MICROSTRUCTURE OF TRACHEIDS OF NYMPHAEA

Edward L. Schneider,1,* Sherwin Carlquist,∗ and C. Barre Hellquist1

*Santa Barbara Botanic Garden, 1212 Mission Canyon Road, Santa Barbara, California 93105, U.S.A.; and
†Biology Department, Massachusetts College of Liberal Arts, North Adams, Massachusetts 01230, U.S.A.

Hand sections of root and stem xylem of diverse species of Nymphaea (including Ondinea) were studied with SEM in order to explore the diversity of wall structure within the genus. Lateral walls of root tracheary elements are untextured at the magnifications used, but end walls suggest that lysis of pit membranes leaves a cellulosic network with a large reticulum. Stem tracheids have lateral walls that are untextured or have a few prominent fibrils, but end walls have a dense accretion of coarse fibrils. These fibrils form a spongiform or compressed network overlying the pit membranes. In addition, on the lumen side of pit membranes, coarse fibrils cross the pits in an axial direction. These coarse fibrils are connected to pit borders, and they may extend onto wall surfaces of the tracheary elements. These fibrillar accretions are considered here to fulfill criteria as secondary-wall components. Variation within the genus with respect to the fibrillar accretions may occur, but there may be more extensive diversity within any given sample than among species. Root tracheary elements could be considered to be vessel elements because of the extensive removal of wall material from the end-wall pit membranes. Stem tracheary elements qualify as tracheids because, rather than showing lysis of pit-membrane portions, end-wall pit membranes bear fibrillar accretions that are only sparsely porose. The absence of coarse fibrillar accretions on lateral walls of stem tracheids (on tracheid-to-parenchyma interfaces) provides difficulty for any hypothesis that would relate wall thickenings to turgor pressure. The patterns of fibrillar accretion observed in Nymphaea include all patterns thus far reported from stem tracheids in the other genera of the family, and they must be considered to be a characteristic of Nymphaeaceae.

Keywords: microstructure, SEM, secondary walls, tracheids, vessel elements, xylem.

Introduction

Nymphaeales (Nymphaeaceae, Cabombaceae, Hydatellaceae) have often been considered to contain numerous features judged to be ancestral for dicotyledons. Accordingly, these families have attracted considerable study. As the largest genus, Nymphaea (ca. 40 spp.; Schneider and Williamson 1993) has attracted the most attention; however, the work published on stem and root anatomy is still minimal. Gwynne-Vaughan (1897) provided careful descriptions and illustrations of poly-stelate, bundle arrangement, and bundle anatomy in the genus. Weidlich (1976a, 1976b) focused on three-dimensional bundle distribution, nodal anatomy, and mesarchy in bundle construction. Weidlich (1976b) was obviously impressed with the differences between the features of stem vascularization as compared with that of typical eudicots. He stated that, “On the basis of the development and organization of the vascular system in the stems of Nymphaea, these plants are considered to be highly specialized and modified.” Both Gwynne-Vaughan and Weidlich illustrate in their figures a lack of secondary growth in stems of Nymphaea, although they do not discuss this feature or its possible significance.

There has been unanimous consent by those who have used light microscopy that xylem of Nymphaeales consists of tracheids. That conclusion may be valid, depending on the definition one uses for “vessel element” and “tracheid.” Schneider et al. (1995) used the term “vessels” for root tracheary elements of Nymphaea, but that interpretation was based on the occurrence of small porosities in pit membranes of tracheids. The tracheary elements in roots of Nymphaeaceae cross the borderline between tracheids and vessel elements. If one defines vessel elements as having end-wall pit membranes with an area that is more than half devoid of primary wall material, Nymphaeaceae may be said to have vessel elements in its roots but only tracheids in its stems. Because of these degrees of intermediacy in design of tracheary elements, Nymphaeaceae are of inherent interest.

Availability of SEM equipment capable of greater resolution has induced us to revisit the question of the nature of tracheary elements in Nymphaeaceae. In doing so, we have employed sectioning techniques we believe offer a better basis for interpretation. Our newer studies began with Nuphar (Carlquist et al. 2009). In Nuphar, we found highly porous pit membranes in end walls of root tracheids. In the stems of Nuphar, however, study of inner wall surfaces of tracheary elements revealed distinctive microstructure—not previously reported in any vascular plant—on end walls of tracheids. Our study of Nuphar (Carlquist et al. 2009) included one collection of Nymphaea for purposes of comparison. The stem tracheids of that Nymphaea proved to have a microstructure comparable to that of Nuphar on the inner surfaces of end walls. Because Nymphaea is a much larger genus than Nuphar, with a wider geographical and ecological range (Borsch et al. 2008), a reconnaissance of Nymphaea tracheid microstructure seemed to be highly desirable.

