Botanical Journal of the Linnean Society, 2009, **159**, 572–582. With 24 figures

Do tracheid microstructure and the presence of minute crystals link Nymphaeaceae, Cabombaceae and Hydatellaceae?

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Received 16 December 2008; accepted for publication 11 February 2009

Original scanning electron microscopy (SEM) observations are presented for stems of *Brasenia schreberi* and *Cabomba caroliniana* of Cabombaceae and three species of *Trithuria* of Hydatellaceae. End walls of stem tracheids of *Brasenia* have the same peculiar microstructure that we have reported in *Barclaya, Euryale, Nuphar, Nymphaea* (including *Ondinea*) and *Victoria* of Nymphaeaceae. This feature unites Cabombaceae with Nymphaeaceae. The minute rhomboidal crystals on the surfaces of stellate parenchyma cells of *Brasenia* reported by Solereder (1906. Oxford: University Press), but not noticed since, are figured. They are like the minute crystals of the often-mentioned astrosclereids of Nymphaeaceae. Neither of these two features has been observed in Hydatellaceae. If the absence of these two features can be confirmed, the reason may be more related to ecology, development, habit and anatomical organization than to degree of phylogenetic relationship as shown by molecular studies. Anatomical observations on the stem anatomy of *Trithuria* are offered on the basis of paraffin sections prepared for a paper by Cheadle & Kosakai (1975. *American Journal of Botany* **62:** 1017–1026); that study is notable for a discrepancy between an illustration of a specialized vessel element on the one hand and tabular data indicating long scalariform perforation plates on the other. Long scalariform perforation plates are mostly found in scalariformly pitted vessels of monocots, whereas the tracheary elements of *Trithuria* mostly have helical or annular thickenings. We were unable to demonstrate the presence of vessels in Hydatellaceae. *Botanical Journal of the Linnean Society*, 2009, **159**, 572–582.

ADDITIONAL KEYWORDS: basal angiosperms – *Brasenia* – Nymphaeales – pit – *Trithuria* – xylem.

INTRODUCTION

In a series of four papers, we have recently examined the tracheary tissue of all genera of Nymphaeaceae with scanning electron microscopy (SEM): *Victoria* Lindl. and *Euryale* Salisb. (Carlquist & Schneider, 2009); *Nuphar* Sm. (Carlquist, Schneider & Hellquist, 2009); *Barclaya* Wall. (Schneider & Carlquist, in press); and *Nymphaea* L., including *Ondinea* Hartog, (Schneider, Carlquist & Hellquist, in press). Our SEM studies revealed peculiar microstructures on the end walls of tracheids in the stems of all these genera. The microstructure consists of coarse microfibrils, apparently secondary wall material, laid down in a spongy reticulum across the pit membranes of end wall pits. On top of this network (facing the lumen), there are usually axially oriented strands composed of coarse fibrils. No such coarse microfibrils, or only a few, are present on the pit membranes of lateral walls of tracheids.

No such coarse fibrils have been reported in tracheids elsewhere in angiosperms, except possibly in *Cabomba* Aubl. (Schneider & Carlquist, 1996a). The appearances we figured for stems of *Cabomba* were not clearly definable because of the low resolution of the analogue SEM used in that study. Our discovery of coarse microfibrils in all genera of Nymphaeaceae with better digital SEM equipment has led us to re-examine

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stems of *Cabomba* and to examine stems of *Brasenia* Schreb., which we had not examined earlier.

The occurrence of the peculiar stem tracheid microstructures that we illustrated for Nymphaeaceae is of special interest with respect to phylogeny. In recent trees for Nymphaeaceae and allies, Cabombaceae are the sister group of Nymphaeaceae (Löhne, Borsch & Wiersema, 2007; Borsch, Löhne & Wiersema, 2008) and, thus, ascertaining whether coarse microfibrils occur in Cabombaceae and finding what variations might occur became an important goal of our SEM studies. The recent inclusion of Hydatellaceae as a third family of Nymphaeales and as the sister group of Cabombaceae plus Nymphaeaceae (Rudall *et al.*, 2007; Saarela *et al.*, 2007; Borsch *et al.*, 2008) has made study of tracheary tissue of Hydatellaceae highly desirable. Nymphaeales are a sister group to all angiosperms except for *Amborella* in DNA-based studies (Parkinson, Adams & Palmer, 1999; see Löhne *et al.*, 2007 for other references). Thus, whether coarse fibrils in tracheids are a primitive character in angiosperms in general or an apomorphy within Nymphaeales becomes a significant question. In either of these scenarios, correlations between such microstructures and ecology, habit and physiology become significant considerations.

Tracheary elements of one species of Hydatellaceae, *Trithuria filamentosa* Rodway were studied by Cheadle & Kosakai (1975) in a paper that seems to have eluded notice by most authors during the recent surge of interest in Hydatellaceae. There is, in that paper, a curious discrepancy between an illustration and the data in the text and the table regarding that species. Cheadle & Kosakai (1975) suggested that very primitive vessel elements (i.e. with exclusively scalariform perforation plates) occur in both early and late metaxylem of roots, stems, inflorescence axes and leaves of *T. filamentosa*. Their illustration for a vessel element from a root of *T. filamentosa*, however, shows a simple perforation plate. Thus, a reinvestigation of their materials (both liquid-preserved plants and microscope slides were still available for study) was in order.

Astrosclereids in the walls of which small rhomboidal crystals are embedded are a feature long known for Nymphaeaceae and have been reported in roots, stems and leaves (Metcalfe & Chalk, 1950; Rao & Banerjee, 1979; Seago, 2002). In stems, these sclereids occur along air channels in all genera except *Euryale* (Rao & Banerjee, 1979). Small rhomboidal crystals were reported for surfaces of aerenchyma of *Brasenia* (Cabombaceae) by Solereder (1906), although that report has seemingly been forgotten in recent years. The crystals in *Brasenia* have never been illustrated with SEM. Because of the systematic implications of inclusion of Hydatellaceae in Nymphaeales, we were interested in searching for the occurrence of any crystals in Hydatellaceae.

