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Distinctive tracheid microstructure in stems of *Victoria* and *Euryale* (Nymphaeaceae)

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Scanning electron microscopy (SEM) photographs of thick sections from liquid-preserved stems of *Victoria cruziana* and *Euryale ferox* show accretions of coarse fibrils on pit membranes of tracheids. The first-deposited fibrils are randomly orientated; on top of them (facing the tracheid lumina) are axially orientated coarse fibrils. The two systems are interconnected. Axially orientated fibrils were more extensively observed in *Euryale* than in *Victoria* and tips of fibrils in *Euryale* extend over the pit apertures onto secondary wall surfaces. Tracheid–parenchyma interfaces bear rudimentary coarse fibrils on the tracheid side. End walls of *Victoria* tracheids have highly porose pit membranes, thinner and less complex than those of the lateral intertracheid walls. The structures reported in *Victoria* and *Euryale* are consistent with those concurrently reported for stems of other Nymphaeaceae. Although also present in Cabombaceae, the coarse fibrils are otherwise not reported for stems of angiosperms and are not yet reported in roots of any species. Pit membrane remnants in perforation plates of various woody dicotyledons represent a nonhomologous phenomenon. The accretions of coarse fibrils in stem tracheids of Nymphaeaceae do not appear to enhance conduction, although they do contain porosities interconnecting tracheids. Removal of pit membrane remnants from perforation plates of primitive dicotyledon woods by hydrolysis does, on the contrary, suggest conduction enhancement.

ADDITIONAL KEYWORDS: aquatics – early branching angiosperms – *Nuphar* – *Nymphaea* – pit membranes – xylem.

INTRODUCTION

Our earlier studies of xylem in Nymphaeaceae (Schneider & Carlquist, 1995; Schneider *et al.*, 1995) were based on studies of roots of the five commonly recognized genera of Nymphaeaceae. For those studies, we used paraffin sections, some examined with light microscopy, some with scanning electron microscopy (SEM). Our results showed differentiation in secondary wall architecture between end walls and lateral walls of tracheids in roots, a feature suggestive of an incipient stage in vessel evolution. We reported (as 'perforations') small porosities in pit membranes of end walls of *Nymphaea* L. and *Victoria* Lindl. root tracheids. Use of the thin paraffin sections resulted in fracturing of pit membranes, so that the porosities, although present, had to be shown in the context of torn portions of pit membranes, along with 'striations' (i.e. buckling of the pit membranes produced by the electron beam). In addition, the SEM equipment that we used was marginal in resolution capacity.

The availability of better SEM equipment has induced us to revisit the topic of tracheid microstructure in Nymphaeaceae. In doing so, we have also adopted a simple method of preparation: that of thick sections cut with razor blades, which minimizes damage to pit membranes by virtue of the support offered by the thickness of the sections. In studying xylem of *Nuphar* Sm. (Carlquist, Schneider & Hellquist, 2009) we found that porosities were clearly revealed in end walls of root tracheids of *Nuphar*, but not in the lateral walls, confirming our earlier observations. However, we now regard those stem tracheary elements as tracheids, not vessel elements, and reserve the term vessel element for cells with pit membrane remnants (perforations of perforation plates mostly clear of primary walls) rather

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than porous sheets of primary wall as pit membranes. We have followed that definition in revisiting the xylem of ferns (Carlquist & Schneider, 2007), only a few genera of which we now regard as having vessels, although tracheid structure with end wall differentiation is evident in a number of fern stems.

