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Conducting tissues and phyletic relationships of bryophytes

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Internal specialized conducting tissues, if present, are restricted to the gametophytic generation in liverworts while they may occur in both generations in mosses. Conducting tissues are unknown in the anthocerotes. Water-conducting cells (WCCs) with walls perforated by plasmodesma-derived pores occur in the Calobryales and Pallaviciniaceae (Metzgeriales) among liverworts and in *Takakia* among mosses. Imperforate WCCs (hydroids) are present in bryoid mosses. A polarized cytoplasmic organization and a distinctive axial system of microtubules is present in the highly specialized food-conducting cells of polytrichaceous mosses (leptoids) and in less specialized parenchyma cells of the leafy stem and seta in other mosses including *Sphagnum*. A similar organization, suggested to reflect specialization in long-distance symplasmic transport of nutrients, also occurs in other parts of the plant in mosses, including rhizoids and caulonemata, and may be observed in thallus parenchyma cells of liverworts. Perforate WCCs in the Calobryales, Metzgeriales and *Takakia*, and hydroids in bryoid mosses, probably evolved independently. Because of fundamental differences in developmental design, homology of any of these cells with tracheids is highly unlikely. Likewise, putative food-conducting of bryophytes present highly distinctive characteristics and cannot be considered homologous with the sieve cells of tracheophytes.

Keywords: hydroids; sieve elements; food-conducting cells; leptoids; tracheary elements; water-conducting cells

1. INTRODUCTION

The presence of an embryo, i.e. a stage in the life cycle during which the sporophyte is associated with and depends on the gametophyte, is perhaps the most important unifying character of plants. For this reason the term 'embryophytes' is receiving increasing favour as a more appropriate name for 'plants', or 'land plants'. The bulk of morphological biochemical and molecular information indicates that the embryophytes form a monophyletic group together with charalean green algae (Garbary *et al.* (1993) and literature therein).

Further subdivision of embryophytes separates vascular plants from bryophytes, essentially on the basis of two major characters concerning sporophyte development and vascular tissues. In vascular plants, the embryo phase is relatively short and the sporophyte soon establishes direct contact with the substrate, thus becoming independent from the gametophyte. Moreover, the sporophyte develops specialized vascular tissues, the xylem and phloem. In particular, the xylem contains water-conducting cells (WCCs), the tracheids and vessel elements, whose developmental pattern includes the deposition of a secondary lignified wall and final cytoplasmic lysis.

The bryophytes traditionally include those embryophytes in which the sporophyte is permanently associated with the gametophyte and never establishes direct contact with the substrate. Traditionally, the bryophytes are set apart from vascular plants also on the basis of the lack of vascular tissues. Indeed, a number of bryophytes do contain vascular tissues including highly specialized WCCs which, like tracheids and vessel elements, undergo programmed cytoplasmic lysis. These cells, however, do not form lignified walls. For this reason the term 'tracheophytes' appears to be preferable to vascular plants when emphasis is put on vascular tissue as a diagnostic feature.

The bryophytes, as above defined, include three major groups: the anthocerotes (hornworts), mosses and liverworts. Phylogenetic links among these groups are largely a matter of speculation as there is no general agreement about whether the bryophytes sensu lato are a mono- or paraphyletic group (Garbary *et al.* 1993; Garbary & Renzaglia (1998) and literature therein). Their current taxonomical ranking spans from three classes in the same division (Pearson 1995) to three separate divisions (Bold *et al.* 1987). In this paper, we will avoid formal names as these might reflect a prejudicial assumption of relationships.

Relationships between the three bryophyte groups and tracheophytes are also far from resolved. Cladistic analyses setting the bryophytes as a paraphyletic group identify the anthocerotes (Sluiman 1985; Garbary & Renzaglia 1998) or the liverworts (Mishler & Churchill 1984, 1985; Bremer *et al.* 1987) as the sister group to the rest of embryophytes and either a clade of mosses plus liverworts (Garbary & Renzaglia 1998) or the mosses alone (Mishler & Churchill 1984, 1985) as the sister

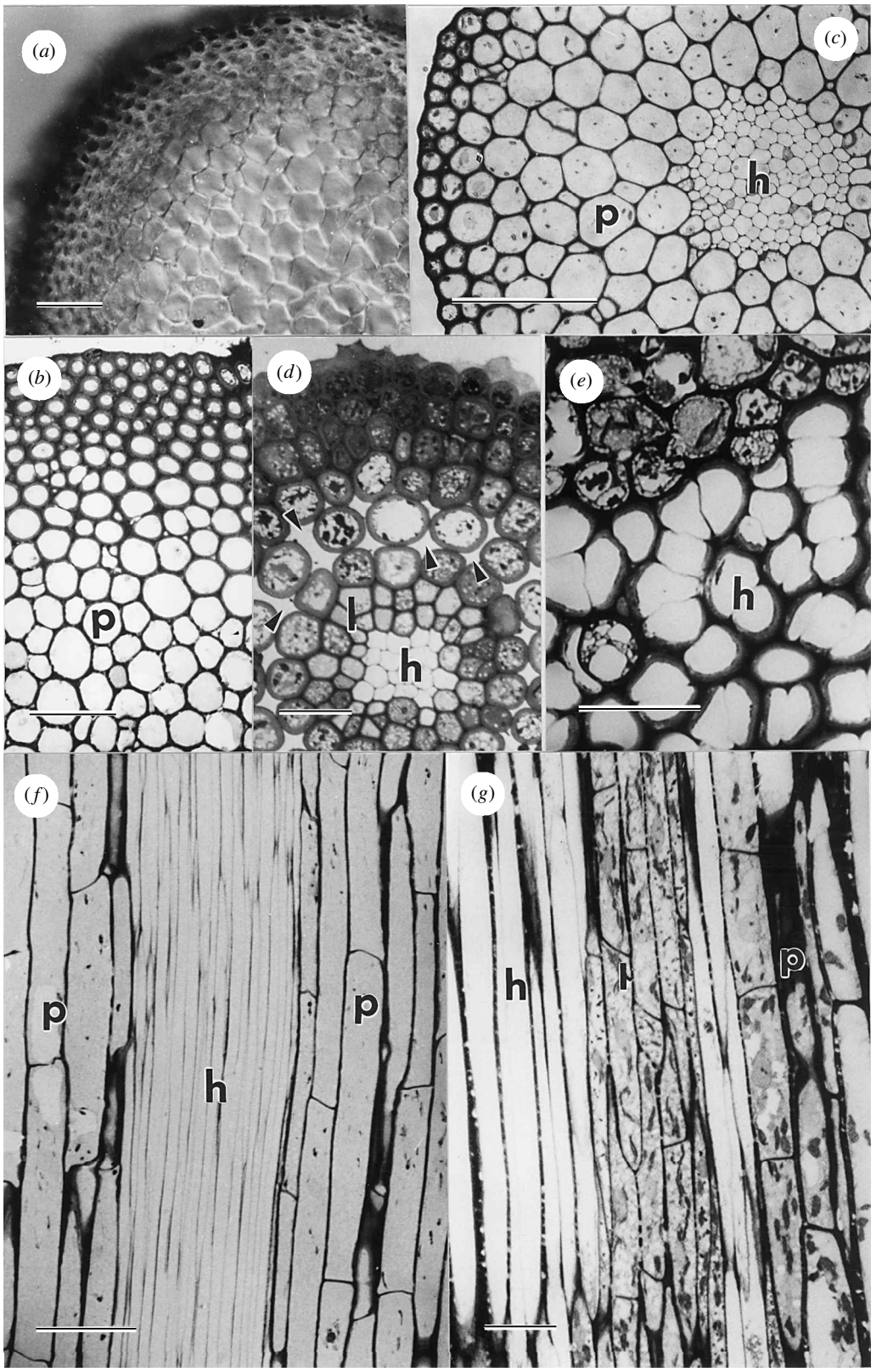

Figure 1. Light micrographs showing details of conducting tissue differentiation in mosses. (*a*) *Andreaobryum*, leafy stem; thick-walled cortical cell layers but no internal tissue differentiation. (*b*) *Neckera crispa*, leafy stem; no apparent internal tissue differentiation and hydroids absent. (*c*) *Plagiomnium undulatum*, leafy stem with a prominent hydroid strand. (*d*) *Pogonatum aloides*, sporophyte foot. Note the intercellular spaces (arrowed) between the parenchyma cells. (*e*) *Polytrichum commune*, details of the hydroids with thick and thin walls. (*f*) *Plagiomnium undulatum*, longitudinal section. Note the extreme lengths and thin walls of the hydroids. (*g*) *Polytrichum commune*, longitudinal section. Note the thick and thin walls of the hydroids. h, hydroids; l, leptoids; p, parenchyma. Bar lines: *a–c*, *f* = 50 μm, *e*, *g* = 10 μm.

