

Piecing together the eophytes – a new group of ancient plants containing cryptospores

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Summary

- The earliest evidence for land plants comes from dispersed cryptospores from the Ordovician, which dominated assemblages for 60 million years. Direct evidence of their parent plants comes from minute fossils in Welsh Borderland Upper Silurian to Lower Devonian rocks. We recognize a group that had forking, striated axes with rare stomata terminating in valvate sporangia containing permanent cryptospores, but their anatomy was unknown especially regarding conducting tissues.
- Charcoalified fossils extracted from the rock using HF were selected from macerates and observed using scanning electron microscopy. Promising examples were split for further examination and compared with electron micrographs of the anatomy of extant bryophytes.
- Fertile fossil axes possess central elongate cells with thick walls bearing globules, occasional strands and plasmodesmata-sized pores. The anatomy of these cells best matches desiccation-tolerant food-conducting cells (leptoids) of bryophytes. Together with thick-walled epidermal cells and extremely small size, these features suggest that these plants were poikilohydric.
- Our new data on conducting cells confirms a combination of characters that distinguish the permanent cryptospore-producers from bryophytes and tracheophytes. We therefore propose the erection of a new group, here named the Eophytidae (eophytes).

Introduction

The nature of the first land flora is an enduring mystery in which the principal players have never been seen in their entirety, yet much can be inferred about their characteristics from what they have left behind. The plant tree of life provides one perspective on the nature of this flora and the timeframe of early plant evolution (Morris *et al.*, 2018; Puttick *et al.*, 2018; Leebens-Mack *et al.*, 2019). Spores dispersed in sediments provide another, and they are the principal source of fossil evidence (Gray, 1985, 1993; Strother *et al.*, 1996, 2015; Wellman *et al.*, 2013). The earliest and most distinctive of these spores are called cryptospores, a term that encapsulates their cryptic nature and acknowledges their obscure affinities (Gray & Boucot, 1971; Strother & Traverse, 1979; Johnson, 1985; Richardson, 1988, 1996; Wellman, 1996; Steemans, 2000; Strother, 2000). The most common configurations of dispersed cryptospores are permanent tetrads, permanent dyads or hilate monads, which possess a circular hilum that marks the contact area between naturally dissociating dyads. Permanent tetrads and permanent dyads are sometimes enveloped by an additional wall. Cryptospores dominate fossil assemblages for some 60 million years, first appearing about 470 million years ago (Ma) during the Ordovician Period and

diversifying rapidly. This period was referred to as the Eoembryophytic epoch by Gray (1993). Cryptospores declined abruptly in taxonomic diversity and abundance about 410 Ma, during the latest Lochkovian (Early Devonian), with only a few forms persisting through to the Emsian (late Early Devonian) (Wellman *et al.*, 2013). By contrast, monads, bearing a trilete mark produced from the natural dissociation of tetrads, began their diversification some 430 Ma during the latter part of the Silurian Period, rising to dominance and persisting as the major element in dispersed spore assemblages (Wellman *et al.*, 2013). While trilete monads are widely considered to be derived from vascular plants, cryptospores have no close modern analogues, except perhaps for some spore configurations in a handful of liverworts (Renzaglia *et al.*, 2015a,b), so the nature of the plants that produced them remains a great unsolved problem in evolutionary botany.

The cryptospore-producing plants were most probably primitive land plants (i.e. embryophytes). This seems likely given their general form and development (Wellman & Gray, 2000; Wellman *et al.*, 2003), the ultrastructure of their spore walls (Taylor, 1995, 1996, 1997) and their chemical composition (Steemans *et al.*, 2010), but analysis of spore walls can get us only so far. The earliest clue to their nature came from spore masses

recovered from a borehole through Ordovician rocks in Oman (Wellman *et al.*, 2003). Although very fragmentary, these fossils showed that cryptospores developed within small sporangia. A more complete picture emerged from minute fossils preserved in charcoal in much younger rocks (Upper Silurian to Lower Devonian) of the Welsh Borderland, UK (Wellman *et al.*, 1998a,b; Edwards *et al.*, 1999, 2014; Habgood, 2000; Edwards *et al.*, 2012a,b; Morris *et al.*, 2018). These sites fall within the latter part of the stratigraphic range of the cryptospores, so they capture the nature of these plants shortly before their demise. By this time vascular plants were also part of the flora, but the cryptospore-producing plants proved to be distinctly different and significantly smaller. Due to the brittle nature of charcoal these fossils are highly fragmentary, so piecing together whole plants is a significant challenge. Among the most informative elements are tiny sporophytes measuring only a few millimetres in length. A distinct group have simple forking axes with distinctive longitudinal ridges that terminate in sporangia in which permanent cryptospore dyads and tetrads, similar to those dispersed in sediments, were observed *in situ* (Edwards *et al.*, 2012a; Morris *et al.*, 2012). Sporangia are mostly valvate with rare stomata. Based on limited data from these partial sporophytes, a sister group relationship with tracheophytes was considered plausible for some species (Edwards & Kenrick, 2015). However, even though the anatomical features are very well preserved in charcoal, the data are not sufficiently complete to resolve relationships with confidence. Whether or not clearly separating dyads (hilate monads) were derived from this group remains uncertain. Further information on the life cycle and on internal anatomy, especially the character of the vascular system, is needed both to clarify the affinities of these plants and to understand better their nature and how they functioned.

Here we document a selection of new materials screened from hundreds of fragments of charcoal. These include specimens in which internal tissues are preserved, including subcellular structures. We compare these fossils to cell types in extant bryophytes and lycophytes. Some fossilized cells exhibit properties associated with food transport that are comparable to features observed in modern food-conducting cells (leptoids) and sieve elements. Together, these new data reveal a suite of characteristics that distinguishes the permanent cryptospore-producing plants from both bryophytes and vascular plants, hinting at the existence of a novel and hitherto unknown major group of early land plants.

Materials and Methods

Fossil specimens

Minute and fragmentary fossils preserved in charcoal, here termed mesofossils, were extracted from fluvial grey siltstone beds exposed in a stream section north of Brown Clee Hill, Shropshire, UK (Edwards *et al.*, 1994). The rock sequences are part of the Ditton Formation, now known as the Freshwater West Formation (Barclay *et al.*, 2015). Dispersed palynological assemblages were studied by John Richardson, who discovered the locality and assigned it to the middle subzone of the *micromatus* –

newportensis Sporomorph Assemblage Biozone (Richardson & McGregor, 1986), indicating an early Lochkovian (Early Devonian) age (*c.* 410–419 Ma).

Organic fractions were extracted from the siltstone first by gentle disaggregation in water followed by maceration treatments in HCl and HF. The fossils were recovered from the macerates by sieving and sorted under a stereo light microscope, followed by screening by scanning electron microscopy (SEM). Reflectance values measured from the plant remains indicate that they were partially charred by low-temperature wildfire (Glasspool *et al.*, 2006). Charcoalification has allowed for some three-dimensional preservation, but they have also been subjected to varying degrees of shrinkage, compression and homogenization (Edwards & Axe, 2004), which tends to be greatest in the narrower and perhaps younger axes. Pyrite crystals fill some cell lumens and intercellular spaces, which sometimes prevent further collapse (or compression), but can also damage wall surfaces (fig. 8 in Edwards & Axe, 2004).

Internal tissues were examined by fracturing the charcoal. Typically, the wider axes were split longitudinally using a razor blade, but with the narrower axes this approach proved to be ineffective, resulting in shattering or haphazard fracturing of the tissues rather than ‘clean’ longitudinal breaks. An alternative technique involved covering the specimen on the stub with a carbon disc, applying slight pressure and then tearing the disc off with a pair of tweezers in the hope that the specimen would split longitudinally, but again in most cases axes shattered. Achieving consistently good results proved impossible, so for the majority of specimens we have little information on the three-dimensional organization of internal tissues. Where these methods were applied successfully, cells exposed were rarely intact. Typically, they were compressed or present irregular fractured views of wall and lumen.

Specimens with numbers prefixed with the letter ‘V’ are housed in the Natural History Museum, London (NHMUK).

Extant bryophytes

For SEM, freshly collected sporophytes of the mosses *Mnium hornum* Hedw. and *Polytrichum juniperinum* Hedw. were placed in 10% ethanol to extract the cytoplasm, cut longitudinally, fixed in 3% glutaraldehyde, dehydrated through an ethanol series, critical-point dried using CO₂ as the transfusion fluid, sputter coated with 20 nm palladium–gold and viewed using an FEI Quanta scanning electron microscope (FEI, Hillsboro, OR, USA). A full protocol for the transmission electron microscopy (TEM) images of the moss *Polytrichum formosum* Hedw. and the liverwort *Haplomitrium gibbsiae* (Steph.) R.M. Schust. used in this account can be found in Ligrone & Duckett (1994).

Results

Fossils – general morphology and cell walls of conducting tissues

The fossil materials documented here were selected from hundreds of fragments of charcoal extracted from a few kilograms of

Table 1 Summary of characteristics of fossil specimens.

