# Resistant tissues of modern marchantioid liverworts resemble enigmatic Early Paleozoic microfossils

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**Absence of a substantial pretracheophyte fossil record for bryophytes (otherwise predicted by molecular systematics) poses a major problem in our understanding of earliest land-plant structure. In contrast, there exist enigmatic Cambrian–Devonian microfossils (aggregations of tubes or sheets of cells or possibly a combination of both) controversially interpreted as an extinct group of early land plants known as nematophytes. We used an innovative approach to explore these issues: comparison of tube and cell-sheet microfossils with experimentally degraded modern liverworts as analogues of ancient early land plants. Lower epidermal surface tissues, including rhizoids, of *Marchantia polymorpha* and *Conocephalum conicum* were resistant to breakdown after rotting for extended periods or high-temperature acid treatment (acetolysis), suggesting fossilization potential. Cell-sheet and rhizoid remains occurred separately or together depending on the degree of body degradation. Rhizoid break-off at the lower epidermal surface left rimmed pores at the centers of cell rosettes; these were similar in structure, diameter, and distribution to pores characterizing nematophyte cell-sheet microfossils known as *Cosmochlaina*. The range of *Marchantia* rhizoid diameters overlapped that of *Cosmochlaina* pores. Approximately 14% of dry biomass of *Marchantia* vegetative thalli and 40% of gametangiophores was resistant to acetolysis. Pre- and posttreatment cell-wall autofluorescence suggested the presence of phenolic compounds that likely protect lower epidermal tissues from soil microbe attack and provide dimensional stability to gametangiophores. Our results suggest that at least some microfossils identified as nematophytes may be the remains of early marchantioid liverworts similar in some ways to modern *Marchantia* and *Conocephalum*.**

Discerning the structural nature of the earliest land plants has been a paleontological challenge. For example, nematophytes (Nematophytales) were first described from Late Silurian remains from the Welsh borderland by Lang (1), who regarded them as an extinct group of early land plants having unusual body construction. The nematophyte body was described as an "encrusting thalloid plant with tubular anatomy, covered by resistant cuticle with pseudocellular patterning" (1). This material included tubes of at least two width types, apparent sheets of cells or adherent parts of cells (described as cuticle), and spores, the latter suggesting that the hypothetical plants reproduced by dispersed spores, as do modern basal embryophytes. Although similar tubes and cell sheets attributed to nematophytes have now been found in Cambrian–Devonian deposits, no later studies have confirmed that the tubes, cell sheets, and spores were attached (2–5). Thus, the original concept of a nematophyte with a body that included all three components (1) remains hypothetical. Even so, this perception of a distinct lineage of early thalloid land plants that left no modern descendants has persisted. Currently, depending on the author, nematophytes are thought to consist of tubular entities (3, 4), termed "nematoclasts" (6), or thick, anisomorphic cellular sheets, described as "cuticles" (7), and to represent different types of organisms.

Another perplexing problem related to early land-plant structure is discrepancy between molecular systematic data indicating that liverwort-like plants almost certainly appeared earlier than any lineage of vascular plants that has extended to the present time (8, 9) and the megafossil record. Late Ordovician (Caradoc, ≈450 million years old) spores having distinctive layered walls that among modern plants are found only among liverworts (occurring within fragments of enclosing material interpreted as sporangial epidermis) are interpreted as liverwort microfossils (10). However, the earliest megafossils (of Late Silurian age, ≈425 million years ago) that are accepted as remains of bryophytes, with many cellular features matching those of liverworts (11), considerably postdate the earliest fossils attributed to vascular plants or pretracheophyte polysporangiophytes (12, 13). The supposed absence from liverworts of decay-resistant tissues such as lignified xylem, typical of vascular plants and their fossils, is usually invoked to explain the paucity of fossil evidence for earliest liverwort-like plants.