Our earlier studies on xylem of Nymphaeaceae focused on roots, which we studied based on the presumption that, in sympodial plants with adventitious roots, such as monocotyledons, roots are more likely to have vessels than stems. Certainly this was evident in the work of Cheadle (1942), especially in his results on aquatic monocotyledons. Our studies of *Nuphar* (Carlquist *et al.*, 2009) validated that assumption to the degree that end walls of root tracheids have porose pit membranes, although, as mentioned, these do not constitute vessels in our present thinking. The stem tracheids of *Nuphar*, however, revealed an unexpected pit membrane microstructure when studied with SEM equipment capable of good resolution. We found systems of coarse fibrils that comprise the pit membranes of lateral walls of tracheids. The coarse fibrils traverse the pit apertures and pit cavities. The occurrence of this phenomenon in *Nuphar* motivated us to study other Nymphaeaceae: is this distinctive microstructure limited to *Nuphar*? *Nuphar* is of phylogenetic interest by virtue of its position as sister to the remainder of Nymphaeaceae (Les *et al.*, 1999; Löhne, Borsch & Wiersema, 2007). What variations in distribution and appearance of the coarse fibrils occur in stem tracheids of other Nymphaeaceae? Does this microstructure extend to other families of plants, particularly aquatic ones?

Fortunately, material used in our earlier studies of *Victoria* and *Euryale* Salisb. was still in existence. The results presented here offer new dimensions in our understanding of this phenomenon. *Victoria* and *Euryale* are now considered to be nested within *Nymphaea* (Löhne *et al.*, 2007). *Victoria* and *Euryale* remain sister groups in that scheme, however, confirming the ideas of Schneider (1976). We confine this study to these giant-leaved waterlilies, which form a convenient unit for presentation.

MATERIAL AND METHODS

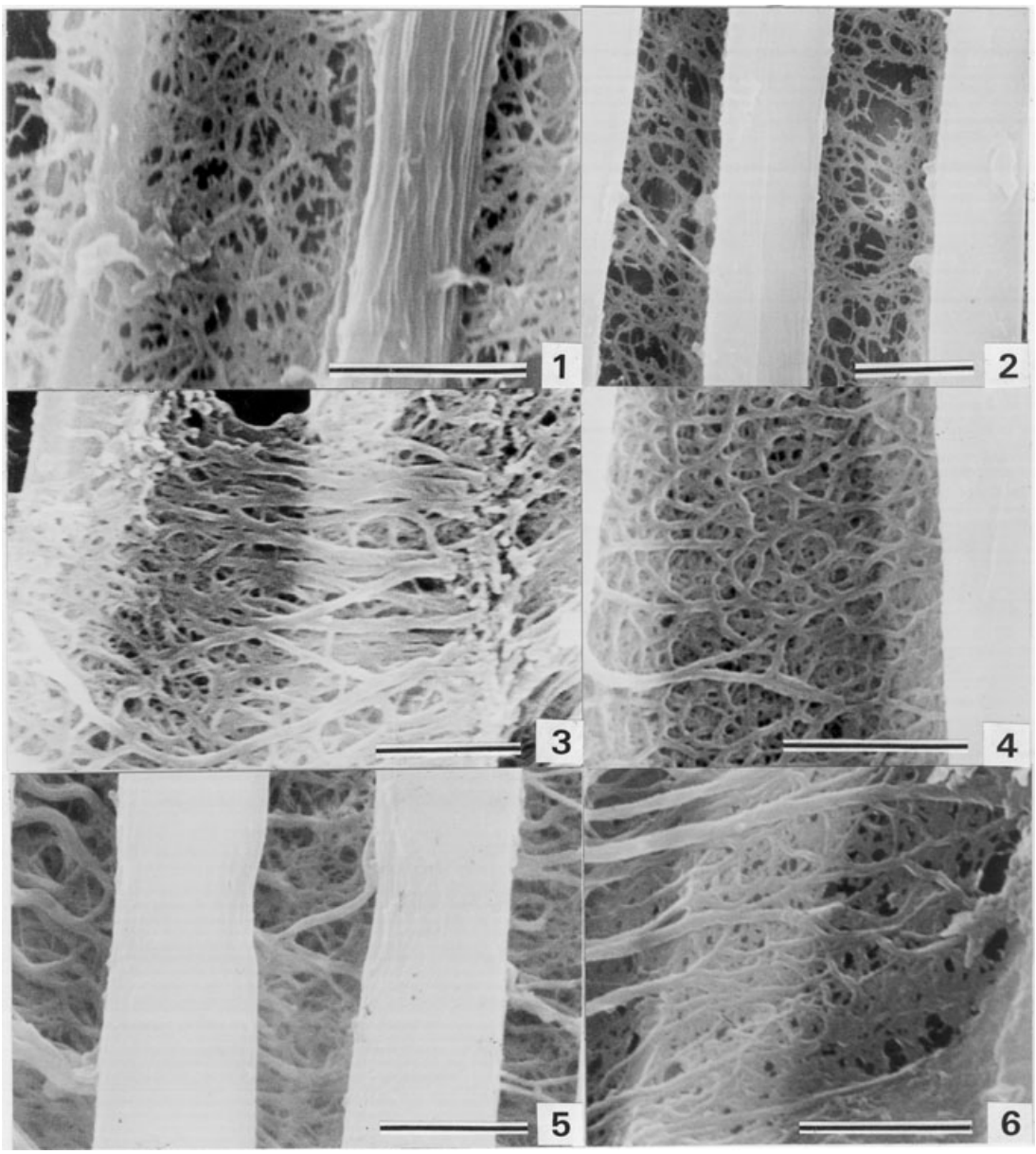
Stems of *Victoria cruziana* d'Orb. and *Euryale ferox* Salisb. were collected in September, 1990, from plants cultivated at the Lilypons Garden in Brookshire, Texas, USA. Stems were preserved in 70% aqueous ethanol. Voucher specimens are housed at the Santa Barbara Botanic Garden (SBBG).