Specimen	Illustrations	Branching	Anatomy of central cells	Spores	Stomata
V 68851	Fig. 1(a–k)	–	Strands and globules	✓ (tetrads)	–
V 68852	Fig. 2(a–h)	✓	Strands	✓ (tetrads)	–
V 68853	Fig. 2(i–o)	✓	Globules	✓ (dyads)	–
V 68854	Fig. 3(a–e)	–	Globules, granular and pits	–	–
V 68855	Fig. 3(f–n)	–	Globules and granular	✓ (dyads)	✓
V 68856	Fig. 4(a–c)	–	Globules?	?	✓
V 68857	Fig. 4(d,l)	–	Globules	–	–
V 68858	Fig. 4(d, m)	–	Globules	–	–
V 68859	Fig. 4(f)	✓	Globules and pits	–	–
V 68860	Figs 4(g), 5(d,e)	✓	Globules and pits	–	–
HD(L)117/06	Figs 4(h), 5(a)	–	Globules, strands and pits	–	–
HD(L)127/01	Figs 4(i), 5(b, c)	✓	Globules and pits	–	–
V 68861	Figs 4(j), 5(f–h)	✓	Globules and pits	–	–
V 68862	Figs 4(k), 5(i,j)	✓	Globules and pits	–	✓

rock at one geological site. They are of similar size and mode of preservation, and they show the range of external form and tissue types, including epidermis, vascular system and spores (Table 1). We divided the material into fertile elements (i.e. those with attached remnants of sporangia and spores) and sterile axes. Although it is not provable that these unconnected fragments were part of an individual plant or perhaps even a single species, all elements bear similarities that lead us to conclude that they are representative of a distinctive group of plants. They show similarities in their sporangia, as far as can be determined because these are incomplete, and in the general nature of their spores. The fertile and sterile remains can also be linked by their highly distinctive epidermis that has ridges that run longitudinally. The ridged features are not cellular, but they do reflect the underlying walls of the elongate peripheral cells of the axis, being formed as protrusions of these. It is our view that these are natural features of the plants or that they reflect natural underlying features rather than being taphonomically induced. They are therefore of taxonomic value. Fertile and sterile remains also bear similarities in their vascular systems. The sterile materials were selected to illustrate the type of branching and aspects of the vascular system.

Fertile specimens Specimen V 68851 is an unbranched axis (Fig. 1a) with typical superficial ridges running longitudinally and intervening horizontal wrinkles. It illustrates how the peripheral tissues within the axis become homogenized during the charcoalification process, losing definition of their cellular composition (Fig. 1b). The axis terminates in a hollow funnel-shaped structure (Fig. 1c) that contains poorly preserved, compressed clusters of fused spores termed polyads. These are probably the remains of spores destined to be dispersed as permanent tetrads that have become partially separated into their individual elements (Fig. 1d,e). A fused line of attachment that transitions into two free spore walls is visible in Fig. 1(f) (arrow i), which is a magnification of the tetrad in Fig. 1(d). Another example shows a monad that has been torn away from the tetrad, revealing a laevigate proximal surface with an irregular ‘hilum’ which is approximately half of the total spore diameter (Fig. 1g). Individual units of the tetrad measure *c.* 35 µm in diameter. The distal walls of

most are ornamented by grana of variable size (average 2 µm in width, 1 µm in height) that are moderately to closely spaced (Fig. 1f). While the grana appear to be continuous with the spore walls (arrow ii, Fig. 1f), in places they cluster together and are discontinuous, indicating that this ornament might be tapetal residue. All spore surfaces are also covered by a minute granular substance, probably a fluoride precipitate (Fig. 1f,g). A transverse fracture of the stem shows that the robust cell walls of the peripheral tissues are fused with the less distinctive cell walls of centrally located tissues (Fig. 1b,h). Longitudinally fractured specimens show that the internal walls of most cells are smooth (Fig. 1i), but some bear growths in the form of strands that sometimes traverse the lumen (Fig. 1j), globules (Fig. 1j,k) and small irregular holes (arrows in Fig. 1k).

Specimen V 68852 illustrates that axes bearing sporangia also dichotomized (Fig. 2a,b). This axis has the typical longitudinal ridging and intervening minute ornament (Fig. 2c) and wrinkling (Fig. 2d), which is particularly evident when viewed obliquely. One branch terminates in a cup-shaped feature that contains a few spores. This is interpreted as the remnants of the base of a broken sporangium. The spores are small, *c.* 24 µm in diameter, and because no haplotypic features were observed we conclude that they are permanent polyads (Fig. 2e). It is difficult to determine the configuration of these polyads, but the thick folds that separate slightly (arrow in Fig. 2e) are interpreted as the junctions between the individual units: thus, they resemble tetrads. The walls have a thin and crumpled appearance, producing an irregular, fine folding or reticulum (Fig. 2f) that might represent the presence of an enclosing envelope. The distal walls are also sculptured with micrograna, *c.* 0.2 µm in width and 0.1 µm in height, that are widely spaced (1.5 µm apart). In the dispersed record, *Velatitetras* was erected to accommodate adherent tetrads enclosed within an envelope (Burgess, 1991); *V. anatoliensis* Steemans *et al.* (1996) is the only species to have an envelope ornamented with grana, but they are more closely packed and larger in width and height (0.5 µm) than the *in situ* spores documented here. Fractured sections of axis reveal that tissues of the interior had uniformly homogeneous cell walls (Fig. 2g). A few show strands extend into the lumen or adpressed granules (Fig. 2h).

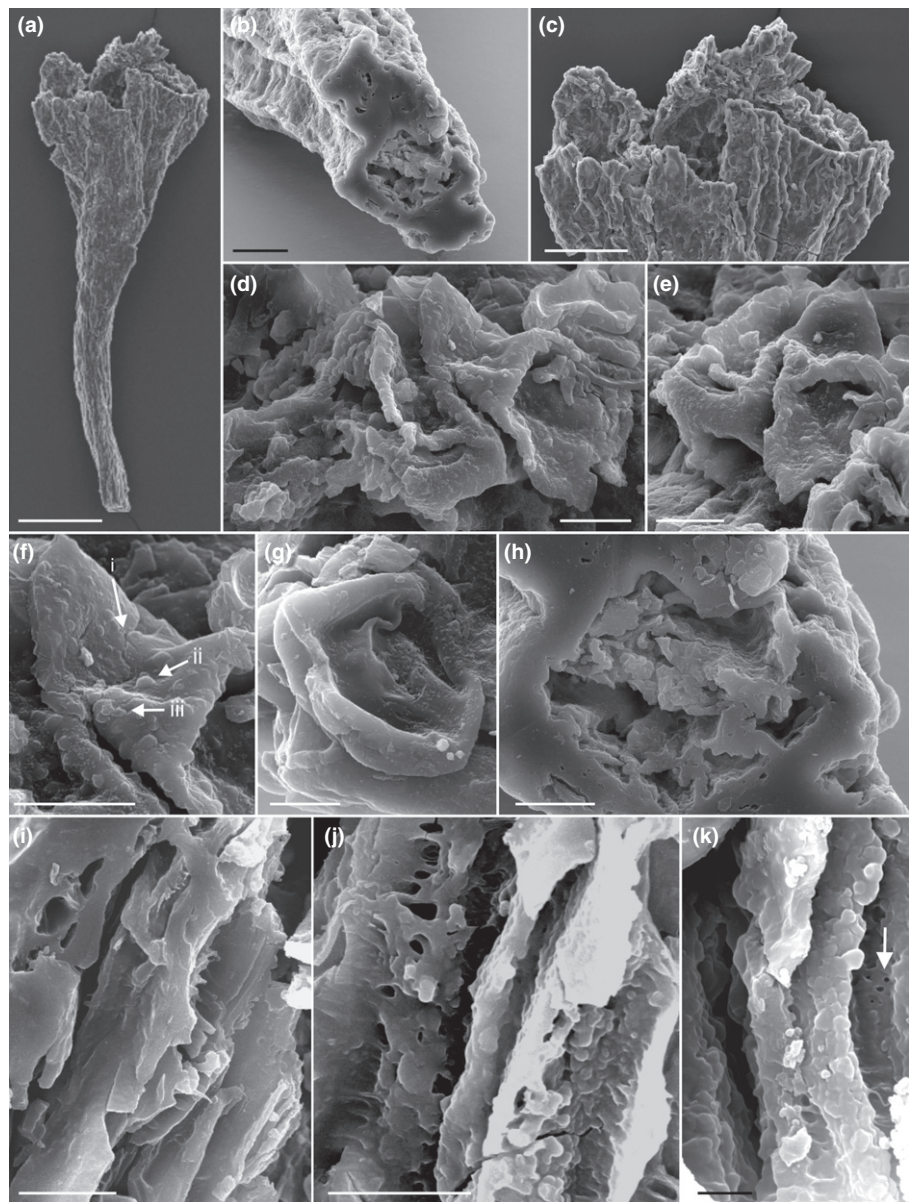


Fig. 1 Fertile specimen, V 68851, Lochkovian Welsh Borderland. (a) Specimen as isolated, base of terminal possibly valvate sporangium. (b) Transversely fractured base of axis with homogenized peripheral region. Surface on left shows typical ridging. (c) Distal parts of sporangium magnified from (a). (d–g) Poorly preserved clusters of polyads/tetrads. Arrows in (f): (i) line of attachment; (ii), granum (?) continuous with surface; (iii), cluster of grana. (h) Central area of axis, magnified from (b). (i–k) Longitudinal fracture of central region. (i) Smooth walled fragments, superficial cells top left. (j) Walls with globules and strands (latter possibly taphonomic). (k) Walls with globules and small pits (arrow). Bars: (a) 200 µm; (b) 20 µm; (c) 100 µm; (d, e, h, i, j) 10 µm; (g) 5 µm; (k) 2 µm.

Specimen V 68853 also dichotomizes (Fig. 2i) and it shows the typical ridged and wrinkled surface features (Fig. 2j). One branch terminates in a shallow saucer-shaped sporangial base, the inner wall of which is covered with an amorphous folded layer of tissue to which a few spores adhere (Fig. 2k). Both the spores and the sporangial wall are covered with microgranular globules, probably being tapetal residue (Fig. 2l,m). The spores are thin-walled and folded permanent polyads, with no haplotypic features. Where fractured, the internal surfaces of the spores are smooth, with no evidence of any internal cross walls. The sporangial features of this specimen are similar to those of *Ficoiditheca* (Morris *et al.*, 2012), particularly the nature of the spore-bearing layer and the spores. In *Ficoiditheca*, sectioning of the spores revealed that they were permanent dyads, but of a form not recognized in the dispersed record. Cellular preservation of tissues is

particularly complete (Fig. 2j). Epidermal cells are large with uniformly thickened walls (Fig. 2n), but views of the inner wall surface are hindered by pyrite crystals (Fig. 2o). Central cells are more compressed, which obscures their form and structure (Fig. 2j, arrowed in Fig. 2n); they possess internal walls with traces of tiny mounds.

