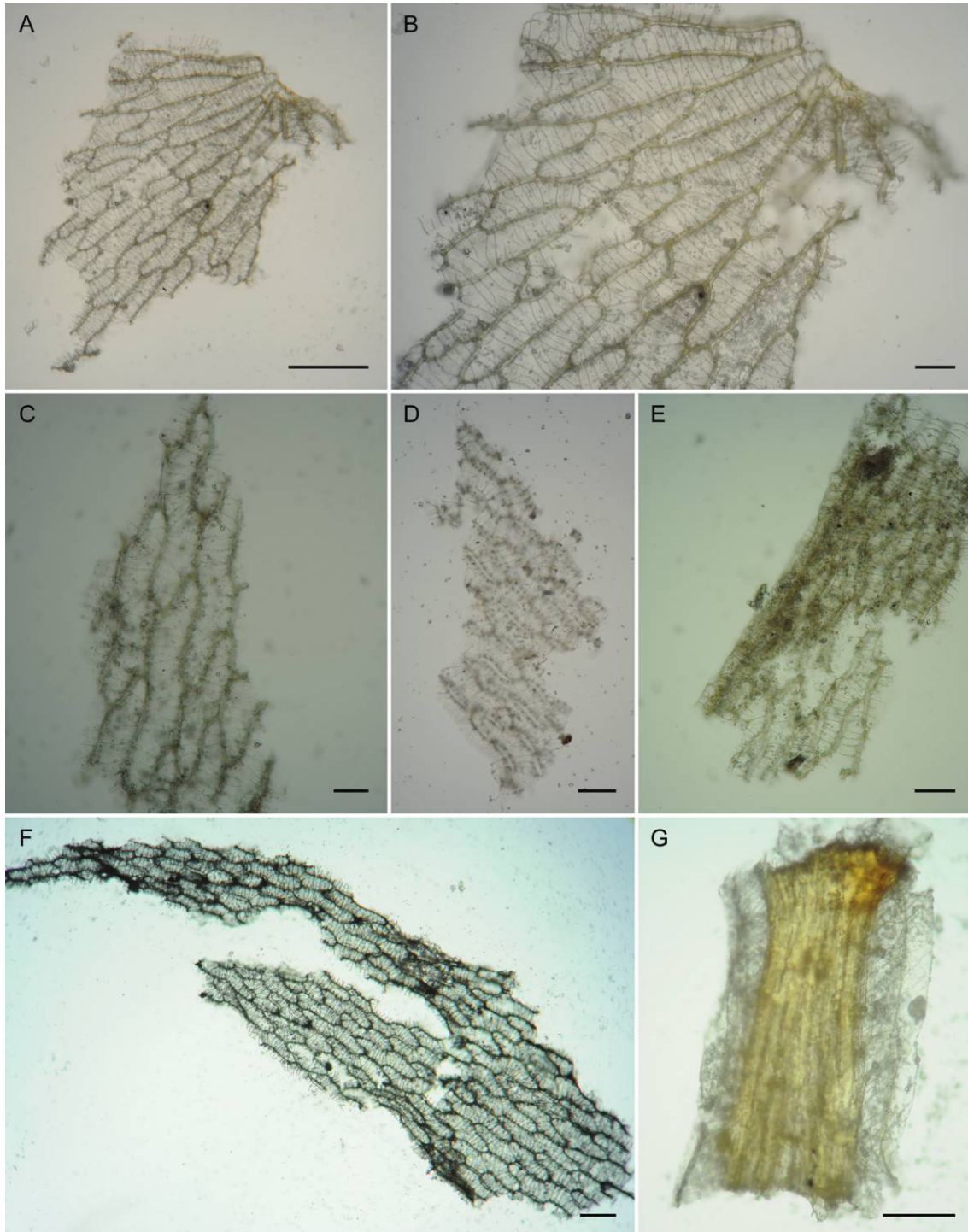


Fig. B1 Additional examples of pale multicellular Ordovician microfossils interpreted as fragments of leaves or stem of a peat moss-like early land plant. *A–F*, Leaflike fragments consistently displayed wider and narrower cells arranged in a monostromatic network, absence of cell contents and epibiota, and consistent presence of surface mineral particles. *B*, Enlargement of microfossil shown in *A*. *G*, Microfossil interpreted as stem fragment showing yellowish core surrounded by colorless tissue. Scale bars: *A* = 150 μm ; *B*, *C* = 50 μm ; *D–G* = 100 μm .