In order to restore firmness to these stems, soft as a result of long storage, they were transferred to absolute alcohol for 1 week. Longitudinal sections about 2 mm thick were prepared using single-edged razor blades. The sections were transferred through three changes of warm (50 °C) distilled water. Washed sections were then placed between clean glass slides, subjected to light pressure to assure flatness and dried on a 50 °C warming table. Dried sections that appeared to show vascular tissues were mounted on aluminum stubs, sputter coated and observed with a Hitachi S-2600N SEM (tungsten filament). We know of no reports that these methods result in artefact formation where the pit membranes of Nymphaeaceae we describe are concerned.

RESULTS

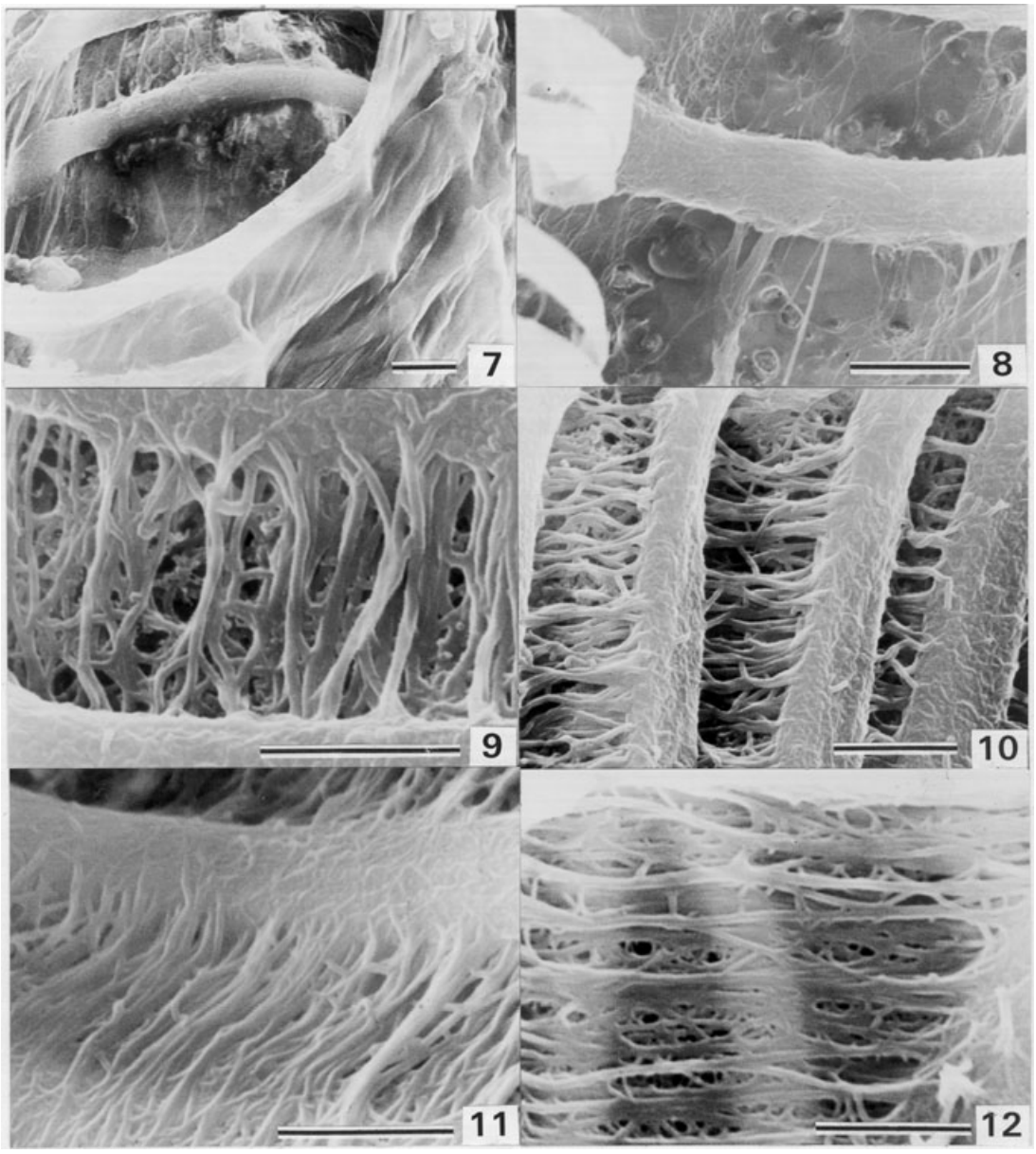
Victoria cruziana (Figs 1–6). All of the views shown here are of inside surfaces of tracheids; the long axis of the tracheids is orientated horizontally in order to show larger areas of pit membrane. The pit membranes in Figures 1 and 2 represent end wall areas of tracheids. In Figure 1, pit membranes are composed of randomly orientated fibrils. One bar of secondary wall material (Fig. 1, right) is intact, whereas a nearby bar (Fig. 1, left) has been torn away by sectioning, revealing a narrow strip of non-reticulate primary wall. In Figure 2, bars of secondary wall material are present. The porosities in both