Better preserved central cells are present in specimen V 68854 (Fig. 3a–e). This ridged and wrinkled axis has a distinct twist, and it terminates with the remnants of the base of a broken sporangium that lacks spores (Fig. 3a). The fractured end of the axis reveals abundant disorganized cell walls, some of which appear to have fused together (Fig. 3b), a feature that is typical of charcoal. The lumen-facing walls of the central cells have abundant globules, fused either singly or clustered (Fig. 3c–e). A few minute pits are present (arrow in Fig. 3e). Other cells appear to have

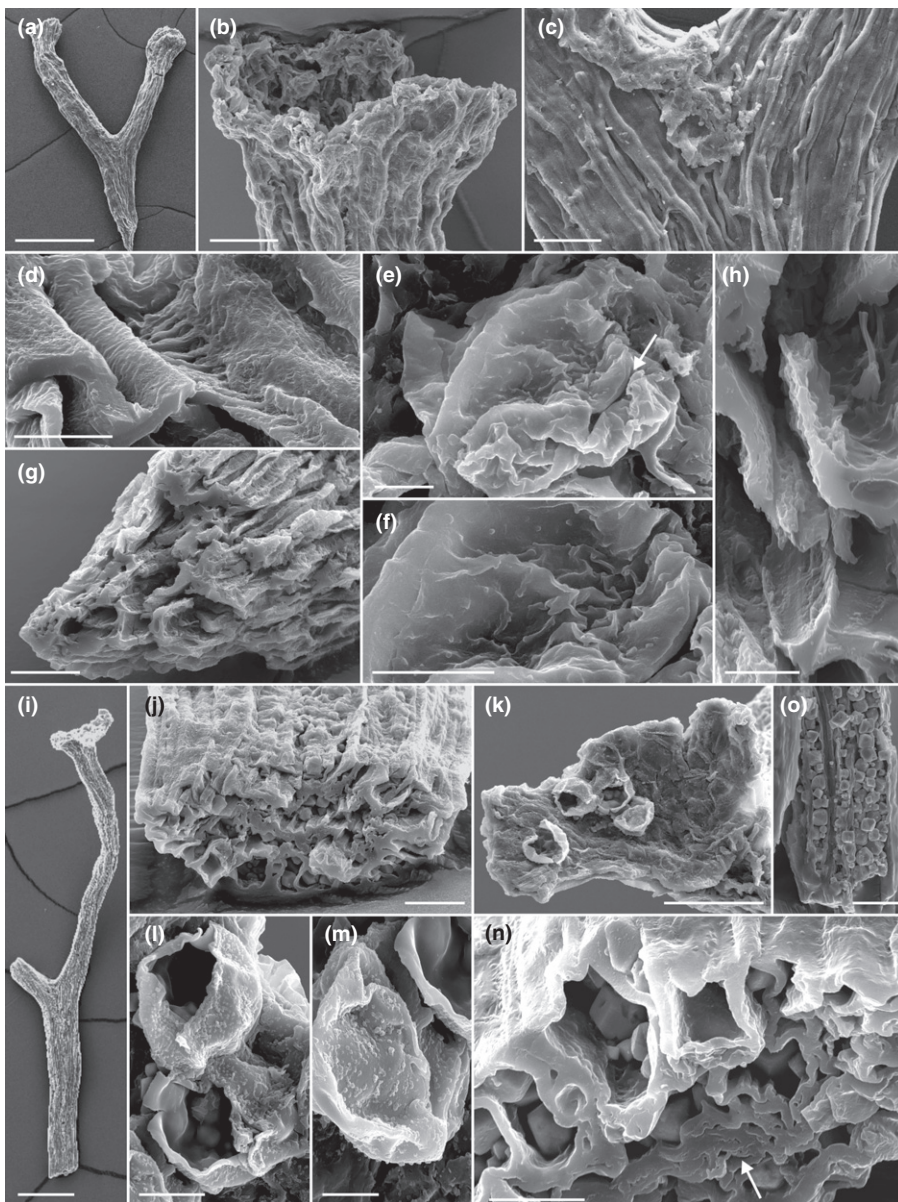


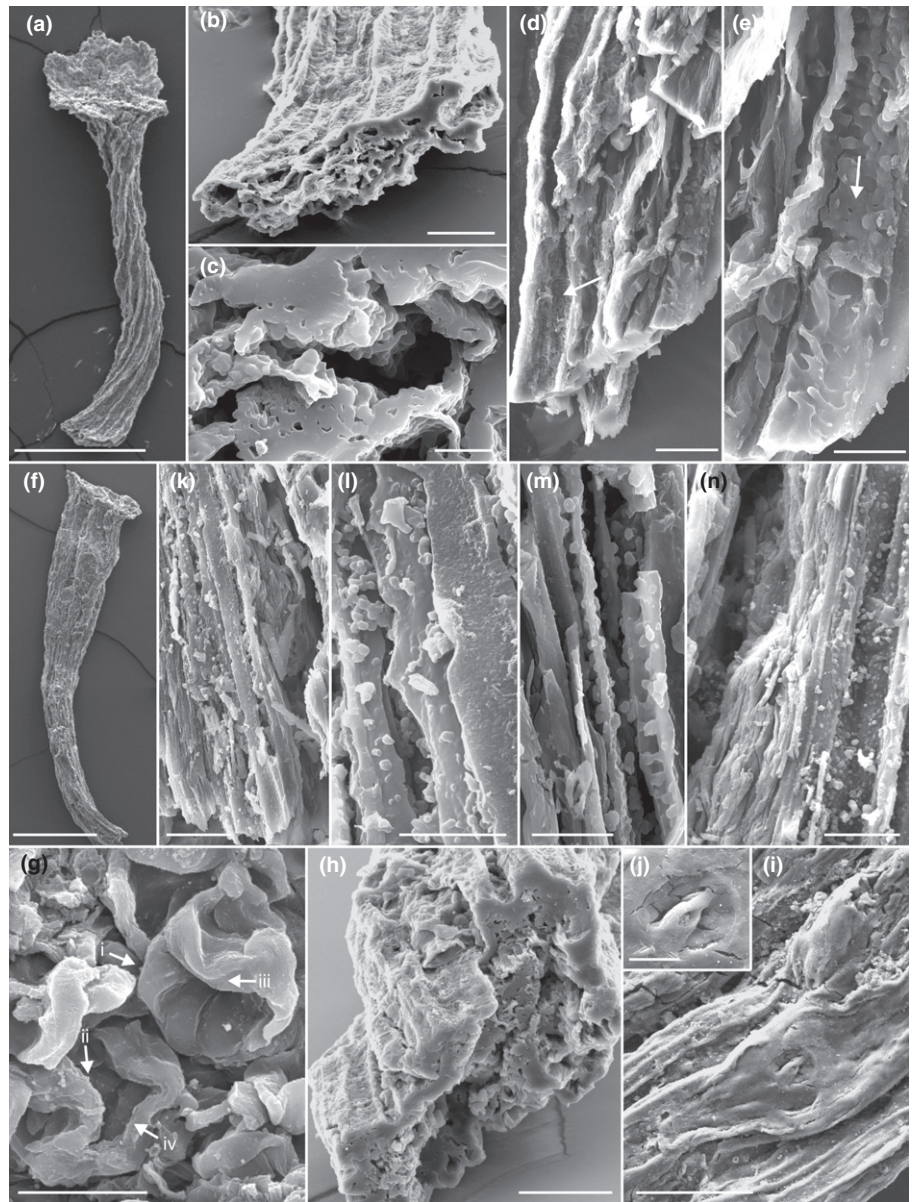
Fig. 2 Fertile specimens: (a–h) V 68852 and (i–o) V 68853, Lochkovian Welsh Borderland. (a) Bifurcating axis with bases of solitary terminal sporangia. (b) Base of right-hand sporangium. (c) Branch point with longitudinal ridging. (d) Magnified area of axis. (e, f) Finely folded surface of spore, interpreted as the remains of an envelope. (g) Fractured end of axis. (h) Somewhat featureless walls of internal cells. (i) Bifurcating axis with base of single sporangium. (j) Transversely fractured axis. (k) Surface of sporangial base with spores. (l) Cluster of spores. (m) Microgranular granules on spore surface interpreted as tapetal residues. (n) Transversely fractured axis. Peripheral cells with large lumina containing pyrite crystals. Arrow indicates more compressed central tissue with traces of globules. (o) Longitudinally fractured axis, showing lumina filled with pyrite crystals. Bars: (a) 500 µm; (b, c, k) 50 µm; (d, n) 10 µm; (e–g, l, o) 20 µm; (h, m) 5 µm; (i, j) 200 µm.

smooth or slightly granular walls (arrow in Fig. 3d). Due to compression of the axis, the relationship among these central cell types remains unclear.

A similar range in structure is seen in specimen V 68855, where a stout, unbranched, longitudinally ridged axis terminates in the base of a sporangium (Fig. 3f), with a few adhering spores (Fig. 3g). This is an atypical specimen because the apparent increase in axis width from base to apex has a taphonomic basis. The base of the axis shows three-dimensional cellular preservation (Fig. 3h), but more distally it becomes flattened. We interpret this axis as part charcoal and part more conventional coalified preservation. In the former, the surface shows characteristic longitudinal ridging and intervening wrinkles (Fig. 3h), but in the latter these features are less distinctive. The cell walls of almost all peripheral tissues are fused (Fig. 3h,i), but a single poorly preserved stoma with collapsed guard cell outer walls remains visible

(Fig. 3i,j). The spores are broadly folded polyads, *c.* 25 µm in diameter, with overall laevigate distal surfaces, but covered in a residue with very occasional microelements (arrows i and ii, Fig. 3g). No haplotypic features are observed; however, running through the centre of some of these folds are discrete furrows, interpreted as fused junctions between individual units of the polyad (arrow iii, Fig. 3g). If the broad folds are fused junctions, their arrangement, and subsequent collapse of the distal walls, suggests that these spores are permanent dyads. One dyad has been partially torn apart, producing a rough 'hilum' (arrow iv, Fig. 3g). Cells within the axis are elongate. Some central cells have smooth walls (Fig. 3k), but others have internal surfaces covered with irregular granular contents or attached globules of various shapes and aggregations (Fig. 3l,m,n). Other elongate, longitudinally orientated linear features are interpreted as the remains of thin, compressed and folded cell walls (Fig. 3n).

