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ORIGINS AND NATURE OF VESSELS IN MONOCOTYLEDONS: 8. ORCHIDACEAE[1]

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Xylem of the orchids studied provided unusually favorable material to demonstrate how conductive tissue evolves in monocotyledons. In the end walls of tracheary elements of many Orchidaceae, remnants of pit membranes were observed with scanning electron microscopy and minimally destructive methods. The full range from tracheids to vessel elements, featuring many intermediate stages, was illustrated with SEM in hand sections of fixed roots, stems, and inflorescence axes of 13 species from four subfamilies. Pit membranes in end walls of tracheary elements are porose to reticulate in roots of all species, but nonporose in stems of Cypripedioideae and Vanilloideae and porose to reticulate in stems of Orchidoideae and Epidendroideae. The distribution pattern of pit membranes and pit membrane remnants in end walls of tracheary elements of orchids parallels the findings of others. The position of Cypripedioideae and Vanilloideae as outgroups to Orchidoideae and Epidendroideae, claimed by earlier authors, is supported by clades based on molecular studies and by our studies. Little hydrolysis of pit membranes in tracheary element end walls was observed in pseudobulbs or inflorescence axes of epidendroids. The pervasiveness of network-like pit membranes of various extents and patterns in end walls of tracheary elements in Orchidaceae calls into question the traditional definitions of tracheids and vessel elements, not merely in orchids, but in angiosperms at large. These two concepts, based on light microscope studies, are blurred in light of ultrastructural studies. More importantly, the intermediate expressions of pit membranes in tracheary element end walls of Orchidaceae and some other families of angiosperms are important as indicators of steps in evolution of conduction with respect to organs (more rapid flow in roots than in succulent storage structures) and habitat (less obstruction to flow correlated with a shift from terrestrial to epiphytic).

Key words: cell wall hydrolysis; Orchidaceae; pit membrane remnants; tracheids; vessel elements; xylem evolution.

Tracheids, with primary walls intact in all pits, have been thought to have given rise evolutionarily to vessel elements in which primary walls are absent in pits of the end walls (termed perforations). In monocotyledons, the products of this process can be seen even within particular species. According to Cheadle (1942), evolutionary specialization in tracheary elements begins in roots, then spreads upward to stems, inflorescence axes, and leaves. This sequence probably has a physiological significance in that roots of monocotyledons are adventitious and thus may be short-lived, so that more specialized end walls in root xylem are an adaptation to more rapid flow in roots (Carlquist, 1975). Orchids offer favorable material for demonstration of this sequence, because orchids have vessels with various degrees of specialization in roots, but only tracheids in stems and leaves of some genera (Cheadle, 1942). Study of this sequence by Cheadle, made on the basis of light microscopy, depends on the ability to detect primary walls in end walls of tracheary elements. Light microscopy cannot reveal the presence of delicate pit membranes or pit membrane remnants in end walls of tracheary elements that are intermediate in morphology between tracheids and vessels. Cheadle's conclusions about the presence of vessel elements or tracheids in monocotyledons were based not on the resolution of pit membranes, but on whether the architecture of the secondary wall of a tracheary element did or did not reveal a probable perforation plate. Scanning electron microscopy can reveal primary wall presence in end walls decisively, if preparation methods are suitable. In our earlier SEM studies on tracheary elements of monocotyledons, we selected families most likely to show what could be called earlier stages in the evolution of vessel elements; alternatively, the tracheary elements of these families could often be described as intermediate between tracheids and vessel elements, with reticulate vestiges of primary walls present in end walls. The families that we studied were Acoraceae (Carlquist and Schneider, 1997); Lowiaceae (Carlquist and Schneider, 1998a); Araceae (Carlquist and Schneider, 1998b; Schneider and Carlquist, 1998); Hanguanaceae (Schneider and Carlquist, 2005a); Haemodoraceae and Philydraceae (Schneider and Carlquist, 2005b); and Juncaginaceae and Scheuchzeriaceae (Schneider and Carlquist, 1997). These families show minimal differentiation between lateral walls and end walls of vessel elements in terms of the secondary wall skeleton. Pit membranes in end walls of vessel elements (so identified on the basis of light microscopy) contain porosities or even thread-like membranes rather than unbroken sheets of primary wall material. Relatively few porosities or holes were present in pit membranes of end walls of presumptive vessel elements in Acoraceae (Carlquist and Schneider, 1997). In end walls of many of the Araceae studied (Carlquist and Schneider, 1998b; Schneider and Carlquist, 1998), primary walls were absent in perforations of vessel elements of roots or present only as remnants. Our preparation methods clearly showed primary walls in pits of lateral walls of vessel elements of Araceae, as one might expect. Orchidaceae have been selected for study because in degree of primitiveness of xylem evolution (Cheadle, 1942), they fall into that small number of nonaquatic monocotyledon families that have tracheary elements with the range of tracheary element expressions just described for Acoraceae and Araceae.

Cheadle (1942) reported on 20 species of 15 genera of Orchidaceae. Although our sampling is much more limited that that of Cheadle (1942), we have attempted to sample Orchidaceae so as to represent the subfamilies Vanilloideae, Cypripedioideae, Orchidoideae, and Epidendroideae, based on

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TABLE 1. Orchid species and their characteristic habitats.

Species	Voucher/accession no.	Habitat
Vanilloideae		
Vanilla chamissonis Klotzch	SBBG 61065	epiphytic
V. fragrans (Salisb.) Ames	SBBG 61069	epiphytic
Cypripedioideae		
Paphiopedilum × *leeanum*	SBBG 66915	terrestrial
Phragmipedium besseae (Dodson & J. Kuhn) V.A. Albert & Börge Pettersson × *P. pearcei* Rchb. f.	SBBG 66801	terrestrial
Orchidoideae		
Spiranthes odorata Lindl.	SBBG 66881	terrestrial
Stenoglottis longifolia Hook. f.	SBBG 67354	terrestrial
Epidendroideae		
Cymbidium pumilum Rolfe cv. Summer Nights	SBBG 67849	terrestrial
Dendrobium nobile Lindl.	SBBG 76713	epiphytic
Epipactis gigantea Dougl. ex Hook.	SBBG 82916	terrestrial
Epidendrum radicans Pav. ex Lindl.	SBBG 84050	epiphytic
Odontoglossum grande Lindl.	SBBG 89759	epiphytic
Phalaenopsis amabilis Blume	SBBG 68592	epiphytic
Sobralia macrantha Lindl.	SBBG 95422	terrestrial

Note: The specimens of *Dendrobium*, *Epidendrum*, and *Sobralia* were provided by John Bleck; the remainder of the materials was purchased from Santa Barbara Orchid Estates. Herbarium specimens of flowering plants of the above species, prepared by the first author, have been deposited in the herbarium of Santa Barbara Botanic Garden (SBBG).

the molecular phylogeny of Freudenstein et al. (2004). Within Epidendroideae, we have attempted to study terrestrial and epiphytic species in about equal numbers. The subfamily Apostasioideae has been consistently hypothesized as an outgroup to the remainder of Orchidaceae (Judd et al., 1993; Freudenstein et al., 2004; Kocyan et al., 2004). We did make and examine preparations of xylem of *Apostasia* and *Neuwiedia*. We confirmed that these genera contain peculiar simple perforations embedded in end walls that otherwise bear alternate pits. This peculiar structure, probably an autapomorphy within Apostasioideae, led Cheadle and Tucker (1962, p. 165) to say that apostasioids "can not have been the origin of Orchidales: the vessels are too specialized." In addition, we deemed our material of apostasioid xylem not well enough preserved to yield good illustrations. Our sampling of the four other orchid subfamilies is obviously very small in comparison with the size of Orchidaceae as a family, but we believe that it represents a suitable preliminary framework for showing the ultrastructural nature of tracheary elements in the family.