1 Author for correspondence; e-mail: eschneider@sbbg.org.

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Nymphaeales are a group of special phylogenetic interest because, in recent phylogenetic trees, they are the sister group to all angiosperms other than Amborellales (Solís et al. 2000; Borsch et al. 2008). Nymphaeales include not only Cambiocarpaceae and Nymphaeaceae, but Hydatellaceae as well (Saarela et al. 2007). The tracheid patterns in Nymphaeaceae we have thus far identified with SEM (Carlquist and Schneider 2009; Carlquist et al. 2009) are of special significance. Do root tracheids throughout Nymphaeaceae have highly porous end walls, suggesting a vessel-like configuration? Are the strandlike and spongiform wall accretions of coarse fibrils on stem tracheid end walls found throughout Nymphaeaceae? What variations on these patterns exist? *Nymphaea* is a key group because its species number and ecological diversity exceed those of the other genera of the family (Schneider and Williamson 1993).

Recently, Löhne et al. (2007) have provided phylogenetic trees that show the interrelationships of most of the subgenera of *Nymphaea*. Interestingly, *Ondinea* is shown to be nested within *Nymphaea* subgenus *Anechphyta*. We therefore included *Ondinea* in this study. The giant water lilies *Victoria* and *Euryale* form a branch within the *Nymphaea* clade and were studied concurrently (Carlquist and Schneider 2009). We have developed information on the microstructure of stem tracheids of the genus *Barclaya* (E. L. Schneider and S. Carlquist, unpublished data). We have included in this study representatives of the subgenera *Nymphaea*, *Brachyceras*, *Hydrocalis*, and *Lotos*, which is a coverage approximately the same as that of Löhne et al. (2007). The recently described subgenus *Confluentes* (Jacobs and Porter 2007) is not included here. The ecological range of the species studied is extensive: *Nymphaea rudgeana* G. Mey. is native from Cuba to southern Brazil (Wiersema 1987), whereas *Nymphaea odorata* Ait. extends from eastern North America into southern Canada (Conard 1905). If the coarse fibrils of stem tracheid end walls are related to ecology or are diversified with respect to phylogeny within the genus, the plants in this study should reflect such sources of variation.

The fibrils of the stem tracheid end walls in Nymphaeaceae should be considered to be secondary-wall components. Reasons for this interpretation include the fact that the fibrils are laid down parallel to each other, rather than in a random network. A random network of microfibrils composes the pit membranes in pits of lateral walls of stem tracheids, where the distinctive coarse fibril accretions are lacking. The coarse fibrils we describe are many times thicker than the microfibrils of the pit membranes of lateral walls in dicotyledons at large (Schmid 1965). Furthermore, they are intercontinuous with secondary-wall portions, such as the pit cavity surfaces and the secondary-wall portions facing the tracheid lumina, as observed with SEM. Hand sections from fixed material with a safranin–fast green stain combination in *Nuphar* suggest that the coarse fibrils are not portions of the primary wall. There are no known reports of primary walls formed within secondary walls, except for those instances in which subdivision of cells occurs (e.g., tyloses, septate fibers). Therefore, the study methods we have adopted are the same as those for such secondary-wall portions as helical thickenings, vesture pits, or trabeculae. Study by means of TEM is to be welcomed, as for any plant structure. Our SEM studies can be considered to offer a basis for such TEM studies by surveying the systematic and anatomical distribution of coarse fibrils within Nymphaeales. Because of its high resolution, TEM is not as useful as SEM for screening instances of coarse fibril occurrence. By screening for the occurrence of coarse fibrils within Nymphaeales, we hope to have offered a choice of starting points for TEM studies.