Fig. 3 Fertile specimens: (a–e) V 68854 and (f–n) V 68855, Lochkovian Welsh Borderland. (a) Specimen as isolated with twisted, typically ridged axis and empty base of sporangium. (b) Transversely fractured end of compressed axis. (c) Magnified area, most cells homogenized, but some globules present. (d) Fragment of longitudinally fractured tissue. Arrow indicates minutely granular wall. (e) Right-hand region of (d) magnified to show lumen-facing walls with abundant granules and a few small pits (arrow). (f) Unbranched axis where increases in diameter relate to differential compression. Wider extremity contains the base of a sporangium. (g) Polyads with essentially laevigate surfaces. Arrows: (gi) and (gii) indicate areas of residue with very occasional microornament; (giii) fused junctions between individual elements of the proposed dyad; (giv) rough hilum following tearing apart of elements of dyad. (h) Transversely fractured axis at proximal end. (i) Surface of distal region of axis with stoma; note the collapsed guard cells. (j) Poorly preserved stoma magnified from (i). (k–n) Longitudinally fractured central tissues from basal region, some with smooth walls, others with granules or globules. Bars: (a, f) 500 μm ; (b, h) 50 μm ; (c) 5 μm ; (d, g, j, k, n) 20 μm ; (e, l, m) 10 μm ; (i) 100 μm .



Specimen V 68856 (Fig. 4a) is included here because, although it is very poorly preserved, the remains of two stomata, both with collapsed guard cells (Fig. 4b), can be observed on the lower surface of the bowl-shaped base of a sporangium. One possible attached spore was observed, but it is too poorly preserved to exhibit any recognizable features. Details of the surface of the sporangium and the axis are obscured by pyrite, microfolding and a possible microbial film (Fig. 4b). Internal cellular features are barely discernible within the transverse fractured section, which presents a more or less homogeneous appearance (Fig. 4c). Two central channels might represent the remains of conducting cells.

Sterile specimens Of the hundreds of specimens subjected to SEM analysis, about 10% were sufficiently well preserved and successfully prepared to reveal details of the lumen-facing walls of

the central cells. Fig. 4(d–k) shows a range of these. The central cells have walls that are continuous or minutely pitted and many have an additional overlying layer of material in the form of globules that protrude into the lumen, either as single entities or in chains or clusters. Individual globules measure 0.12–0.87 μm and they can be so dense as to obscure the structure of the underlying walls (V 68857, Fig. 4l; V 68858, Fig. 4m). There is considerable variation even within a specimen. Globules are absent from some areas, revealing that the underlying wall has minute pits (HD(L)117/06, Fig. 5a). In others the minutely pitted layer is overlain by adhering globules that may be few in number or numerous (HD(L)127/01, Fig. 5b,c). In some specimens the central cells have globules exclusively in chains or clusters (e.g. NMW99.20G.17, fig. 179 in Edwards *et al.*, 2003; V 68857, Fig. 4l; V 68858, Fig. 4m). The pits are too small to measure accurately from SEM images, but they fall within plasmodesmata

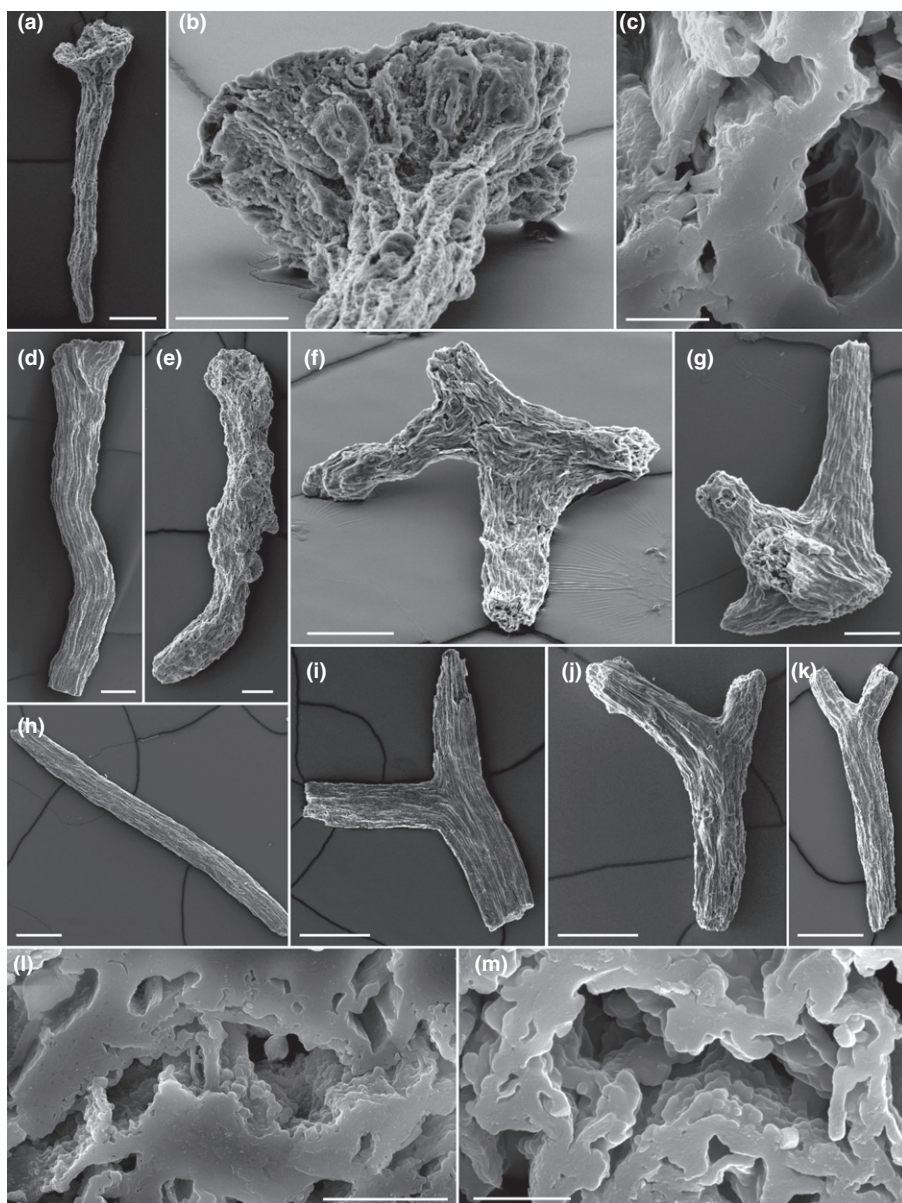


Fig. 4 (a–c) Fertile specimen V 68856, Lochkovian Welsh Borderland. (a) Unbranched axis with typical striations and base of empty sporangium. (b) Lower surface of sporangium with stoma; note the collapsed guard cells. (c) Transverse section through largely homogenized axis. (d–k) Sterile specimens: diversity in morphology of axes with typical surface striations. Specimens: (d) V 68857; (e) V 68858; (f) V 68859; (g) V 68860; (h) HD(L)117/06; (i) HD(L)127/01; (j) V 68861; (k) V 68862. (l) Transversely sectioned axis in (d). Homogenized walls with remaining lumina covered by globules. (m) Centred cells of transversely fractured axis (in e) with numerous globules. Surface of axis unusual in possession of small mounds. Bars: (a, d–g) 200 μ m; (b) 100 μ m; (c) 5 μ m; (h–k) 500 μ m; (l) 10 μ m; (m) 2 μ m.

dimensions. In most examples, the pitted layer overlies a continuous wall, but in others the pits also perforate a detached inner layer. In neither case are there continuous channels between cells (V 68860, Fig. 5d,e). Fragments of cells with smooth walls are sometimes present, but they are rarely of any length. Specimen V 68861 is an exception where smooth walls (Fig. 5f) are associated with cells with pitted walls and globules (Fig. 5g). As in fertile specimens, stomata are very rare. Specimen V 68862 is unique in that a single stoma (Fig. 5i) occurs on a dichotomously branching axis with traces of pitted walls internally (Fig. 5j).

Living bryophytes – cell walls of conducting tissues

Our cytological analyses of food-conducting cells (FCCs) in extant mosses reveals that their inner walls are generally covered by an additional, highly irregular layer of material, which is

visible under both SEM (Fig. 6) and TEM (Fig. 7). This layer varies in appearance from being dense and completely covering the underlying wall (Figs 6b, 7a–c) to consisting of globose (Figs 6c,d, 7d) or, at the end walls, labyrinthine protrusions (Figs 6e, 7e). The globules range in size from 0.1 to 0.7 μ m in diameter and are irregularly distributed. At the end walls, the presence of plasmodesmata also in the additional layer (Fig. 7e) indicates that this must comprise wall material. The characteristic appearance of their inner walls unambiguously distinguishes FCCs from water-conducting cells (WCCs), as WCCs are consistently thin-walled and lack any additional layer (Figs 6g, 7f).