We have introduced a new method for preparation of xylem of monocotyledons, a method free from oxidative solutions and therefore more likely to produce an accurate picture of tracheary elements in the living plant (Sano, 2005). We used a similar method in our study on Acoraceae (Carlquist and Schneider, 1997). Our earlier works (see Literature Cited) relied on macerations made with Jeffrey's solution. This method separates large numbers of xylem cells and is efficient for revealing the secondary wall architecture, but it may result in deterioration of primary wall ultrastructure. Some roots and stems of monocotyledons macerate readily with little primary wall damage, but others may require longer times for maceration (particularly if fibrous bundle sheaths are involved), with some consequent primary wall dissolution.

MATERIALS AND METHODS

Plants selected for study were living, cultivated specimens (Table 1). Only mature functional plant portions were taken for study. Roots and stem portions of all species were fixed in 50% aqueous ethanol. In epidendroids that showed differentiation between a horizontal rhizome and an upright stem or pseudobulb, both portions were sampled. Inflorescence axes were studied only for *Phalaenopsis amabilis*. Suitable fixed portions were sectioned with razor blades. Longitudinal sections were made for all specimens except for stems of *Vanilla chamissonis*. Transections of *V. chamissonis* stems were studied due to the large diameter of metaxylem tracheary elements and their sparseness. Sections of all materials were relatively thick (ca. 1–2 mm) so that the thickness of the section offered support, minimizing damage to delicate wall portions and showing more extensive portions of tracheary elements. Sections were air dried between clean glass slides. Macerations were prepared for roots of *V. fragrans* and *Odontoglossum grande* to observe end wall architecture. Preparations were mounted on aluminum stubs, sputter-coated with gold-palladium, and observed with a Hitachi (Tokyo, Japan) S2600N scanning electron microscope. Virtually all of our photographs contain some degree of tearing of pit membranes or pit membrane remnants in tracheary element end walls. At times, the pit membranes were so delicate that we observed real-time occurrence of damage during observation, regardless of beam current or accelerating voltage settings.

The term "porosities" to designate holes less than ca. 1 μm in diameter in pit membranes is used here to avoid confusion with the term "pore" as used in dendrology or in the study of sieve plates and to avoid confusion with "micropores" that may correspond to plasmodesmatal strands.

RESULTS

Vanilloids—Material of *Vanilla fragrans* roots (Figs. 1–4) had marked differences between the scalariform perforation plates of end walls (Figs. 1, 3, 4) and the mostly opposite pitting of the lateral walls (Fig. 2). Network-like pit membranes (Figs. 3, 4) were observed in end walls of tracheary elements of roots. In the transections of stem materials of *V. chamissonis*, end walls of the tracheary elements, which are notably wide, have a scalariform pattern (Fig. 5, above; Fig. 6, upper left). Lateral wall pitting is either transitional (Fig. 5, below) or opposite (Fig. 6). No porosities were observed in the scalariform end walls of *V. chamissonis* stem tracheary elements.

Cypripedioids—Roots of both *Phragmipedium* (Figs. 7, 8) and *Paphiopedilum* have reticulate to porose pit membrane remnants in end walls of tracheary elements (Figs. 7, 8). End wall architecture is not appreciably different from lateral wall pitting (Fig. 7) in secondary wall architecture. Both end and lateral walls show scalariform to transitional type of secondary wall patterns.

Stems of *Phragmipedium* have scalariform pitting on both end walls and lateral walls of tracheary elements (Figs. 9, 10). The pit membranes of tracheary element end walls in stems have extremely minute porosities (Fig. 10).

Orchidoids—In roots of *Stenoglottis longifolia* (Figs. 11, 12) and *Spiranthes odorata*, end walls of tracheary elements have a scalariform or scalariform-like pattern (Fig. 11). Pit membranes of these end walls are markedly porose or reticulate (Fig. 12). One interface between a tracheary element and a parenchyma cell in which portions of pit membranes were shaved away to various degrees was observed to have

porosities on the tracheary element side of these pits but nonporose pit membranes on the parenchyma cell side. Longisections of stems revealed tracheary elements with highly porose pit membranes in end walls. Because stems of *Stenoglottis* are highly condensed, we encountered difficulty in discriminating in all cases between tracheary tissue of the stems and that of the roots embedded in the stem.

Epidendroids—In *Cymbidium* roots, end walls (Fig. 13) are in marked contrast to lateral walls (Fig. 14). End walls feature reticulate pit membrane remnants in which the fine reticulate nonhydrolyzed portions are much greater in area than extensive hydrolyzed areas. Lateral walls of tracheary elements (Fig. 14) have no porosities. In rhizomes, end walls of tracheary elements have pit membranes with typically smaller hydrolyzed porosities relative to the intact areas; this applies to pseudobulbs of *Cymbidium* (Fig. 15) as well.

Epidendrum radicans has similarity between end walls of tracheary elements of the roots (Fig. 16) and those of the upright, cane-like stem (Fig. 17). In both, pit membrane remnants occur in the form of linear strands, oriented axially in the roots (Fig. 16), but randomly reticulate in the stems (Fig. 17). Occasional nonporose portions of the pit membrane are illustrated in Fig. 16.

Odontoglossum grande roots presented technical problems because of their slenderness. Razor blade sections could not provide reliable views of secondary wall architecture. Macerations of roots revealed vessel elements with scalariform end walls (Fig. 18). The absence of pit membrane remnants from the end walls may be a result of the oxidative nature of the maceration process. Pseudobulbs of *O. grande* have tracheary elements with relatively small, circular porosities in pit membranes of end walls (Fig. 19).

Phalaenopsis amabilis (Figs. 20–25) proved ideal material for demonstrating degrees of presence of pit membranes in end walls of tracheary elements. The contrast between the end walls (Fig. 20, left) and lateral walls (Fig. 20, right) in roots is clear. Pit membranes in the root end walls consist of reticulate strands of primary wall material spanning relatively large areas in which cell wall hydrolysis has occurred. In stems of *P. amabilis*, tracheary element end walls have pit membrane areas with numerous circular porosities (Figs. 22, 23). The nonporous membrane areas are approximately equal to the porosities in total area. A contrast between the end wall of a tracheary element (Fig. 23, bottom two-thirds) and a tracheary element/parenchyma interface (Fig. 23, top third) is evident in secondary wall architecture and in the condition of the primary walls. Tracheary elements of *P. amabilis* inflorescence axes (Figs. 24, 25) show similarity between end walls and lateral walls in terms of secondary wall architecture. The transition between end wall (Fig. 24, left) and lateral wall pitting (Fig. 24, right) is gradual rather than abrupt. Parts of the end wall (Fig. 25) retain pit membranes with their characteristic porosities despite the stress involved in the sectioning process.