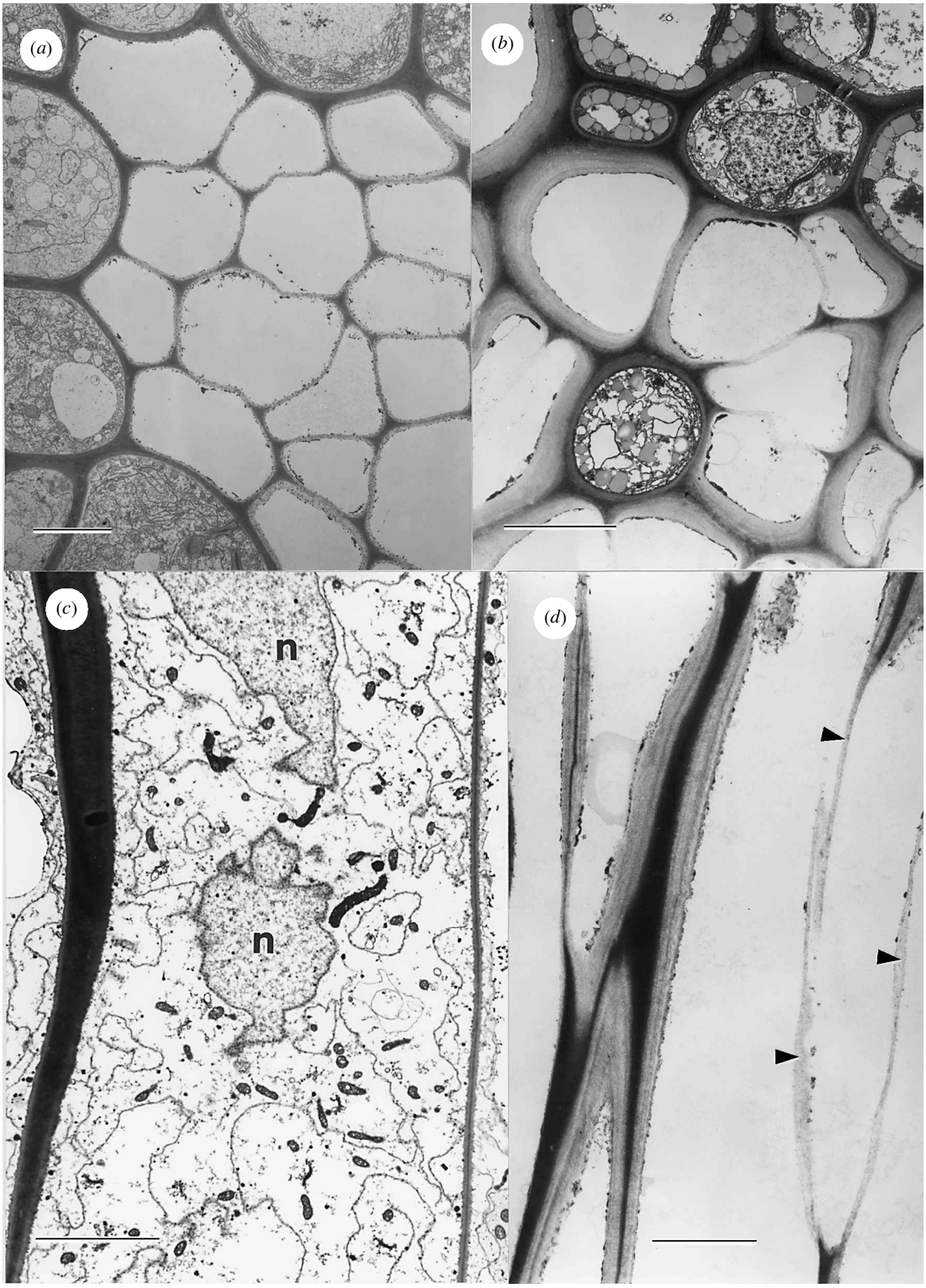

Figure 2. Transmission electron micrographs of moss hydroids. (*a*) *Mnium hornum*, sporophyte foot; hydroids with uniformly thin walls. (*b*) *Polytrichum juniperinum*, leafy shoot; hydroids with unevenly thickened walls. (*c*) *Polytrichum formosum*, leafy shoot; differentiating hydroid. Note the large pleomorphic nucleus (n); the longitudinal wall on the left is becoming thickened whilst the original transverse wall on the right is very thin. (*d*) *Polytrichum formosum*, leafy shoot; mature hydroids. Note the low electron opacity and absence of pores in the originally transverse walls (arrowed). Bar lines = 5 μm.

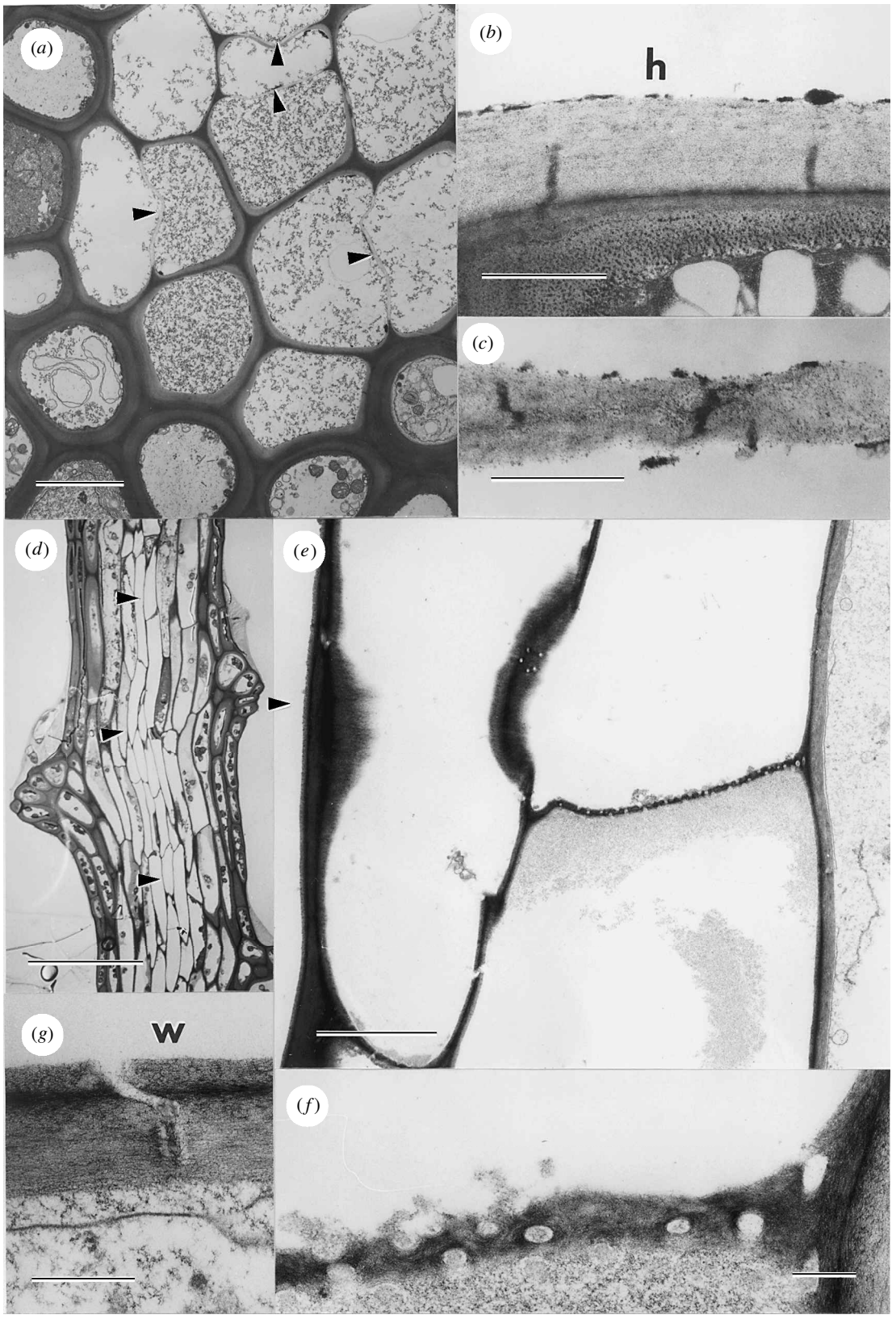

Figure 3. (*a–c*) Details of polytrichalean hydroids. (*a*) *Polytrichum formosum*, sporophyte foot. Note the thick longitudinal walls and the thin, originally transverse walls (arrowed). (*b*,*c*) *Polytrichum formosum*, leafy shoots. (*b*) Obliterated plasmodesmata between a hydroid (h) and an adjacent cortical cell. (*c*) Remnants of plasmodesmata in an originally transverse wall of a differentiating hydroid. (*d–f*) *Takakia*, water-conducting cells. (*d*) Light micrograph, longitudinal 1 μm section. The central water-conducting cells (arrowed) are similar in size and shape to the adjacent cortical cells. (*e*) In longitudinal sections, the water-conducting cells have transverse septa containing numerous plasmodesmata-derived pores. (*f*) Details of the pores in an end wall. (*g*) Detail of the wall between a water-conducting cell (w) and an adjacent cortical cell. Bar lines: *a*, *e* = 5 μm; *b*, *c* = 1 μm; *d* = 100 μm; *f* = 0.5 μm; *g* = 0.25 μm.

Table 1. *Taxonomic distribution and main features of internal water-conducting cells in bryophytes*

anthocerotes	unknown
liverworts	
Calobryales	slightly elongate cells with thin walls and large pores produced by lysis of primary wall associated with modified plasmodesmata
Metzgeriales (Pallaviciniaceae)	elongate cells with thickened walls and large pits produced by dissolution of secondary wall material associated with modified plasmodesmata; polyphenols in cell walls
Metzgeriales (*Moerckia*)	slightly elongate cells with swollen walls. Remnants of plasmodesmata visible occasionally
Marchantiales s.l.	unknown[a]
Jungermanniales	unknown
mosses	
Takakia	slightly elongate cells with thin walls and small pores derived from plasmodesmata
Andreaeidae	unknown
Sphagnidae	unknown
Bryidae	imperforate and very highly elongate cells with thick and/or thin walls; thin walls loosely textured; polyphenols in cell walls

[a]See text for discussion of possible water-conducting elements in *Conocephalum* (Kobiyama & Crandall-Stotler 1999).

group to tracheophytes. Among the most relevant morphological characters considered are stomata, present in the sporophyte in some anthocerotes and mosses, and internal conducting tissues, occurring in the majority of mosses and few liverwort taxa.

The present paper analyses ultrastructural and developmental features of water- and food-conducting tissues in bryophytes, with emphasis on their possible significance in the context of phyletic interrelationships of embryophytes. Much of the present knowledge on this issue is due to the seminal work by the late Charles Hébant, to whom this paper is dedicated.

2. WATER-CONDUCTING TISSUES

(a) *Mosses*

Three major moss groups are currently recognized, i.e. the Andreaeidae, Sphagnidae and Bryidae, the last (peristomate mosses) being considered advanced relative to the first two (Crosby 1980). An internal strand of WCCs is lacking in both the Andreaeidae, including the newly introduced genus *Andreaeobryum* (Murray 1988) (figure 1*a*), and Sphagnidae (Ligrone & Duckett 1998*a*). The latter are characterized by specialized dead cells, the hyalocysts, forming an effective external water-conducting system (Mozingo *et al*. 1969).