Examination of published transmission electron micrographs of sieve elements in the lycophytes *Selaginella kraussiana* (Burr & Evert, 1973) and *Huperzia lucidula* (*Lycopodium lucidulum*) (Warmbrodt & Evert, 1974) reveals inner cell walls similar in appearance to those observed in moss FCCs, with invaginations

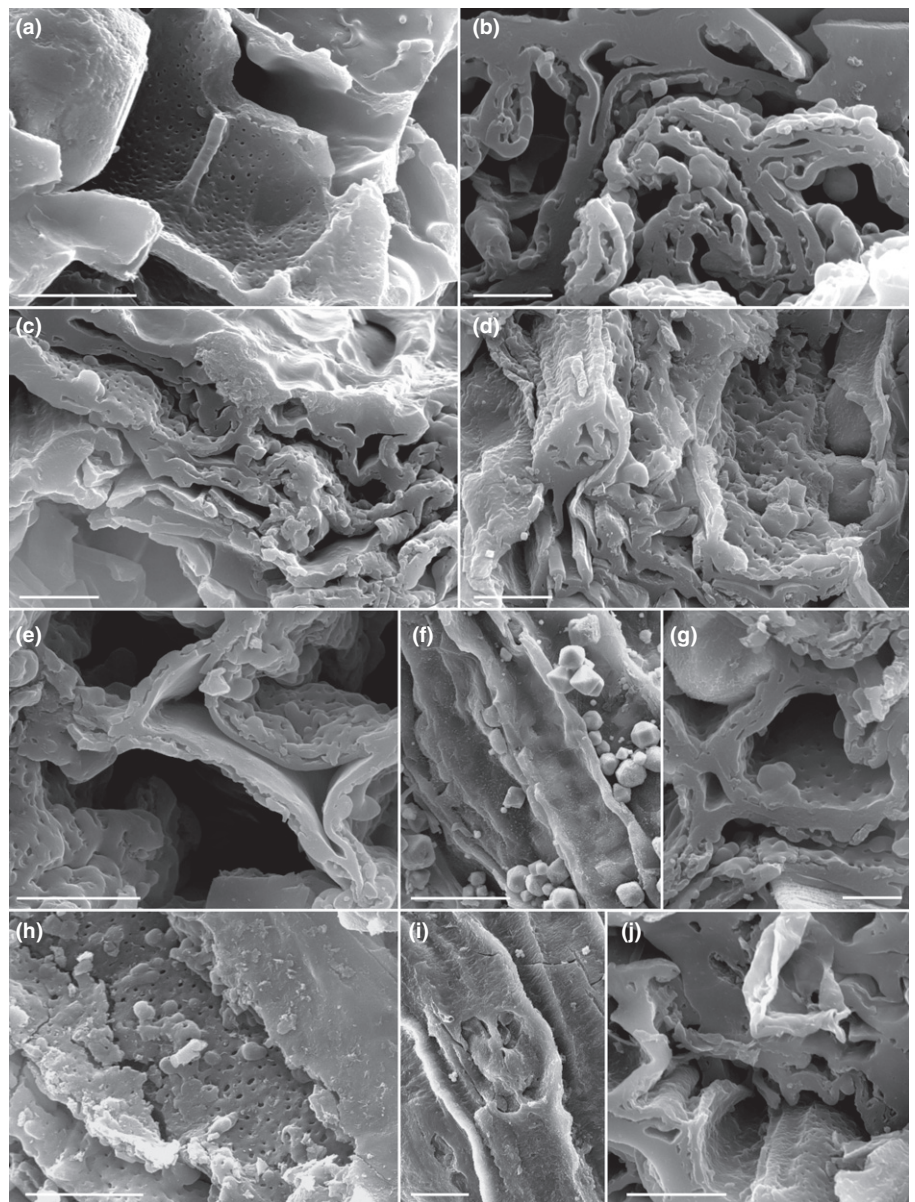


Fig. 5 Sterile specimens, Lochkovian Welsh Borderland. Anatomy of centres of sterile axes. (a) Pitted surface with single projections, surrounded by fragments with occasional globules. HD(L)117/06. (b, c) Contorted cells with numerous globules and pitted surfaces (arrow c). HD(L)127/01. (d, e) Pitted walls in surface view (d) and in section (e). In the latter, pitted wall is separate from a continuous outer one, here adjacent to an intercellular space. V 68860. (f–h) Cells with diverse wall types in central area. (f) Longitudinally fractured cells with smooth walls and imprints of pyrite crystals. Note their presence in adjacent cells. (g) Transversely fractured cells with pitted walls, occasional globules and intercellular space. (h) Pitted wall with occasional globules. V 68861. (i) Stoma with collapsed guard cells on axis. V 68862. (j) Transverse fracture of central cells with pits on axis with stoma. V 68862. Bars: (a) 5 μ m; (b) 2 μ m; (c–e, g, h) 5 μ m; (f, i) 20 μ m; (j) 10 μ m.

of the plasmalemma forming small globules and projections in the cell lumen.

Systematics

We propose the descriptive informal name eophyte for the group, which is derived from Ancient Greek ἑώς (ēōs, 'early in the day') + -phyte ('plant'). We further propose a formal name above the rank of family in accordance with article 16.1b of the Shenzhen Code (Turland *et al.*, 2018). This is given at the subclass level to be consistent with ranking in the classification of Chase & Reveal (2009). Because the name is descriptive, and therefore not automatically typified, a diagnosis is provided. Gray (1993) coined the term Eoembryophytic to describe an evolutionary land floral epoch or evolutionary level, when the dispersed spore record was dominated by spore tetrahedral tetrads (and some associated

permanent dyads and early records of trilete spores), between the mid-Ordovician and Early Silurian. Here we use the term eophytes to define a distinct group of plants with valvate sporangia that contained permanent cryptospores (tetrads and dyads). The eophytes were one component of Gray's concept of an eoembryophytic land flora.

Class Embryopsida Engler ex Pirani & Prado (2012)

Subclass Eophytidae Edwards, Morris, Axe, Duckett, Pressel & Kenrick, **subcl. nov.**

Diagnosis. Tiny plants with simple, leafless, predominantly dichotomizing axes that have a plicate surface. Spore capsules terminal, formed as extensions of the axes, and fusiform, infundibular or globose, typically valvate, with rare stomata. Spores of the cryptospore type, dispersed as permanent dyads or tetrads. Centrally located vascular system of food-conducting cells with minutely pitted walls overlain by globules.

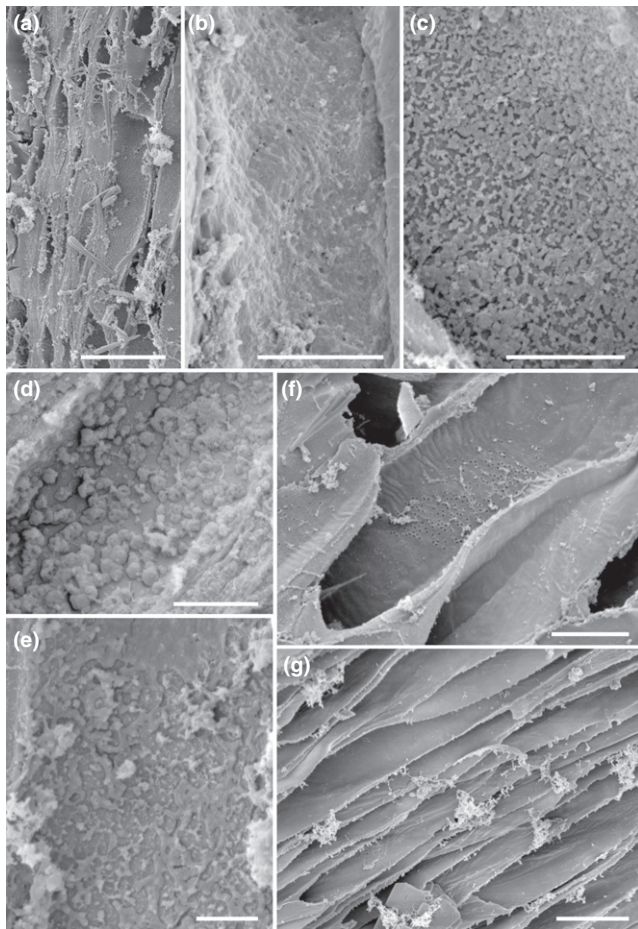


Fig. 6 Scanning electron micrographs of living bryophytes. Longitudinal sections of moss setae: (a–e, g) *Mnium hornum*; (f) *Polytrichum juniperinum*. (a) Elongate food-conducting cells with typical thick wall and oblique end walls. (b–e) Under higher magnifications their inner walls have an additional, highly irregular, overlying layer of material which is either very dense so as to completely obscure the underlying wall (b); or consists of globules varying in density, from very dense (c) to somewhat more sparse (d); or have a labyrinthine appearance (e), especially at the end walls. (f) When the additional inner layer is absent, minute pits are clearly visible in the oblique end walls. (g) Water-conducting cells have invariably thin and smooth walls. Bars: (a) 20 µm, (g) 10 µm; (b, c, f) 5 µm; (d, e) 2 µm.

Circumscription: In addition to the fossils illustrated here, the group includes many dispersed permanent cryptospores and cryptospore-producing mesofossils that were informally termed cryptophytes (Edwards *et al.*, 2014) or cryptosporophytes (Wellman & Ball, 2021) (Table 2). However, because this term is already applied to algae in the Cryptomonada (Kingdom Chromista, Cavalier-Smith, 2018), to avoid confusion we here change the informal name to eophyte. Dispersed cryptospores include: permanent dyads *Cymbobolates horridus*, *C. cymosus* and *Dyadospora* (Edwards *et al.*, 2012a) and *Chelinobolates erraticus* (Morris *et al.*, 2012); enveloped dyads in the genera *Segestrespora*/*Abditusdyadus* (Wellman *et al.*, 1998); and enveloped tetrads *Velatitetras* (Edwards *et al.*, 1999) and *Cheilotetras* (Edwards *et al.*, 2012b, 2014). Charcoal-preserved mesofossil members include the genera *Fusiformitheca* (Wellman *et al.*, 1998; Xue &

Wang, 2011), cf *Salopella* (in part, e.g. Edwards *et al.*, 1994), *Grisellatheca* (Edwards *et al.*, 1999) and *Ficoiditheca* (Morris *et al.*, 2012) as well as specimens that have been published but not yet formally named (Habgood, 2000). Possible other affiliated genera are *Partitatheca* (Edwards *et al.*, 2012a) and *Cululitheca* (Wellman *et al.*, 1998), but this is less certain because these genera are based on sporangia with limited evidence on the form and anatomy of subtending axes. We emphasize that dyads found in the eophytes were permanently fused (i.e. they never separated before dispersal), but due to the vagaries of taphonomic processes may have become torn apart. Therefore, we exclude *Lenticulatheca* (Morris *et al.*, 2011), sporangia of *Cooksonia*-type discoidal morphology containing separating dyads (hilate monads of the genus *Cymbobolates*) and other specimens known to contain hilate monads, such as *Laevolancis* (Wellman *et al.*, 1998a), and consider these to be more closely related to basal tracheophytes. We also exclude *Tortilicaulis* (Edwards *et al.*, 1994) on the basis that it contains trilete monads and possesses tracheids, although it is similar in other respects including size, plicate surface and sporangia.