MATERIAL AND METHODS

The collections studied were as follows: Cabombaceae. *Brasenia schreberi* J. F. Gmel.: north end of Mill Kale at McClure Road, Waterloo State Recreation Area, Washtenaw County, Michigan; Gary and Laura Hannan, viii.2008 (EMU). *Cabomba caroliniana* A. Gray: Aquarena Springs at the headwaters of the San Marcos River, Hays County, Texas; E. L. Schneider vi.2000 (SWT). Hydatellaceae. *Trithuria filamentosa* Rodway: Lake Dobson, Mt. Field National Park, Tasmania, Australia; V. I. Cheadle and W. Jackson, 10.ii.1960 (UCSB). *Trithuria lanterna* D. A. Cooke: Wanganult Springs, 17°25′S, 120°19′E, Kimberley Range, Dampier Peninsula, Western Australia, Australia; Kevin F. Kenneally 9045B, 18.vi.1984 (PERTH). *Trithuria submersa* Hook.f.: Bangham Conservation Park, South Australia, Australia; J. C. Conran 961, 3.xi.1998 (ADU); A. Doust 1123 J. I. Davis and D. W. Stevenson (MELU).

The materials of *Cabomba* were of the nodal plates of the stem. Stem portions of *Brasenia* near points where adventitious roots depart and have more mature xylem were selected; elongate stem portions (runners) of *Brasenia* may contain immature tracheary elements. Materials were preserved in 70% aqueous ethanol. Longitudinal sections were obtained with hand-held razor blades. Stems of Hydatellaceae are very small, so that stems yielded only one or two such sections.

Sections were cleansed of alcohol and extraneous debris by transferring them through three changes of water at 50 °C. The sections were dried between glass slides, clamped in order to prevent curling of the sections and dried on a warming table at 50 °C. Dried sections were mounted on aluminum stubs, sputter-coated with gold and observed at 15 kV with a Hitachi SZ2600N scanning electron microscope.

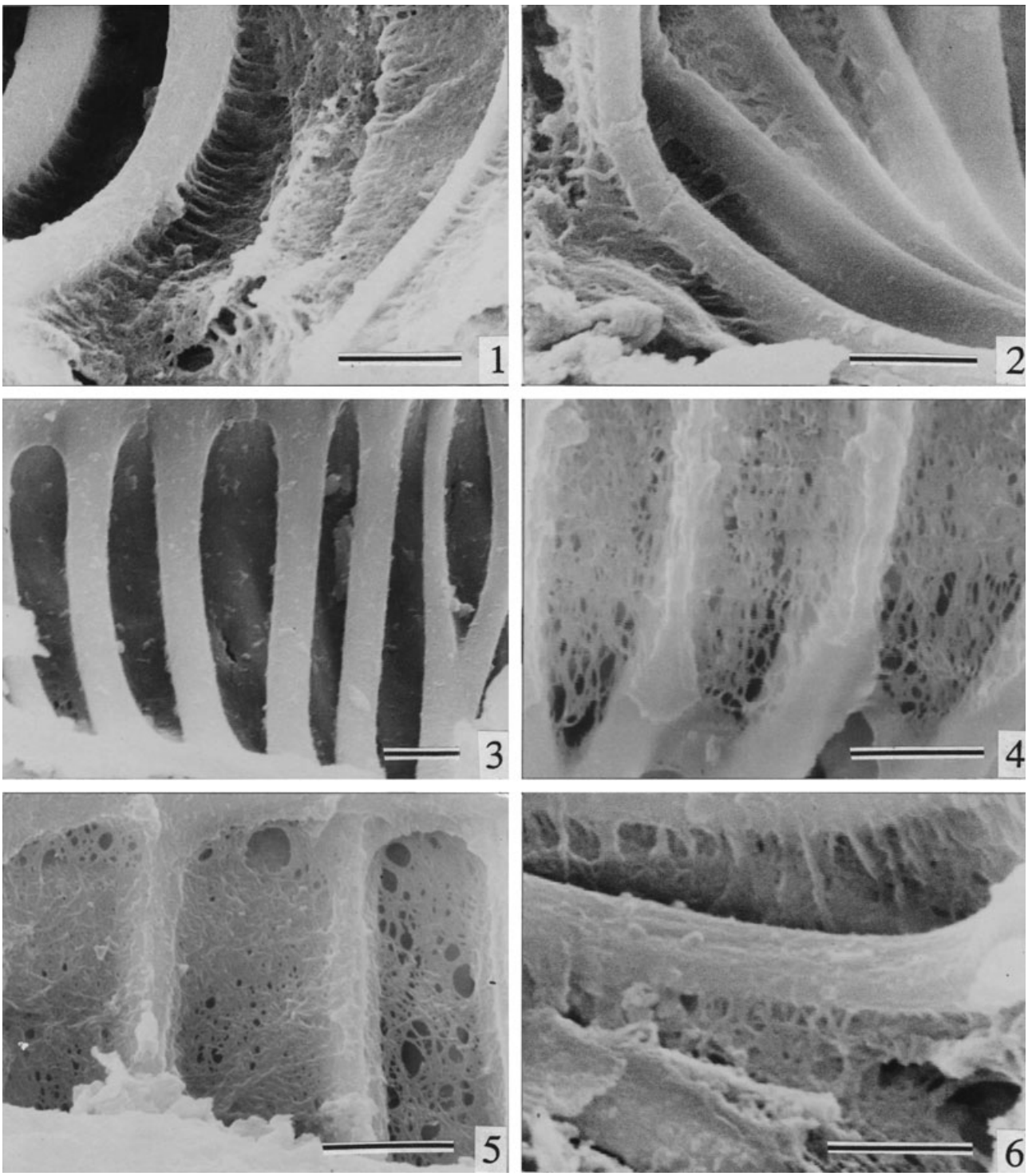
Classification of Hydatellaceae follows that of Sokoloff *et al.* (2008), who recognized only a single genus, *Trithuria* Hook.f.