An internal water-conducting system occurs in the Bryidae (figures 1, 2 and 3*a–c*) and is considered as a distinctive character of this group. This consists of highly elongate cells lacking cytoplasmic contents at maturity. Traditionally these cells are called hydroids, a term introduced by Potonié (1883) and first used for mosses in Tansley & Chick's (1901) classical paper on conducting tissues of bryophytes. The hydroids, alone or associated with stereids (thick-walled living cells), form a central strand of varying size (figure 1) in the leafy stem of the gametophyte and/or in the sporophyte seta, which is sometimes referred to as hydrom. In many instances, hydroids also occur in the leaf nerve, where they are usually associated with stereids and specialized parenchyma cells referred to as deuters (Hébant 1977). Absence of a central strand in certain genera (figure 1*b*) and species of Bryidae is frequent (Hébant 1977, 1979) and is interpreted as a result of reduction (Schofield 1985). In line with this notion is the fact that the more primitive members of the Bryidae, i.e. the nematodontous order Polytrichales (including the Dawsoniaceae), have highly developed conducting strands. Conversely, a tendency towards a reduction of the water-conducting system is observed in the more advanced groups. Some moss species are known where a strand of conducting cells occurs in the seta but is lacking in the gametophyte (Hébant 1977). The converse is true in *Grimmia pulvinata*, where the developing sporophyte remains almost entirely ensheathed by the upper leaves of the parental shoot.

The hydroids in the leafy stems of mosses arise from the inner cell produced by the first division of the apical derivatives (Berthier 1972). The initials are shortly rectangular cells with numerous plasmodesmata in their oblique terminal walls and fewer in the longitudinal walls. Hydroid differentiation is very fast and involves pronounced cellular elongation (figure 1*f*,*g*) during which the cellular ends become so strongly tapered that the demarcation between longitudinal and transverse walls is solely indicated by the presence of Y-shaped junctions (figure 2*d*). The nucleus becomes elongate and pleomorphic (figure 2*c*) and may undergo endopolyploidization (Hallet 1972). Very early in development the plastids lose their starch content and become relatively small and irregular in shape. In some members of the Polytrichales (figures 2*b*,*c* and 3*a*) new wall material is deposited along longitudinal walls, but it is not clear if this is to be considered as a secondary wall (Hébant 1974). The newly deposited material causes the obliteration of the plasmodesmata between the hydroids and adjoining cortical cells (figure 3*b*). Hydroids in bryalean mosses have uniformly thin walls (figure 2*a*). Differentiation concludes with cytoplasmic degeneration and the alteration of walls, including the disappearance of all the plasmodesmata.

The modified walls have amorphous appearance with low electron opacity and may contain remnants of disrupted plasmodesmata (figure 3*c*) but always lack pores. Because of their loosely fibrillar texture (figure 2*d*) in transmission electron micrographs, it has been assumed that similar to tracheary elements (O'Brien 1974), maturational changes in the walls of hydroids involve

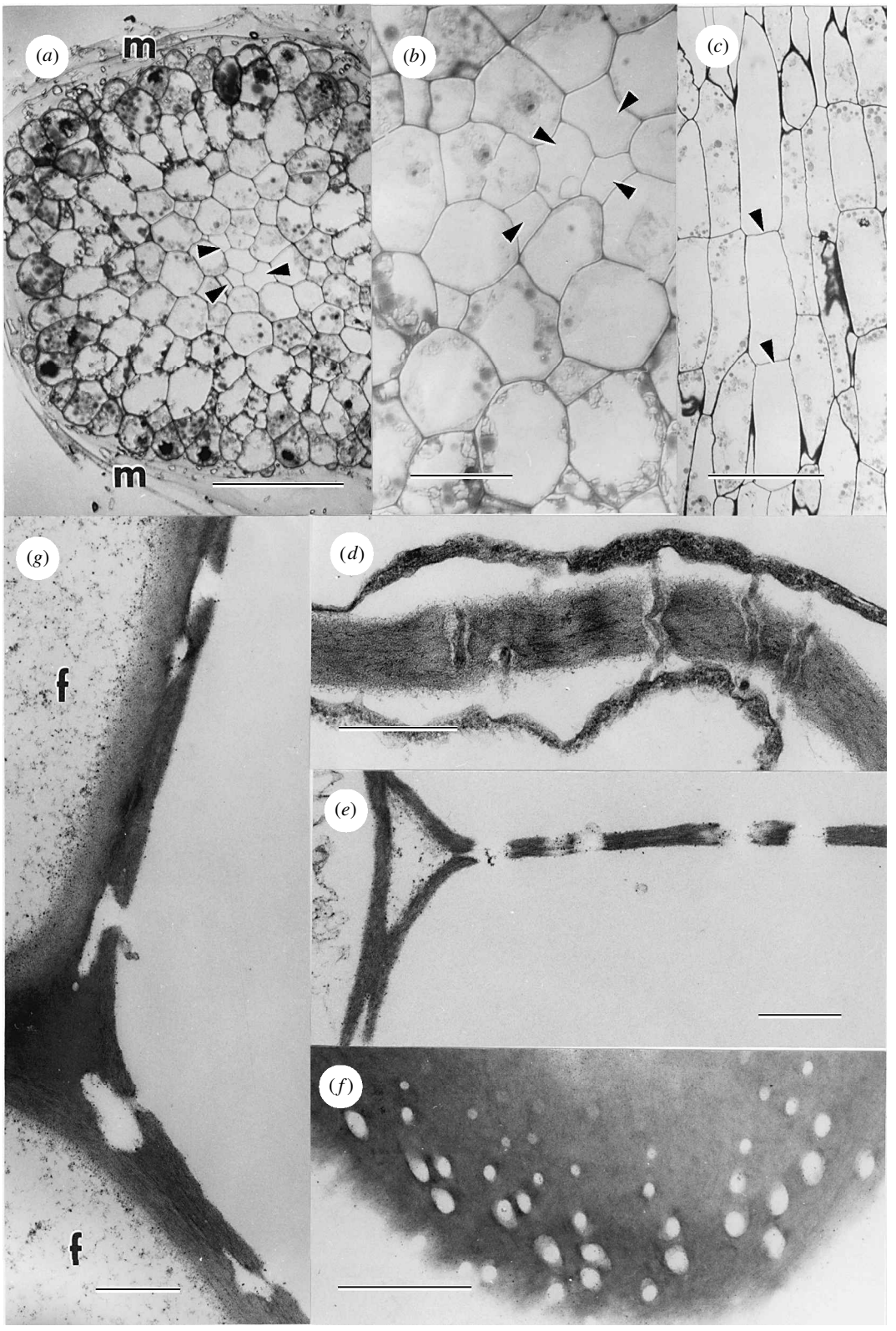

Figure 4. Conducting cell differentiation in *Haplomitrium hookeri*. (*a*) Transverse section of an underground axis. Note the investitive of mucilage (m) and the small central strand of water-conducting cells (arrowed). (*b*) At higher magnification the smaller size and absence of contents distinguishes the water-conducting cells (arrowed) from the adjacent food-conducting elements. (*c*) Longitudinal section showing the transverse end walls of the water-conducting cells. (*d*) Plasmodesmata in the end wall between two differentiating water-conducting cells. (*e–g*) Details of the plasmodesmata-derived perforations in mature water-conducting cells. (*e*, *f*) Longitudinal and transverse sections through end walls. (*g*) Pits ending blindly against adjacent food-conducting cells (f). Bar lines: *a*, *c* = 100 μm; *b* = 25 μm; *d*, *f*, *g* = 1 μm; *e* = 0.25 μm.

