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# Xylem of early angiosperms: Novel microstructure in stem tracheids of *Barclaya* (Nymphaeaceae)<sup>☆</sup>

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#### ABSTRACT

Pit membranes of stem tracheids of all recognized species of Barclaya, an Indomalaysian genus of Nymphaeaceae, were studied with scanning electron microscopy (SEM). Pit membranes of the tracheids are composed of two thick layers, both constructed of fibrils much larger than those of tracheary elements of angiosperms other than Nymphaeaceae. The outer (distal) layer, which comprises the continuous primary wall around the tracheids, is spongiform, perforated by porosities of relatively uniform size, and confined to or most prominent on end walls of stem tracheids. The second layer consists of thick widely spaced fibrils that are oriented axially and are laid down proximally (facing the cell lumen) to the first (outer) layer, although continuous with it. These axial fibrils are attached at their ends to the pit cavities. This peculiar microstructure is not known outside Nymphaeaceae except in Brasenia and Cabomba (Cabombaceae, Nymphaeales), and has not been previously described for Barclaya. The longitudinally oriented threads and strands in perforation plates of secondary xylem of wood and stems of a variety of primitive woody angiosperms (e.g., Illicium) are not homologous to the pit membrane structure observed in stem tracheids of Barclaya, which, like other Nymphaeaceae, has only primary xylem and no perforation plates. The tracheid microstructure reported here is different from pit structures observed in any other group of vascular plants, living or fossil. The tracheid stem microstructures of Barclaya and other Nymphaeaceae appear to be a synapomorphy of Nymphaeaceae and Cabombaceae, and need further study with respect to ultrastructure and function.

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#### 1. Introduction

Barclaya consists of four species, ranging from New Guinea, western Borneo, Thailand, the Andaman Islands, and Burma. Malaya is a center for the genus (Ridley, 1922; Schneider and Williamson, 1993; Williamson and Schneider, 1994). A fifth named species (B. pierreana Gagnep., Gagnepain, 1908) from Vietnam may be referable to B. longifolia Wall. Barclaya tends to grow in clear running streams, except for B. rotundifolia, which occurs as a nearterrestrial plant with aerial leaves on muddy forest flats. B. longifolia has submersed leaves. B. kunstleri and B. motleyi have the habits typical of most Nymphaeaceae; they grow in shallow slowflowing streams and have submersed or partially floating leaves. This range of habits and habitats is of possible interest with respect to xylary features.

In an earlier series of papers (Schneider and Carlquist, 1995a,b; Schneider et al., 1995), we offered observations on microstructure

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of root and stem tracheids of Nymphaeaceae, based on macerations and paraffin sections of liquid-preserved Nymphaeaceae. Our focus was mainly on the end walls of tracheids. The end walls of tracheids of roots of Nymphaeaceae appeared to have porose pit membranes. The lateral wall pits appeared to lack porosities. Our limited observations on stem tracheids did not clearly show porosities or any other unusual features. Access to better scanning electron microscopy (SEM) equipment and to more diverse collections of nymphaeacous taxa, including all known species of Barclaya, have induced us to revisit xylem of Nymphaeaceae. Our previous studies of Nuphar (Carlquist et al., 2009), Victoria and Euryale (Carlquist and Schneider, 2009), Nymphaea (Schneider et al., in press), and Cabomba and Brasenia (Carlquist and Schneider, in press) have led us to inquire into the systematic distribution of this feature in Nymphaeales. In this connection, Barclaya is a key genus. Nuphar is the sister group to the remainder of Nymphaeaceae (Les et al., 1999; Borsch et al., 2007; Löhne et al., 2007); Barclaya forms the sister group to the genera other than Nuphar. The genera Euryale, Ondinea, and Victoria are nested within Nymphaea, according to Borsch et al. (2007) and Löhne et al. (2007).