RESULTS

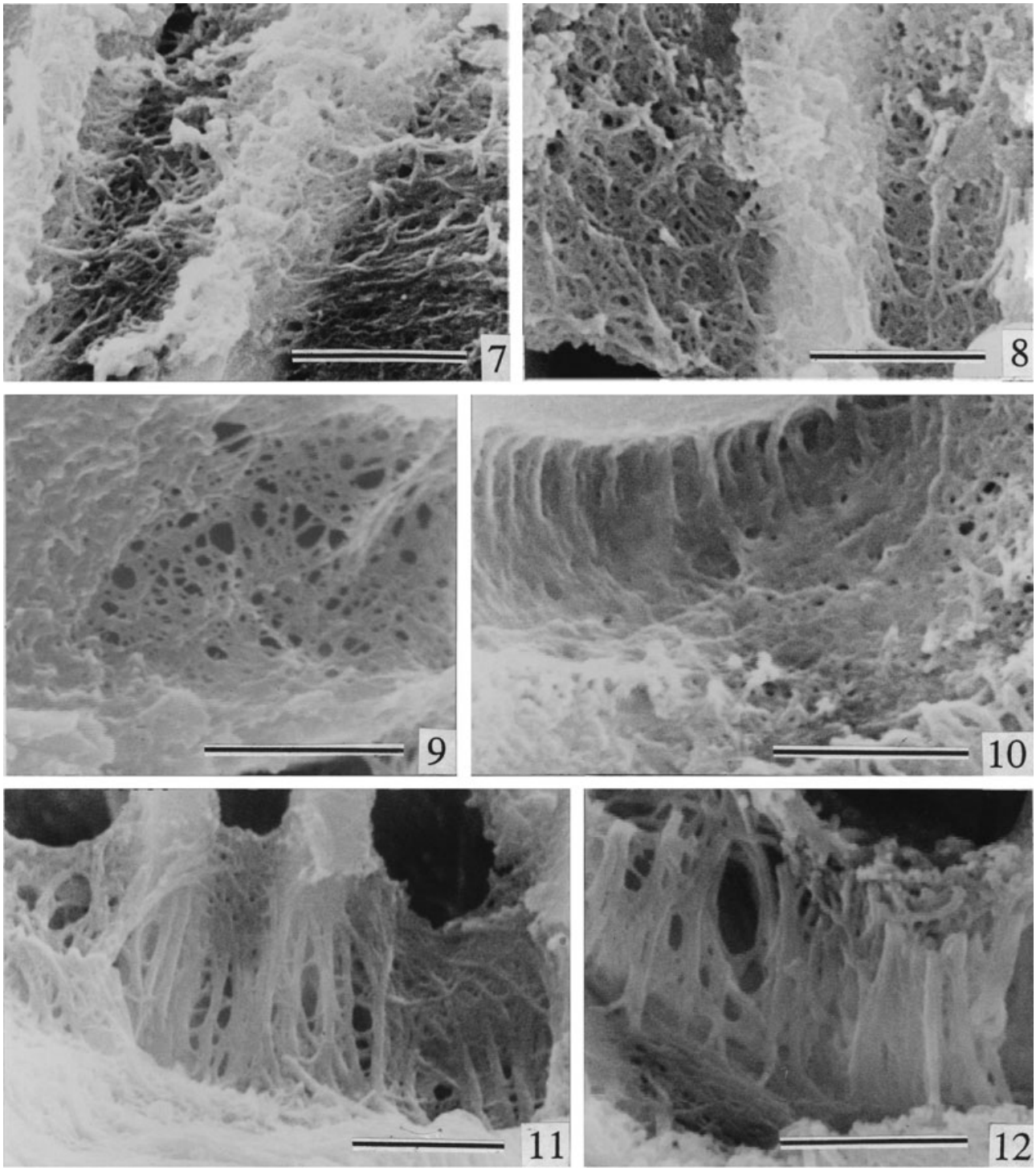
CABOMBACEAE: STEM TRACHEIDS

Brasenia schreberi

Tracheids from stems of *Brasenia* (Figs 1–12) viewed from the inner (lumen) surface, bear coarse fibrils on their end walls (Figs 1, 2). These may easily be seen in face view of an end wall, but are also apparent even in oblique views (Figs 1, 2) between gyres of the helical thickenings of early metaxylem cells. The



Figures 1–6. Scanning electron microscopy (SEM) micrographs of tracheids of *Brasenia schreberi*; Figures 1–2, 4–6 show the end walls; Figure 3 shows the lateral wall. Fig. 1. Oblique view of the inside of a tracheid, one gyre removed by sectioning; axially orientated strands are present. Fig. 2. Oblique view of the inside of a tracheid; the broken portion of microstructure, upper left, reveals the spongiform pattern. Fig. 3. Pit membrane surface of lateral walls pits is untextured. Figures 4–5. Tracheid surface seen from outside of tracheid, pit membrane partially sectioned away. Fig. 4. Porosities of various sizes. Fig. 5. Porosities and meshwork-like configurations. Fig. 6. Inner portion of tracheid surface; axially orientated coarse fibrils above, meshwork-like pattern below. Scale bars for all figures, 2 μm.