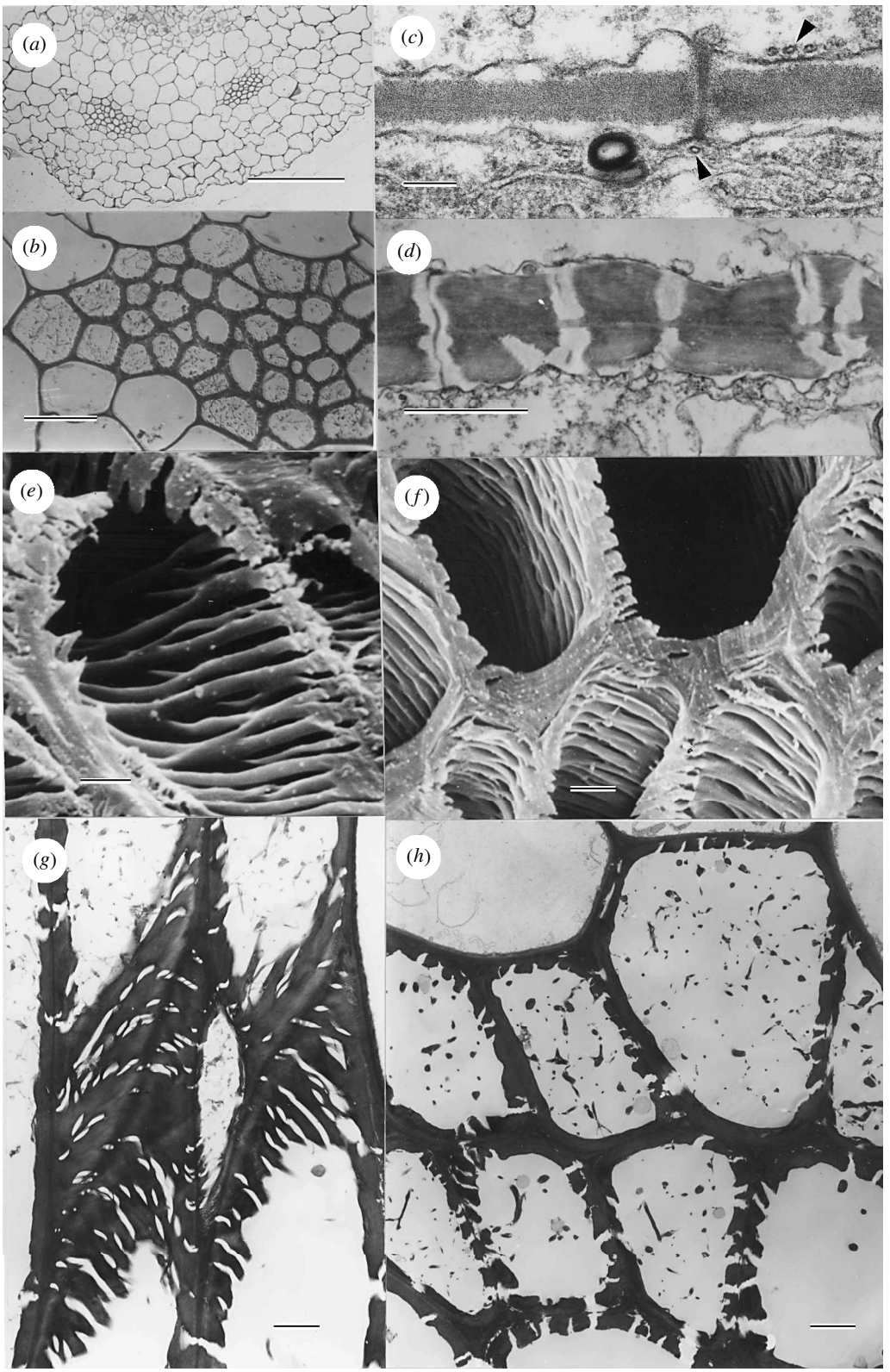

Figure 5. Water-conducting cells in *Symphyogyna*. (*a*,*b*) Light micrographs. (*a*) Two strands of narrow water-conducting cells are visible in a recently bifurcated thallus. (*b*) Higher magnification. Note the thick walls of the conducting cells. (*c*,*d*) Transmission electron micrographs showing details of wall differentiation. (*c*) At the onset of cell maturation the thin walls contain normal plasmodesmata and numerous cortical microtubules (arrowed) are present. (*d*) Thickening wall. Collars of electron-transparent material are associated with the plasmodesmata. (*e*,*f*) Scanning electron micrographs of mature water-conducting cells showing the elongated pits in the thickened walls. (Micrographs kindly supplied by Dr W. Frey). (*g*,*h*) Transmission electron micrographs of mature conducting cells showing the large pits in the now highly electron-opaque walls. Bar lines: $a = 100\,\mu m$; $b = 10\,\mu m$; $c = 0.2\,\mu m$; d, $e = 0.1\,\mu m$; f–$h = 20\,\mu m$.

enzymatic removal of non-cellulosic polysaccharides (Hébant 1977). However, cytochemical evidence for such wall 'hydrolysis' has never been produced. The apparently 'hydrolysed' appearance of the walls may be simply a consequence of cell extension. Only unthickened walls or wall areas undergo these structural alterations in polytrichaceous mosses, while in *Timmiella* (Ligrone *et al*. 1980) and other bryalean mosses the walls are modified throughout except at the cellular corners. Where the hydroids abut parenchyma cells the wall alteration stops at the middle lamella region of these cells (Ligrone *et al*. 1980). In no case has lignin been detected in hydroids (Hébant 1974, 1977), although histochemical evidence points to the presence of polyphenolic compounds (Scheirer 1980). Hydroids also lack secondary wall patterns such as spirals, bands or pitting. A chemical study of whole plants confirms that mosses do not contain lignin as defined in angiosperms and gymnosperms but may produce other types of polyphenolic compounds (Miksche & Yasuda 1978).

Mature hydroids are very variable in size, ranging from 200 to 1500 μm in length and 10 to 25 μm in width, according to the species and organs. The overlap contact area of adjacent hydroids may be as large as several hundred micrometres.

External water conduction by capillarity is of utmost importance in mosses (Proctor 1979). Nevertheless, numerous experiments using a range of different techniques have demonstrated that the inner strand of hydroids, when present, is an effective preferential way for water transport (Hébant (1977) and literature therein). Water supply to the growing capsule must usually rely entirely on internal conduction along the seta, as this lacks an external capillary system and is covered by a water-repellent cuticle. However, the capillary system formed by the ensheathing leaves in *Grimmia pulvinata* may have a role in water supply to the growing sporophyte, thus accounting for the lack of hydroids in the seta of this moss.

In a distinct position relative to the other mosses stands *Takakia*, an enigmatic taxon recently transferred from liverworts, where it had been placed since the discovery of its gametophyte by Mitten (1861) and the circumscription of the genus (Hattori *et al*. 1968). With the recent discovery of the sporophytic generation and antheridial plants, it has become undeniable that *Takakia* is a moss with affinities with the Andreaeidae (Smith & Davison 1993), a conclusion also supported by ultrastructural studies (Garbary *et al*. 1993; Ligrone *et al*. 1993; Renzaglia *et al*. 1997). As first reported by Hébant (1973), and in sharp contrast to other mosses, however, *Takakia* possesses WCCs with plasmodesmata-derived pores, a feature formerly considered to support its classification in the liverwort group of Calobryales (Schuster 1984; Schofield 1985). These cells form a small, central strand both in the gametophyte shoot and in the sporophyte seta (Renzaglia *et al*. 1997). They are of about the same size and shape as the adjoining cortical parenchyma cells, i.e. *ca*. 12 μm in width and 80 μm in length, but have much thinner walls (*ca*. 0.3 μm) (figure 3*d*,*e*). The terminal walls are nearly perpendicular or slightly oblique to the long cellular axis. Like the hydroids, the central strand cells in *Takakia* undergo cytoplasmic autolysis, with a strong peak of acid-phosphatase activity (Hébant 1975), and have no cytoplasmic contents at maturity. The plasmodesmata show no apparent modification during differentiation and when disrupted leave perforations in the walls, which do not normally exceed 120 nm in diameter (figure 3*f*). Most perforations are concentrated in the terminal walls but are also frequent in longitudinal walls. The plasmodesmata connecting WCCs with adjoining cortical cells break down only on the side of the dead cell, while on the other side remains a clearly recognizable desmotubule (figure 3*g*).

(b) *Liverworts*

Among the liverworts, an internal strand of specialized WCCs occurs in the Calobryales and in few genera of the Metzgeriales (simple thalloid liverworts), in both cases being restricted to the gametophyte (Burr *et al*. 1974; Smith 1966; Hébant 1977, 1978, 1980). The Marchantiales (complex thalloid liverworts) and Jungermanniales (leafy liverworts) have no internal strand of water-conducting cells (Hébant 1977; Kobiyama & Crandall-Stotler 1999). Like hydroids and tracheids, WCCs in liverworts are dead and lack cytoplasmic contents at maturity. However, they are distinct from both as their walls contain numerous pores arising from plasmodesmata.

As in *Takakia*, WCCs in calobryalean liverworts (figure 4) are similar in shape to ordinary parenchyma cells (*ca*. 20 μm wide and 50–60 μm long) with thin walls (0.25–0.50 μm) and transverse or slightly oblique terminal septa. Different from *Takakia*, at a late stage of differentation swelling of the middle region of plasmodesmata is observed (figure 4*d*,*e*). Moreover, the wall material immediately adjoining the plasmodesmata is removed during terminal cytoplasmic dissolution, thus producing pores much wider (300–600 nm) than those in *Takakia*.

By contrast, WCCs in the metzgerialean genera *Symphyogyna* (figure 5), *Hymenophyton* and *Pallavicinia* are thin and elongate (*ca*. 8 μm wide and up to 300 μm long) with tapering ends and thickened walls (1–1.7 μm) perforated throughout by numerous pits (250–600 μm in diameter). The walls are strongly electron-opaque (figure 5*g*,*h*) and probably contain polyphenolic compounds because they are autofluorescent when examined in blue light, though tests to reveal lignin are negative (Smith 1966). The pits arise from plasmodesmata (figure 5*c*,*d*) through a mechanism closely recalling the genesis of pores in sieve elements (Esau & Thorsh 1985; Lucas *et al*. 1993). This process has recently been described in detail in *Symphyogyna* (Ligrone & Duckett 1996). Following an elongation phase that terminates when the cells have reached their definitive sizes, the cell walls are thickened by deposition of electron-opaque material, on extraplasmodesmatal areas, and of electron-transparent material forming collars around plasmodesmata (figure 5*d*). The newly deposited wall material differs cytochemically and structurally from the original wall (Smith 1966; Ligrone & Duckett 1996) and is therefore to be considered a secondary wall. Eventually, the cells undergo cytoplasmic dissolution and the electron-transparent wall material around the plasmodesmata is removed. Only a minority of the pits are completely open, while most appear to be occluded by a plug in the middle (Frey *et al*. 1996). This arises from the thin,