This article describes an innovative approach toward understanding the structure of early land plants: experimental degradation of liverworts as modern analogues and comparison of their remains with Cambrian-to-Devonian microfossils. Our results show that although modern liverworts vary in degree of degradation resistance, the widespread extant marchantioid liverworts *Marchantia polymorpha* and *Conocephalum conicum* possess lower epidermal tissues that are highly resistant to decay or high-temperature acid hydrolysis. Additionally, we found that these resistant liverwort tissues closely resemble tubular or cell-sheet remains attributed to the nematophytes. On the basis of this evidence, we propose the hypothesis that at least some of the tubular and cellular sheet microfossils attributed to nematophytes may well be the remains of early liverwort-like land plants. We also determined that at least one modern liverwort produces substantial amounts of resistant organic carbon, suggesting the possibility that Early Paleozoic relatives might have contributed to $CO_2$ sequestration in early terrestrial ecosystems.

## Materials and Methods

**Degradative Treatments.** We subjected water-washed portions of herbarium specimens or greenhouse-grown or field-collected specimens of the early divergent liverwort *Blasia pusilla* and later divergent (14) liverworts *Lunularia* sp., *Sphaerocarpos* sp., *M. polymorpha*, *Preissia* sp., and *Ricciocarpos natans* to two treatments designed to mimic decay and burial processes. These treatments included mechanically disruptive high-temperature acid hydrolysis (standard acetolysis) (15) and a milder treatment, rotting in moist soil for at least 3 months (16). In addition, gametophytes of the marchantioid liverwort *C. conicum* were subjected to the same rotting procedure. *Marchantia* and *Conocephalum* were distinctive in that the vegetative thallus' lower epidermis and rhizoids and (in the case of *Marchantia*) components of the erect gametangiophores of fertile thalli survived degradative procedures. In contrast, little more than spores (and smooth gemmae, in the case of *Blasia*) was recovered from the

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other liverworts examined. Subsequently, quantitative high-temperature acetolysis (17) was performed separately on 10 samples each of *Marchantia* vegetative thalli and mature archegoniophore stalks and heads (which included dehiscent sporophytes) as a way of conservatively estimating the percentage of hydrolysis-resistant carbon in bodies of *Marchantia*, excluding spores and elaters.

**Fossil Remains.** Representative cellular scrap microfossils obtained by maceration (6) from Early Devonian volcanic ash-derived sediments near Atholville, NB, Canada, were used for comparative morphological studies. Tubular microfossils were not used for direct comparison, because they vary sufficiently such that choosing valid representatives could not be accomplished objectively. Rather, comparisons were made with the use of published images of tubes attributed to nematophytes.

**Imaging.** Bright-field and fluorescence images of liverwort tissues before and after rotting and high-temperature acetolysis treatments and of fossil cell scraps were made with the use of an epifluorescence microscope (Axioplan, Zeiss) equipped with violet and UV excitation filter sets. Scanning electron micrographs were obtained by examining dried, gold-coated, rot-degraded *Marchantia* with a scanning electron microscope (Cambridge Stereoscan 240, Altran, Boston).

## Results

**Pretreatment Attributes.** Large numbers of unicellular rhizoids arise by tip growth of specialized cells of the lower epidermis of marchantioid liverworts, particularly along the thallus midline, in which lower epidermal tissues may be deeply pigmented (Fig. 1*A*). The mean width of 40 *Marchantia* rhizoids was 26.8 $\mu$m (range, 12.5–62.5 $\mu$m). [In comparison, the mean width of 40 pores from the fossil cell scrap (Fig. 1*J*) was 14 $\mu$m (range: 7.5–22.5 $\mu$m).] Liverwort rhizoids may extend perpendicularly or be packed in arrays parallel to the lower thallus surface by unistratose, pigmented, multicellular scales (Fig. 1*B*). Rhizoids and lower epidermal tissues from which rhizoids arise exhibited yellow-green autofluorescence in violet excitation (Fig. 1*C*) and blue-white autofluorescence in UV excitation. Core rhizoids and other cells composing the stalks of archegoniophores (typical of fertile *Marchantia* thalli) were also autofluorescent (Fig. 1*D*). The mean length of 20 oven-dried archegoniophore stalks that were used in subsequent quantitative acetolysis procedures was 3 cm.