Figures 7–12. Scanning electron microscopy (SEM) micrographs of stem tracheids of *Brasenia schreberi*, showing patterns of the coarse fibrils. Figures 7–8. Spongiform structure of end wall pit membrane revealed by cutting away of adjacent tracheid. Fig. 7. Coarse microfibrils broken by sectioning. Fig. 8. Meshwork-like appearance of coarse fibrils. Fig. 9. Probable tracheid-to-parenchyma contact, with porose wall revealed on tracheid side of the pit membrane by sectioning. Fig. 10. Inside surface of tracheid end wall; axially oriented coarse fibrils join gyre, above. Figures 11–12. Axially orientated coarse fibrils from tracheid end walls. Fig. 11. Interconnections between the main fascicles of coarse fibrils. Fig. 12. Three-dimensional nature of the coarse fibrils (note fibrils broken by sectioning, upper right). Scale bars for all figures, 2 μm.

coarse fibrils form a dense spongiform layer (Fig. 1, right) on top of which (toward the cell lumen) is superimposed a series of longitudinally oriented strands (Figs 1, 2). Lateral walls of tracheids bear pits that have smooth pit membranes, devoid of the coarse fibrils (Fig. 3). Outer surfaces of the tracheids show porose pit membranes (Figs 4, 5). The pores vary in size depending on how much of the pit membrane has been scraped away. Thus, pits adjacent to each other on a tracheid wall can show various degrees of pore presence (Fig. 5).

The essentials of the coarse fibrils and their modes of occurrence can be seen in Figure 6. Part of a helical gyre is torn away from the section (Fig. 6, bottom). The spongiform coarse fibrils are attached to the gyres, also shown on the intact gyre (Fig. 6, middle). The axially oriented coarse fibrils are also attached to the secondary wall gyres (Fig. 6, top).

If the sectioning process removes the helical gyres of the secondary wall, the spongiform layer of coarse fibrils is exposed (Figs 7, 8). Some cut or broken ends of this three-dimensional reticulum are exposed. The random orientation of the coarse fibrils is evident. Sectioning may remove the spongiform layer, exposing an outer layer of pit membrane (Fig. 9). Such a pit membrane portion contains pores of various sizes.

The reticulate coarse fibrils and the axially oriented coarse fibrils are attached the secondary wall bars or gyres (Fig. 10). The coarse fibrils are attached to the pit borders and the edges of the pit apertures. The sectioning away of a gyre or gyre portions is associated with breakage of the coarse fibril reticulum.

There is no clear demarcation between the spongiform layer of coarse fibrils and the layer of axially oriented coarse fibrils laid down distally (toward the lumen) on top of the spongiform layer (Figs 1, 12). The two layers are interconnected at numerous points (Fig. 11). The axially oriented strands coalesce in places (Figs 11, 12). Thicker areas of the coarse fibrils where broken may be seen in Figure 12 (bottom right). Sizes of pores and holes within the meshwork of coarse fibrils vary greatly in size (Figs 7, 9, 11, 12) and some areas lack pores, at least at the magnifications we employed (Figs 9–12).

Cabomba caroliniana

Cabomba stem metaxylem tracheids are most readily seen in the nodal plates. The stem tracheids have mostly annular or helical gyres (Schneider & Carlquist, 1996a). In our current studies, we observed the primary wall (pit membrane) of annular tracheids much stretched and consisting of threads in places (Fig. 12). Where the least stretching had occurred, some areas of tracheids viewed from the inside showed a meshwork of coarse fibrils rather like those of the *Brasenia* tracheids in Figure 14. Where stretched or scraped by sectioning, the outer surfaces of a *Cabomba* tracheid show such a meshwork to be thinner and with larger pores (Fig. 15).

Cabombaceae: crystal presence in stem

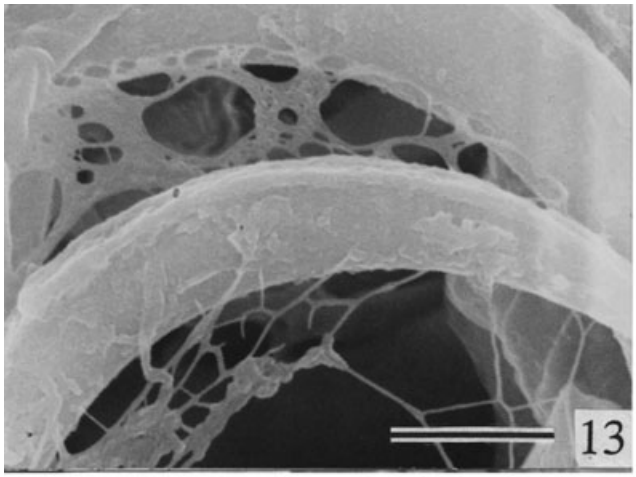
Brasenia schreberi

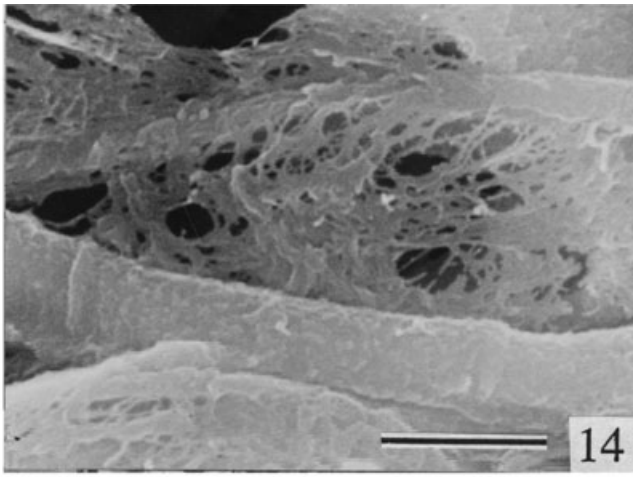
As seen in the stem transection portion (Fig. 16), stems of *Brasenia* have radiating plates of stellate parenchyma that interconnect the vascular core with the outer parenchyma (Fig. 16). The parenchyma cells that form these plates can be called stellate parenchyma because they have radial arms (Fig. 17). These and other parenchyma cells adjacent to the air spaces in *Brasenia* stems are thickly covered with crystals (Fig. 17). At higher magnifications, these crystals show a typical rhomboidal form (Fig. 18). The crystals are less abundant on the arms of the stellate parenchyma cells than on the central surfaces (Figs 17, 18). Where crystals have been dislodged from the parenchyma cells, small corresponding depressions in the cell wall may be seen. Thus, the crystals are embedded in the walls, but not deeply. Crystals were not observed in the stems of *Cabomba*.