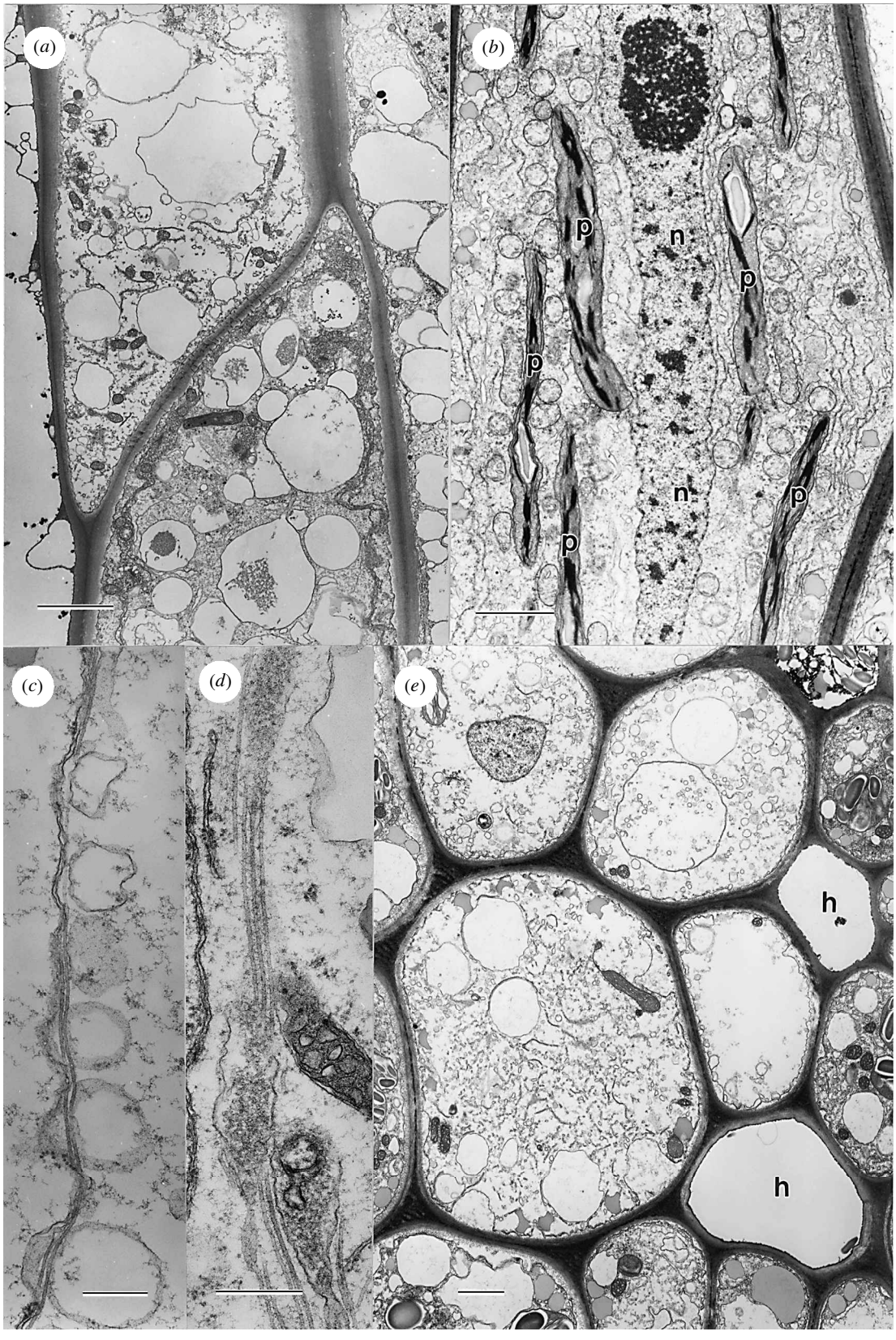

Figure 6. Cytological details of moss food-conducting cells. (*a*) Cytoplasmic polarity in a leafy stem of *Plagiomnium undulatum*; most of the organelles are at the distal (top) end of the lower cell. (*b*) *Mnium hornum*, seta showing the longitudinal alignment of elongated plastids (p) and the highly elongated nucleus (n). (*c*,*d*) Longitudinal arrays of microtubules associated with tubules and vesicles in a leafy stem of *Plagiomnium undulatum* (*c*) and *Polytrichum juniperinum* (*d*). (*e*) Transverse section of leptoids and adjacent hydroids (h) in a stem of *Polytrichum commune*. Note the numerous vesicles and membrane profiles throughout the electron-transparent cytoplasm. Bar lines: $a = 4.0\,\mu m$; *b*, $e = 2.0\,\mu m$; *c*, $d = 0.5\,\mu m$.

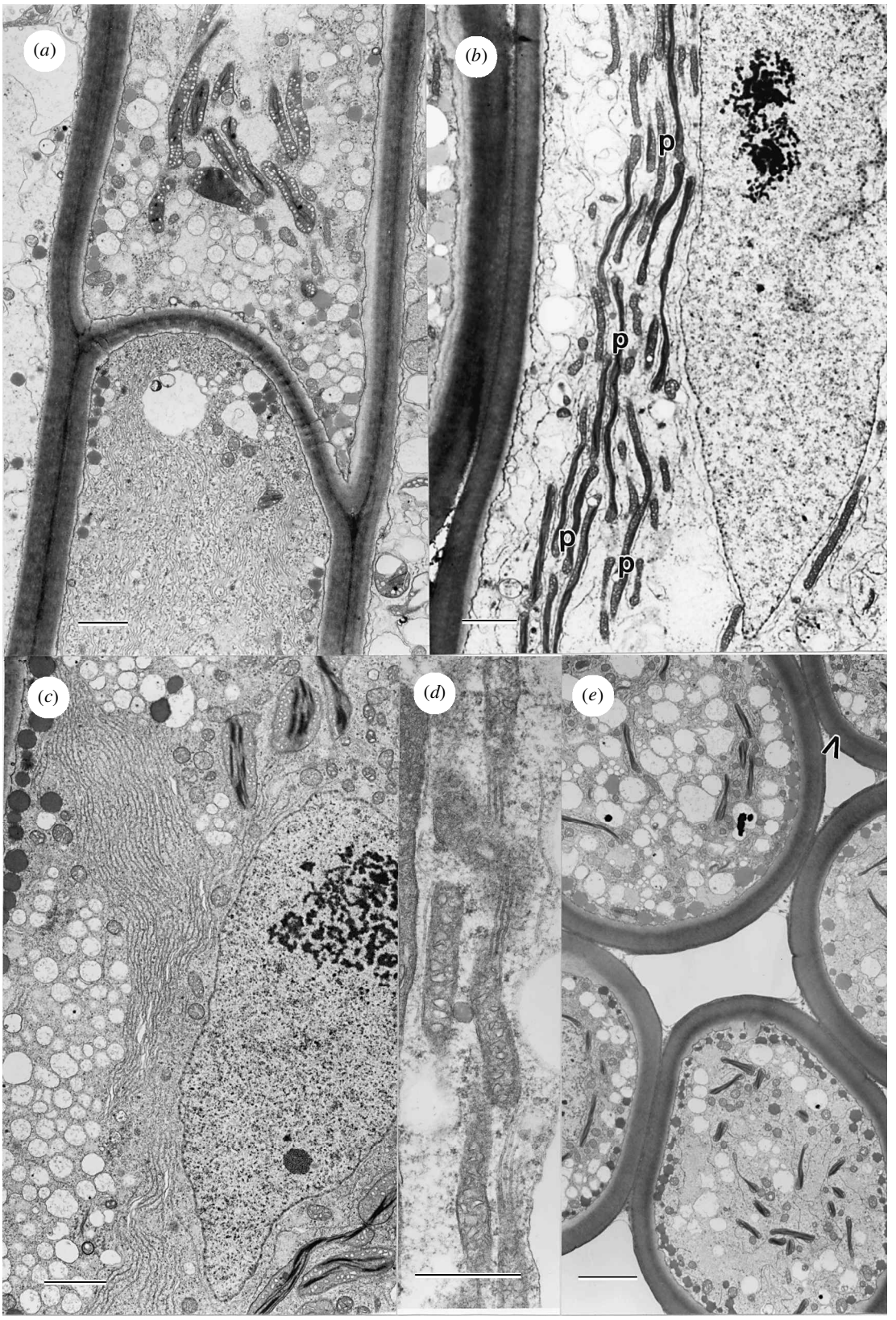

Figure 7. Cytological details of leptoids in *Polytrichum juniperinum* sporophytes. (*a*) Cytoplasmic polarity in a foot. The distal cellular end of the lower cell is packed with endoplasmic reticulum. (*b*) Longitudinally aligned plastids (p) and a spindle-shaped nucleus in a seta. (*c*) Sheets of endoplasmic reticulum adjacent to the nucleus. (*d*) Endoplasmic microtubules associated with elongate mitochondria. (*e*) Conducting parenchyma in a seta. Note the thick walls and intercellular spaces. Bar lines: *a–c* = 2.0 μm; *d* = 10 μm; *e* = 4.0 μm.

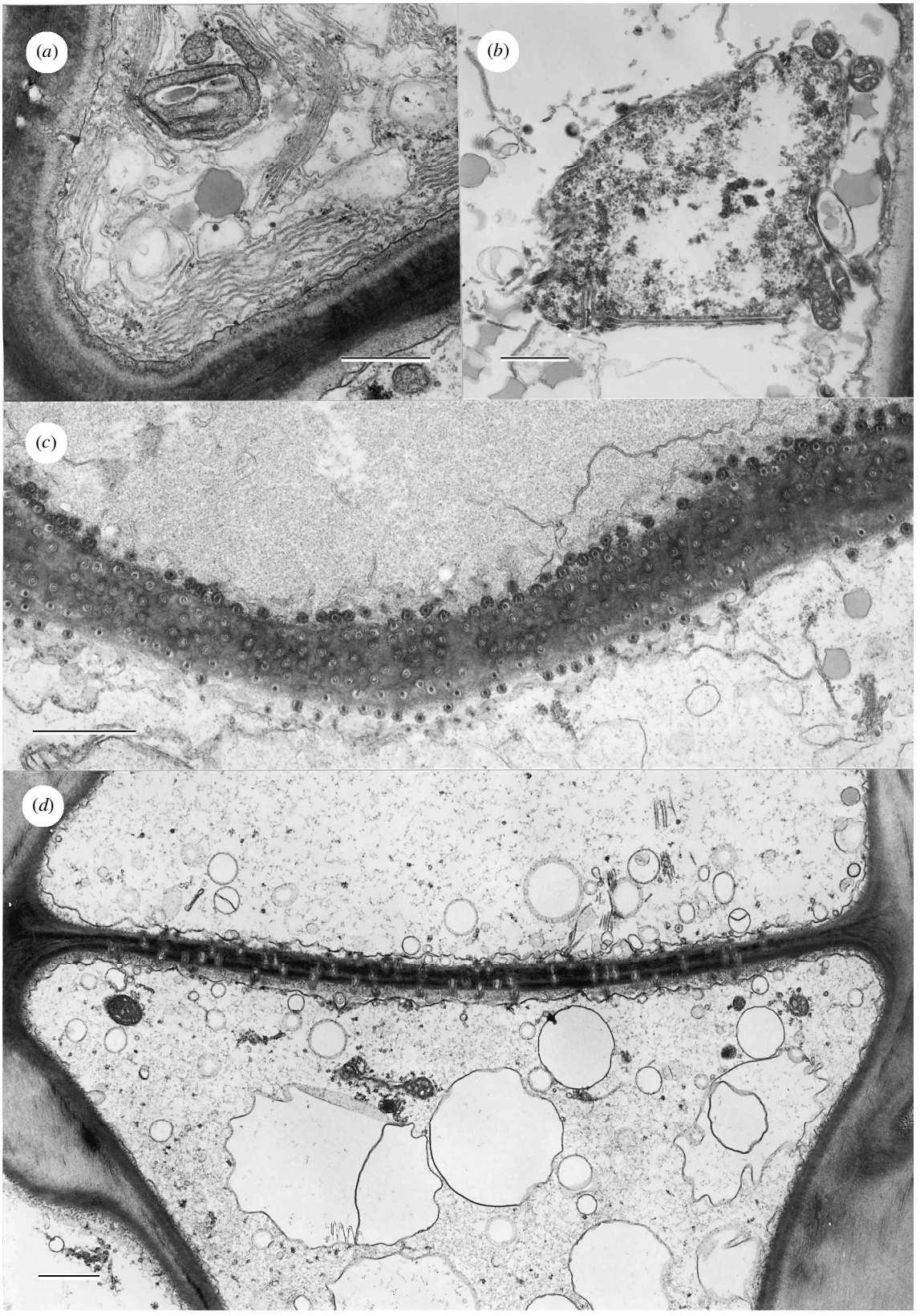

Figure 8. Cytological details of moss food-conducting cells. (*a*) Abundant endoplasmic reticulum in a leafy shoot of *Polytrichum juniperinum*. (*b*) Degenerating nucleus in a shoot of *Polytrichum juniperinum*. (*c*,*d*) Abundant plasmodesmata in the end walls between food-conducting cells in *Neckera crispa* and *Sphagnum cuspidatum*. Note the trumpet-shaped cell ends in the latter. Bar lines: $a = 5\,\mu m$; $b–d = 10\,\mu m$.

primary wall area being not completely removed along with the electron-transparent collar lying above it. However, the dissolution of the desmotubule and plasmalemma leaves an open, albeit small, pore in the primary wall. The pits show a spiral arrangement that mirrors that of cortical microtubules present during cell differentiation, and often merge together to form larger compound pits. Because of the spiral arrangement of pits and their tendency to fuse during development, the *Symphyogyna*-type fully differentiated WCCs exhibit a striking similarity to tracheids (figure 5*e*,*f*) (Frey *et al*. 1996). Experimental evidence using eosin as a tracer in living plants has demostrated that water moves in the central strand at a much faster rate than in neighbouring parenchyma cells (Smith 1966).