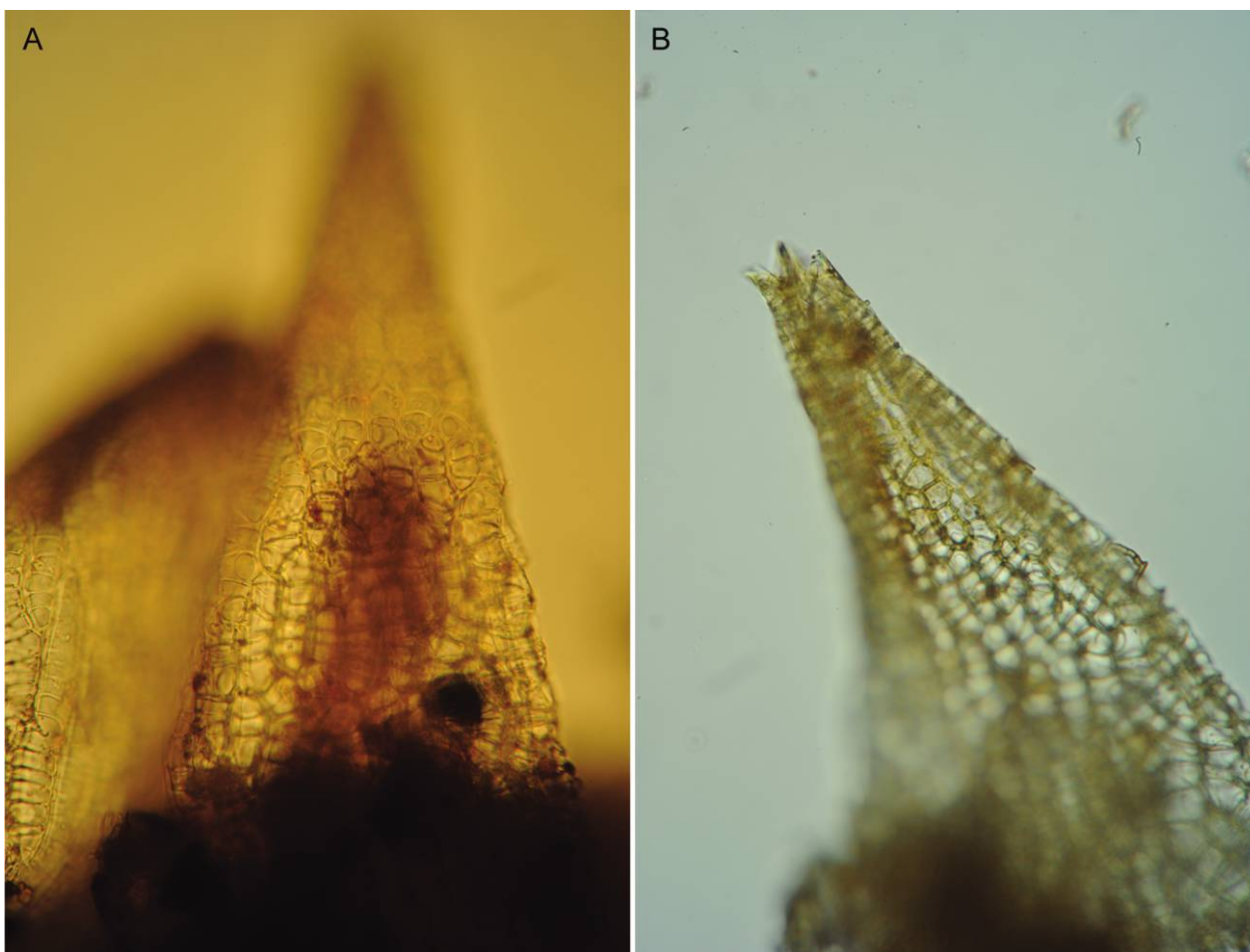


Fig. B2 Commercial peat samples viewed by LM after treatment with HCl. *A, B*, Materials were consistently deeply colored, a feature distinguishing them from actively growing peat moss tissues as well as multicellular microfossils described from Ordovician carbonates. HCl-treated peat samples showed no evidence of abundant surface mineral particles.