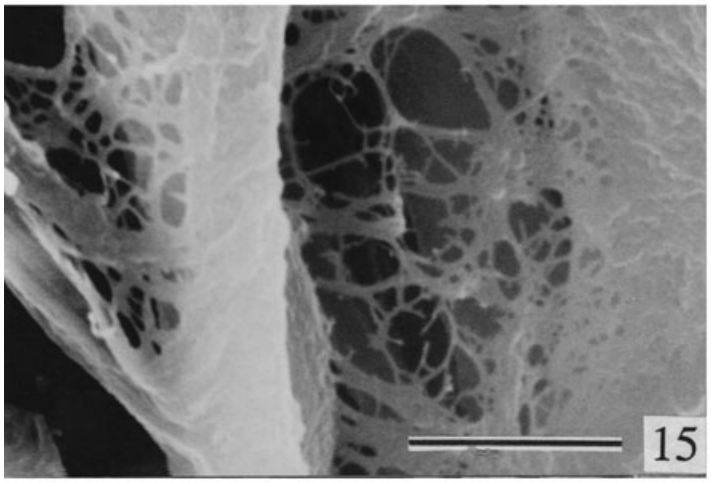
Hydatellaceae: stem histology and tracheid structure

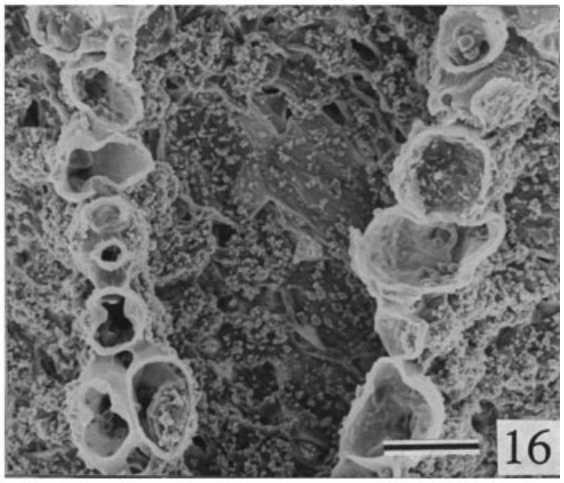
Transverse sections of *T. filamentosa* stems, as examined by light microscopy, have a central core of xylem and phloem surrounded by a well-demarcated endodermis with a brightly staining Casparian strip. The vascular core can be regarded as a 'vitalized protostele' because there are approximately equal numbers of tracheary elements and parenchyma cells randomly arranged in the vascular core. Strands of phloem were observed at the periphery of the xylary core. One to three files of tracheids (mostly annular) depart from the stele into each of the monarch roots of the plant. The stem tracheids vary in shape from elongate (Fig. 19) to isodiametric and contorted (Fig. 20), although the latter are much less common. The elongate tracheids bear helical thickenings but, on some cell edges, fine strands of secondary wall material interconnect the gyres (Fig. 19), which thus show a modest degree of transition to scalariform conformation. The isodiametric tracheids (Fig. 20) have a pattern referable to scalariform, but the pits vary considerably, depending on the size and shape of the facet. Absence of pit membranes in the tracheid portions shown (Figs 19, 20) is an artefact caused by sectioning.

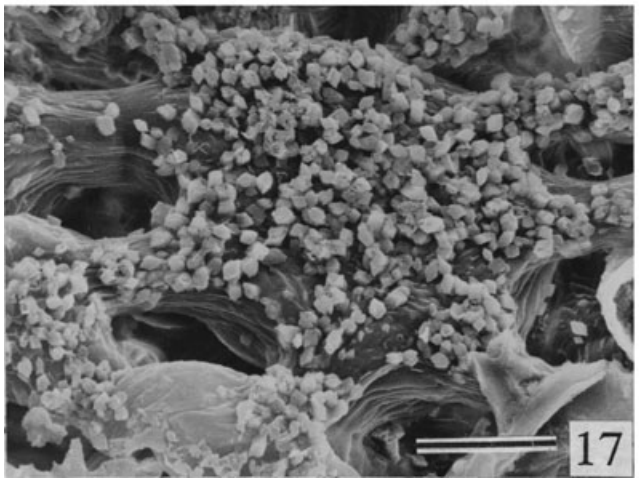
The range in pitting types in stem tracheids of *T. lanterna* (Fig. 21) is similar to that of *T. filamentosa*. A near-helical pattern is shown in the tracheid above in Figure 21, whereas a second inconspicuous