The tracheary elements of *Dendrobium nobile* showed the same patterns described for *Cymbidium pumilum*, whereas tracheary elements of *Epipactis gigantea* and *Sobralia macrantha* closely resembled the ultrastructural details illustrated for *Epidendrum radicans*.

DISCUSSION

Tracheary element definition—Tracheary elements of Orchidaceae more frequently show conditions intermediate between tracheids and vessel elements than distinct conditions typical of these two cell types. One can then ask what the distinctions between these two cell types are. Understandably, the definitions are derived from light microscopy and therefore from secondary wall architecture, which is revealed much more clearly than pit membranes by light microscopy. For many angiosperms, such as those with simple perforation plates, the presumptive absence of pit membranes in perforations plates is confirmed at the ultrastructural level. However, in scalariform perforation plates of some dicotyledons (e.g., Carlquist, 1992) and some monocotyledons (Carlquist and Schneider, 1997,

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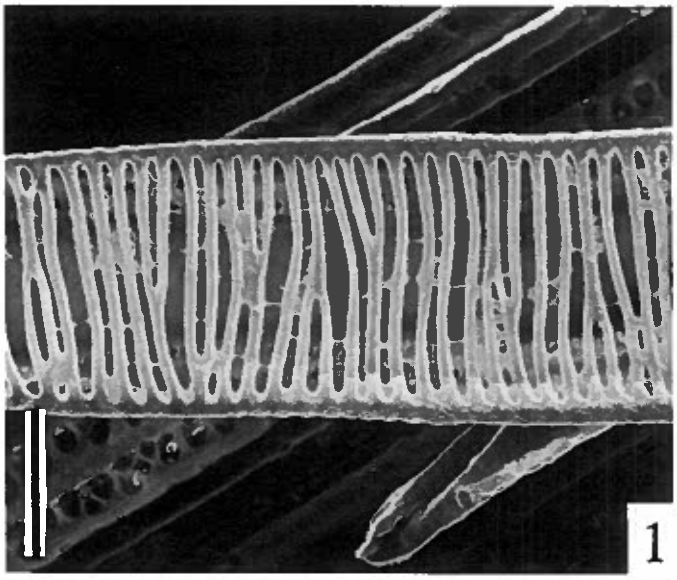
Figs. 1–6. Tracheary elements of *Vanilla*. 1–4. Portions of roots from *Vanilla fragrans*. **1.** Portion of vessel element from a maceration, showing a scalariform perforation plate. 2–4. Portions of razor-blade longisections. **2.** Lateral wall of vessel element, showing opposite to transitional or scalariform pitting. 3–4. Pit membrane remnants in end wall. **3.** Sparse reticulate remnants. **4.** Remnants form dense networks. 5–6. Imperforate tracheary elements from transections of a *Vanilla chamissonis* stem. **5.** Scalariform end wall (above) and transitional lateral wall pitting (below). **6.** Scalariform end wall and opposite lateral wall pitting. Fig. 1, bar = 25 μm; Figs. 2–6, bar = 5 μm.

Figs. 7–12. End walls from razor-blade longisections of tracheary elements of Cypripedioideae (Figs. 7–10) and Orchidoideae (Figs. 11–12). 7–t0. Sections from roots (Figs. 7–8) and stems (Figs. 9–10) of *Paphiopedilum* × *leeanum*. 7. Lateral wall (extreme left) and end wall. **8.** Reticulate and porose pit membranes in end wall. 9–10. Sections from stem. **9.** Both lateral and end walls have a scalariform pattern. **10.** End wall portion with minutely porose pit membranes. 11–12. End wall of tracheary elements from root of *Stenoglottis longifolia*. **11.** Pitting pattern is variously scalariform. **12.** Pit membranes are reticulate to porose. Figs. 7–9, 11–12, bar = 5 μm; Fig. 10, bar = 2 μm.

Figs. 13–19. End walls from maceration (Fig. 18) and razor-blade longisections (Fig. 13–17, 19) of xylem of Epidendroideae. 13–15. *Cymbidium pumilum* tracheary elements. **13.** End wall from root; pit membrane consists of a sparse reticule. **14.** Lateral wall; pit membranes are intact. **15.** End wall from base of rhizome–pseudobulb transition; pit membrane is intermediate between porose and reticulate. 16–17. *Epidendrum radicans* tracheary elements. **16.** End wall from root; pit membrane remnants are mostly linear. **17.** End wall from stem; pit membrane pattern is markedly reticulate. 18–19. *Odontoglossum grande* tracheary elements. **18.** Scalariform end wall from root. **19.** Minutely porose end wall from stem. Figs. 13, 15–18, bar = 2 μm; Figs. 14, 18, bar = 5 μm.