The rhizomes of *Barclaya* are commonly thick and condensed, not unlike those of some species of *Nymphaea*, but long runners are also present (Williamson and Schneider, 1994). We discovered coarse

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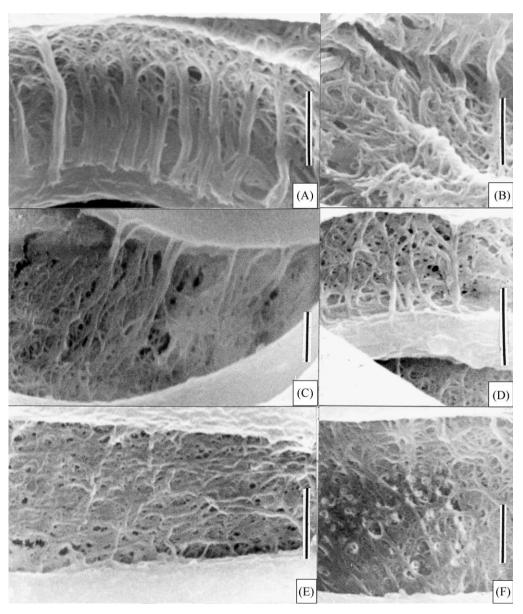
Corresponding author.

fibrillar microstructure in pit membranes in stems of *Cabomba* (Schneider and Carlquist, 1996), *Nuphar* (Carlquist et al., 2009), Nymphaea (Schneider et al., in press) and *Victoria* and *Euryale* (Carlquist and Schneider, 2009) but an issue of critical importance is formed by the distribution of such pit features within the family as a whole. Any variations in the nature of such patterns with respect to ecology, systematics, or organography are of potential significance.

The xylem of stems of Nymphaeaceae contains primary xylem with tracheids only, and therefore cannot be compared directly to the secondary xylem of woody angiosperms (Carlquist et al., 2009). Secondary xylem of woody angiosperms with taproots contains such features as division of labor into conductive, mechanical, and storage systems: such division of labor is not present in the roots or stems of Nymphaeaceae. However, the occurrences of distinctive coarse microfibrillar structure in pit membranes of *Barclaya*, reported here, do invite explanation precisely because they have not been observed in plants other than Nymphaeaceae and Cabombaceae.

Amborella is now considered to be the sister group to the remainder of the angiosperms (e.g., Soltis et al., 2000). Nymphaeales or Austrobaileyales are the sister group to the remainder of the angiosperms other than Amborella (Soltis et al., 2000; Löhne et al., 2007). Nymphaeales are now considered to include Hydatellaceae as well as Nymphaeaceae and Cabombaceae (Rudall et al., 2007; Saarela et al., 2007).

The coarse fibrils that we describe are many times thicker than the microfibrils of pit membranes of dicotyledons at large (Schmid, 1965). The coarse fibrils are intercontinuous with secondary wall portions, such as the pit cavity surfaces and the secondary wall portions facing tracheid lumina, as seen with SEM. The coarse fibrils are probably secondary wall material because there are no known reports of primary walls formed within secondary walls, except for those instances in which subdivision of cells with secondary walls occurs (e.g., tyloses, septate fibers).



**Fig. 1.** A–F: SEM micrographs of inner surfaces of stem tracheids of *Barclaya*. A, B: *B. kunstleri*. A: Portion of pit membrane showing prominent axially oriented coarse microfibrils. The sectioning process has cut the fibrils at the bottom of the micrograph. B: Portion of pit membrane with spongiform structure; folds in the membrane are artifacts produced by handling. C–F: *B. longifolia*. C: Pit membrane in which some axially oriented coarse fibrils are fused with the layer in which the fibrils are randomly oriented. Some tears are present. D: Pit membrane in which the three-dimensional nature of the coarse fibrils is evident. E: Porosities in a pit membrane portion; axially oriented fibrils are nearly absent. F: Porosities in a pit membrane portion in which the coarse microfibrils are not strongly three-dimensional. A–F: scale bars = 2 μm.