Justification for new taxon: Although the overall form and life cycle of the eophytes remain poorly understood, there are sufficient known characters to distinguish them from other major clades of land plants. They can be distinguished from bryophytes in the Setaphyta – the clade containing liverworts (Marchantiidae) and mosses (Bryidae) (Renzaglia *et al.*, 2000; Renzaglia & Garbary, 2001; Puttick *et al.*, 2018; Sousa *et al.*, 2020) – and from hornworts (Anthocerotidae) based on the presence of dichotomizing axes subtending the spore capsule and axes with a plicate surface. Furthermore, liverworts lack stomata, although based on recent phylogenies (Puttick *et al.*, 2018; Sousa *et al.*, 2020; see also Fig. 8) this absence may represent secondary loss rather than a plesiomorphy. The spores of bryophytes typically are dispersed as single units, some of which possess trilete marks, but dispersal of cryptospore-like dyads is documented in the liverwort *Haplomitrium gibbsiae* (Renzaglia *et al.*, 2015a), and spores are dispersed or released as tetrads in the liverworts *Sphaerocarpos* (Sphaerocarpaceae), *Riccia* subgenus *Thallocarpus* (Marchantiales) (Renzaglia *et al.*, 2015b) and the subterranean mycoheterotrophic species of *Aneura*, formerly *Cryptothallus* (Mezgeriales) (Wickett & Goffinet, 2008). It should be noted, however, that developmental differences show that the dyads in extant *Haplomitrium* (Renzaglia *et al.*, 2015a) are probably unrelated to cryptospore dyads. Extant *Haplomitrium* produces tetrads following meiosis. The tetrad then disaggregates into two dyad pairs – both of which show very clear scars (see fig. 2 in Renzaglia *et al.*, 2015a) where the two dyads were joined together in a tetrad. Cryptospore dyads never show such scars, presumably because they formed by successive meiosis with a clean division in between.

Eophytes are distinguished from tracheophytes by their sporangia that developed permanent cryptospores and their very narrow axes with a plicate surface. Eophytes did not possess WCCs; a vascular system of FCCs combined with occasional epidermal stomata is a unique feature of the group and of some extant mosses (Renzaglia *et al.*, 2020).

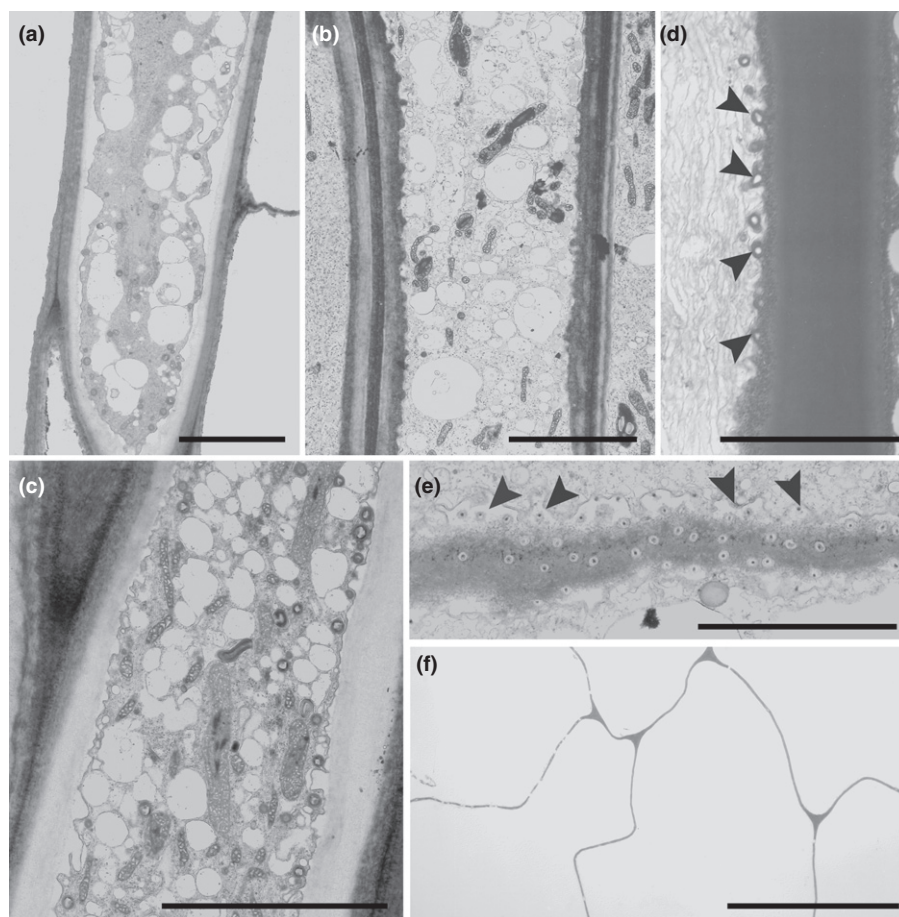


Fig. 7 Transmission electron micrographs of living bryophytes. (a–e) Longitudinal sections of food-conducting cells in the moss *Polytrichum formosum* showing a highly irregular, additional overlying layer of material on the lateral (a–d) and oblique end walls (a, e). This additional layer is either continuous (a–c), sometimes labyrinthine (e), or consists of small, globose projections into the cytoplasm (arrowed) (d), and can be either electron-transparent (a, c, e) or electron-dense (b, d) in appearance. (e) At the oblique end walls plasmodesmata extend from the wall proper into the additional layer (arrowed). (f) Smooth, uniformly thin-walled water-conducting cells in the liverwort *Haplomitrium gibbsiae*. Bars: (a–c) 5 μm ; (d, e) 2 μm ; (f) 10 μm .

Phylogenetic position: The eophytes possess a distinctive suite of characteristics, but many aspects of their morphology and their life cycle are still unknown, which renders their phylogenetic position speculative. Recent phylogenetic treatments of living plants recognize a clade comprising mosses and liverworts (setaphytes) that is either the sister group to tracheophytes or sister group to hornworts (Fig. 8; Renzaglia *et al.*, 2000; Renzaglia & Garbary, 2001; Puttick *et al.*, 2018; Sousa *et al.*, 2020). Plausible placements for the eophytes are the stem groups of these more inclusive clades of basal land plants. One possibility is that eophytes are stem group tracheophytes. Here, they could fit with the original concept of the polysporangiophytes (Kenrick & Crane, 1997), a clade that includes living tracheophytes and extinct stem group plants that lacked tracheids but which developed multiple sporangia on branched axes. The fossils *Aglaophyton majus* and *Horneophyton lignieri* were interpreted as the earliest diverging taxa in the polysporangiophytes. Their vascular systems are thought to contain both WCCs and FCCs, but their WCCs differ from typical tracheids in lacking well-developed internal thickenings (Edwards, 2003, 2004). The absence of WCCs would therefore indicate that eophytes diverged earlier than *A. majus* and *H. lignieri*. Crucial to resolving the relationships of the eophytes is a detailed comparison of their FCCs with those of other early polysporangiophytes. Even though the anatomy of the Rhynie chert plants is well preserved, the structure of the cell walls of FCCs in key early fossils remains

unknown in detail. Further study of the Rhynie chert plants could supply the missing information.

Other details crucial to phylogenetic placement of the eophytes include the nature of their gametophyte generation and whether the sporophyte was nutritionally dependent on the gametophyte, as in modern bryophytes (i.e. matrotrophic). The plants were undoubtedly much smaller than contemporaneous vascular plants, in size more comparable to small mosses. The sexual phase of the life cycle remains conjectural, but it is possible that thalloid fossils with copious transfer cells extracted from the same geological site represent the fragmentary remains of the gametophyte generation (Edwards *et al.*, 2021). This tentative hypothesis is based on the observation that similar putative transfer cells are also present in eophyte fertile aerial axes and on their striking similarities with placental transfer cells of extant bryophytes. Matrotrophy is therefore plausible even though still unproven. If the eophytes were matrotrophic, this would be a second characteristic that distinguishes them from other polysporangiophytes.

Discussion

Eophyte plants possessed conducting cells in the spore-producing phase of their life cycle

Previous research on charcoalfied mesofossils from this unique Early Devonian locality focused on documenting sporangia and

their *in situ* cryptospores (e.g. Wellman *et al.*, 1998a,b; Habgood, 2000; Morris *et al.*, 2011, 2012; Edwards *et al.*, 2012a,b, 2014). This was accompanied by a comprehensive survey of associated sterile axes of unknown affinity targeting the anatomy of cells and tissues thought to be associated with water transport and structural support (Edwards & Axe, 2000; Edwards *et al.*, 2003). One key discovery was the variety of ultrastructural features present in the lateral cell walls of these minute axes (end walls were not observed). Whereas in some cells the wall layer appeared homogeneous, in others it was distinctly two-layered, and invaginations of the inner layer caused an array of folds or projections of various shapes and sizes to develop within the lumens. In many cells, the lumen-facing inner wall layer was peppered with plasmodesmata-derived pores, but these did not penetrate through the outer layer of the wall to form direct links between neighbouring cells. In addition to features that appeared to be fundamental components of the cell wall, sheets or globules of material overlying the layer with the plasmodesmata were interpreted as structures similar to those found in desiccation-tolerant FCCs of modern mosses (Pressel *et al.*, 2006). These various features, combined in different ways, produced a diversity of cell types that seem beyond that seen in the WCCs of the vascular plants.

The results here show for the first time that a similar array of cell types are specifically found within sporophyte stems bearing sporangia that contain permanent cryptospores, further refining the concept of the whole organism.