**Material and Methods**

Living material of actively growing plants was available for the following species: *Nymphaea caerulea* Savigny (subgenus *Brachyceras*), *Hellquist 17107* (UMASS); *Nymphaea lotus* L. (hybrid; subgenus *Lotos*) *Hellquist 17108* (UMASS); *Nymphaea micrantha* Guill and Perr. (subgenus *Brachyceras*) *Hellquist 17106* (UMASS); *Nymphaea odorata* Aiton (subgenus *Nymphaea*) *Hellquist 17109* (UMASS); *N. odorata* ‘Sulphurea Grandiflora’ (cultivated Alice Keck Park, Santa Barbara, CA); *Nymphaea rudgeana* G. Mey. (subgenus *Hydrocalis*) *Hellquist 17105* (GH); *Ondinea purpurea* den Hartog, E. L. Schneider July 8, 1988 (PERTH, SBBG), Kalumburu Mission, Western Australia (Schneider 1983; Williamson et al. 1989). The *Hellquist* collections were cultivated by C. B. Hellquist in North Adams, Massachusetts. Roots and stems of all collections were preserved in 70% aqueous ethanol. Sections of stems of all species were prepared by hand sectioning with razor blades; sections of roots were prepared for only two collections of *N. odorata* in which the roots were large enough in diameter to be sectioned using this technique. Roots were not sectioned for the majority of species because our surveys of root and stem anatomy of the other genera of Nymphaeaceae have revealed no variation in the basic pattern reported in *Nuphar* (Carlquist et al. 2009). Hand sections were prepared because the thickness (1–2 mm) tends to support delicate cells during handling and because larger portions of tracheids are exposed, compared with the surfaces that are exposed by rotary micromtome sectioning. Sections were washed in three changes of warm (60°C) distilled water and were then dried on a warming table under fumigation for three minutes. Sections were sputter-coated with gold, and examined with a Hitachi S2600N scanning electron microscope. These methods were used because they are entirely appropriate for use when studying secondary walls and secondary-wall structures (warts, helices, grooves, vesutres) in angiosperms. There is no literature, to our knowledge, that would suggest that techniques such as critical-point drying, environmental SEM, etc., are necessary or even desirable for the secondary-wall structures we have studied. All of the tracheary elements illustrated here qualify as metaxylem, either by virtue of scalariform lateral-wall pitting or lack of elongation of a wall pattern that is transitional between helical and scalariform. “Stem” is used as a synonym for “rhizome” throughout this study. The terms “axially oriented” and “longitudinally oriented” refer to coarse fibrils that run parallel to the long axis of the trach (and therefore also to the longitudinal axis of the rhizome).

The illustrations are arranged according to subgeneric groupings. Because *Ondinea* is now placed within *Nymphaea* (*Ondinea* is included within *Nymphaea* subgenus *Anechphyta* by Löhne et al. [2007]), illustrations of its tracheids are placed within the sequence of *Nymphaea* subgenera.
Results

Root Tracheids

Our photographs of root tracheids (fig. 1A–1D) are based on Nymphaea odorata. This material proved to be convenient because the large-diameter roots in our collections of this species could be readily sectioned according to the methods described above. Metaxylem tracheary elements have scalariformly pitted lateral walls (fig. 1A). The secondary-wall architecture frequently shows oblique, thin interconnections between the major horizontally oriented wall bands (fig. 1A). All observed pit membranes of lateral walls showed untextured laminar pit membranes of the type widely reported for lateral-wall pitting in vessels (fig. 1A). The tendency of the axially oriented coarse fibrils to be attached to pit borders and pit cavities is often observed (fig. 2A). Longitudinally oriented fibrils may fade into the reticulate appearance of the pit-membrane surface (fig. 4F). Some longitudinally oriented fibrils are gathered in fascicles, but finer fibrils run between the fascicles.

Stem Tracheids

Lateral walls. Lateral walls of metaxylem tracheary elements in Nymphaea as seen from the inner (lumina) surfaces bear smooth, little-textured pit membranes (fig. 1E, 1F). As seen from the inside of the tracheid, some lateral-wall pit membranes bore no texturing, although undulating contours attributed to drying or handling were present (fig. 1E). More commonly, we found pit membranes of tracheary-element lateral walls to be overlaid with fine, threadlike fibrils superimposed on the smooth pit-membrane surface (fig. 1E, 1F). These threadlike fibrils are reduced versions of the coarse fibrils found on pit membranes and secondary walls of stem tracheid end walls (figs. 2–5). The lateral walls of tracheary elements appear to consist mostly of tracheary element–parenchyma interfaces.

