

INVITED SPECIAL PAPER

VESSELS IN FERNS: STRUCTURAL, ECOLOGICAL, AND
EVOLUTIONARY SIGNIFICANCE¹

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We have studied macerated xylem of ferns, supplemented by sections, by means of scanning electron microscopy (SEM) in a series of 20 papers, the results of which are summarized and interpreted here. Studies were based mostly on macerations, but also on some sections; these methods should be supplemented by other methods to confirm or modify the findings presented. Guidelines are cited for our interpretations of features of pit membranes. Fern xylem offers many distinctive features: (1) presence of numerous vessels and various numbers of tracheids in most species; (2) presence of vessels in both roots and rhizomes in virtually all species; (3) presence of specialized end walls in vessels of only a few species; (4) multiple end-wall perforation plates in numerous species; (5) lateral-wall perforation plates in numerous species; (6) porose pit membranes associated with perforation plates in all species; and (7) pit dimorphism, yielding wide membrane-free perforations alternating with extremely narrow pits. Multiple end wall perforation plates and lateral wall perforation plates are associated with the packing of tracheary elements in fascicles in ferns: facets of tips of elements contact numerous facets of adjacent elements; all such contacts are potential sites for conduction by means of perforations. This packing differs from that in primary xylem of dicotyledons and monocotyledons. Porosities in pit membranes represent a way of interconnecting vessel elements within a rhizome or root. In addition, these porosities can interconnect rhizome vessel elements with those of roots, a feature of importance because roots are adventitious in ferns as opposed to those of vascular plants with taproots. Fully-formed or incipient (small-to-medium sized porosities in pit membranes) perforation plates are widespread in ferns. These are believed to represent (1) ease of lysis of pit membranes via pectinase and cellulase; (2) numerous potential sites for perforation plate formation because of fasciculate packing of tracheary elements; (3) evolution of ferns over a long period of time, so that lysis pathways have had time to form; (4) lack of disadvantage in perforation plate presence, regardless of whether habitat moisture fluctuates markedly or little, because ferns likely have maintaining integrity of water columns that override the embolism-confining advantage of tracheids. Although all ferns share some common features, the diversity in xylem anatomy discovered thus far in ferns suggests that much remains to be learned.

Key words: ecological anatomy; ferns; incipient perforation plates; intermittent perforation plates; lateral wall perforation plates; multiple perforation plates; pit dimorphism; pit-membrane porosities; pit membrane remnants; vessel definitions; vessel evolution, xylem.

Vessels were first reported for the ferns *Nephrodium filix-mas* (L.) Schott and *Pteridium aquilinum* (L.) Kuhn by Rus-sow (1873); vessel presence in *Pteridium* was confirmed by Bliss (1939). Reports of vessels in ferns by Gwynne-Vaughan (1908) were discounted by Bancroft (1911). However, distinctive vessels were reported in *Marsilea* (White, 1961), a report confirmed by Loyal and Singh (1978). The possibility that vessels might occur in other ferns was hinted by White (1962). With light microscopy, White (1962) found end walls in tracheary elements of *Astrolepis*, *Phlebodium*, *Polystichum*, and *Woodsia* that were distinct and suggestive of perforation plates, but he could not demonstrate presence of perforations with his methods. In deciding to use SEM to determine presence and nature of perforations, we began with these genera. The usefulness of SEM in determining presence of pit membranes and pit membrane remnants has been demonstrated repeatedly in woody dicotyledons by Meylan and Butterfield (1978) and by Carlquist (1992, 1997). We have investigated vessels of monocotyledons with SEM and illustrated porose pit membranes and pit membrane remnants (see Schneider and Carlquist, 1998a, and Carlquist and Schneider, 1998a, and the papers cited therein). When our earlier studies on fern tracheary elements revealed that the distinctive end walls cited by

White (1962) were, in fact, perforation plates (whereas lateral walls of tracheary elements did show well-preserved pit membranes, we extended our studies to other genera and families. We soon concluded that vessels might be much more widespread in ferns than had been suspected, and we also found a series of structural features of interest in fern tracheary elements. The component papers in this series are listed below, so that the numerous citations of species and genera can be made without listing the relevant paper at each mention. The occurrence of vessel elements or incipient vessel elements in so many taxa might arouse skepticism in those who have not had the opportunity to view a large number of preparations such as ours. We have attempted to take into account any possibilities of artifact formation. We have, however, offered a discussion of preparation methods and interpretation below in order to clarify the bases for our reports.