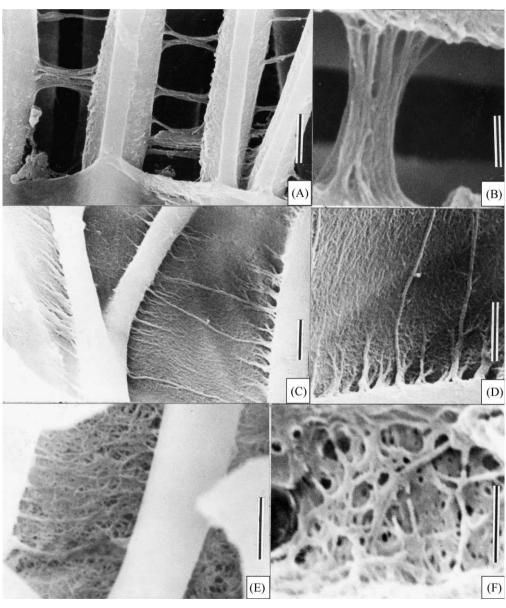
The coarse fibrils on end walls of stem tracheids of *Barclaya* should not be confused with the pit membrane remnants found on end walls of vessels (Carlquist, 1992) in angiosperms with more numerous symplesiomorphic features. By demonstrating the occurrence of coarse microfibrils in *Barclaya* stem tracheids, we hope to clarify the systematic and ecological significance of this distinctive feature in Nymphaeales. By being able to study materials of all four species of *Barclaya*, we are able to discern variations within the genus, an opportunity not possible in our studies of the large genera *Nuphar* (Carlquist et al., 2009) and *Nymphaea* (Schneider et al., in press).

### 2. Materials and methods

Plants of the four *Barclaya* species were collected in the wild and preserved in 70% aqueous ethanol. The collections are as follows:

B. kunstleri (King) Ridley, E. L. Schneider 801; B. longifolia Wall., E. L. Schneider 802; B. motleyi Hook. f, E. L. Schneider 803; B. rotundifolia Hotta, E. L. Schneider 804. Specimens are housed at SBBG.

Stems were successfully sectioned by hand with single-edged razor blades. The thickness of these sections makes them preferable to microtome sections because they often display larger portions of tracheary elements as rendered by SEM, and because the thickness of the sections tends to minimize damage to microstructure by handing. Sections were transferred through three changes of distilled water, then dried on a 50 °C warming table between clean glass slides clamped to create pressure to prevent curling of sections during the drying process. Dried sections were mounted on stubs, sputter coated with gold, and examined with a Hitachi S2600N SEM. The observations reported below apply to metaxylem tracheids. The secondary wall structure



**Fig. 2.** A–F: Pit membrane portions of *B. motleyi*. A, B: Views of axially oriented fibrils in tracheid pits, seen from the outer surface of a tracheid; the outer layer of the pit membrane has been cut away. A: View of several adjacent pits; pit borders are shown. B: Enlarged portion of axially oriented fibrils. The fibril strand is composite. The pit border (top of micrograph) has a rough appearance because the randomly oriented fibrils have been broken away by the sectioning process. C, D: Pit membrane portions seen from the inside of a tracheid; a parenchyma cell faces the wall portion shown. C: Axially oriented strands fade into the layer of randomly oriented fibrils; porosities are not observable. D: The axially oriented strands branch in a dendritic fashion where they are attached to the pit border. E, F: Pit membrane portions seen from the proximal (lumen side) of the tracheid. E: Axially oriented fibrils merge into the layer composed of randomly oriented fibrils. F: Portion to show the spongiform nature of the layer composed of randomly oriented fibrils. A, C, E: scale bars = 2 μm; B, D, F: scale bars = 1 μm.

of the tracheids studied suggests that they represent various types of pitting, some reminiscent of helical thickenings, but with enough interconnections between the gyres so that the tracheids are probably not extensible and therefore qualify as metaxylem rather than protoxylem tracheids.

#### 3. Results

### 3.1. B. kunstleri (Fig. 1A and B)

On the inner surfaces (facing the lumen) of stem tracheid pits, coarse axially oriented fibrils are characteristic of pits (Fig. 1 A). The fibrils form dendritic branches that intersect with the reticulate spongiform pit membrane distal to the fibrils. The reticulate layer is composed for coarse randomly oriented fibrils (Fig. 1 B) and may occur without the overlay of axially oriented fibrils.