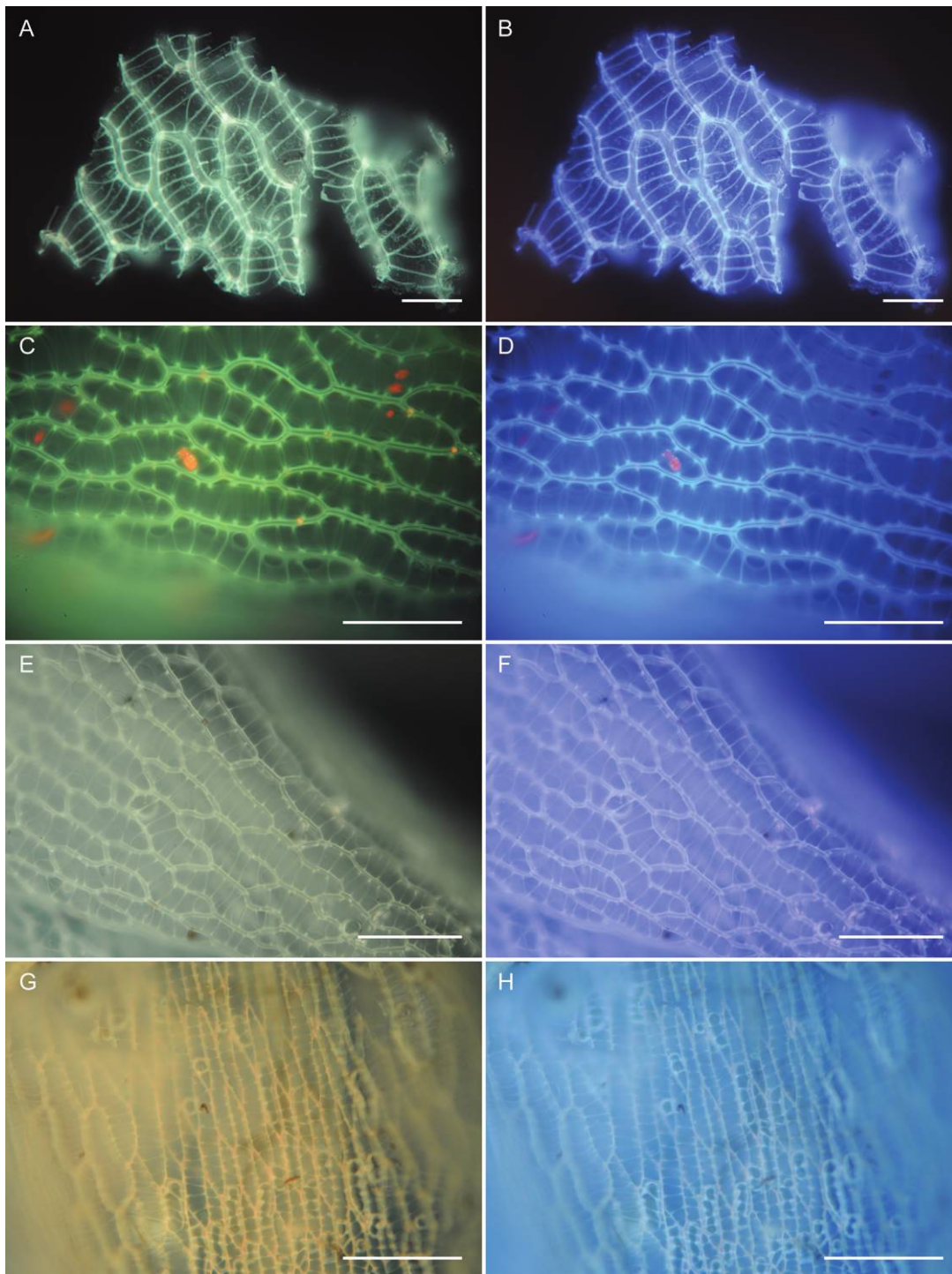


Fig. B3 Epifluorescence of microfossil leaflike fragments compared to that of peat moss leaves from fresh and >150-yr-old herbarium specimens. *A*, Epifluorescence of representative microfossil leaf fragment in violet excitation. No fluorescence evidence for epibionts. Scale bar = 50 μm . *B*, Epifluorescence of representative leaflike fragment in UV excitation. No fluorescence evidence for epibionts. Scale bar = 50 μm . *C*, Bright yellow-green epifluorescence of representative older (nongreen) leaf of fresh *Sphagnum fimbriatum* in violet excitation. Note red fluorescence of photosynthetic epibionts. Scale bar = 100 μm . *D*, Comparatively bright blue-white epifluorescence of representative older (nongreen) leaf of fresh *S. fimbriatum* in UV excitation. Note red fluorescence of photosynthetic epibionts. Scale bar = 100 μm . *E*, Epifluorescence in violet excitation of a leaf from a herbarium specimen of *Sphagnum squarrosum* collected from the Penokee Iron Range near Lake Superior by I. A. Lapham in 1858. Scale bar = 100 μm . *F*, Epifluorescence in UV excitation of the same specimen shown in *E*. *G*, Epifluorescence in violet excitation of the base of a leaf of *Sphagnum wulfianum* from a herbarium specimen collected from the Penokee Iron Range in 1858 by I. A. Lapham. Scale bar = 100 μm . *H*, Epifluorescence in UV excitation of the same specimen described in *G*. Scale bar = 100 μm .