Eophyte sporophytic axes lacked specialized WCCs

A natural inference to draw about the primary functions of these varied cell types is that they played roles in solute transport and structural support (Edwards *et al.*, 2003). Considering possible functions, we need to take account of the extraordinarily small size of the plants. Their axes rarely exceed 1 or 2 mm in diameter, and their length, although unknown, is unlikely to exceed a couple of centimetres. Extremely small size limits the variety of tissues and cell types that can be accommodated, creating physiological constraints (Raven, 1999; Boyce, 2008). Furthermore, although the same physical laws affect all organisms, the physiological and biomechanical consequences of those laws differ depending on size (Raven, 1977, 1993; Niklas, 1992; Proctor *et al.*, 2007). Like many modern bryophytes, the eophytes would probably have been poikilohydric (i.e. unable to regulate cell and tissue water content). Their size would mean that they rapidly equilibrated with their environments, implying that they had a desiccation-tolerant physiology (Raven, 1999; Proctor *et al.*, 2007). Some of the cell wall features observed suggest a role in structural support. The walls are comparatively thick, and the presence of invaginations projecting into or traversing the lumen could act to resist cell collapse during desiccation in a similar manner to the protrusions within the pegged rhizoids of complex thalloid liverworts (Duckett *et al.*, 2014). Furthermore, several features of these cells suggest a role more akin to food conduction (i.e. leptoid/phloem function) than strictly water transport (i.e. hydroid/tracheid function). The presence of plasmodesmata-

derived pores in the cell walls is not by itself diagnostic of function. They have been documented in one form of early fossil tracheary element (S-type cell of Kenrick & Crane, 1991; Kenrick *et al.*, 1991). Among the liverworts, the Haplomitriales and a few genera of the Metzgeriales possess an internal strand of specialized WCCs, in both cases restricted to the gametophyte (Ligrone & Duckett, 1996; Ligrone *et al.*, 2000) and with smooth and thin or thick walls respectively, all generally perforated by plasmodesmata-derived pores. In mosses, the monogeneric Takakiales stands out as the only group in which WCCs are also perforated by plasmodesmata-derived pores, similar to those of the Haplomitriales but occurring in both gametophyte and sporophyte generations. Otherwise WCCs are absent in the early divergent Sphagnopsida and Andreaopsida, and in other mosses are invariably imperforate (Ligrone *et al.*, 2000). By contrast, plasmodesmata-derived pores are a constant feature of bryophyte FCCs, which are present in a few liverworts, including *Haplomitrium*, but common through the mosses. Here we show that moss FCCs possess a lumen-facing wall layer that takes the form of sheets, globules or strands of material overlying a plasmodesmata-rich layer, strongly resembling the wall structure seen in the fossils. The fact that pits and globules are characteristic of the FCCs of extant bryophytes makes it highly unlikely that they might be taphonomic artefacts in the fossils. It should also be noted that whilst the pits in the fossils have a size range compatible with a plasmodesmal origin, their diameters are quite variable. Whereas pits on some walls in extant bryophytes tend to be highly uniform in size (e.g. *Polytrichum*, see Fig. 6f) others associated with conducting cells may be just as variable as in the fossils (Ligrone & Duckett, 1996; Ligrone *et al.*, 2000) and similarly those in the phloem of *Lycopodium* (Warmbrodt & Evert, 1974) and ferns (Miller & Duckett, 1979; Warmbrodt & Evert, 1979), not to mention angiosperms (Robinson-Beers & Evert, 1991).

It is the combination of thick cell wall, plasmodesmata-derived pores and additional overlying sheets, strands or globules of material that favour comparisons to the desiccation-tolerant FCCs of mosses, although similar inner walls appear to be also a feature of lycophyte sieve elements (Burr & Evert, 1973; Warmbrodt & Evert, 1974). We therefore conclude that there were no strictly WCCs in the eophytes. Their vascular system was composed of living cells principally involved in food transport. We suggest that, as in many modern bryophytes, hydration was largely serviced through external surface water. The eophytes equilibrated rapidly with the water potential in their surroundings being either fully hydrated and metabolically active or desiccated and quiescent.

One of the more puzzling features of the walls of the eophyte conducting cells is that the plasmodesmata-derived pores appear to be confined to the inner, lumen-facing part of the two-layered wall. In other words, they do not seem to form direct channels between adjacent cells. In the FCCs of modern mosses, the plasmodesmata-derived pores create channels between cells. There is continuity of the plasmalemma through these pores and direct cytoplasmic connection. However, across land plants there are numerous examples where plasmodesmatal continuity is

Table 2 Published eophytes: intact sporangia-bearing permanent cryptospores, from the Lower Devonian Brown Clee Hill locality.

Taxon/specimen*	Sporangium		Stem morphology		Stem anatomy		
	Shape	Spore taxa/description	Branching	Stoma	Strands	Globules	Pits
Containing permanent dyads							
<i>Culullitheca richardsonii</i> ¹	Beaker	<i>Dyadospora murusdensa</i>	Unbranched	—	?	?	?
<i>Fusiformitheca fanningiae</i> ¹	Fusiform	<i>Segestrespora laevigata</i> / <i>Abditusdyadus laevigatus</i>	Isotomous	—	?	?	?
<i>Ficoiditheca aenigma</i> ²	Fig-shaped, valvate	Permanent; microgranulate	Isotomous	✓	?	?	?
<i>Partitatheca splendida</i> ⁴	Globular, valvate, stomata	<i>Cymbohilates horridus</i> var. <i>splendens</i>	?	?	?	?	?
<i>Partitatheca horrida</i> ^{4,5}	Elongate, valvate	<i>Cymbohilates horridus</i> var. <i>horridus</i>	Isotomous	✓	?	?	?
<i>Partitatheca densa</i> ⁴	Elongate, valvate	<i>Cymbohilates horridus</i>	?	?	?	?	?
<i>Partitatheca cymosa</i> ⁴	Fusiform, valvate	<i>Cymbohilates cymosus</i>	?	?	?	?	?
cf <i>Partitatheca cymosa</i> ⁴	Elongate, valvate	<i>Cymbohilates cymosus</i>	?	?	?	?	?
<i>Partitatheca</i> sp. ²	Elongate, valvate	<i>Chelinohilates erraticus</i>	Unbranched	—	?	?	?
NMW97.42G.1 ^{1,6}	Valvate	<i>Dyadospora murusdensa</i>	?	?	?	?	?
(? <i>Partitatheca</i>)							
V 68853 ⁸	Saucer-shaped	permanent; microgranular	Isotomous	?	?	✓	?
V 68855 ⁸	?	permanent; laevigate, ?microgranular	Unbranched	✓	?	✓	?
Containing permanent tetrads							
<i>Grisellatheca salopensis</i> ³	Elongate, bifurcating	<i>Velatitetras</i> sp.	Isotomous	?	?	?	?
NMW2012.17G.3 ^{7,6}	Valvate	<i>Cheilotetras</i> sp.	?	?	?	?	?
(? <i>Partitatheca</i>)							
NMW99.11G.2 ⁷	Elongate, bivalved	<i>Cheilotetras</i> sp.	?	?	?	?	?
NMW99.19G.2 ⁵	Elongate, bivalved	<i>Velatitetras</i> sp.	?	?	?	?	?
NMW98.23G.2 ³	?	<i>Tetrahedraletes</i> sp.	Isotomous	?	?	?	?
NMW96.11G.3 ³	?	<i>Velatitetras</i> sp.	Isotomous	?	?	?	?
NMW2012.17G.5 ⁷	Valvate	?permanent, conic, micro-conic, -grana	?	?	?	?	?
V 68851 ⁸	Funnel, valvate	permanent; grana, micrograna	Unbranched	?	✓	✓	?
V 68852 ⁸	Cup shaped	permanent; micrograna, folded?envelope	Isotomous	?	✓	?	?

We exclude genera and specimens that produced hilate cryptospores, a product of naturally separating dyads.

*References: ¹Wellman *et al.* (1998a); ²Morris *et al.* (2012); ³Edwards *et al.* (1999); ⁴Edwards *et al.* (2012a); ⁵Habgood (2000); ⁶Edwards *et al.* (2014); ⁷Edwards *et al.* (2012b); ⁸this work.

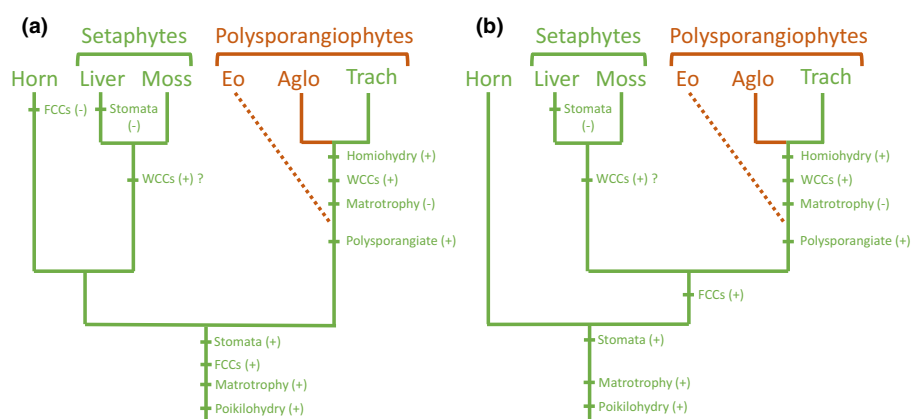


Fig. 8 The two hypotheses on the relationships among living bryophytes (Hornwort, Liverwort, Moss) and tracheophytes (Trach) with the most significant support in molecular phylogenetic analyses by Puttick *et al.* (2018). Superimposed is the inferred position of the early fossil plant *Aglaophyton majus* (Aglo) and one of several possible phylogenetic positions of the eophytes (Eo). Proposed gains (+) and losses (-) of various characteristics of the clades are plotted on branches. This is a highly simplified framework to help visualize the discussion in the text. For example, we assume that stomata are plesiomorphic in mosses and have been lost iteratively within the clade (not shown); we assume that food-conducting cells (FCCs) are plesiomorphic in mosses and liverworts and have been lost iteratively within these clades (not shown). WCCs, water-conducting cells.

blocked by the deposition of additional layers of wall material. Though structurally very similar to the FCCs of modern mosses there may have been functional differences in eophytes. At

present, the structure of the end walls remains unknown. How these adjoin to neighbouring cells and the nature of their plasmodesmata also requires further investigation.