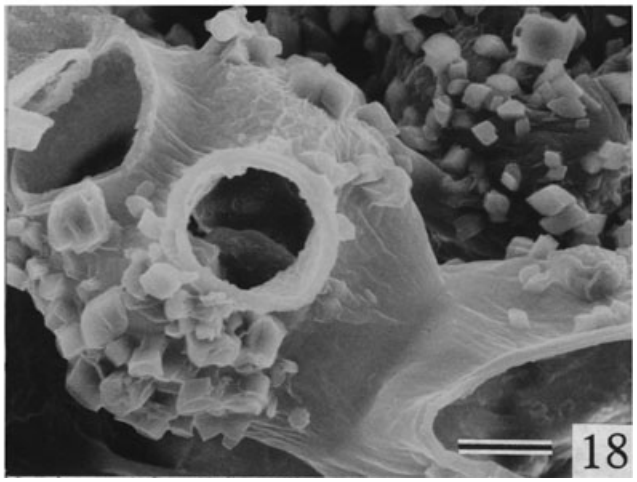




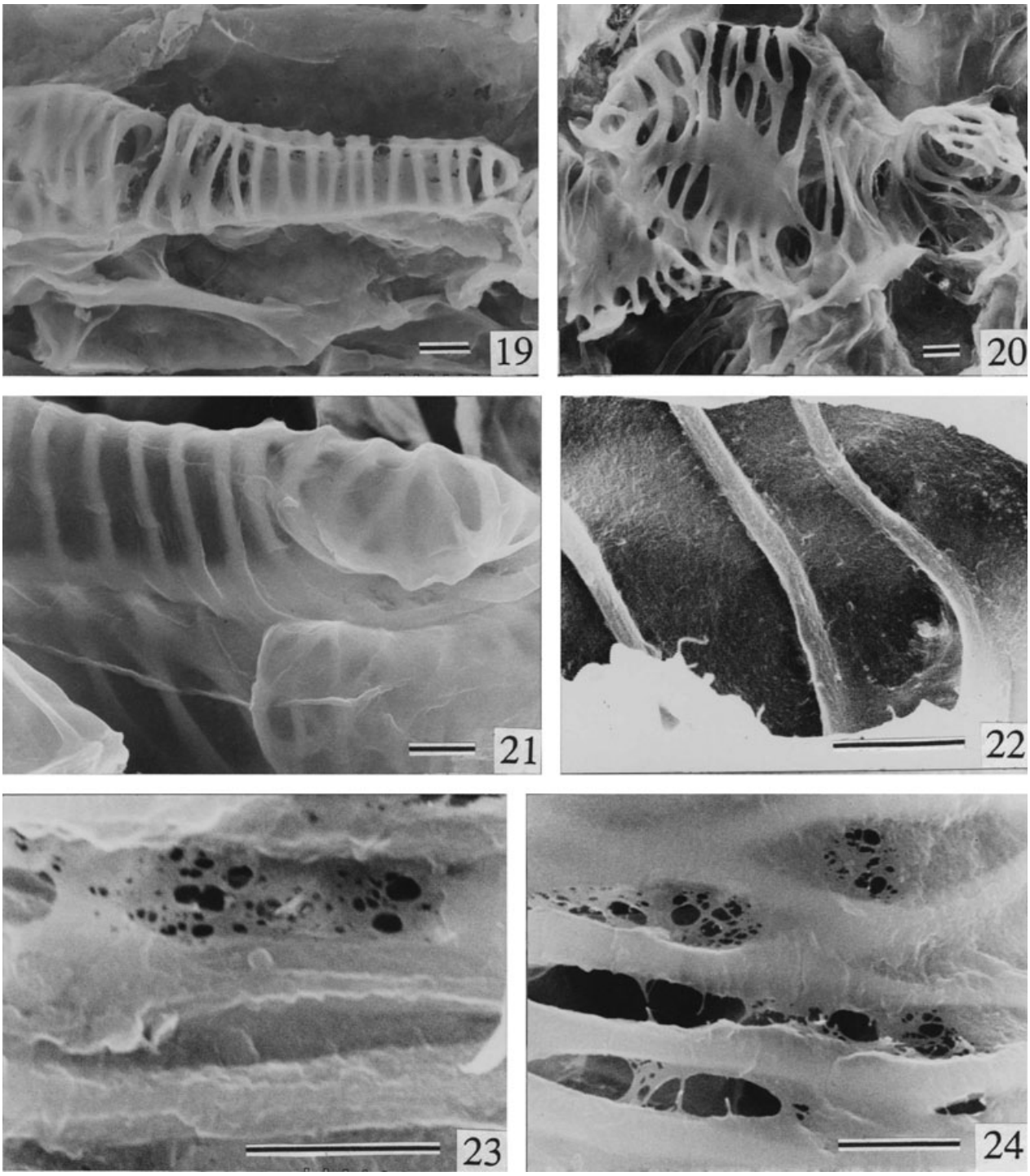








Figures 13–18. Scanning electron microscopy (SEM) micrographs of stems of Cabombaceae. Figures 13–15. *Cabomba* nodal plate tracheids. Fig. 13. Primary wall of protoxylem tracheid stretched into thread-like portions by cell elongation. Figures 14–15. Probable end walls of metaxylem tracheids, portions of pit membranes removed by sectioning. Fig. 14. Meshwork-like pattern of coarse fibrils. Fig. 15. Relatively open network of coarse fibrils. Figures 16–18. Sections from aerenchyma of *Brasenia* stem. Fig. 16. Transverse section of stem of *Brasenia*, showing two diverging plates of stellate parenchyma cells. Fig. 17. Stellate parenchyma cell of *Brasenia*, showing radiating arms. Fig. 18. Stellate parenchyma cell, higher power, to show calcium oxalate crystals on outer surfaces of stellate parenchyma cells. Scale bars: Figs 12–15, 2 μm; Fig. 16, 50 μm; Fig. 17, 20 μm; Fig. 18, 7 μm.



Figures 19–24. Scanning electron microscopy (SEM) micrographs of tracheids from longitudinal sections of stems of Hydatellaceae. Figures 19–20. *Trithuria filamentosa.* Fig. 19. Elongate tip of tracheid; breaks in the pit membranes are artefacts. Fig. 20. Unusually shaped metaxylem tracheid with ellipsoid pits; membrane absence is a probable artefact. Figures 21–22. *T. lanterna*. Fig. 21. Portions of tracheids; elongate tracheid above and in background, below; blunt cell at lower right may also be a tracheid; pit membranes are intact. Fig. 22. Inner surface of tracheid; pit membrane finely textured. Figures 23–24. *T. submersa* tracheids believed to represent tracheid-to-parenchyma contacts, with the primary walls sectioned variously, thereby revealing porosities on the tracheid side of the common pit membrane, untextured wall on the parenchyma side. Fig. 23. Helically thickened tracheid. Fig. 24. Ellipsoidal pits in metaxylem tracheid. Scale bars in all figures, 2 μm.

tracheid, below in Figure 21, has a blunt end and irregularly arranged pits. The helices of secondary wall materials in a tracheid of *T. lanterna* are seen in Figure 22 from the inside of the tracheid. Views of the inner surfaces of tracheid walls of *T. lanterna* did not reveal any coarse fibrils at magnifications comparable with those which showed coarse fibrils so conspicuously in *Brasenia* tracheids.