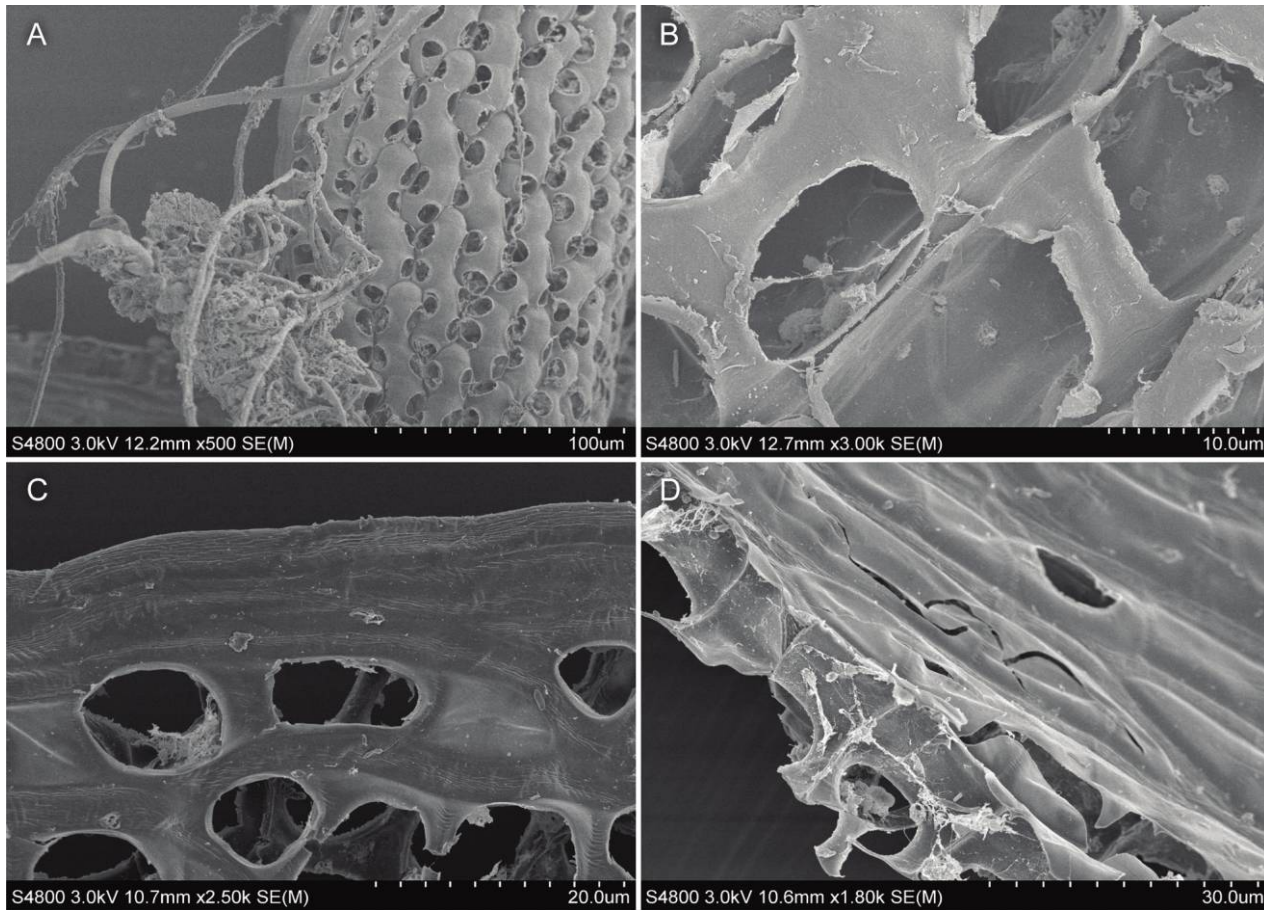


Fig. B4 SEM views of surfaces of modern *Sphagnum fimbriatum* leaves sampled from nature. *A*, Note cells with conspicuous cell wall pores, a feature diagnostic for peat mosses that was shared with multicellular microfossils. *B–D*, Although epibiotic microbes may be present on surfaces of modern peat mosses, including the inner walls of porose cells, abundant mineral particles such as those observed on multicellular microfossils were not present. Scale bar measurements included in SEM data.