Figures 1 and 2 are relatively large and varied in shape. The portions shown are judged to be relatively free of artefacts, although there is some fragmentation of the fibrils. The pit membranes of Figure 1 suggest layering of the fibrils, whereas the pit membrane in Figure 2 suggests a single layer of fibrils.

The pit membranes of Figures 3–6 are from lateral intertracheid walls. The pit membrane in Figure 3 is composed of a dense deposition of coarse fibrils. Those fibrils deposited earlier appear more nearly reticulate, but, on top of them, a pattern of predominantly linear fibrils running axially is superimposed. Somewhat spongiform patterns of coarse fibrils were observed in intertracheid pit membranes (Fig. 4). Variations observed in pit membranes of lateral pits representing tracheid to tracheid contacts include various mixtures of reticulate and axially oriented coarse fibrils (Figs 5, 6). Some of the fibrils in Figure 5 are notably coarse. The pit membrane in Figure 6 contains small porosities prominently. The lateral walls shown here (Figs 3–6) have fewer and smaller pores in pit membranes than do the end walls (Figs 1, 2). The coarse fibrils in *Victoria* stem tracheids do not extend onto the secondary wall surfaces ('bars') facing the lumen. Parenchyma cells in the stems of *V. cruziana* do not show any texturing in primary walls at the magnifications used in the present study.