In longitudinal sections of stem tracheids of *T. submersa*, tracheary elements with pitting intermediate between helical and scalariform is present (Figs 23, 24). The tracheary elements show patches of porose pit membrane; elsewhere, pores are lacking in the pit membranes. This may represent zones where sectioning has scraped away part of the thickness of a pit membrane. Alternatively, the porose portions may belong to a tracheid and the untextured portions to an overlying parenchyma cell.

We were not able to demonstrate in Hydatellaceae, with light microscopy or with SEM, the coarse fibrils we have reported in stems of *Brasenia* or of Nymphaeaceae. Also, we were unable, with either method, to demonstrate areas we could designate as perforation plates in any of the tracheids of *Trithuria* that we studied.

Stem sections in the stained paraffin section preparations of *T. filamentosa* made by Hatsume Kosakai show that most of the stem is composed of aerenchyma. The shapes of cells in this aerenchyma are irregular or 'spongy' rather than referable to 'stellate' or some other more precise descriptive term.

The aerenchyma cells bear what appear to be pectic warts facing the air spaces. These warts are sparse and well spaced from each other and small (mostly 0.2–0.5 μm in diameter). Some of the pectic warts are stalked, although most are sessile. The warts stain with fast green rather than with the safranin in the safranin–fast green combination used by Kosakai. Therefore, these warts represent primary wall material and seem clearly referable to the phenomenon reviewed by Kisser (1928) and more recently by Carlquist (1956). No crystals were observed in the stems of any of the species of *Trithuria* examined.

DISCUSSION

We have demonstrated the occurrence of a peculiar microstructure in stem tracheid end walls of all genera of Nymphaeaceae (Carlquist & Schneider, 2009; Carlquist *et al.*, 2009; Schneider & Carlquist, in press; Schneider *et al.*, in press) and Cabombaceae (*Cabomba*: Schneider & Carlquist, 1996a; results for *Brasenia* given above). We were unable to observe any such microstructure in the stem of the three species of *Trithuria* (Hydatellaceae) that we studied. Absence of a structure or character is difficult to prove, but we can tentatively conclude that Hydatellaceae lack this feature. It should be noted that the coarse fibrils are represented in metaxylem, usually scalariformly pitted metaxylem. There is a possible functional correlation here. The dense spongiform reticula of coarse microfibrils, probably composed of secondary wall material, are probably non-extendable, in accord with the non-extendable nature of metaxylem. The xylem of Hydatellaceae consists mostly of tracheids with helical thickenings. Even if formed late in the sequence of maturation of xylem cells, such metaxylem retain many characteristics of protoxylem.

Is the presence of coarse fibrillar microstructure related to ecology? It probably is, but not in any obligate sense. We looked for such microstructure in stems of aquatic monocotyledons (*Acorus* L., *Alisma* L., *Echinodorus* Rich. & Engelm. ex A. Gray) and did not find it; likewise, we could not find it in stems of an aquatic dicot, *Nelumbo* Adans. Tracheid microstructure of this sort has never been reported in woody dicotyledons, despite intensive study with SEM of secondary xylem of early angiosperms. The occurrence of pit membrane remnants in perforation plates of angiosperms is an entirely different phenomenon (see Carlquist & Schneider, 2009; Carlquist *et al.*, 2009).

Minute crystals, presumably calcium oxalate, occur in walls of astrosclereids of Nymphaeaceae, as noticed previously (Metcalfe & Chalk, 1950; Rao & Banerjee, 1979; Seago, 2002). The occurrence of similar crystals in *Brasenia* stems, shown here for the first time with SEM, had gone unnoticed in recent times, although Solereder (1906) observed them. Crystals are absent in the stem aerenchyma of the three species of Hydatellaceae we studied. The occurrence of minute crystals probably deters herbivores, as in the wood of *Ephedra* L. (Carlquist, 1989, 1992). The occurrence of minute crystals in *Ephedra* secondary xylem and phloem is not an indication of relationship to angiosperms, any more than the presence of similar crystals in fungi is. The obvious homoplasious distribution of such features discourages one from using them in the construction of data matrices to be used for phylogenetic analysis in general, but within smaller groupings such as Nymphaeales they may be indicative of relationships.

The closeness of Nymphaeaceae to Cabombaceae has never been questioned. Indeed, the two genera of Cabombaceae were included in Nymphaeaceae until the second half of the 20th century (see Schneider & Williamson, 1993; Williamson & Schneider, 1993). Segregation of Cabombaceae was based on a few distinctive characters. Cabombaceae is recognized as the sister group to Nymphaeaceae (Löhne *et al.*, 2007). In phylogenetic trees in which Hydatellaceae are included, Hydatellaceae become the sister group to the remainder of Nymphaeales (Saarela *et al.*, 2007).

Assuming that nymphaeaceous stem tracheid microstructure and minute calcium oxalate crystals are never found in Hydatellaceae, are these symplesiomorphies or apomorphies? Because Hydatellaceae are a plant group with so many structural alterations (presumably mostly reductions related to ephemeral habits), such analysis is difficult and, perhaps for some characters, moot. Symplesiomorphy and apomorphy are more easily designated in groups with large numbers of species and genera and in which only small number of characters distinguish the component species. Against a background in which more species are present, patterns of character change become more evident. In groups such as Hydatellaceae, in which structure has probably been extensively re-patterned over long periods of time, the phyletic meaning of particular characters is often not evident.