Figs. 20–25. Portions of razor-blade longisections of tracheary elements from *Phalaenopsis amabilis*. 20–21. Portions from root. **20.** End wall (left) damaged in sectioning, revealing lateral wall (right) behind it. **21.** Reticulate to porose end wall portion. 22–23. Portions from stem. **22.** Porose pit membranes. **23.** Porose to reticulate end wall portions plus (above) intact membranes from a tracheary element/parenchyma interface. 24–25. Tracheary element portions from an inflorescence axis. **24.** Transition region between lateral wall (right) and end wall (left); porosities are more abundant in the central portion of the end wall. **25.** Oblique view of sectioned end wall, showing porose membranes little disturbed by the sectioning process. Figs. 20, 21, 24, bar = 5 μm; Figs. 22, 23, 25, bar = 2 μm.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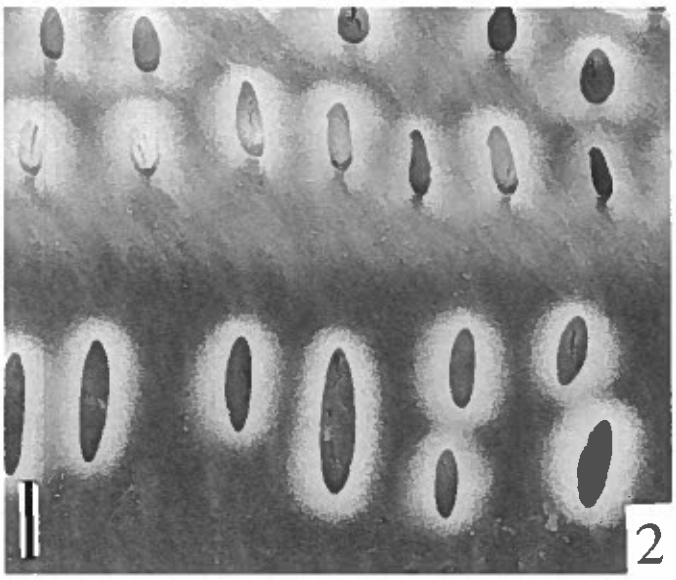
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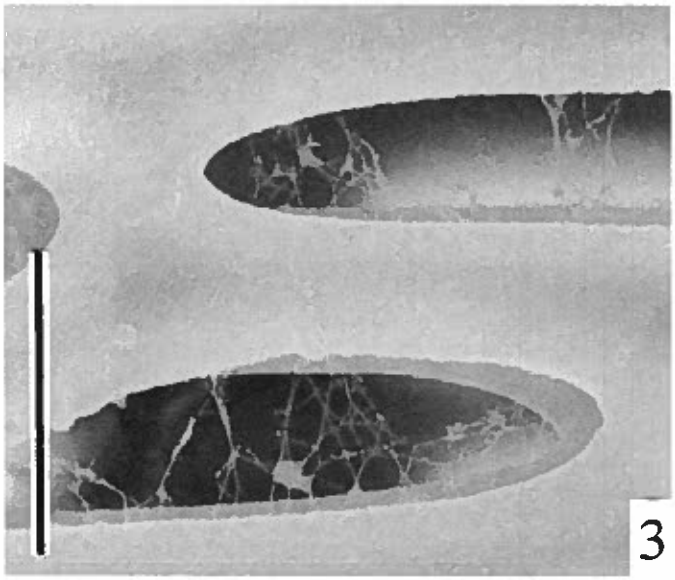
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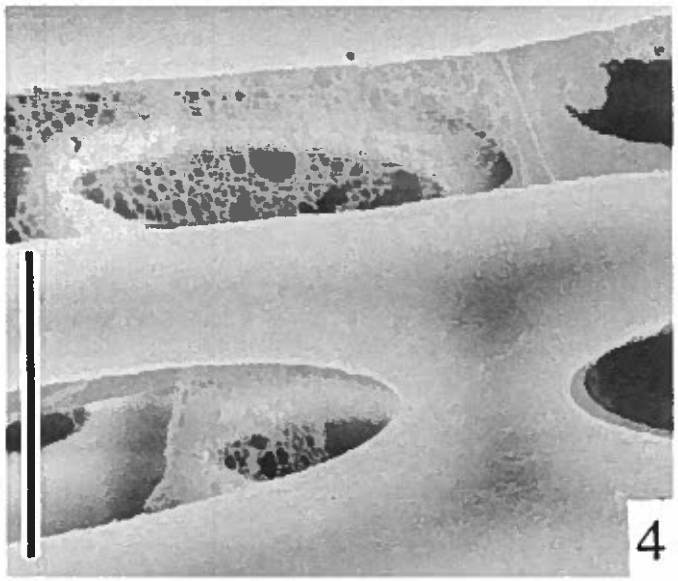
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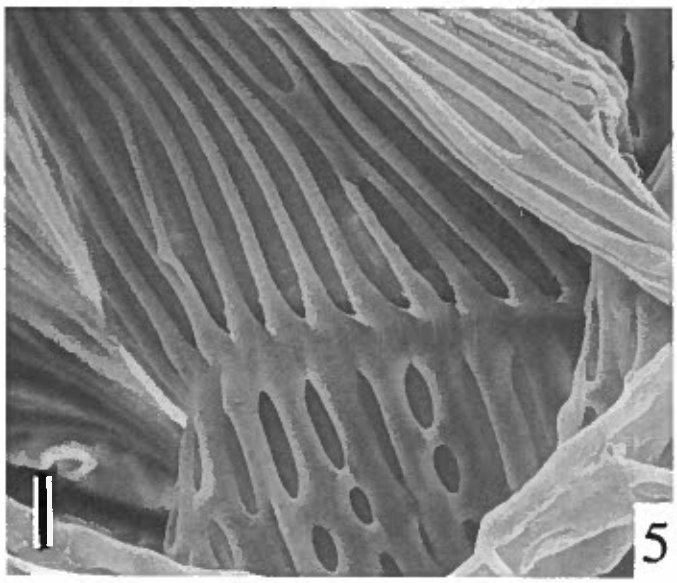
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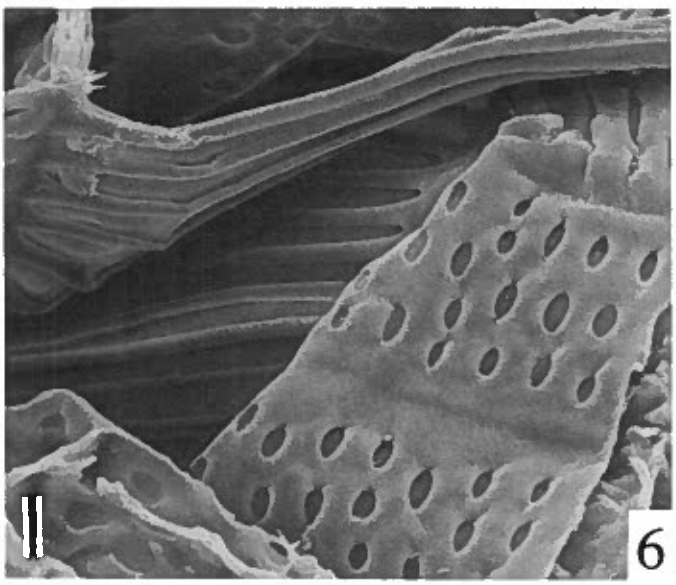
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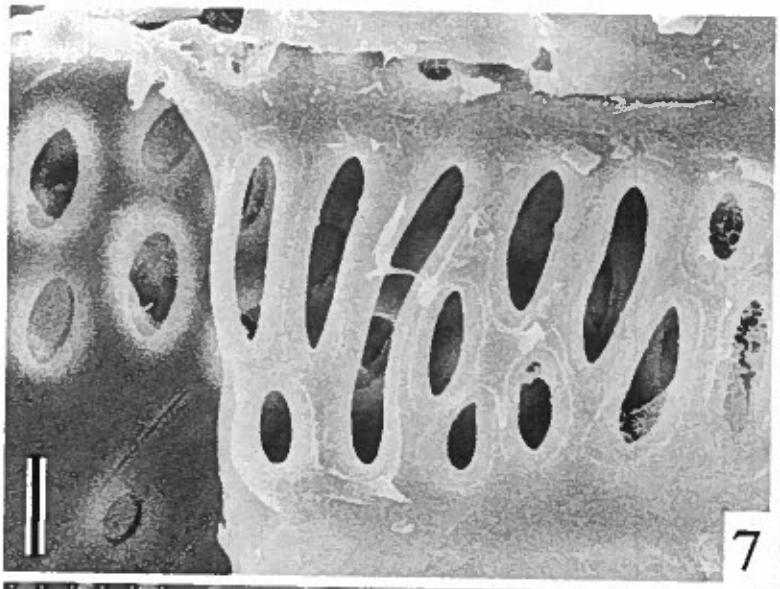
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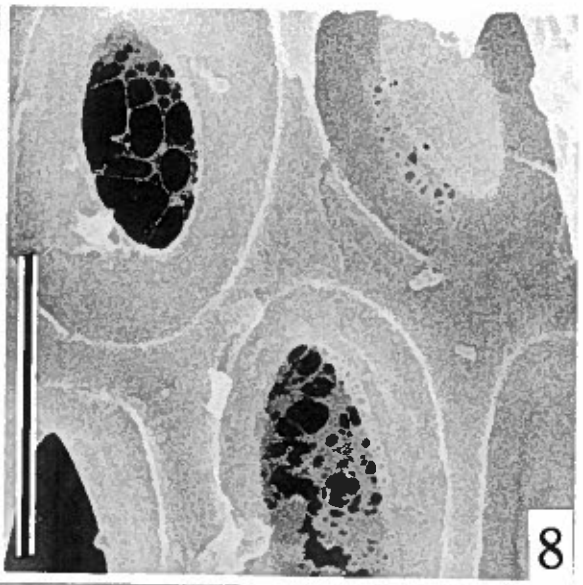
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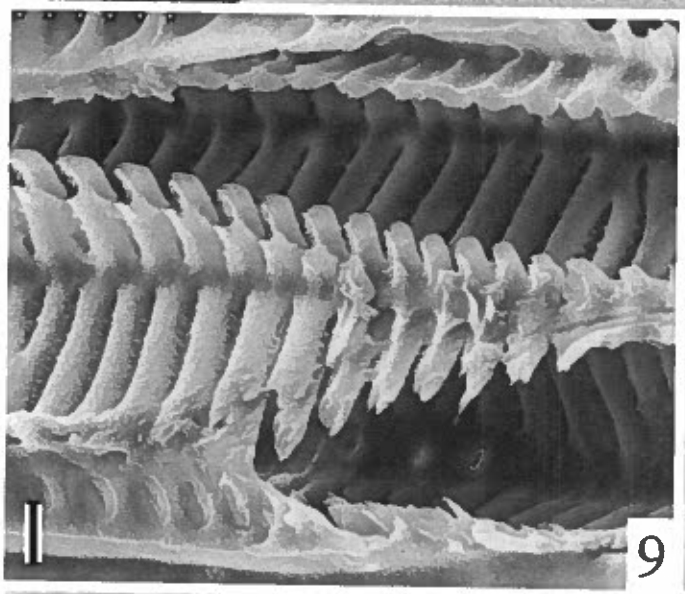
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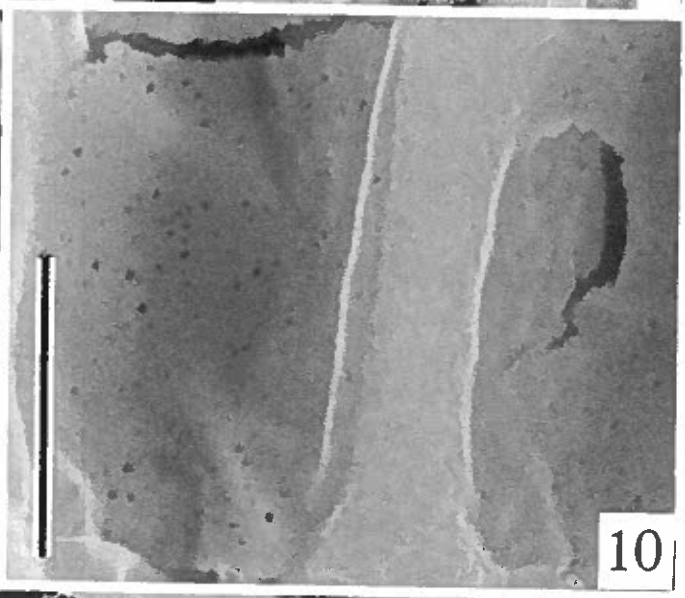
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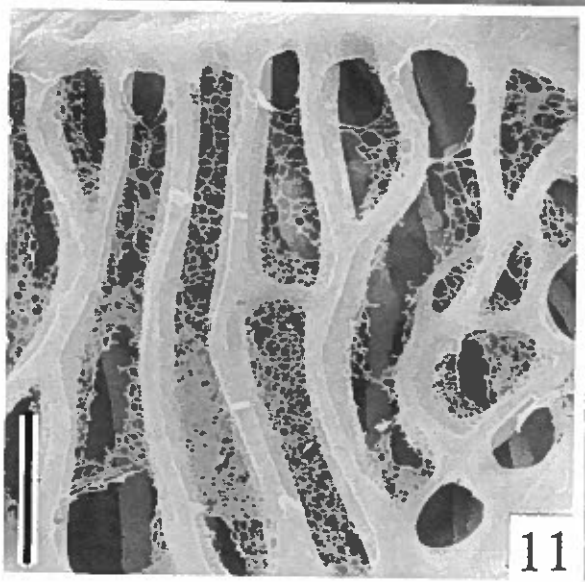
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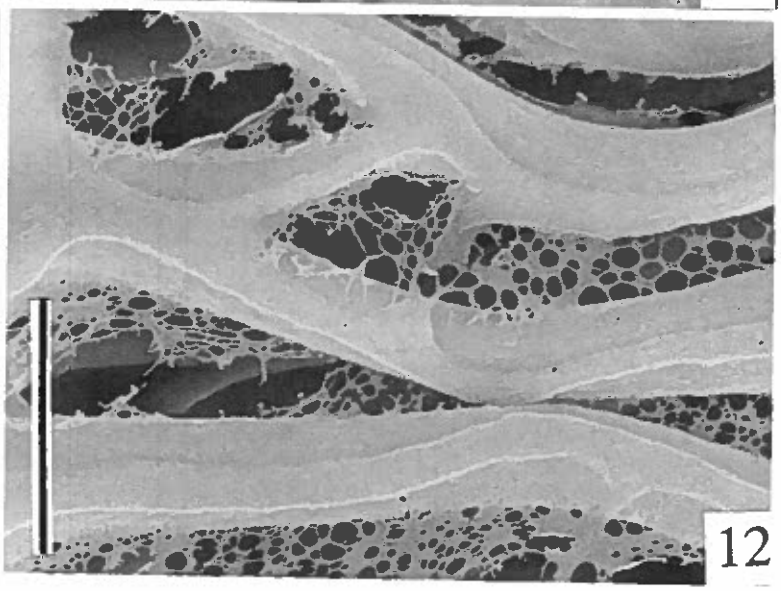
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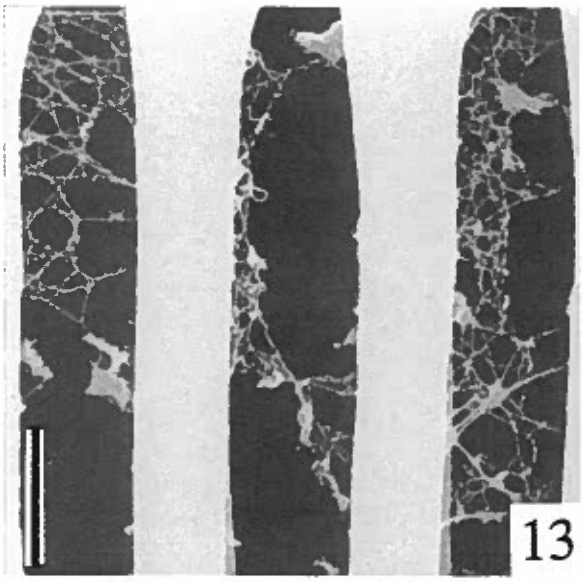
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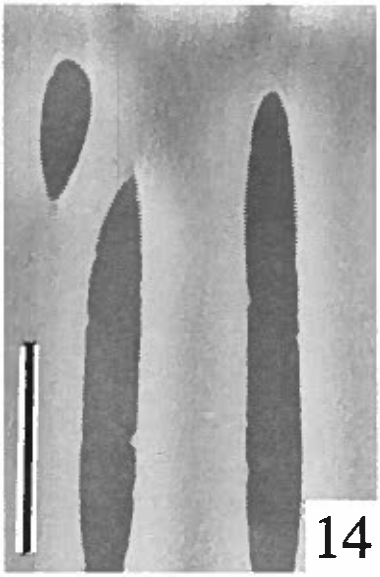
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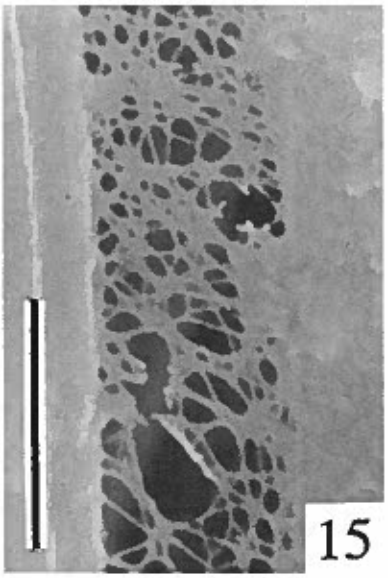