Figures 1–6. Scanning electron micrographs of inside surfaces of stem tracheids of *Victoria cruziana*. Figures 1 and 2. Pit membranes from end walls. Fig. 1. Bar of secondary wall sectioned away at left, revealing non-textured strip of primary wall material; bar at right present, striate surface probably because of drying. Fig. 2. Pit membranes showing characteristic large size of porosities in end walls. Figures 3–6. Pit membrane portions from intertracheid pits of lateral tracheid walls. Fig. 3. Predominently axially oriented coarse fibrils. Fig. 4. Predominantly randomly oriented coarse fibrils. Fig. 5. Narrow pits with wide thick fibrils; fibrils do not extend onto secondary wall surface between pits. Fig. 6. Axially oriented coarse fibrils overlie a meshwork of randomly oriented fibrils; porosities present within the meshwork. Scale bars, 2 μm.

Euryale ferox (Figs 7–12). The tracheid in Figure 7 has been broken by the sectioning process, revealing both the outside (right and below) and the inside (upper left) surfaces. The outer surface shows a series of opposite pits, indicating where files of parenchyma cells were adjacent prior to sectioning. The outside wall surface of the tracheid does not show any texturing of the wall on the parenchyma side. The inner wall surface of the *Euryale* tracheid shows little texturing, but some coarse strands are present.



Figures 7–12. Scanning electron micrographs of surfaces of stem tracheids of *Euryale ferox*. Fig. 7. Broken end of tracheid, showing outer surface with parenchyma–cell imprints in foreground, inside surface with textured appearance above, left. Fig. 8. Enlarged portion of tracheid–parenchyma interface, seen from inside of tracheid; coarse fibrils are sparse and various in size. Figures 9–12. Pit membranes of intertracheid pits from lateral tracheid wall, seen from inside of tracheid. Fig. 9. Fibrils extend from pit membrane onto surface of secondary wall (top). Fig. 10. Fibrils run predominantly in an axial direction. Fig. 11. Fibrils fade into surface of secondary wall at margin of pit. Fig. 12. Porosities evident in the reticulate layer that underlies the coarse axially running fibrils. Scale bars, 2 μm.

Porosities are absent in the pit membranes of the tracheid in Figure 7. A similar tracheid–parenchyma interface, at greater magnification (Fig. 8), shows a few coarse strands.

In the pit membranes of tracheid-to-tracheid interfaces of the lateral wall tracheid of *Euryale* (Figs 9–12), coarse fibrils are common and are laid down in a three-dimensional, sometimes spongiform, fashion. The pit membrane of Figure 9 shows a tendency for the coarse fibrils to be more randomly oriented distally (outer surface of a pit membrane, facing a neighbouring tracheid), but superimposed on the proximal surface (facing the lumen) are longitudinally oriented coarse fibrils. The coarse fibrils appear not to be

confined to the pit cavity; their tips extend onto the lumen-facing surface of the secondary wall (Fig. 9, top; Figs 10, 11). Where the coarse fibrils extend onto the secondary wall surfaces, they branch and fade into the secondary wall (Figs 9–11). Porosities of various sizes may be observed within the reticulate portions of coarse fibrils of the pit membranes (Fig. 12). The porosities evidently interconnect adjacent tracheids.