For example, potential predation of the thick, starch-rich stems of Nymphaeaceae would be different from that facing the small, ephemeral stems of Hydatellaceae. In colonies of *T. filamentosa*, there are thick mats of freshwater diatoms (original observation based on fixed material), the sturdy silica frustules of which might serve as deterrents to predation of *T. filamentosa* by small aquatic herbivores.

The peculiar microstructure of end walls of stem tracheids of Nymphaeaceae and Cabombaceae poses similar problems for phylogenetic analysis. The functional significance of these microstructures is not evident at present (Carlquist & Schneider, 2009; Carlquist *et al.*, 2009; Schneider *et al.*, in press). The localization of the meshwork and strands of coarse fibrillar wall material exclusive to the end walls of tracheids is curious. The superimposition of the spongiform network onto secondary wall structures is not unique: vestured pits have strands or networks of secondary wall material attached to the pit border and pit cavity (and even tracheary element lumen surface). Vestured pits, however, are an entirely different phenomenon. They are probably related to wood xeromorphy, but not exclusively so, and often present throughout a family (Carlquist, 1983).

Stems of Nymphaeaceae and Cabombaceae do not possess vessels. The pores on the end wall pits, like those on end wall pits in stems of orchids (Carlquist & Schneider, 2006) and ferns (Carlquist & Schneider, 2007), are too small and too few to allow passage of air bubbles from one tracheid to a superadjacent or subadjacent one. At most, the tracheary elements of Nymphaeaceae and Cabombaceae should be considered pre-vessels. The end walls of tracheary elements of roots of Cabombaceae (Schneider & Carlquist, 1996b) were shown to have prominent lysis of pit membranes, resulting in the formation of conspicuous pores, and that characterizes roots of *Nuphar* (Carlquist *et al.*, 2009), *Barclaya* (Schneider & Carlquist, in press) and *Nymphaea* (Schneider *et al.*, in press). One could claim that such porose end walls in these root tracheary elements do, in fact, qualify such cells as vessel elements. Because definitions of tracheids and vessel elements have been based on light microscopy, disagreement on this point is entirely possible. Not all morphological expressions can be contained within the 0–1 binary system of data matrices. To us, the functional significance of pre-vessels, as in the stems of Cabombaceae and Nymphaeaceae, or putative vessels, as in the roots of those two families, outweighs the demands of cladistic methodology. Tracheary elements of the two families presumably represent enhanced conduction capabilities with respect to pore size and presence, while retaining the ability to confine air bubbles within individual cells, thereby preventing embolisms from spreading the length of a vertical file of superposed tracheary elements.

With respect to vessel presence, Hydatellaceae remain uncertain. Our figures show some porosities in pit membranes of tracheary elements (*T. submersa*), but the pores observed were not ones that could be observed to extend from one tracheary element into the adjacent one, a criterion we feel is important, and which guided our interpretations in orchids (Carlquist & Schneider, 2006) and ferns (Carlquist & Schneider, 2007).

Cheadle & Kosakai (1975) stated that '*Trithuria filamentosa* has the most primitive vessels throughout the plant . . .'. The comparison is with *Gaimardia* Gaudich. (Centrolepidaceae), for which they illustrated scalariform perforation plates in roots, so one would expect that they were referring to scalariform perforation plates even longer and with more numerous bars than those of *Gaimardia*. In a table, they rated tracheary elements of both early and late metaxylem of not only roots, but also stems, inflorescence axes and leaves of *T. filamentosa* as '1.0', which they defined as '[vessels with] only scalariform perforation plates.' The presence of tracheids but no vessel elements would be scored '0.0' in their system. However, their figure for a root vessel element of *T. filamentosa* (Cheadle & Kosakai, 1975, Fig. 11) shows a metaxylem vessel with a simple perforation plate, which would rank much higher in their system and is not in accord with their text descriptions.

Observing long scalariform perforation plates on end walls of vessel elements of monocotyledons with light microscopy, either in sections or in macerations, is extraordinarily difficult, because lateral walls can look much the same as end walls and the presence or absence of pit membranes or primary walls, which usually do not stain deeply, cannot be demonstrated clearly. This is especially true where metaxylem tracheary elements have helical thickenings rather than

scalariformly pitted end walls or lateral walls. Hydatellaceae have mostly helical thickenings. In fact, using SEM, we have not seen convincing presence of scalariform perforation plates in tracheary elements with helical thickenings in any vascular plants we have studied, with the exception of roots of *Brasenia* (Schneider & Carlquist, 1996b). We have viewed the microslides prepared by Kosakai and, although the sections were excellent (macerations are no longer usable), we cannot confirm the presence of vessel elements from light microscopy of those paraffin sections.

Studies of Hydatellaceae combining SEM and transmission electron microscopy (TEM) are needed for understanding the nature of tracheary elements in this intriguing family. The number of individuals studied must be sufficient to observe end walls of tracheary elements and differentiate them from lateral walls, not an easy task when the shape of tracheary elements is irregular and when planes of sectioning are often not what is needed. TEM studies will help to clarify the peculiar microstructures on end walls of tracheary elements in Cabombaceae and Nymphaeaceae. Likewise, TEM studies can tell us more about the nature of the pectic warts in Hydatellaceae. Although such studies may be motivated by anticipation of better phylogenetic understanding, there will be other dividends that comparative anatomy typically yields: understanding of structure in relation to ecology and physiology. Study of key species from varied ecological settings rather than a single species from any of these families seems to us highly desirable.

ACKNOWLEDGEMENTS

Kevin Kenneally kindly provided us with liquid-preserved specimens he collected of *T. lanterna*. Likewise, Gary and Laura Hannan responded to our request for materials of *Brasenia* and collected specimens with optimal maturation characteristics. Jennifer Thorsch deserves thanks for providing us access to the slides and macerations made by Hatsume Kosakai for the Cheadle and Kosakai (1975) study, as well as to liquid-preserved material of *T. filamentosa* collected by Vernon I. Cheadle in the field. Dennis Stevenson generously provided material of *T. submersa*.

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