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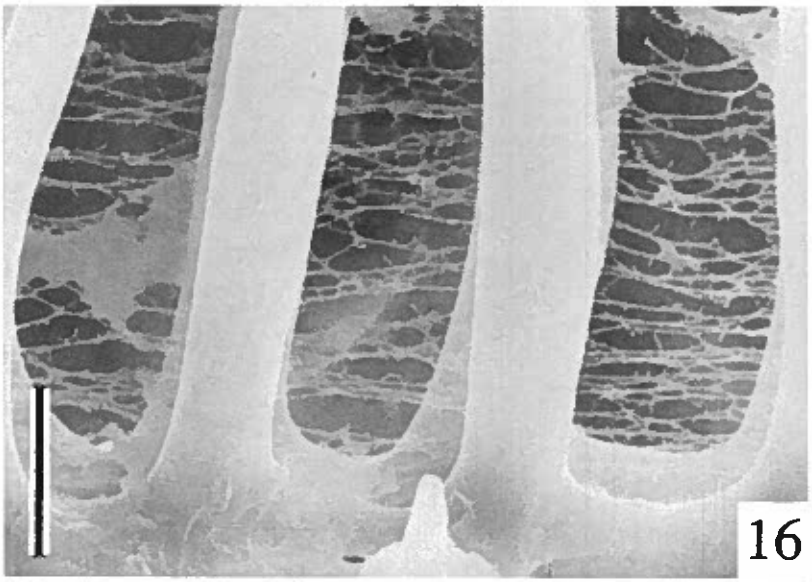
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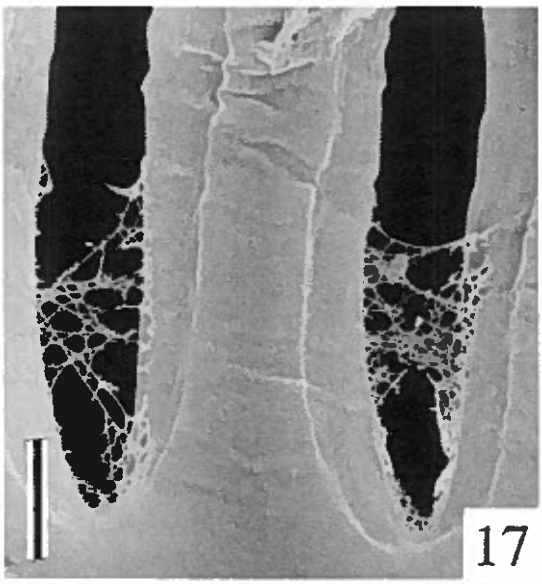
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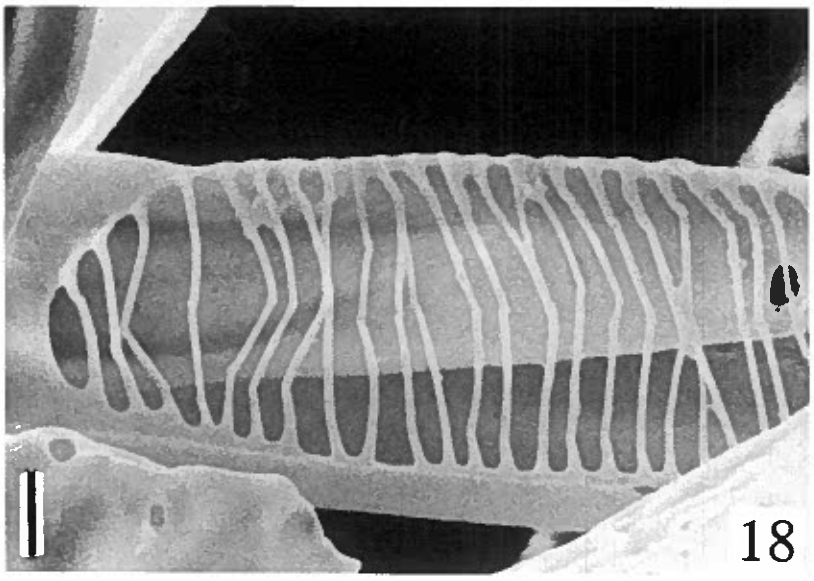
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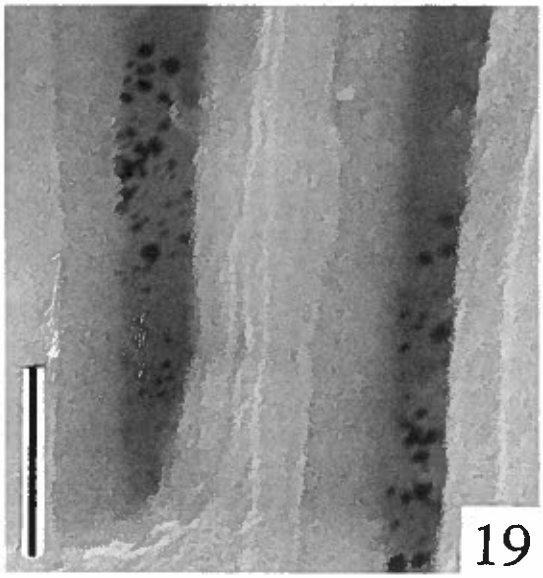
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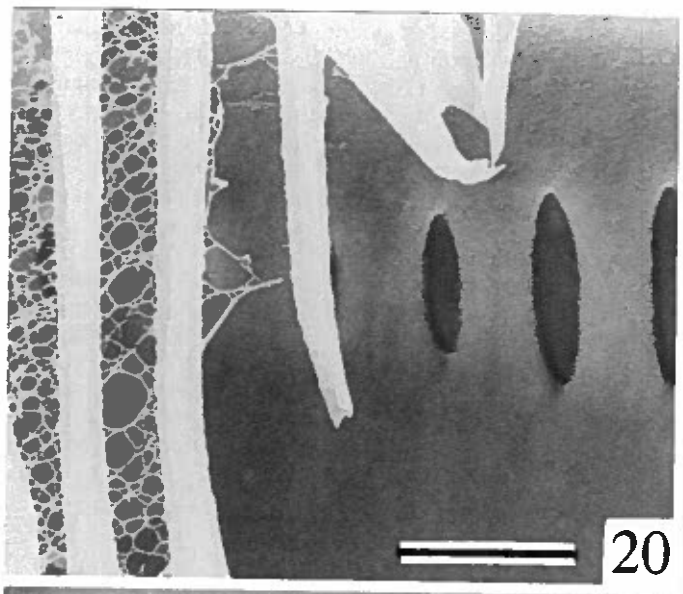
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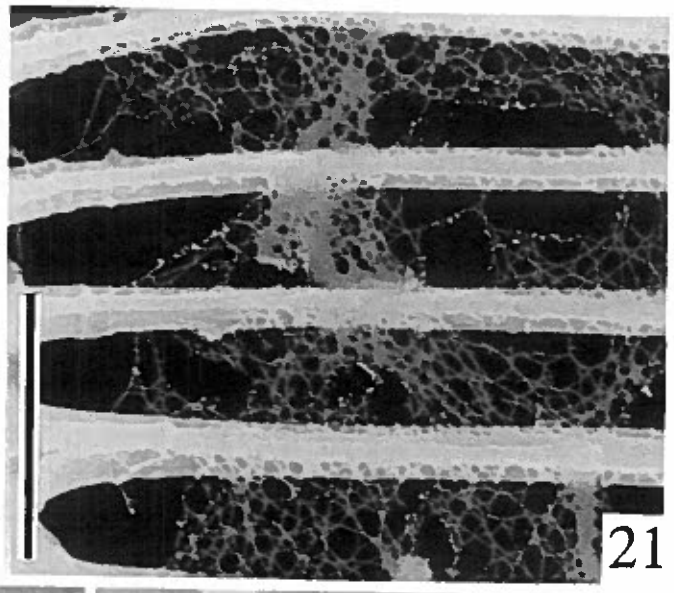
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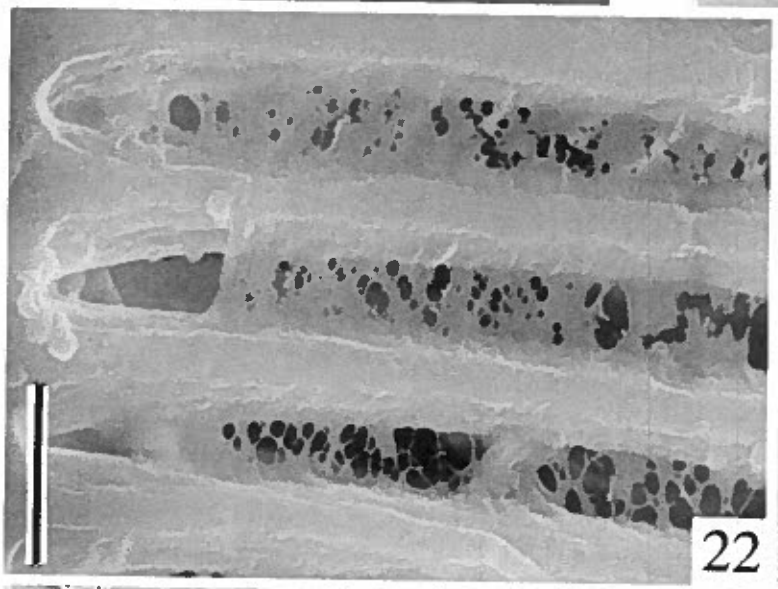
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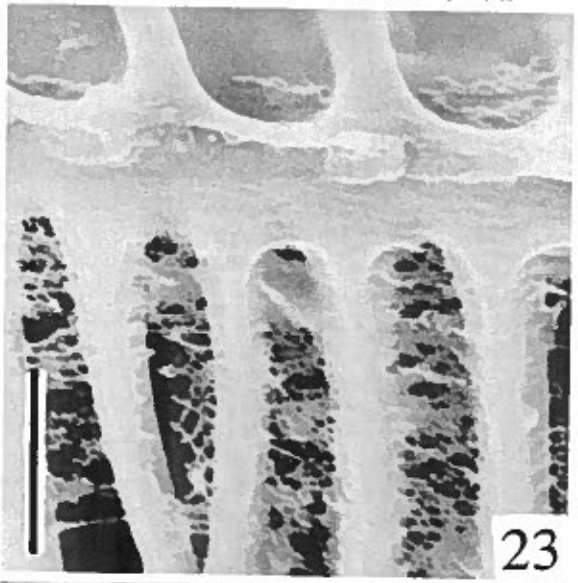
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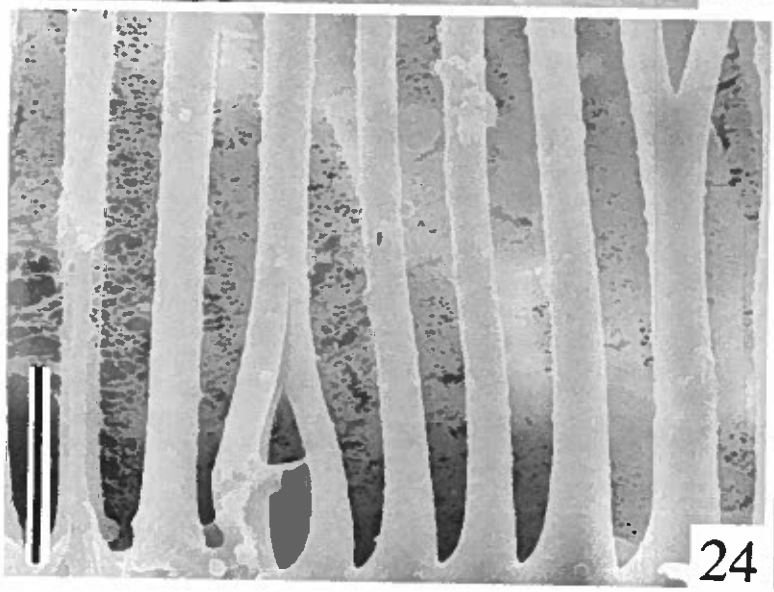
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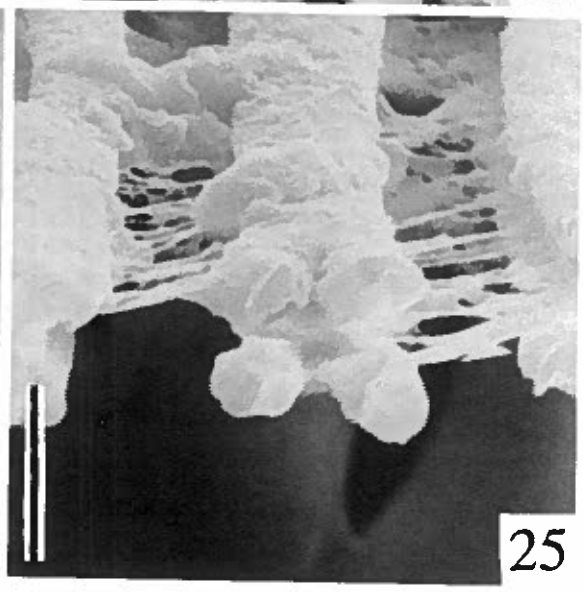
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1998a, b; Schneider and Carlquist, 1997, 1998, 2005a, b), various degrees of pit membrane presence blurs the distinction. A notable example is seen in *Vanilla chamissonis* stems. Tracheary elements in *V. chamissonis* are wide with a marked difference between the opposite lateral wall pitting and the scalariform end wall pitting; the latter would have been called perforation plates using light microscopy, However, our studies show intact pit membranes in the scalariform end walls. In fact, Cheadle (1942) did report vessel elements in stems of *Vanilla*. The inflorescence axis tracheary elements of *Phalaenopsis amabilis* also show very little difference between secondary wall architecture of end walls and lateral walls. There are only a few porosities in perforation plates. The roots have vessel elements in which extensive hydrolysis of pit membranes has occurred, and stem tracheary elements are intermediate. Thus, within a single plant, a continuum exists from what could be called vessel elements to what could be called tracheids. The terms "perforation" and "perforation plate" also are based on light microscopy and are dubiously applicable in *Phalaenopsis* and other orchids.