FCCs of bryophytes and phloem in tracheophytes share a common origin

The evolutionary origins of the WCCs and the FCCs of bryophytes and vascular plants have been widely debated (reviewed in Ligrone *et al.*, 2000, 2012; Renzaglia *et al.*, 2000; Edwards *et al.*, 2003; Raven, 2003; Lucas *et al.*, 2013). FCCs are taxonomically more widespread than WCCs in moss gametophytes, suggesting that translocation of photosynthate and other metabolic products can be more challenging in small desiccation-tolerant plants than is water transport. In mosses, FCCs are characterized by a distinctive cytological organization. Their key attributes include enlarged plasmodesmata in the end walls, cytoplasmic polarity (i.e. alignment of plastids, mitochondria, endoplasmic reticulum-derived vesicles along arrays of endoplasmic microtubules) and breakdown of the vacuole. FCCs are most well developed in the so-called leptoids of the large Polytrichales, in which there is degeneration or loss of various organelles including the nucleus (Héban, 1977). In these respects, FCCs of Polytrichales resemble the sieve cells in the phloem of vascular plants (Scheirer, 1980, 1990), differing principally in the distinctive cytoplasmic polarization of the former and the greater degree of organelle degeneration in the latter. This highly distinctive moss FCC cytology was also documented in complex thalloid liverworts (Marchantiales) (Ligrone & Duckett, 1994), some simple thalloid liverworts (Metzgeriales) (Ligrone *et al.*, 2000) and in the axes of *Haplomitrium* (Edwards *et al.*, 2003), but not in leafy liverworts. FCCs are absent from the hornworts. The distribution of FCCs within and among the three major clades of bryophytes and the vascular plants could therefore be interpreted in evolutionary terms as either several independent acquisitions or a shared ancestral feature of land plants that has been lost in some modern groups (e.g. leafy liverworts, hornworts, the moss *Andreaea*) (Ligrone *et al.*, 2012) (Fig. 8).

The vascular system of the early fossil eophytes is consistent with an ancestral role for FCCs in land plants, at least for the common ancestor of the setaphytes (i.e. liverwort + moss clade) and the vascular plants (Fig. 8b). Homologies are best considered in terms of specific characteristics of FCCs and sieve elements. At a basic level, cell elongation, enlarged plasmodesmata, breakdown of the vacuole and symplastic transport are features common to FCCs of bryophytes and to the sieve elements of the vascular plants (Ligrone *et al.*, 2000). Desiccation tolerance in FCCs is thought to be the ancestral state in land plants (Pressel *et al.*, 2009), an idea that is consistent with the structure of the conducting cell walls in the eophyte fossils. This would imply that the capacity for desiccation tolerance has been lost in the phloem sieve elements of the vascular plants, perhaps when this group acquired their ability to regulate cell and tissue water content (Proctor *et al.*, 2007). The distinctive cytoplasmic polarity that is widely seen in the FCCs of the setaphytes is apparently absent from the sieve elements of the vascular plants, implying either an independent gain in the former or loss in the latter. Organelle degeneration is not ubiquitous in the setaphytes. It occurs only in the FCCs of large, derived mosses of the Polytrichales (Ligrone *et al.*, 2000). This characteristic is better developed in the sieve

elements of the vascular plants, where it was probably acquired independently. Thus, certain characteristics of FCCs of setaphytes and the sieve elements of vascular plant phloem could be homologous whereas others might not. The ancestral FCC would not necessarily have shared all the characteristics of its living descendants.

Our documentation of an additional wall layer comprising sheets, stands or globules of material in the FCCs of extant mosses raises the question of whether this is also a feature of sieve elements in the vascular plants. As mentioned earlier, similar inner cell walls are also a feature of sieve elements in early diverging vascular plants, namely *Selaginella kraussiana* (Burr & Evert, 1973) and *Huperzia lucidula* (*Lycopodium lucidulum*) (Warmbrodt & Evert, 1974), and perusal of published transmission electron micrographs of sieve elements in other tracheophyte groups, including flowering plants, reveals that these too often have an additional irregular layer covering their inner walls (e.g. Evert & Mierzwa, 1989). Thus, we posit that this may well be a characteristic feature of all FCCs in plants, not restricted to those of the early diverging lineages but possibly extending across the entire land plant phylogeny. Clearly, further targeted cytological analyses of food-conducting tissues in living and extinct vascular plants are needed to test this hypothesis.

The stomata of eophyte plants did not play a role in transpiration-driven water transport

The presence of stomata sparsely distributed in the epidermis of the minute eophyte plants on axes and associated sporangia provides evidence that their role in the earliest plants was not primarily related to water transport and photosynthesis, as in modern vascular plants. The presence of FCCs in the eophytes implies symplastic transport in a manner resembling the FCCs in modern bryophytes and the sieve elements of the vascular plants. In phloem, the mechanism of fluid transport is osmotically generated pressure flow driven by accumulation of sugars in sources (e.g. leaves) and consumption in sinks (e.g. roots) (i.e. the Münch Hypothesis) (Van Bel, 2003; Jensen *et al.*, 2016), and the sieve elements can be loaded with sugars in several different ways (Turgeon, 2010). Stomata play no roles in these processes. Also, because there are no WCCs in the eophytes there can be no passive water transport driven by transpiration through stomatal pores (i.e. cohesion–tension theory). Comparisons with modern mosses are illuminating. During the evolution of mosses, stomata have been lost on many occasions in different groups, and their occurrence is not linked to the presence or absence of conducting cells (Renzaglia *et al.*, 2020). The moss *Takakia* has WCCs in the sporophyte but no stomata. In the Polytrichaceae, sporophytes of *Polytrichum* have stomata, whereas they are absent from *Atrichum*, yet in both genera there is a well-developed conducting strand of hydroids and leptoids. The Orthotrichaceae (Bryidae) possess stomata but do not have water-conducting tissue in the sporophyte, and there are different combinations of these features in other mosses (Renzaglia *et al.*, 2020). Furthermore, it has been shown that in very large mosses of the Polytrichales, water transport via transpiration can be actively regulated in the

gametophyte in the absence of stomata (Brodribb *et al.*, 2020). Additionally, hornworts, the only other bryophyte group bearing stomata, completely lack WCCs. The weight of recent evidence indicates that, where stomata do occur, they play roles in capsule maturation, drying, dehiscence (Field *et al.*, 2015; Merced & Renzaglia, 2017; Duckett & Pressel, 2018; Pressel *et al.*, 2018; Renzaglia *et al.*, 2020) and, to a lesser extent, carbon acquisition (Kubásek *et al.*, 2021). Since the stomata of the eophytes and other early mesofossils preserved in charcoal resemble in their distribution and numbers those of the sporophytes of some modern mosses and hornworts (Renzaglia *et al.*, 2017, 2020), they may have functioned in similar ways. Indeed, collapsed guard cells, as seen today in hornworts and some mosses, the result of maturational cell death locking the stomatal pores in an open position and thus aiding sporophyte desiccation, are also a common feature of earliest fossil stomata, including those of the eophytes described herein, but are absent in tracheophytes (Renzaglia *et al.*, 2017). Given the extremely minute size of eophytes, it is likely that a putative role of stomata on sporangia in desiccation for spore release may have extended to those adorning the subtending axes. Also pertinent to these considerations is whether eophyte sporophytes may have been physiologically dependent on a parental gametophyte, as in mosses and other bryophytes, as proposed, albeit speculatively, by Edwards *et al.* (2021).

Conclusions

Eophytes are a distinctive ancient group of land plants that shed light onto vegetation that preceded and grew alongside the earliest herbaceous vascular plants. The small size of these permanent cryptospore-producing plants might explain the lack of compelling fossil evidence of stem group bryophytes and embryophytes. Such minute fossils are easily overlooked, and they will lack preserved tissue systems in many sedimentary settings (e.g. Tomescu *et al.*, 2014). Furthermore, when diagnostic features are absent, such fragmentary organic materials can be misinterpreted, leading to implausible attributions (e.g. Retallack, 2019). Eophytes possess novel combinations of features that could inform our developing understanding of the evolution of the land plant body plan. Here we show that they possessed a vascular system composed of food-conducting cells resembling those modern bryophytes and that there was no internal water-conducting system. Their size and anatomy indicate that like modern bryophytes they were desiccation-tolerant plants (i.e. poikilohydric). We infer that they equilibrated rapidly with the water potential in their surroundings being either fully hydrated and metabolically active or desiccated and quiescent. The anatomy of their conducting tissues suggests an ancestral role for desiccation-tolerant FCCs in land plants and a role for stomata that was more like that of the modern mosses and hornworts than the vascular plants. Earlier phylogenetic treatments of basal land plants interpreted the absence of stomata in liverworts as plesiomorphic (e.g. Mishler & Churchill, 1985; Kenrick & Crane, 1997). Now, genomic evidence points to a single origin of stomata in the last common ancestor of land plants (Harris *et al.*, 2020). The developing phylogenetic picture and the new

genomic and fossil evidence point to an ancestral land plant that was small, yet it possessed a greater complement of cell types than previously supposed. Whether the sexual phase of its life cycle comprised a thalloid gametophyte, as tentatively suggested in Edwards *et al.* (2021), requires further investigation.

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Author contributions

DE conceived the concept and together with PK coordinated the writing of the paper. LA and JLM undertook the maceration of fossil specimens and SEM; SP and JGD performed TEM and SEM on extant bryophytes. Except for LA, all authors contributed to the text (DE, JLM, PK on palaeobotany; SP, JGD on bryophytes).

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Data availability

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

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See also the Commentary on this article by Tomescu, 233: 1018–1021.