End walls. Material of N. odorata (fig. 2) provides a convenient introduction to the nature of tracheary-element end walls in stems of Nymphaea. Coarse fibrils overlies the pit membranes, but appearances depend on the plane of sectioning of any given tracheary element. Pit membranes, as seen from the inner surface (fig. 2A–2C), bear both rather sparse, longitudinally oriented fibrils (fig. 2B, 2C) and densely spongy coarse fibrils (fig. 2A). The reticulate coarse fibrils are in contact with the pit membrane, whereas the longitudinally oriented fibrils are more distal (toward the tracheid lumina) from the pit membrane. If one slices the pit membrane away (fig. 2A, right), one sees the pit borders of pits of the facing tracheid. The rough surfaces of the pit borders (fig. 2A, right) represent stubs where the coarse fibrils were broken away from the pit border by sectioning. If one views undisturbed surfaces of tracheary-element end walls from the inside of the tracheid, one sees longitudinally oriented coarse fibrils predominantly; behind these (more proximal to the pit membrane), the dense spongiform reticulum can be seen. Within the spongiform reticulum, tiny porosities can be seen. The tendency of the axially oriented coarse fibrils to be attached to pit borders and pit cavities is often observed (fig. 2C).

If more of the end wall is cut away by sectioning, revealing the outer surfaces of the pit membrane of the adjacent tracheid (fig. 2D–2F), one can see the dense spongiform network of coarse fibrils. Depending on the portion sectioned, one can see porosities within these fibrils (fig. 2E). The reticulum of coarse fibrils is attached to the surfaces of the pit border (fig. 2F).

Nymphaea caerulea (fig. 3) shows a wide range of expressions of longitudinally oriented coarse fibrils. In some tracheary-element end-wall pits, the fibrils are relatively inconspicuous and are fused with the porose layer (fig. 3A). Fibrils fade out as they terminate on the secondary-wall surface between the pits (fig. 3A). At an opposite extreme, longitudinally oriented coarse fibrils may be largely separate from the plate-like pit membrane; the longitudinally oriented fibrils may occur as irregularly distributed fascicles when viewed from the inside of a tracheid (fig. 3B). When viewed from the outside of a tracheid, a section in which the pit membrane has been removed shows the longitudinally oriented fibrils crossing the pit cavity between pit borders (fig. 3C). A tracheary end wall that was sectioned such that the bars of secondary-wall material are removed and that is observed from the inside of the tracheary element (fig. 3D) shows the longitudinally oriented fibrils in face view; it also shows broken ends of the fibrils (fig. 3D, center, top) that were attached to the pit borders. Longitudinally oriented fibrils may vary in thickness (fig. 3E); an unusual appearance is shown (fig. 3F): the longitudinally oriented fibrils are gathered in fascicles, but finer fibrils run between the fascicles.

Two contrasting photographs of end-wall pit membranes of Nymphaea micrantha as seen from the inside of a tracheary element are presented. In the first (fig. 4A), pores in a relatively solid membrane are the most clearly delineated feature. In the second (fig. 4B), the longitudinally oriented coarse fibrils are conspicuous.

Several photographs of end walls of Ondinea purpurea tracheary elements (fig. 4C–4F) show subtle variations in deposition of the coarse fibrils. At a lower magnification (fig. 4C), the reticulate appearance of the pit-membrane surface is evident. At higher magnifications, one can see that coarse fibrils form a more spongiform reticulum, especially where they join the bars of the secondary wall in pit cavity areas (fig. 4D). Some coarse fibrils terminate on bands of secondary-wall material between the pits; the fibrils fade into the surfaces of those bands (fig. 4E). Some longitudinally oriented fibrils are evident on the pit-membrane surface (fig. 4F); together with departure of the strands to the pit, border areas may be evident, with lacunae prominent between the strands where they contact the pit border areas.