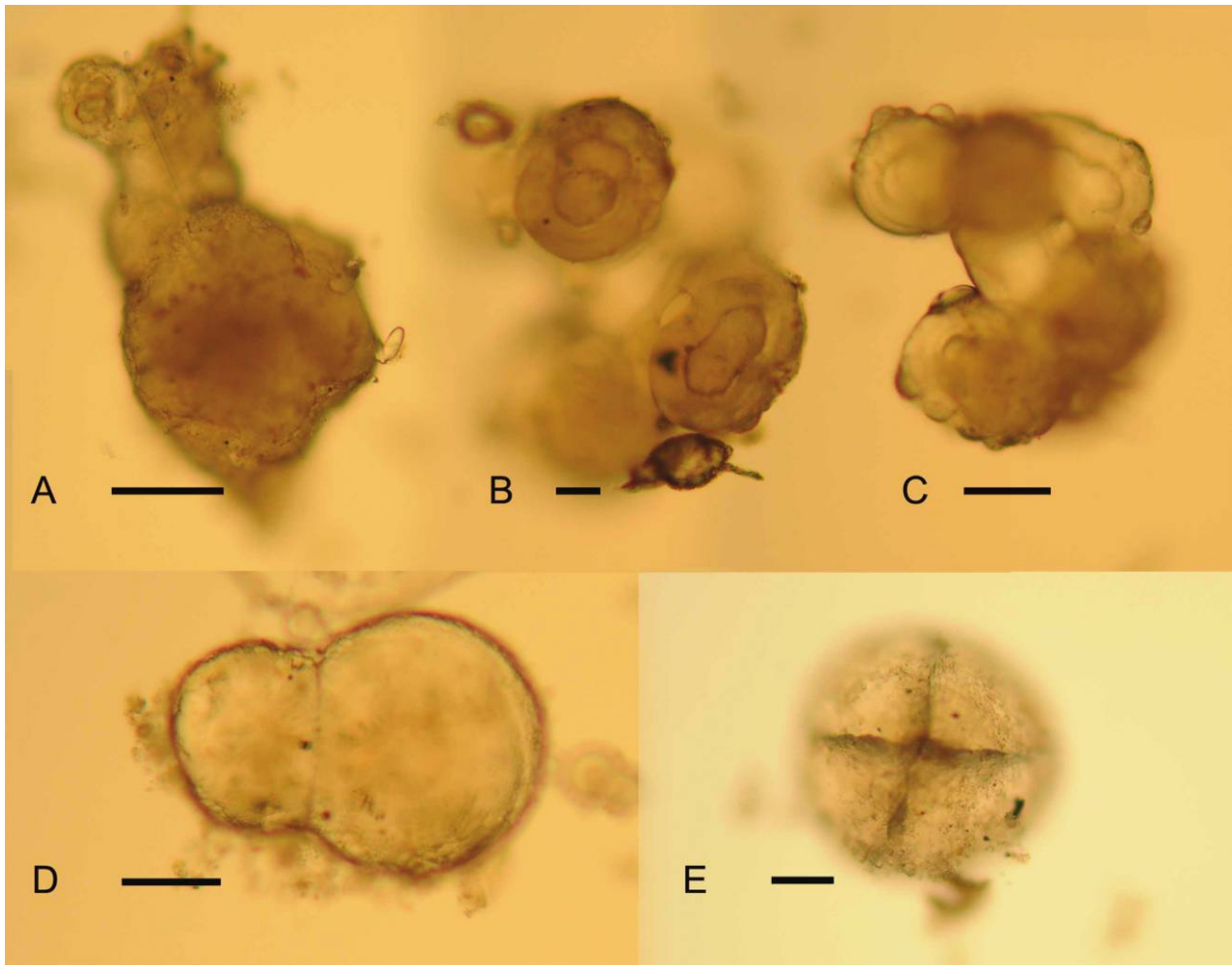


Fig. B5 Bright-field images of representative foraminifera-like microfossils associated with Ordovician multicellular remains. Overall form and dimensions were consistent with chambered agglutinated foraminifera previously reported for the Platteville Formation (Gutschick 1986). Pale color indicates low thermal maturity, i.e., that organic materials had not been much heated after deposition. Scale bars: A = 50 μm ; B = 5 μm ; C, D = 10 μm ; E = 7.5 μm .