**Posttreatment Remains.** Portions of the *Marchantia* thallus that survived high-temperature hydrolysis or rotting treatments included scraps of the lower epidermis with attached rhizoids (Figs. 1*E* and 2 *A* and *B*) or thick-rimmed pores from which rhizoids had become detached (Figs. 1 *F* and *G* and 2 *A* and *D*). Broken-off rhizoids occurred singly (Fig. 1*H*), particularly when the degradation process was mechanically harsh (acetolysis), or in clumps (Fig. 1*I*), especially under the milder conditions of rotting. Resistant *Marchantia* lower epidermis and emergent rhizoids retained preexisting autofluorescence properties after acetolysis or rotting (Fig. 1 *E* and *F*). Occlusions were observed in some pores of both modern degraded material (Fig. 1*G*) and the microfossil (Fig. 1*J*). Some rhizoids exhibited autofluorescent, resistant internal cell-wall ornamentations (Fig. 1*E*), and some appeared spiraled (Fig. 2*E*). At the point of rhizoid emergence from lower epidermal cells, cell walls were particularly brightly autofluorescent (Fig. 1*E*), as were the rims of pores (Fig. 1*F*). Rhizoid-producing lower epidermal cells occurred at the center of a rosette of epidermal cells (Fig. 1*E*), as did pores formed by rhizoid detachment (Figs. 1*G* and 2 *B–D*) and those of the microfossil cell sheets (Fig. 1*J*). Similar rosettes were observed in the lower epidermal remains of rotted *C. conicum* (data not shown). In addition to lower epidermal tissues, the rhizoids that occur in clusters at cores of *Marchantia* gametangiophore stalks (Fig. 1*D*) survived rotting or acetolysis, occurring as single tubes or clusters of tubes. Quantitative acetolysis revealed that 14.4% of the dry biomass of *Marchantia* vegetative thalli, 39.8% of gametangiophore stalks, and 40.7% of gametangiophore heads are composed of resistant organic materials. Resistance and autofluorescence properties were consistent with the presence of cell-wall-bound polyphenolics.

In summary, we observed that the resistant lower epidermis of marchantioid liverworts can appear as a sheet of cells devoid of rhizoids, with pores where rhizoids have broken off, when degradation has been sufficiently extreme (Fig. 1 *F* and *G*). We also observed that rhizoids of degraded liverwort may occur singly (Fig. 1*H*) or as masses (Fig. 1*I*) lacking organic attachment to lower epidermal cell sheets.

## Discussion

Our innovative degradation and comparative process revealed that modern liverworts vary in resistance properties: the marchantioid liverworts *Marchantia* and *Conocephalum* are more resistant and thus yielded more remains than other liverworts tested. Additionally, among the tissues present in *Marchantia* (excluding spores and elaters), only the lower epidermis (including tubular rhizoids that extend from it) and gametangiophore components originating from the lower epidermis survived degradation procedures. Our results show that the remains of rotted or hydrolyzed *Marchantia* (and rotted *Conocephalum*) resemble some of the presumed sheets of cells or cuticle and some of the tubular entities that have been assigned to the nematophytes. A synopsis of recent interpretations of microfossils (cell sheets and tubes) attributed to early land plants and comparison of these fossil entities with degraded liverworts follow.