End walls of stem tracheary elements of Nymphaea rudgeana, as viewed from the lumen side of the wall (fig. 5A, 5B), show a predominance of axially oriented coarse fibrils. The fibrils may be sparse (fig. 5A) or grouped into fascicles and more densely placed (fig. 5B). When the outer surface of a tracheary-element end wall is exposed by sectioning, however, the reticulate nature of the pit membrane is revealed (fig. 5C). In end walls of N. lotus tracheary elements as viewed from the lumen side (fig. 5D–5F), several variations are evident merely on the basis of sampling within the stems that were studied. The longitudinally oriented fibrils may fade into the reticulate spongiform portion of the pit membrane (fig. 5D). A some-
Fig. 1  Tracheary-element surfaces of *Nymphaea* as seen with SEM. *A*, Lateral-wall pitting of the root of a *Nymphaea odorata* tracheary element, showing subdivisions of scalariform pitting; pit membranes are nonporous and untextured at this magnification. *B–D*, End-wall pit membranes of *N. odorata* root tracheary elements; some tears in the membranes are evident. *B, C*, End walls of *N. odorata* ‘Sulphurea Grandiflora’; the reticula are relatively large in size. *D*, End wall of *N. odorata* subsp. *tuberose*; porosities are larger in the center of the pit membrane. *E, F*, Lateral walls of tracheary elements from stems, as seen from the lumen side of the cell. *E, Nymphaea odorata* ‘Sulphurea Grandiflora’ pits relatively free of coarse fibrils. *F, Nymphaea micrantha*, detail of inconspicuous fibrils on surface pit membrane. Scale bars = 2 μm.
Fig. 2 SEM micrographs of inner (A–C) and outer (D–F) surfaces of stem tracheid end walls of *Nymphaea odorata* ‘Sulphurea Grandiflora.’ A, Dense covering of pit membranes with coarse fibrils (left); at right, the pit membrane has been sectioned away, revealing the pit borders of the facing tracheid. B, Portions of four pit membranes with numerous axially oriented coarse fibrils. C, Numerous axially oriented coarse fibrils on pit membranes, illustrating their three-dimensional distribution. D, Portions of several pits with the axially oriented fibrils removed, revealing the spongiform nature of the underlying coarse fibril accretions on the pit membrane. E, Portions of several pits; the more open appearance in the top pit is related to shaving away more of that pit membrane during sectioning. F, Portions of three pits; the three-dimensional nature of the spongiform coarse fibrils is shown. Scale bars = 2 μm.
Fig. 3  SEM micrographs of pit-membrane portions from stem tracheid end walls of *Nymphaea caerulea*. A, Pit membrane with axially oriented fibrils closely adherent to the pit membrane. B, Pit membrane with coalescent axially oriented fibrils (left) and holes (white dots) in the denser secondary-wall accretion layer. C, Tracheid seen from outer face; the pit membrane has been sectioned away, showing only the axially oriented fibrils traversing the pit cavity. D, Coarse fibril accretions on pits as seen from the lumen side of the tracheid; the bars (gyres) of the secondary wall have been sectioned away, exposing the cut ends of the spongiform layer of coarse fibrils, broken away from the pit borders. E, Portion showing axially oriented coarse fibrils abundant and overlying the meshwork. F, Abundant axially oriented coarse fibrils, which are gathered into fascicles; some coarse fibrils extend between the axial fascicles. Scale bars = 2 μm.
Fig. 4 SEM micrographs of pit-membrane portions from stem tracheid end walls of Nymphaea micrantha (A, B) and Ondinea purpurea (C–F) as seen from the lumen side of the cell. 

A, Pit-membrane portions in which porosities (some appear ringed by white) are conspicuous. B, Pit-membrane portions in which axially oriented coarse fibrils (somewhat oblique) are evident. C, Oblique view into tracheid showing a meshwork pattern of coarse fibrils. D, Detail showing coarse fibrils traversing the pit cavities, with their ends fading into the bar (gyre) surface of the secondary wall. E, Pit-membrane portion with meshwork of the reticulate accretion layer extending over the smooth secondary-wall bars. F, Pit-membrane portion with porosities in the spongiform layer, axially oriented layer of fibrils, and conspicuous strands traversing the pit cavity areas. Scale bars = 2 μm.
Fig. 5 SEM micrographs of pit-membrane portions from stem tracheid end walls of *Nymphaea rudgeana* (A–C) and *Nymphaea lotus* (D–F). A, Sparse distribution of axially oriented fibrils as seen from the lumen side of the tracheid. B, Coarse fibrils abundant, some coalescent into fascicles, as seen from the lumen side of the tracheid. C, Pit membrane exposed by sectioning, its reticulum overlying the pit borders and pit cavity. D, Intermingling of axially oriented and reticulate coarse fibrils on a pit membrane, as seen from the lumen side of the tracheid. E, Oblique view of the inside of the tracheid, revealing transitions between pit membranes; coarse fibrils fade into the smooth secondary wall in these transition zones. F, Oblique view of the inside of the tracheid; spongiform fibril meshwork at left; at right, sinuous ends of coarse fibrils taper over the secondary-wall bars (gyres). Scale bars = 2 μm.
what oblique view of a tracheary-element end wall (fig. 5E) illustrates a tendency for many of the coarse fibrils to fade into the bars (or gyres) of the secondary wall. Another wall portion (fig. 5F) is notable for showing the three-dimensional nature of fibril deposition over the pit-membrane area (fig. 5F, left) as well as a consistent reticulate pattern of fibrils on the surfaces of the bars (gyres) of the secondary walls.