WCCs in *Moerckia*, a genus closely allied to *Pallavicinia* (Renzaglia 1982), appear to stand apart from those in other metzgerialean liverworts, for these cells show swollen, apparently hydrolysed walls with no pits (Hébant 1973). However, different from moss hydroids, traces of plasmodesmata have occasionally been encountered in these walls (Hébant 1977). It is not possible to establish from the data available whether WCCs in *Moerckia* are a variant of perforate WCCs or represent a distinct type.

A recent study in the marchantialean liverwort genus *Conocephalum* has produced a detailed description of pitted cells scattered in the parenchyma tissue in the thallus nerve and in the stalk of archegoniophores (Kobiyama & Crandall-Stotler 1999). These cells possess reticulate primary wall thickenings of cellulosic nature and prominent pit fields, the latter occurring in both lateral and terminal walls in *C. conicum* but only in lateral walls in *C. japonicum*. The cells have large central vacuole(s) and very thin peripheral cytoplasm. Nuclear loss and partial cytoplasmic lysis are reported to occur in some of the cells, though no ultrastructural evidence is produced. Kobiyama & Crandall-Stotler (1999) also claim that tests with methylene blue suggest that these cells facilitate absorption of water from the rhizoids but again supporting micrographic data are lacking. The authors' claim that these cells are a unique kind of water-conducting element must therefore be viewed with considerable caution; Kobiyama & Crandall-Stotler's micrographic evidence indicates that these cells are much more likely to be the food-conducting elements previously described in the centre of the thalli of complex thalloid liverworts (Ligrone & Duckett 1994*b*).

(c) *Anthocerotes*

The anthocerotes contain no specialized WCCs in the gametophyte nor in the sporophyte. In some genera, e.g. *Phaeoceros* and *Anthoceros*, the sporophyte contains a central strand of elongate cells, referred to as the columella, which bears a superficial resemblance to the stele in lower tracheophytes (Campbell 1925). Associated with spores are elaters with spirally arranged wall thickenings similar to those in tracheids but lacking lignin (Proskauer 1960). The columella cells lack cytoplasmic contents at maturity but apparently have no special role in water transport (Isaac 1941). No ultrastructural study of these cells has been reported to date.

3. FOOD-CONDUCTING TISSUES

(a) *Mosses*

The Polytrichales have long been known to possess, besides hydroids, specialized cells with marked morphological similarity to the protophloem sieve cells in tracheophytes. These cells were called 'leptoids' by Tansley & Chick (1901), who apparently derived this term from the term 'leptom', introduced by Haberlandt in 1879 for the whole system of phloem-like tissue in polytrichaceous mosses (table 2).

Leptoids in polytrichaceous mosses (figures 6–8) occur both in the gametophyte and the sporophyte seta. They attain the highest degree of structural specialization in the leafy stem (table 3), where they are intermingled with elongate parenchyma cells to form a ring around the central strand of hydroids. The leptoids are several hundred micrometres long, up to 500 μm in *Dendroligotrichum* (Scheirer 1990), have enlarged extremities and oblique end walls. Major features of mature leptoids that recall protophloem sieve cells are listed in table 4.

The pores in the terminal walls of leptoids are modified plasmodesmata (figure 9) with a desmotubule forming a median enlargement *ca*. 100–200 nm wide but usually still constricted at both ends (Hébant 1976; Scheirer 1978, 1990; cf. Lucas *et al*. 1993). The desmotubule is continuous with tubular endoplasmic reticulum elements forming a prominent network in the adjacent cytoplasm. Tubules of endoplasmic reticulum traversing the sieve area pores are of common occurrence in ferns and *Psilotum* (Evert 1990*b*) as well as in conifers (Schulz 1990), but these do not form desmotubule-type constrictions; the sieve pores are generally completely open in other vascular cryptogams (Evert 1990*b*) and in angiosperms (Eleftheriou 1990, 1996; Evert 1990*a*).

With the use of radioactive tracers, leptoids have been demonstrated to be a preferential route for the translocation of organic nutrients, with translocation rates of up to several tens of centimetres per hour (Eschrich & Steiner 1967; Collins & Oechel 1974; Thomas *et al*. 1988; Schmid 1998).

In all other moss groups apart from the Polytrichales, the cortical tissue of the leafy stem and seta consists of elongate parenchyma cells (150–250 μm). These are likely to function in symplasmic transport of assimilates because of the presence of numerous plasmodesmata (figure 8*c*) in their end walls (10–30 μm^{-2}). As in leptoids, the plasmodesmata in these cells present a median enlargement (figure 9) and often show continuity with endoplasmic reticulum. However, the internal parenhyma cells of non-polytrichaceous mosses lack most of the distinctive features of leptoids and appear to be structurally much less specialized. According to Hébant (1977) they should be referred to as 'conducting parenchyma cells', while the use of the term leptoid should be restricted to the specialized conducting cells of Polytrichales.

Recent ultrastructural research on members of the Sphagnales, Polytrichales and bryalean mosses (Ligrone & Duckett 1994*a*, 1998*a*), as well as preliminary observations on *Takakia*, have revealed that—irrespective of the level of morphological specialization—putative food-conducting cells share a series of morphological characteristics that enable them to be distinguished from normal parenchyma cells at first sight (table 2). This extremely

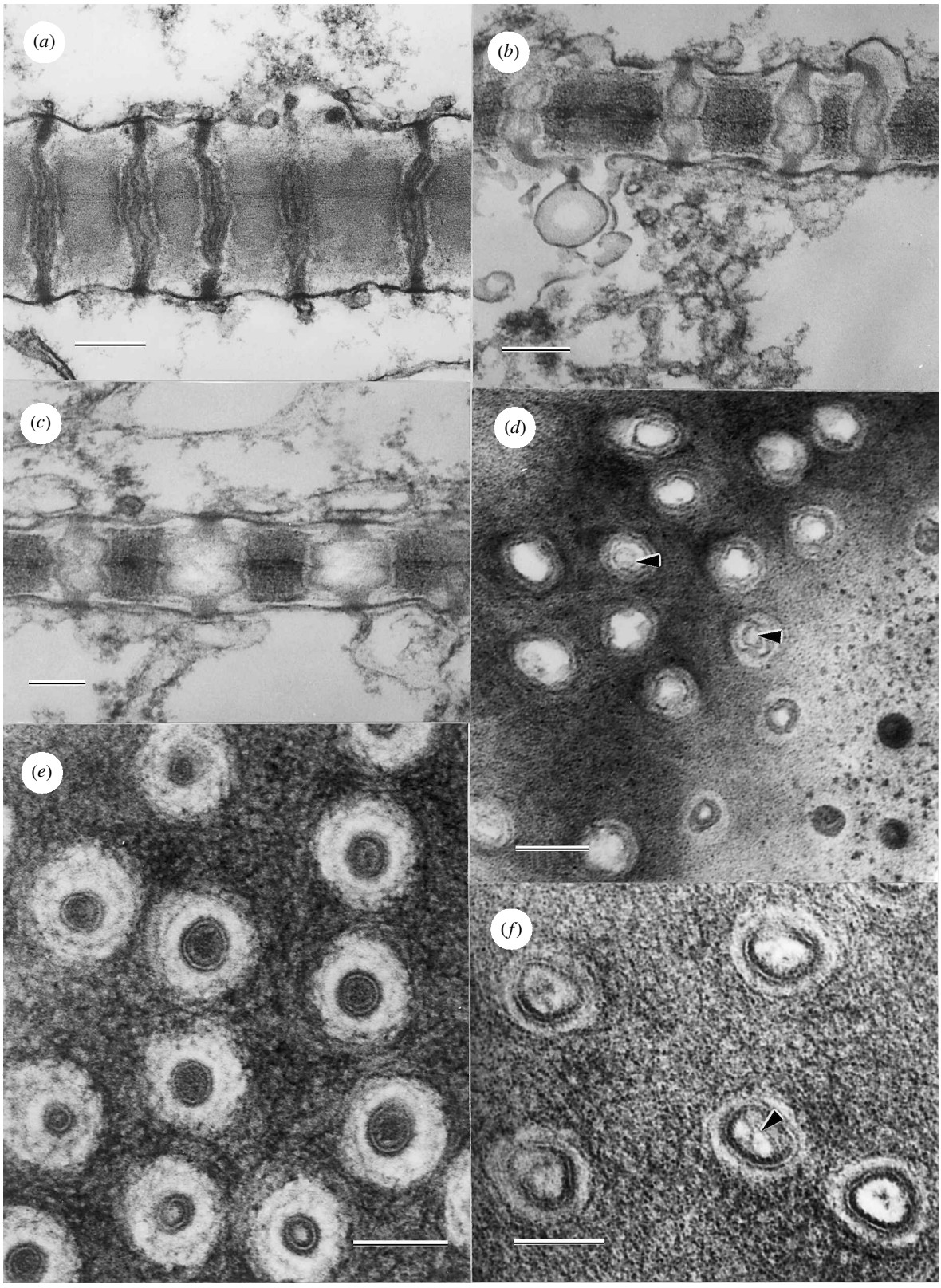

Figure 9. Plasmodesmata between food-conducting cells in mosses. (*a–c*) Longitudinal sections showing median enlargements in a leafy shoot of *Mnium hornum* (*a*) and *Aulocomnium palustre* (*b*,*c*). Note the frequent continuity with endoplasmic reticulum. (*d–f*) Transverse sections of *Polytrichum formosum* (*d*) and *Sphagnum recurvum* (*e*,*f*) showing median chambers usually containing no discernible desmotubule, but occasionally irregular tubular structures (arrowed in *d* and *f*), and subterminal constrictions with clearly defined plasmalemmal and desmotubule profiles. Bar lines: *a–c* = 0.2 μm; *e–f* = 0.1 μm.