#### 3.2. B. longifolia (Fig. 1C-F)

Axially oriented coarse fibrils occur sparsely on some inner surfaces of stem tracheid pits (Fig. 1C and D), but are not present on other pits (Fig. 1E and F). Where present, the axially oriented fibrils are closely integrated into the reticulate layer (Fig. 1C and D). The reticulate layer is composed of densely packed randomly oriented coarse fibrils. Small porosities occur sporadically within the pit membranes (Fig. 1C–F). Artifacts appeared few in our material: a few tears are present in the pit membrane shown in Fig. 1C.

## 3.3. B. motleyi (Fig. 2A-F)

Axially oriented coarse fibrils are prominent on most stem tracheid pits (Fig. 2A–E). The axially oriented fibrils are shown isolated from the reticulate wall layer in A and B, because the

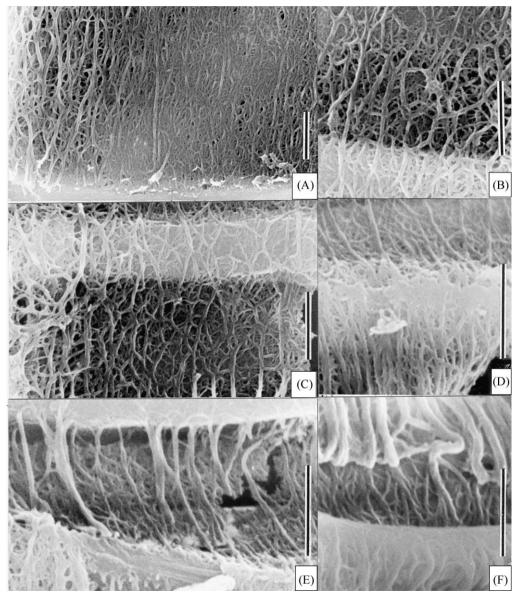


Fig. 3. A–F: Portions of stem tracheid pit membranes of *B. rotundifolia*. A–D: Pit membranes seen from outer surface of tracheids. A: Pit membrane in which a nonporose pit membrane portion (probably an interface between a parenchyma cell and a tracheid) and a porose portion (at left; the nonporose layer shaved away by sectioning) are illustrated. B: Pit membrane portion showing three-dimensionality of the randomly oriented-fibrils. C: Porosities occur in the layer of randomly oriented coarse fibrils. D: Portion of pit membrane overlying a bar of secondary wall material; a narrow zone of nontextured primary wall material (left to right, center) overlies the bar. E, F: Views of pits from inside tracheids. E: Axially oriented coarse fibrils join the bar of secondary wall material of the pit (top). F: Detail to show the coarseness of the axially oriented fibrils (fold in the pit membrane is an artifact). Scale bars = 1 µm.

reticulate layer has been removed by the sectioning process. Note that the axially oriented fibrils tend to be attached to the edges of the pits. The rough surface of the borders (Fig. 2A; Fig. 2B, top) correlated with breakage of the fibrils away from the pit border because of sectioning. The axially oriented fibrils may be aggregated into compound strands (Fig. 2B). As viewed from the inner surface of the tracheid (Fig. 2C-F), the axial fibrils are more prominent where they attach to the pit borders. This apparent prominence is a result of grouping of the fibrils adjacent to the bars (Fig. 2D). Porosities may be infrequent or apparently absent in the reticulate layer of some walls (Fig. 2C and D); this may relate to the nature of the cell on the other side of the pit membrane. For example, walls of parenchyma cells are not porose, and thus the pits of a tracheid facing a parenchyma cell lack the relatively conspicuous porosities of a tracheid-to-tracheid interface. The reticulate layer of the pit membrane can, in places, be seen to form a three-dimensional spongiform structure when viewed at high magnification (Fig. 2F). This contrasts with the planar pit membranes characteristic of woody dicotyledons and in the roots of Nymphaeaceae.