Discussion

Our earlier SEM studies of tracheary elements of *Nuphar, Nymphaea*, and *Ondinea* (Schneider et al. 1995) dealt only with roots. Our reason for this focus was that, if evolution toward a vessel-like configuration was present in Nymphaeaceae, one would expect it in the roots because they are relatively short-lived organs and therefore are designed for more rapid water uptake characteristics (Carlquist 1975). This idea was based on Cheadle’s (1942) data for monocotyledons, which have adventitious roots in a pattern that is very similar to that of Nymphaeaceae. Organs of a shorter duration should have specialization for more rapid conduction, whereas presence of tracheids in stems (e.g., ferns) may correlate with the advantages of an imperforate tracheary element in resisting the spread of embolisms (Carlquist 1975). The thin paraffin sections with which we worked earlier (Schneider et al. 1995) and the low-resolution SEM equipment that was available to us at that time did not permit us to analyze the end walls of nymphaeaceous root tracheary elements with maximal accuracy. We did observe porosities in the pit membranes of end walls; however, we would not consider these cells to be vessel elements today because only a small portion of the pit membranes of the cells we illustrated consisted of holes interconnecting two tracheary elements.

Our current findings in *Nymphaea* roots (on the basis of *N. odorata* only) demonstrate a much greater area of the tracheary-element end walls (certainly >50%) that is devoted to holes (porosities) that are separated by narrow primary wall strands. These end walls are presumably the result of lysis of much of the membrane during maturation of the tracheary element. One could reasonably argue that the tracheary elements we observe in *N. odorata* are vessel elements. Certainly, vessel elements have been designated in woody dicotyledons that have a greater portion of the end walls of these cells devoted to membrane remnants rather than to porosities, although other characteristics (greater width than co-occurring tracheids, difference between end-wall and lateral-wall secondary-wall architecture, length shorter than co-occurring tracheids) support the concept of such tracheary elements as vessel elements. Such vessel elements include those of Chloranthaceae (Carlquist 1987, 1990, 1992a, 1992b; Sperry et al. 2007) and Illiciaceae (Carlquist 1982; Carlquist and Schneider 2002; Sperry et al. 2007). We believe that our SEM illustrations of the end walls of root tracheary elements of *N. odorata* could be reasonably interpreted as either vessel elements or tracheids. The terminology and the attempt to devise a dividing line between cell types is not so important, in our view, as the fact that there is a continuum. End-wall specialization by pit-membrane lysis (as well, perhaps, as lessened deposition of wall materials on end walls of tracheary-element pit membranes) is a pervasive process in the specialization of tracheary elements to achieve more effective conduction.

Ultimately, the most satisfactory division between tracheids and vessel elements may be the ability of the end walls of the former to block passage of air bubbles from one element into the next, whereas air bubbles are capable of passing from one vessel element to the next. This distinction, however, depends on the ability to demonstrate air bubble behavior at cell junctions by experimental means. Perhaps experiments that demonstrate air bubble passage in relation to known thresholds for end-wall porosities can offer the necessary criteria for differentiating between tracheids and vessel elements. Use of tracheary elements known to be intermediate between tracheids and vessel elements, such as those of *Nymphaea* roots, will be desirable in such experiments.