**Sheets of Cells.** Edwards (2) described a number of isolated cuticle fragments showing cellular patterning of Late Silurian age from South Wales, which she considered most similar to *Nematothallus*, and also masses of tubes. She recommended restricting the concept of *Nematothallus* to "cuticular" remains, and assigning another name to those tubes that did not belong to *Prototaxites*, which is composed of aggregations of at least two types of tubes into massive thalli several meters long and up to 1 m in diameter. No formal classification was established for the different types of cuticle and tubes, although she did recognize several morphotypes. Edwards and Rose (7) described cuticular fragments showing cellular patterning from the earliest Devonian of England and referred them to *Nematothallus* but also compared them with remains of spongiophytes. Edwards (18) described cuticle fragments with a distinctive morphology (cellular scraps having numerous pores, each surrounded by a rosette of cell-wall remains) within an artificial classification system as several species of the genus *Cosmochlaina*, these again being of Silurian and Devonian age. Cell sheets similar to *Cosmochlaina* were also described from the Early Devonian of New Brunswick (6).

We argue that lower epidermis remains of at least some marchantioid liverworts closely resemble certain microfossil cell sheets (cuticles) attributed to nematophytes, particularly those known as *Cosmochlaina*. We propose that the pores characteristic of *Cosmochlaina* represent sites of rhizoid break-off. Our results reveal how easily liverwort rhizoids break off, leaving cell sheets with pores similar to those of *Cosmochlaina*. The following features of *Cosmochlaina* and resistant liverwort lower epidermis are held in common: resistance to degradative processes, patterns of evenly spaced perforations, perforations centered within rosette cellular patterns, perforations having a range of diameters, perforations having thickened rims, pores