Table 2. *Cytological characteristics of putative food-conducting cells in bryophytes*

(+, Constant feature; −, not observed; ±, observed occasionally.)

	Takakia	Sphagnidae	Bryidae	liverworts
longitudinal arrays of endoplasmic microtubules associated with:				
spindle-shaped nucleus	+	+	+	±
elongate plastids	−	−	+	−
elongate mitochondria	+	+	+	−
elongate microbodies	+	+	+	−
pleomorphic vacuoles	+	+	+	+
membrane-bound tubules and vesicles	+	+	+	+
endoplasmic reticulum	−	−	+	−
cytoplasmic polarization: the bulk of organelles at the sink end of each cell (i.e. towards the apex of the leafy shoot and capsule in the sporophyte); granular cytoplasm at the opposite end	+	+	+	±
disappearance of large vacuoles (present during differentiation)	+	+	+	+
high frequency of plasmodesmata in end walls; desmotubules forming a median enlargement	+	+	+	+
aggregation of free ribosomes	+	+	+	−
abundant dictyosomes and trans Golgi network	+	+	+	+

distinctive organization is found not only in the cortical cells of the leafy shoot and seta but also in other types of tissues and organs, including mature caulonemal and rhizoidal cells (Duckett *et al.* 1998), where a need for long-distance symplasmic transport of solutes can be postulated (table 5).

An experimental study (Ligrone & Duckett 1996) has shown that polarized organization is suppressed following removal of the source/sink gradient. The longitudinal alignment of the nucleus and organelles is disrupted by oryzalin, a microtubule inhibitor, while it is insensitive to cytochalasin, a drug affecting the actin microfilaments.

Of special interest is the situation recently discovered in the Sphagnales, where the internal parenchyma cells of the leafy stem are morphologically similar to the conducting parenchyma cells of other mosses but, unlike these, arise from a subapical secondary meristem (Ligrone & Duckett 1998*a*,*b*). The internal parenchyma cells of *Sphagnum* are most likely the main route for the symplasmic longitudinal transport of nutrients shown experimentally and thought to reflect nutrient recycling from old to new parts of the plant (Rydin & Clymo 1989). From a developmental standpoint, the conducting parenchyma cells of Sphagnales are not homologous to the equivalent cells in other mosses. The presence of a secondary meristem appears to be a characteristic unique to the Sphagnales, being probably related to the equally unique ecology of these mosses and their success in water-logged oligotrophic habitats.

Major morphological similarities and differences between sieve elements of tracheophytes and food-conducting cells of mosses are summarized in table 5.

(b) *Liverworts*

Until very recently no specialized food-conducting tissue had been described in liverworts. The internal cells of the thallus or of the stem in leafy liverworts are slightly elongate and were typically thought to contain a large central vacuole. In numerous thallose liverworts, the ventral cells may be colonized by endophytic fungi (Read *et al.*, this issue). However, a recent study of *Asterella* (Ligrone & Duckett 1994) revealed that the parenchyma cells of the midrib, between the dorsal photosynthetic tissue and the ventral fungus-containing tissue, are polarized and contain highly pleomorphic vacuoles associated with endoplasmic microtubules. Similar microtubule–vacuole associations have now been detected in other liverworts (table 5), including *Pellia* and *Haplomitrium* (figure 10).

(c) *Anthocerotes*

No morphological specialization for symplasmic transport has been reported in the anthocerotes. However, very little ultrastructural research has been done on this group to date. Considering that the highly distinctive cellular organization of polarized food-conducting cells in mosses has escaped attention until very recently, in spite of the considerable interest focused on this group, careful investigation of the anthocerotes must now be given a high priority.

4. DISCUSSION: EVOLUTIONARY AND FUNCTIONAL CONSIDERATIONS

Making holes in the walls by disruption of plasmodesmata together with the total loss of cytoplasmic contents is perhaps the easiest way to form a WCC under a selective pressure for more efficient apoplastic water transport. Possible homology of perforate WCCs in the calobryalean and metzgerialean liverworts should therefore be considered with caution, especially when set against the multiple origin of vessel elements, a much more complex cell type. WWCs in the two liverwort

Table 3. *Additional features of food-conducting cells in polytrichaceous mosses (leptoids)*

- nacreous walls
- endopolypoidy (also documented in caulonemal cells; Kingham *et al*. 1995; Duckett *et al*. 1998)
- partial degeneration of the nucleus
- refractive spherules
- large endoplasmic reticulum stacks
- abundant tubular endoplasmic reticulum associated with plasmodesmata
- callose associated with plasmodesmata

Table 4. *Sieve elements versus putative food-conducting cells in mosses*

similarities	
absence of vacuoles	
aggregation of ribosomes	
nacreous walls[a]	
nuclear degeneration[a]	
refractive spherules[a]	
callose associated with plasmodesmata[b]	
differences (in sieve elements):	
no cytoplasmic polarization	
disappearance of desmotubules	
oryzalin-insensitive at maturity	
actin bundles prominent during differentiation	
phloem protein	
total nuclear degeneration	

[a]Restricted to the Polytrichales in mosses.
[b]Restricted to the Polytrichales in mosses; absent in some lower tracheophytes.

Table 5. *The occurrence of 'food-conducting cytology' in bryophytes*

mosses
- inner parenchyma cells in the stem of the leafy shoot
- inner parenchyma cells in the sporophyte foot and seta
- parenchyma cells in the leaf nerve
- rhizoids
- caulonemata
- basal cells of mucilage hairs
- stalks of cauline gemmae

hepatics
- inner parenchyma cells in the central thallus of *Conocephalum* and *Asterella* (Marchantiales) and *Pellia* (Metzgeriales)
- inner parenchyma cells surrounding water-conducting cells in the leafy shoot and 'roots' of *Haplomitrium* (Calobryales)

anthocerotes
- unknown

groups present marked structural differences, those in the Metzgeriales being far more specialized than those in the Calobryales. Moreover, while an internal strand of WCCs is a generalized feature of the Calobryales—13 species in the only genus *Haplomitrium* (Bartholomew-Began 1991)—this is restricted to a minority of relatively advanced species within the much larger order Metzgeriales (Renzaglia 1982; Schuster 1984). Basal taxa in liverworts lack WCCs, and the closely allied families Pallaviciniaceae (*Pallovicinia*, *Symphygyne*) and Hymenophytaceae (*Hymenophyton*) have only a very remote link with the Calobryales (Mehra 1968; Schuster 1984; Schofield 1985). Even though Bartholomew-Began (1990) places the Calobryales (Haplomitriales) as an order within the subclass Metzgeriidae, our conclusion is that perforate WCCs have most probably evolved independently in the Calobryales and metzgerialean liverworts.

The general appearance of WCCs in metzgerialean liverworts, notably their thickened walls with elicoidally arranged pits, is strongly reminiscent of tracheids. However, pits in the former develop by removal of secondary wall material closely associated with modified plasmodesmata, while in the latter they arise from the lysis of primary unlignified walls with no direct relation to plasmodesmata, albeit in both cases cortical microtubules appear to have a prominent role in morphogenesis (cf. Ligrone & Duckett 1996; McCann 1997; Chaffey *et al*. 1997, 1999; Seagull & Falconer 1991). The two cell types, therefore, have sharply different developmental designs and homology between them is highly unlikely. The same applies to perforate WCCs in other bryophyte groups.

Reports of cells with helical thickenings from Lower Devonian Rhyniophytina (sensu Edwards 1993), referred to as *Sennicaulis*-type or S-type WCCs, emphasize alleged similarities with metzgerialean WCCs, notably the presence of 'micropores' scattered thoroughout a continuous inner wall layer (Kenrick *et al*. 1991; Kenrick & Crane 1991). As noted by Frey *et al*. (1996) and much earlier by Smith (1966), a more careful comparison of the structure of the two cellular types reveals that the two cellular types have basically different designs.

The WCCs in *Haplomitrium* are reminiscent of those in the moss *Takakia*. Consideration of the possible relationships of these cells becomes highly pertinent to the still unresolved question of phyletic interrelationships between mosses and liverworts. Thus it is intriguing, in the present context, to observe that similar WCCs is but one of several characters that provided the basis for the grouping *Haplomitrium* and *Takakia* in the liverwort order Calobryales (Schuster 1984; Gradstein 1990). The assumption of homology of WCCs in *Takakia* and *Haplomitrium* is consistent with topologies in which mosses and liverworts form a monophyletic group (e.g. Garbary & Renzaglia 1998), with the additional implication of mosses sharing a common ancestor with *Haplomitrium*. Once again, however, it must be emphasized that because of the extreme structural simplicity of WCCs in the two taxa, independent origin of these cells is quite possible. The lack of sporophytic WCCs in the liverworts is a major character separating liverworts from mosses, including *Takakia*. Most likely this is functionally related to the fact that the sporophyte seta in liverworts elongates by cell expansion (Schnepf & Deichgräber 1979) only after spore maturation, while in mosses the seta completes development before capsule maturation. WCCs are dead and no longer capable of expanding at maturity; hence they can develop in the seta of mosses but not of liverworts.