Cheadle (1942) was aware of the limitations of light microscopy and therefore employed tests with transmission of India ink particles (which are ca. 1 μm in diameter) in xylem as confirmations of distinctions based on secondary wall architecture. Today, latex spheroid samples with diameter specifications are now available and could be used to define perforation plates in terms of passage of spheroids of some size other than those of India ink particles. Such criteria might be of interest to those seeking some definition threshold, but would those definitions be relevant to the conductive physiology of a particular plant? Would forcible injection or would natural uptake be a criterion for transmission across perforation plates (the pit membranes of which might be damaged by forcible injection)? Even if passage of particles of a given diameter is accepted for the purposes of definition, how are intermediate tracheary elements to be described? Fahn (1953) used the term "vessel-tracheid" to describe this situation, but his term has not been adopted. The adoption of terms that involve ultrastructural examination or injection of particles before a decision about vessel presence or absence in a particular plant portion would seem impractical and not applicable to dried or even liquid-preserved material. Textbooks and references often refer to evolution of vessel elements from tracheary elements (and entertain the possibility of a reverse phylesis), but almost invariably illustrate clearly distinguishable examples of the two cell types rather than intermediates. Binary definitions of anatomical character states in plants may be appealing, but if misleading or erroneous, they lack merit. Elsewhere, we have attempted to show that there are multiple differences between the two cell types, any or all of which may show various degrees of intermediacy (Carlquist, 1992; Carlquist and Schneider, 2002).