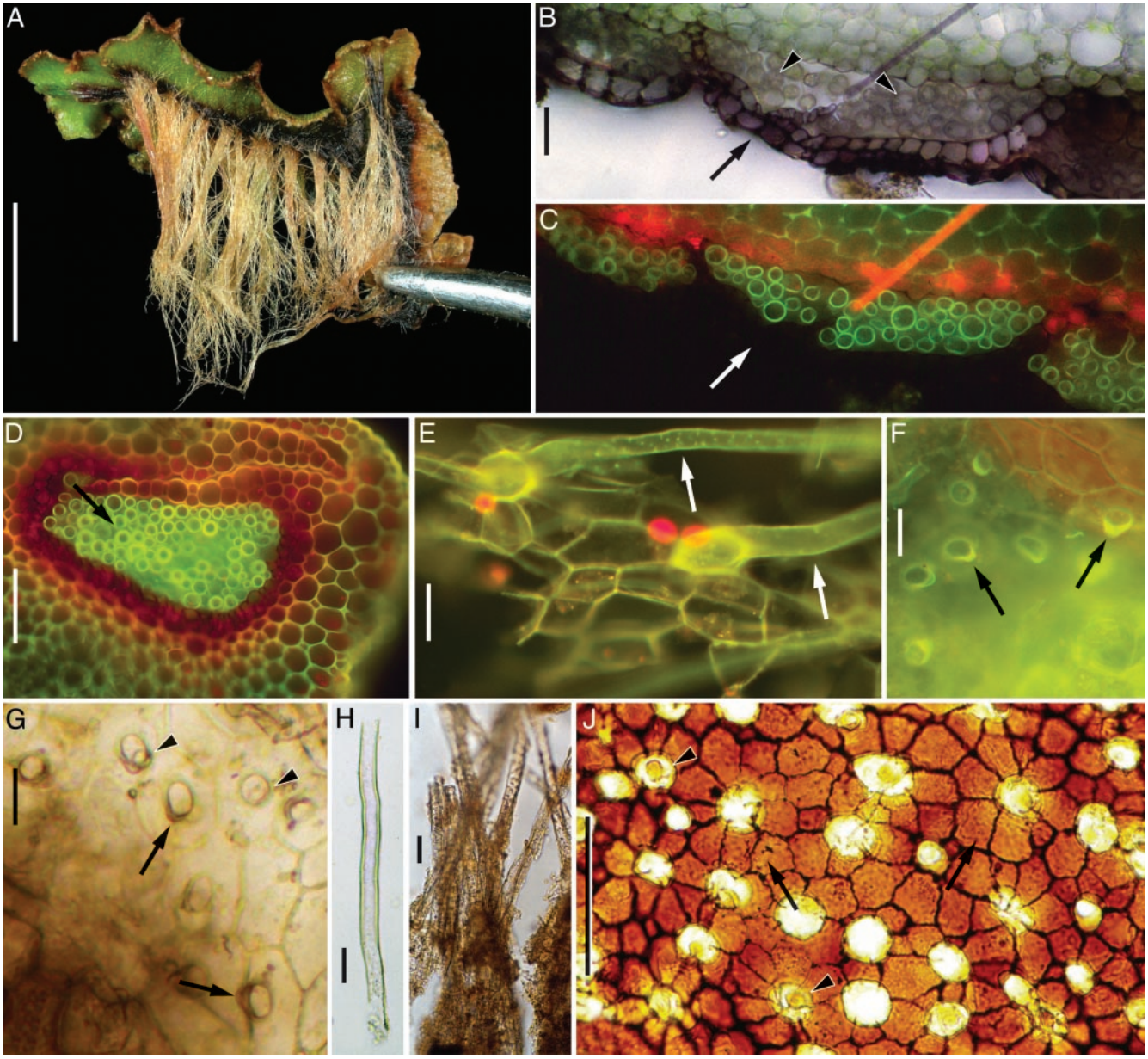


**Fig. 1.** Living and rotted *M. polymorpha* compared with the microfossil *Cosmochlaina*. (*A*) The underside of living *Marchantia* thallus showing large masses of rhizoids especially along the midline. (*B*) Handmade section through living *Marchantia* thallus showing rhizoids (arrowheads) held close to lower epidermis by unistratose scales (arrow). Rhizoids occur in varying diameters and morphology; some exhibit wall ingrowths known as pegs. (*C*) Section shown in *B* viewed in violet excitation. Lower epidermal tissue and rhizoids exhibit yellow-green cell-wall autofluorescence; rhizoids exhibit varying diameters and wall thickness. Scales are not autofluorescent, possibly because of quenching by other cell-wall materials [like rhizoids, lower epidermal scales survive rotting and acetolysis (data not shown)]. The orange filament is a soil cyanobacterium. (*D*) Freehand cross section of living *Marchantia* gametangiophore stalk, which is formed by infolding and upward growth of thallus branch. Rhizoids of varying diameter and wall thickness occur in a cluster at the core (arrow). (*E*) Remains of rotted *Marchantia* thallus, consisting of lower epidermal cells and rhizoids of differing morphology and width (arrows). Rhizoid bases are at the centers of cell rosettes and have thicker walls with brighter fluorescence. (*F*) Lower epidermis of rotted *Marchantia* showing pores with rims that are brightly autofluorescent. Pores are bases (arrows) of rhizoids that have broken off during treatment. (*G*) Same specimen as shown in *F*, viewed with bright-field optics. Arrows point to rims of broken-off rhizoids. Occlusive structures may occur in pores (arrowheads). (*H*) An example of an isolated, broken rhizoid that typically occurs when *Marchantia* bodies are subjected to the extreme degradation process of acetolysis. (*I*) Rhizoids may occur in tangled masses when *Marchantia* bodies are subjected to the milder degradative process of rotting. (*J*) Unistratose cell sheet of the nematophyte *Cosmochlaina* showing pores of differing diameter and morphology surrounded by cell rosettes (arrows). Some pores have a central protuberance (arrowheads). (Scale bars: *A*, 1 cm; *B*, *C*, and *E–J*, 50 $\mu$m; *D*, 100 $\mu$m.)

sometimes occluded, and tubular structures emerging from centers of rosette cell patterns. With reference to the latter feature, some published images (figure 32 in ref. 7 and figure 42 in ref. 18) show a short, tubular protrusion extending from the center of a cell-sheet rosette. These protrusions could be interpreted as developing or broken-off rhizoids.