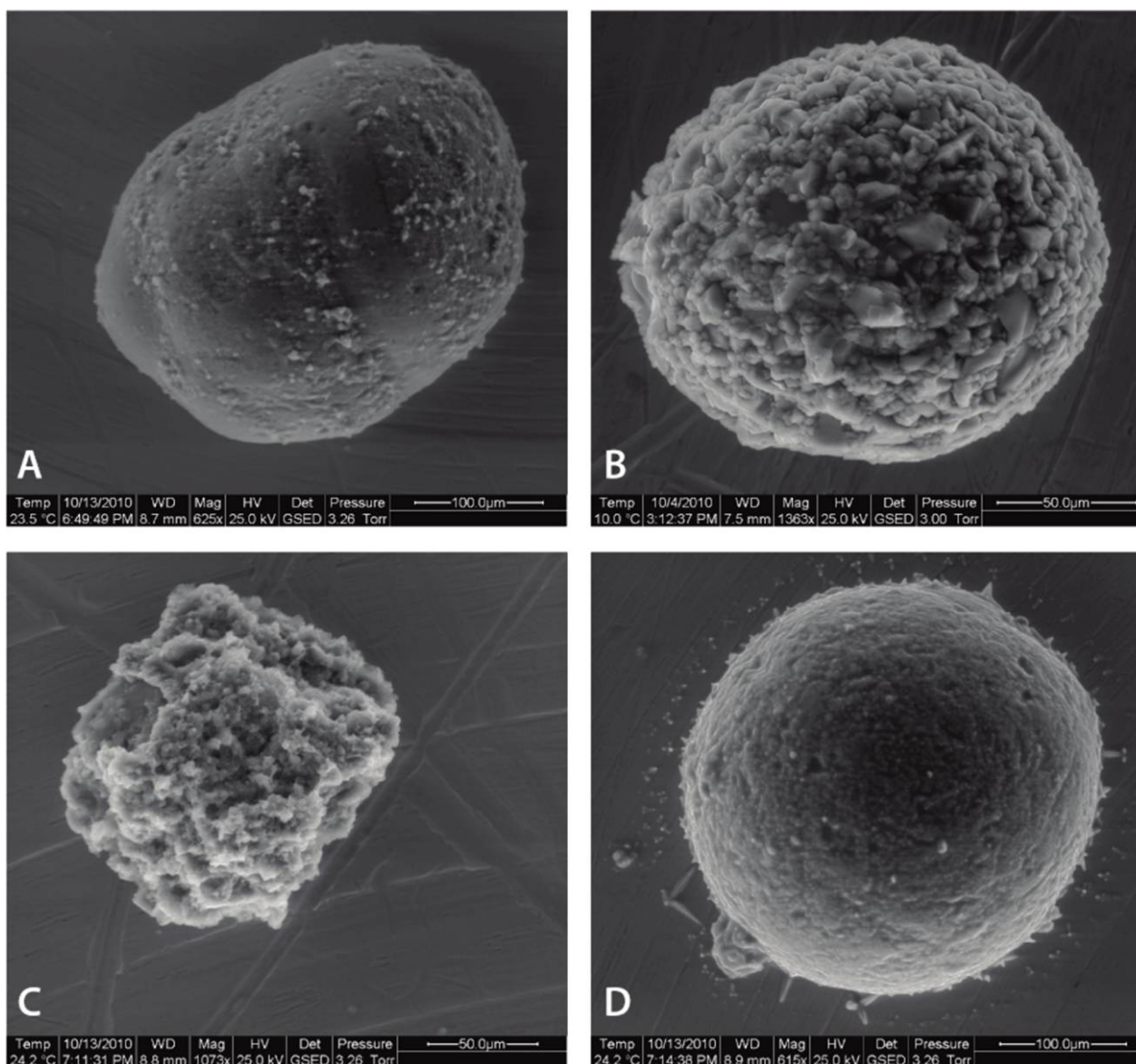


Fig. B6 Environmental SEM views of representative acritarch-like microfossils associated with Ordovician multicellular remains. Dimensions indicate eukaryotic affinities. Scale bar measurements included in SEM data.

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