Tracheary elements in relation to orchid phylogeny—Cheadle (1942) reported that vessels (with scalariform or rarely, simple perforation plates) were present in roots of all the orchids he studied, but that vessels were present in stems of only some of the orchids he sampled. We found markedly porose pit membrane remnants in tracheary elements of roots in Vanilloideae and Cypripedioideae, but stems in those subfamilies have pit membranes nonporose or nearly so. Leaving aside Apostasioideae (the xylem of which shows apomorphies), the Vanilloideae and Cypripedioideae together are outgroups to the remainder of Orchidaceae (Freudenstein et al., 2004; Kocyan et al., 2004). Cheadle (1942) hypothesized that vessels originated first in roots, then in stems, and that root xylem is thus more specialized than stem xylem in monocotyledons, which corresponds with our data as well as current phylogenetic hypotheses of Orchidaceae. Cheadle (1942) thought that vessels were "undoubtedly" present in stems of *Vanilla* because of the large diameter of tracheary elements and the presence of well-defined scalariform end walls. Our observations show that intact pit membranes occur in tracheary element end walls of *Vanilla*. Considering current molecular phylogenies, this would seem to indicate that vessel elements differ from tracheids by means of several characteristics, which may have evolved independently (Carlquist and Schneider, 2002). The presence of porose end walls in tracheary elements of stems of Epidendroideae and Orchidoideae could be a characteristic supporting the sister relationship of these two clades.