**Tubular Entities.** Burgess and Edwards (19) described aggregations of different-sized and patterned tubular remains, also from Late Silurian and Early Devonian of the Welsh Borderland, naming masses in which two particular types of tubes occurred *Nematasketum*. Burgess and Edwards (20) classified isolated and masses of tubular remains by again using an artificial system and

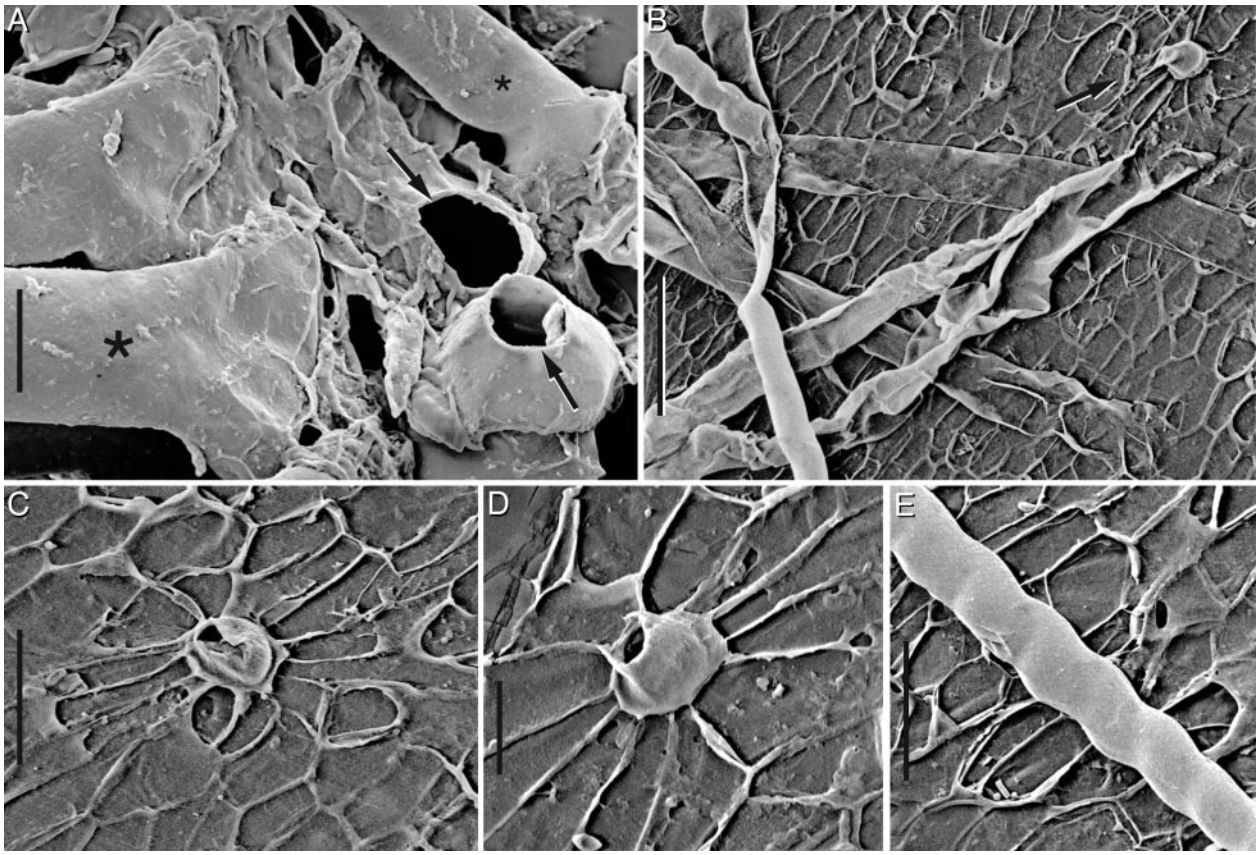


**Fig. 2.** Scanning electron microscopic views of rotted *M. polymorpha* thalli. (*A*) Rhizoids of two sizes (asterisks) and bases of detached rhizoids (arrows), which contain pores of differing size. (*B*) Rhizoids of differing diameter lying on lower epidermis. Some collapsed during the preparative treatment for scanning electron microscopy. Arrow points to a rosette of epidermal cells with the base of a detached rhizoid. (*C*) Lower epidermis rosette with central rhizoid base. (*D*) Higher-magnification view of another rosette with central rhizoid base. (*E*) A spiral rhizoid. (Scale bars: *A* and *D*, 25 $\mu$m; *B*, 100 $\mu$m; *C* and *E*, 50 $\mu$m.)

discussed similarities and differences between *Nematothallus*, *Nemataskетum*, *Nematoplexus*, and *Prototaxites*. Some tubes are smooth-walled, whereas others exhibit different types of thickenings, many being helical. Tubes also vary from unbranched to branched. Gensel *et al*. (6) also presented an informal set of categories of tubular remains. Strother (3) described "thalloid" masses of tubular remains as several species of *Nematothallu*s from the Silurian of Pennsylvania. Although no cuticle or epidermis is shown in actual attachment, he figured one unconvincing specimen with a possible site of attachment to a surficial covering and considered that some kind of protective covering was possible. Strother (4) later provided a taxonomic clarification of the concepts of *Nematothallus* and the Nematophytales. He restricted the genus and order to entities with tubular anatomical construction, the tubes often being of two sizes and sometimes showing complex wall structure in the form of thickenings or fibrils. The masses of tubes were either axial or thalloid. He assigned the Nematophytales to the informal group Paraphyta within protists, i.e., plant-like entities of unknown affinity. Strother established a new family, Nematothallaceae, for small crustose to lobate thalli, with tubular anatomy composed of one or more distinct