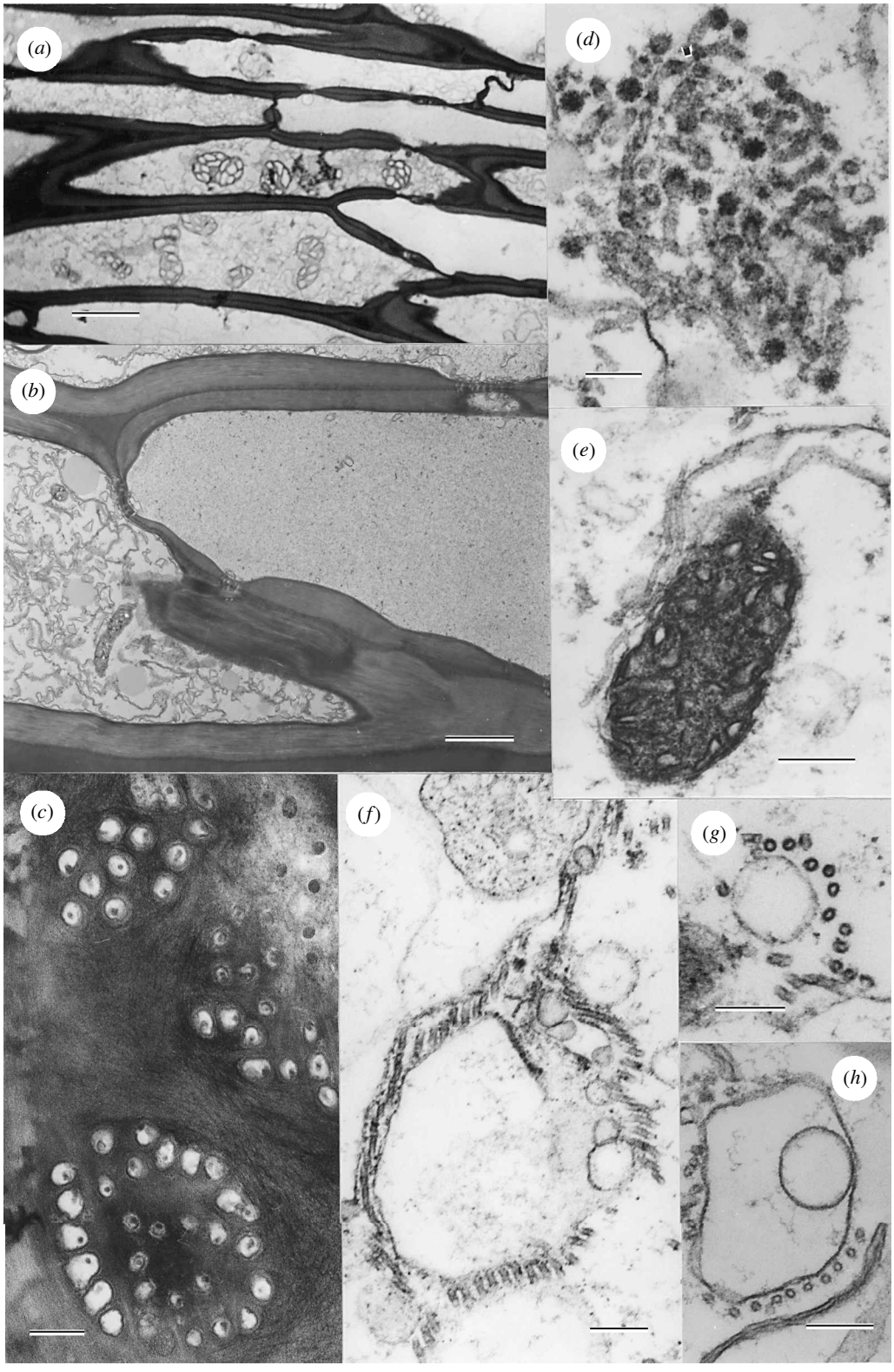

Figure 10. Details of food-conducting cells in liverworts. (*a–c*) *Asterella wilmsii*. (*d*,*e*) *Pellia epiphylla*. (*f*,*g*) *Haplomitrium hookeri*. (*a*,*b*) Light and transmission electron micrographs, longitudinal sections of inner thallus cells showing their thickened, pitted walls and cytoplasmic polarity (*b*). (*c*) Details of the pits showing numerous plasmodesmata with enlarged median chambers containing desmotubules. (*d*) trans Golgi network. (*e–h*) Endoplasmic microtubules associated with a mitochondrion (*e*) and membranous vesicles and tubules (*f–h*). Bar lines: $a = 20\,\mu m$; $b = 2\,\mu m$; $c, f–h = 0.2\,\mu m$; $d, e = 0.4\,\mu m$.

The hydroids in bryoid mosses are a highly distinctive type of WCC. Unlike WCCs in other bryophytes, hydroids have imperforate walls, the plasmodesmata being obliterated during development. Preliminary experimental observations have shown that when dehydrated, hydroids tend to collapse, unlike tracheary elements, and are highly resistant to cavitation (Schmid 1998; A. M. Schmid, unpublished data). Most likely this remarkable property has made possible the reconciliation of dead WCCs with desiccation tolerance, which is a major physiological characteristic of mosses (Proctor 1982; Oliver & Bewley 1984). Desiccation tolerance not only occurs in the Bryidae, but also in the Sphagnidae and Andreaeidae. There are at present no clues as to whether the absence of hydroids in these two groups is derived or primitive, though primitiveness is consistent with the basal position assigned to both groups. The perforate WCCs in *Takakia* might be interpreted either as a derived feature (autapomorphy) of this taxon or as a primitive feature (plesiomorphy) lost in most living mosses.

Nothing is known of the effects of dehydration on perforate WCCs. It is interesting, however, that neither *Haplomitrium* nor *Takakia*, is tolerant to desiccation (Grubb 1970). The same is probably true for the Pallaviciniaceae. Among the liverworts, desiccation tolerance is a widespread feature of the Jungermanniales and also occurs in members of the Marchantiales (Proctor 1982), both of which lack internal WCCs.

Similarities between hydroids and tracheids have been emphasized to support the contention that these two cell types are homologous (Scheirer 1980). In particular, attention has been focused on the mechanism of wall modification, allegedly involving in both cases partial hydrolysis of certain areas of the primary wall, with polyphenols playing a protective role in hydroids similar to that postulated for lignin in tracheids (O'Brien 1974). The assumption of homology of hydroids and tracheids is fundamental to cladograms in which the mosses are the sister group to tracheophytes (Mishler & Churchill 1984, 1985; Mishler *et al*. 1994). A closer look at the developmental design of the two cell types, however, shows several major differences. The deposition of secondary lignified walls is a fundamental event in the morphogenesis of tracheids and is preceded by the establishment of a cortical microtubule array that accurately predicts the sites of formation of wall thickenings and pits (Hogetsu 1991; McCann 1997; Chaffey *et al*. 1997, 1999). No immunocytochemical study of differentiating hydroids has hitherto been carried out, but transmission electron microscopy shows no prepatterned arrangement of microtubules in the cortical cytoplasm underlying expanding walls. Cell wall thickening, most likely of primary nature, only occurs in the hydroids of certain polytrichaceous mosses and here it only affects longitudinal walls. In the large majority of mosses, the hydroids undergo no wall thickening. Moreover, cell wall alteration indiscriminately affects all unthickened walls. This suggests that, at least in part, cell wall loosening results from passive stretching of differentiating and dead hydroids by adjoining living cells. We conclude from this that hydroids are a specialized type of WCC unique to mosses between hydroids and tracheids.

The discovery of unornamented WCCs, referred to as 'hydroids', in the branched sporophyte of the Lower Devonian plant *Aglaophyton major* (Edwards 1986) is unlikely to support the hypothesis of homology. These cells are much wider (*ca*. 40 μm) than moss hydroids and, as Edwards (1993) properly remarks, have no exact counterpart in extant bryophytes.

Indirect support for the notion of independent origin for hydroids and tracheids comes from studies of putative food-conducting cells in mosses. The discovery of a common structural pattern in leptoids and conducting parenchyma cells of mosses, independent of the diverse morphology and varying degree of cytological specialization that may be found in different species and organs, introduces a fundamental distinction from sieve cells of tracheophytes. In the latter cytoplasmic polarity has never been observed, while microtubules, present in the form of conspicuous cortical arrays during development, do not persist beyond maturation of the cell (Eleftheriou 1990, 1996; Evert 1990*a*,*b*; Schulz 1990). The two cell types, therefore, are unlikely to be homologous. The striking similarity of leptoids in polytrichaceous mosses to sieve cells is probably an instance of homoplasy related to the relatively large sizes attained by these mosses and consequent evolutionary pressure for a more efficient transport of assimilates. A similar example of homoplasy is found in the brown algae, notably the Laminariales, which contain highly specialized food-conducting cells bearing striking similarity to sieve elements of tracheophytes (Schmitz 1990). The occurrence in liverworts of cells structurally similar to food-conducting cells of mosses raises the possibility that this type of cell is a plesiomorphy of the liverwort–moss clade. This notion cannot be reconciled with topologies where mosses alone are resolved as the sister group to tracheophytes.

While pointing to conducting tissues as a major issue in embryophyte phylogeny, the present analysis introduces more new questions than answers. A more precise assessment of homology/homoplasy requires further investigation. In particular, useful information is expected from a better characterization of conducting tissues in the fossil record of early embryophytes as well as from comparative cytochemical and immunocytochemical studies of conducting tissues in extant bryophytes. Also required are physiological experiments that compare rates of water and solute transport in the diverse conducting elements found in bryophytes with those in the xylem and phloem of lower tracheophytes.

5. CONCLUSIONS

Perforate WCCs in the Calobryales and metzgerialean liverworts have probably evolved independently.

Perforate WCCs in *Takakia* have no counterpart in other mosses nor in tracheophytes. Similarities with WCCs in Calobryales are most likely a homoplasy.

The imperforate hydroids in bryoid mosses have no counterpart in other living embryophytes and must be considered as an autapomorphy of this group; absence of hydroids in one or both generations in numerous bryoid mosses is due to reduction.

Polarized food-conducting cells of mosses (including leptoids in polytrichaceous mosses) are not homologous with sieve cells of tracheophytes.

Further studies are needed to clarify possible relationships between (i) the WCCs in *Moerckia* and in other

metzgerialean liverworts; (ii) perforate WCCs of *Takakia* and imperforate hydroids in the Bryidae; (iii) cells with 'food-conducting cytology' in liverworts and in mosses.

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