Possible correlations with habit and habitat—A case has been made, based on ecological preferences of the various monocotyledoid families, that types of vessels in those families and their organographic occurrence are related to ecology (Carlquist, 1975). In Alliaceae, for example, vessels with simple perforation plates occur in roots, whereas only tracheids occur in the remainder of the plant. This was interpreted as an adaptation for rapid uptake of water during brief periods of water availability. Orchid roots not so short-lived as those of Alliaceae suggest a correlation with moist habitats (Cypripedioideae) or the attenuation of moisture afforded by a velamen (characteristic of roots in Epidendroideae) or other structural conditions. The pseudobulbs of *Cymbidium* and *Odontoglossum* have tracheary elements with end walls less porose than those of *Epidendrum* and *Sobralia*. One interpretation is that the succulence of pseudobulbs minimizes the value of maximal porosities in end walls because succulent organs would tend to have less rapid conductive rates per unit time than nonsucculent ones (Carlquist, 1975). Succulence is common within Orchidaceae, especially in pseudobulbs and leaves of Epidendroideae. If succulence is related to less porousness of pit membranes in tracheary element end walls in Orchidaceae, then relatively nonporose pit membranes may be of some positive selective value in pseudobulbs. Restriction of embolisms to single xylem cells by having pit membranes that prevent spread of air bubbles is a conceivable functional explanation (Zimmermann, 1983). In this case, one might view pseudobulbs as the conductively "safe" structure that could survive periods when roots might die of desiccation. Once one attaches adaptive significance to vessel types and their organographic distribution in monocotyledons, the issue of directionality of evolution arises. Could relatively nonporose tracheary pit membranes evolve independently many times, or are nonporose pit membranes symplesiomorphic? To what degree is vessel evolution in monocotyledons reversible in a family like Orchidaceae, in which so many xylem cells are intermediate between tracheids and vessel elements?

Because of the sectioning technique used, we have been able to demonstrate delicate reticulate pit membrane remnants occur in end walls of tracheary elements. In sections that revealed lateral wall pits, we were able to observe nonporose pit membranes in one part of a pit and porose pit membranes in another part, whether it was a tracheary element to parenchyma contact or a contact between two tracheary elements. This

finding suggests porose pit membranes may occur on all surfaces of tracheary elements. A pit membrane consists of two adjacent primary walls, but the ultrastructural pattern of one may not match the ultrastructural pattern of the adjacent one—a finding not observed in the literature.

During early stages of perforation plate formation, pit membranes in end walls of tracheary elements appear much like those of the lateral walls, but lysis of pit membranes becomes more active or complete on the end walls. One hypothesis for this might be that fewer cellulosic strands are deposited in end walls than in lateral wall pit membranes. Removal of primary wall remnants by rapid flow between tracheary elements may also be involved. Studies using SEM and transmission electron microscopy are desirable to elucidate the nature of pit membranes in tracheary elements intermediate between tracheids and vessel elements. Orchids offer excellent material to show not only intermediacy in types of tracheary elements, but to demonstrate how the ultrastructural architecture of water conductive cells are adapted in various parts of plants and how such architecture is developed in evolutionary and developmental terms.

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