kinds of tubes. The possibility that these tubular structures may be associated with "cuticle with pseudocellular patterning" or simple cuticle was retained, although again no conclusive evidence for attachment of tubes to cuticles had been obtained. He retained Lang's (1) original generic diagnosis for *Nematothallus* but revised the specific diagnosis of *Nematothallus pseudovasculosa* and designated a lectotype. The revised species diagnosis does not include mention of the cuticle or spores and is as follows (ref. 4, p. 1091): "Wefts of resistant, compressed tubes of two distinct sizes; smaller tubes unaligned, with smooth-walled surface, homogeneous wall structure, *ca*. 2.5 $\mu$m in diameter; larger tubes *ca*. 25 $\mu$m wide, thin-walled with annular thickenings." It was Strother's intent to establish the group, and the genus, as organisms of a fundamentally tubular composition, and we agree with this circumscription of his material.

The majority of tubular remains first appearing in the Ordovician (and extending to the Lower Devonian) of the Anglo-Welsh Basin are considered to be nematophytes (20). Mats of small, sinuous tubes "that could represent plant tissue" have been described from the U.S. Middle Cambrian Bright Angel Shale assemblage and the Ordovician Hadadir Formation of Saudi Arabia, and "pseudocellular cuticular fragments" also thought to be possible land-plant remains occur in the latter deposits (21). Such microfossils suggest that the history of organisms interpreted as nematophytes likely began even earlier than the Silurian and Devonian, from which tubular entities are best known.

In regard to both isolated tubes and masses of tubes, several possible affinities have been suggested. Some, such as tubular elements adhering to the sporangium of *Tortilicaulis* (22) or tubular features on sporangia of a tetrad-containing plant of putative affinity to liverworts (23), may represent fungal hyphae (5). Some might have been components of lichens or the resistant sheaths of cyanobacteria. *Prototaxites* has been attributed to the fungi (24). Similarity of acid-hydrolyzed calyptra remains of the moss *Polytrichum* to particular thick-walled, branched tubes

from Silurian and Devonian deposits suggests that at least some of the latter could represent resistant parts of early mosses (16). Also, as discussed earlier, wefts of tubes of discrete form are attributed to *Nematothallus*.