The stem tracheary elements of *Nymphaea*, like those of *Nuphar* (Carlquist et al. 2009), *Euryale*, and *Victoria* (Carlquist and Schneider 2009, as well as those of *Barclaya* (E. L. Schneider and S. Carlquist, unpublished data), present a picture that is quite different from that of the root tracheary elements in these genera. The stem tracheary elements of these genera, as seen from the inside surfaces, have predominantly smooth pit membranes facing parenchyma cells. The end walls of the tracheids, which represent tracheid-to-tracheid interfaces, bear an accretion of secondary-wall material in the form of coarse fibrils in a somewhat spongiform layer (adjacent to pit membrane) plus longitudinally oriented fibrils (distal from the pit membrane, adjacent to the tracheid lumen). This phenomenon is, to the best of our knowledge, not represented outside of Nymphaeales. The phenomenon of secondary-wall accretion on inside pit-membrane surfaces of end walls of nymphaeaceous tracheids is quite different from the process of primary-wall hydrolysis (Butterfield and Meylan 1982) that is seen in pit membranes of perforation plates of Chloranthaceae, Illiciaceae, and other dicotyledons with primitive wood features. The perforated pit membranes in imperforate tracheary elements of certain dicotyledonous woods (Sano and Jansen 2006) represent a phenomenon that is different from those considered here.

Variation in the patterns of coarse fibrils on end walls of stem tracheids of *Nymphaea* does not seem to be related to subgeneric groupings. Admittedly, our sampling of the genus is limited, although we attempted to include the taxonomically most diverse species (i.e., at least one species from each subgenus except for the recently named Australian subgenus *Confluentes*). It should be noted that exposing end walls of tracheids via any type of sectioning does not yield extensive areas for examination, although the method of hand sectioning we use exposes much greater areas than does sectioning by means of a rotary microtome. Although some of our illustrations do differ appreciably from others, the differences that can be cited are more attributable to plane of sectioning, site within stem, etc., than to variability among individual plants. At present, the amount of variation we see from one species of *Nymphaea* to another is less than the helical thickening variations within vessels of a genus of woody dicotyledons (or even within a single wood sample from a dicotyledonous species that has such thickenings).

What is the functional significance of the peculiar end-wall accretions on the stem tracheid end-wall pit membranes in
**Nymphaea?** Some would say, quite defensibly, that explanations of the functional nature of structures in xylem can be made only with the aid of experimental techniques. However, a choice of hypotheses to experimentally test is best guided by comparative studies, because knowledge of systematic, organographic, and ecological distribution of a xyleary feature can and should guide conceptualization about function. In the case of the coarse fibrils of nymphaeous stem tracheids, one interpretive problem arises from their distribution within the tracheids. If some sort of tensile strength is involved, why are the fibrils deposited only on the end walls and not on the lateral walls, which face parenchyma cells? Both types of wall surfaces ought to equally experience issues related to stress. The distribution of the fibrils within stem tracheids is also a factor that mitigates against any view of the tracheid as contributing to the overall strength of the stem. Fibers, which are notably absent in stems of Nymphaeaceae, would be the expected indication of a means to increase stem strength as a whole or to strengthen any particular region of the stem. Mechanical strength of stems of *Nymphaeae* and other Nymphaeales is surely not a factor, in view of their form, shortness, and tendency to rest on a stream or pond bottom. Turgor pressure and the primary walls of parenchyma cells in such stems would account for the mechanical strength of stems in *Nymphaea* to a much greater extent than the tracheid microstructures we have illustrated. The above-mentioned considerations, however, do not eliminate explanations of the functional significance of the coarse fibrils. They are absent from stems of *Neelumbo*, an aquatic genus with a similar habitat (E. L. Schneider and S. Carlquist, unpublished observations), and they have not yet been reported in any group of terrestrial woody dicotyledons or aquatic or terrestrial monocotyledons. The consistent presence of the coarse fibrils on the end walls of stem tracheids of Nymphaeaceae does suggest some functional explanation, because such localized structures that represent an appreciable cellulosic investment would not be expected to persist over the long periods of geological time during which Nymphaeaceae have existed (Löhne et al. 2008).

**Literature Cited**


Queries

Q1  Is it correct to say “end walls suggest that lysis of pit membranes leaves a cellulosic network with a large reticulum”?
Q2  Please provide the page number(s) of the quote from Weidlich 1976a.
Q3  Is Carlquist et al. 2009 correct (from Carlquist et al. 2008)?
Q4  Is “together with departure of the strands to the pit, border areas may be evident” correct as edited?
Q5  Is it correct to say “One could reasonably argue that the tracheary elements we observe in N. odorata are vessel elements” (from “the tracheary elements we figure for N. odorata...”)?
Q6  Is “helical thickening variations” correct? Or, perhaps, “variation in helical thickening”?
Q7  Is “issues related to stress” correct (from “stresses related to stress”)? Would something else be better?
Q8  Is “an aquatic genus with a similar habitat” correct (from “with a similar habit”)?