DISCUSSION AND CONCLUSIONS

Micrographs of plant pit membranes and other primary wall portions taken by means of transmission electron microscopy (TEM) at high magnification often show a meshwork-like pattern of cellulose fibrils. What we describe here is significantly coarser than such apparently universal primary wall patterns, as can be noted from the magnifications involved. The coarse fibrils of stem tracheids in Nymphaeaceae also differ in several other ways from ultrastructural patterns seen with TEM. The intertracheid pit membranes of *Victoria* and *Euryale* are composed of two layers: a reticulate porose layer of randomly orientated coarser fibrils, on top of which are laid down coarse axially running fibrils. In *Victoria*, the fibrils are less prominent than they are in *Euryale*. The longitudinally orientated fibrils fade into the secondary wall in *Euryale*, whereas such extensions onto lumen-facing portions of the secondary wall were not observed in *Victoria*.

The report of highly porose flat pit membranes in stem tracheid end walls of *Victoria* is new for Nymphaeaceae. The pit membranes of lateral intertracheid pits of *Victoria* and *Euryale* are not as thick and spongiform as corresponding pit membranes of *Nuphar* (Carlquist *et al.*, 2009) or *Barclaya* Wall. (E. L. Schneider & S. Carlquist, unpubl. data). Stem tracheids of a single collection of *Nymphaea* were reported to have coarse fibrillar pit membrane structure (Carlquist *et al.*, 2009). A single collection of *Ondinea* Hartog (a genus now considered nested within *Nymphaea*: Löhne *et al.*, 2007) has been observed to have coarse fibrillar pit membrane structure in stem tracheids (E. L. Schneider & S. Carlquist, unpubl. data). With the present study, all genera of Nymphaeaceae have been shown to have coarse fibrils in pit membranes of stem tracheids. Such coarse fibrils, in a less conspicuous form, appear to occur in the nodal plexus tracheids of stems of *Cabomba* Aubl., but patterns like those of Nymphaeaceae occur in stems of *Brasenia* Schreb. that are mature and bear roots (Schneider & Carlquist, 1996; E. L. Schneider & S. Carlquist, unpubl. data).

Root tracheid pit membranes in *Nuphar* do not have coarse fibrils; the root tracheid pit membranes are untextured at the magnifications we employed. There are, however, porosities visible in pit membranes of end walls of root tracheids of *Nuphar* (Carlquist *et al.*, 2009).

Apart from Nymphaeaceae and Cabombaceae, which are closely allied, no instances of coarse fibrils in tracheids similar to those we have described have been reported. Hydatellaceae are now included in Nymphaeales (Rudall *et al.*, 2007; Saarela *et al.*, 2007), but our observations on material of *Trithuria* Hook.f. (*s. l.*) reveal no coarse fibrillar structure in tracheid walls (E. L. Schneider & S. Carlquist, unpubl. data). Curious types of thickenings and secondary wall conformations have been reported in tracheids of early vascular plants (Kenrick & Crane, 1997; Friedman & Cook, 2000). These structures are clearly different from those in stem tracheids in Nymphaeaceae. The pit membrane remnants ('microfibrillar webs') in perforation plates of dicotyledons with primitive vessels (Carlquist, 1992a), most notably present in Chloranthaceae (Carlquist, 1990, 1992b, 1992c) and Illiciaceae (Carlquist & Schneider, 2002), represent a different phenomenon. These pit membrane remnants apparently involve removal of pit membrane portions by hydrolysis (Butterfield & Meylan, 1982), whereas the coarse fibrils we report represent an accretion of wall material to pit membranes. The difference between these stem tracheid wall accretions and instances of pit membrane remnants have been tabulated elsewhere (Carlquist *et al.*, 2009). The removal of pit membrane portions in perforation plates by hydrolysis (possibly also involving the formation of less primary wall material in the perforations) has been interpreted as an adaptation to facilitate water flow (Carlquist, 1992a). The accretion of secondary wall material on pit membranes of stem tracheids in Nymphaeaceae only thickens the primary walls, thereby potentially providing more impedance to water flow. The explanation for the phenomenon we describe here does not appear to be related to improvement of hydraulic efficiency.

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