We hypothesize another possibility for some of the unbranched tubular entities, particularly those occurring in aggregations. We argue here that liverwort rhizoid remains resemble some nematophytic tubular microfossils in the following ways: resistance to degradative processes, unbranched, lacking transverse walls, some exhibiting regular internal ornamentation, some spiraled, occurrence of tubes of varying widths in the same specimen, and occurrence as masses or individual tubes.

On the basis of our data, we interpret *Cosmochlaina*, originally considered to be of uncertain affinity, as the lower epidermal surface of a marchantioid liverwort and at least some of the tubular aggregations assigned to nematophytes (and possibly to *Nematothallus*) as masses of resistant liverwort rhizoids, sometimes attached to pieces of lower epidermal tissue. Rhizoid aggregates from gametangiophore stalks, which are resistant to decay and sometimes abundant, might explain some occurrences of *Nematothallus*. Resistance of lower epidermal tissues and rhizoids, which are in contact with soil-decay microbes, is likely adaptive and may explain not only the existence of nematophyte cell sheets and tubes but also the perennial persistence of extensive growths of modern marchantioids.

We do not suggest that *Cosmochlaina* and *Nematothallus* are the remains of archaic *Marchantia* or *Conocephalum*, only that some ancient microfossil tubes and cell sheets may well be remains of ancient liverworts that shared some features with modern marchantioids. During the hundreds of millions of years that have passed between the times of earliest liverworts and their modern descendants, innovative features likely evolved. Hence, some specialized characteristics of modern marchantioids, such as pegged rhizoids or flared rhizoid bases, might not be expected to occur in fossils of ancient relatives. Our results show that during degradation, liverwort rhizoids can separate from other lower epidermal tissue when mechanical disruption of the body occurs. This is consistent with the separate occurrence of cell sheets and tubular remains that is typical of the fossil record. Our results do suggest the possibility that fossil liverworts showing clear connection between cell sheets and tubes may yet be found in Cambrian–Silurian deposits formed under favorable conditions.

Our data suggest that not all of the organismal lineages classified as nematophytes have become extinct, nor do they all represent an unusual, unsuccessful body construction type. In our interpretation, at least some of the nematophyte fossils illustrate both typical plant tissue (interpreted as epidermal remains) and the earliest known fossil examples of plant polar tip growth in the form of rhizoids. Tip growth is an important feature of plant morphogenesis that is also exemplified by higher-plant root hairs and pollen tubes. Our observations that resistant epidermal tissue and rhizoids occur at the core of *Marchantia*'s gametangiophore stalks suggest that they may have provided structural support as well as materials transport, analogous to xylem tissues of vascular plants. Our interpretations of nematophyte fossils also suggest that marchantioid liverworts were present from at least the Silurian, hundreds of millions of years earlier than the Early Mesozoic, as previously thought (25). Such interpretations would be consistent with molecular phylogenetic evidence for early divergence of liverworts from the lineage leading to vascular plants (8). Our quantitative studies of resistant carbon suggest that Lilliputian forests of marchantioid liverworts, if present in the Early Paleozoic (as suggested by microfossil nematophytes), may have sequestered resistant organic carbon. If sufficient amounts of this liverwort-produced carbon were buried, there may well have been an impact on early terrestrial carbon cycles, an effect also proposed for early moss-like plants (17), long before dominance of woody land plants. Quantitative determinations of resistant carbon in additional liverwort taxa as well as estimates of cover and productivity for these taxa will be necessary to estimate the possible global carbon-cycle impact of liverwort-like early land plants.


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