#### 3.4. B. rotundifolia (Fig. 3A-F)

The outer surfaces of tracheids are shown in Fig. 3A–D. One can see that the reticulate layer of the primary wall is composed of coarse fibrils that are oriented randomly. Where more of the wall has been sectioned away (Fig. 3A, left), some of the axially oriented fibrils that face the lumen are evident. In the wall portion shown in Fig. 3A, a nonporose pit membrane evidently characterizes the primary wall of the adjacent cells (evident in the right two-thirds of micrograph). A highly porose three-dimensional spongiform network of coarse fibrils is evident in Fig. 3B and C. Pores are less conspicuous in Fig. 3D, perhaps because of the angle from which the pit membrane is viewed.

By contrast, as viewed from the lumen side, the tracheids pits of *B. rotundifolia* stems show prominent coarse axially oriented fibrils or aggregations of such fibrils (Fig. 3E and F).

#### 4. Discussion

The peculiar microstructures described are confined to end walls of stem tracheids only, and are not soluble in water or dilute or absolute ethanol; their distribution and appearance in *Barclaya* are entirely comparable to those in *Nuphar* (Carlquist et al., 2009), *Nymphaea* (Schneider et al., in press), and *Victoria* and *Euryale* (Carlquist and Schneider, 2009, in press) and the consistency of these structures within Nymphaeaceae, their peculiar nature, and their distribution only on end walls of tracheids rule out any possibility that the coarse fibrils are artifacts. Our concept that the coarse microfibrils represent secondary wall rather than primary wall structures would be in agreement with the retention of these structures in stored material.

The end wall of stem tracheids of *Barclaya* consists of two portions. A layer of randomly oriented coarse fibrils, termed the reticulate layer here, is overlain, on the lumen side, by axially oriented strands. The reticulate layer is thick and spongiform compared to primary walls reported for tracheids in other angiosperms. Walls of parenchyma cells in the stems of *Barclaya* do not show this texturing. Porosities that are mostly circular shaped and relatively consistent in size (circa  $0.1~\mu$ ) may be found in the walls of some tracheids. We believe that such porosities interconnect tracheids.

The axially oriented coarse fibril strands proximal to the spongiform layer tend to be spaced from each other by prominent gaps. These gaps may be related to the fact that at their ends where they touch pit borders, these strands are branched, so that the

central portions of the strands are fused together and thus compound. The axially oriented strands were least commonly observed in *B. longifolia*, and most densely present in *B. rotundifolia*. For the present, we prefer not to present a hypothesis for the functional significance of the distinctive stem-tracheid end-wall structure in *Barclaya*, just as we were reluctant to offer a hypothesis for the very similar microstructure of stem tracheid walls in *Nuphar* (Carlquist et al., 2009), *Nymphaea* including *Ondinea* (Schneider et al., in press), *Victoria* and *Euryale* (Carlquist and Schneider, 2009), and *Brasenia* (Carlquist and Schneider, in press).

The axially oriented strands in pit membranes of *Barclaya* stem tracheids may superficially resemble those in the perforation plates of vessels of wood of *Illicium* and other woody angiosperms rich in primitive features (Carlquist, 1992). Because both of these phenomena involve strands oriented parallel to the long axis of the tracheid, those less familiar with xylem features may not readily understand the differences. The structures seen in woody genera represent remnants of primary walls after lysis within secondary xylem, whereas the pit microstructures reported herein are accretion of secondary wall material in primary xylem.

In addition the pit microstructure in Nymphaeales are threedimensional, consisting of a two-layered coarse fibril system, contrasted to the thin, laminar, one layered architecture of woody angiosperms.

The distinctive microstructures of pits in stem tracheids of *Barclaya*, *Euryale*, *Victoria*, *Nuphar*, *Nymphaea*, and *Brasenia* can in no way be likened to distinctive pit membranes of tracheids in any other known group of living or fossil vascular plants (Carlquist et al., 2009). The microstructure patterns found in *Barclaya* represent a probable synapomorphy for the families Nymphaeaceae and Cabombaceae, but may not be present in Hydatellaceae (Carlquist and Schneider, in press) or other aquatic taxa we have studied to date (*Acorus*, *Alisma*, *Echinodorus*, *Nelumbo*). Studies on the physiological significance and ultrastructure (as defined by TEM study) are needed.

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