Bierhorst (1960) presented detailed light microscope studies of tracheary elements of ferns. Not surprisingly, we have been able to see a number of details not figured by Bierhorst. We report, in a later section of this paper, some structures not related to perforation plates that represent new reports in our series.

In the process of surveying xylem in ferns, we searched for correlations between tracheary element structures and systematics or ecology. Although our sampling represents a small proportion of fern species, the correlations are clear: the var-

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ables in structure are few, and therefore relatively easy to interpret. We would like to offer data applicable to understanding of conductive physiology of ferns. However, very little is known about conductive physiology in ferns. We hope that our data on such features as presence of perforation plates on lateral walls of tracheary elements, presence of multiple end wall perforation plates, and highly porose or weblike pit membranes will be taken into account by those who pursue studies on fern physiology.

New data on fern tracheary elements invite comparison with data on xylem of other vascular plants. Such comparisons are complicated by the diversity of opinion concerning the delimitations of ferns and the relationships of ferns (Hasebe et al., 1995; Pryer, Smith, and Skog, 1995; Rothwell, 1996). We have included Psilotaceae in our studies because some authors place Psilotales in ferns (Hasebe et al., 1995), whereas others do not (Rothwell, 1996). Comparisons between a group with primary xylem only (living ferns) and groups with secondary growth (cycads, gnetophytes) offer further interpretive concerns. However, the similarities of tracheary elements among the fern families in our studies outweigh the differences among the families. Do these similarities reflect retention of ancient patterns of ferns (and other vascular plants, such as *Selaginella*: Schneider and Carlquist, in press a, b)? Is this relative uniformity a reflection of growth forms and conductive physiology, or is it a reflection of phylogenetic unity, or a combination of both factors?

Major questions such as these are opened by our SEM studies. We wish to stress that our results are based on a small sample. Also, our methods represent a limited range of techniques, and thus we emphasize the need for further studies, especially those involving TEM and histochemistry.

DATABASE PAPERS

Our papers in order of writing, cited by species and family or families are: *Woodsia obtusa* Spreng. ex Torr. (Dryopteridaceae or Woodsiaceae), [Carlquist, Schneider, and Yatskievych, 1997]; *Pteridium aquilinum* (L.) Kuhn (Pteridaceae) [Carlquist and Schneider, 1997a]; *Phlebodium aureum* (L.) Smith (Polypodiaceae) and *Polystichum acrostichoides* (Mich.) Schott (Aspidiaceae) [Schneider and Carlquist, 1997]; *Astrolepis sinuata* (Lag. ex Swartz) D. M. Benham & Windham (Pteridaceae or Cheilantheaceae) [Carlquist and Schneider, 1997b]; *Woodsia scopulina* D. C. Eaton (Dryopteridaceae or Woodsiaceae) [Schneider and Carlquist, 1998b]; *Woodsia ilvensis* (L.) R. Br. (Dryopteridaceae or Woodsiaceae) [Carlquist and Schneider, 1998b]; *Microgramma nitida* A. R. Smith (Polypodiaceae) [Schneider and Carlquist, 1998c]; *Platyzoma microphyllum* R. Br. (Pteridaceae or Platyzomataceae) [Carlquist, Schneider, and Kenneally, 1999]; *Dicranopteris linearis* (Burm. f.) Underw. (Gleicheniaceae) [Schneider and Carlquist, 1998d]; *Todea barbara* (L.) Moore (Osmundaceae) and *Anemia phyllitidis* (L.) Moore and *Lygodium flexuosum* (L.) Swartz (Schizaeaceae or Lygodiaceae) [Carlquist and Schneider,

1998c]; *Ophioglossum crotalophoroides* Walt., *O. pendulum* L., and *O. vulgatum* L. (Ophioglossaceae) [Schneider and Carlquist, 1999a]; *Angiopteris evecta* Hoffm., *Danaea elliptica* Sm., *D. wendlandii* Reichenb. f., *Macroglossum smithii* (Racib.) D. H. Campbell, and *Marattia fraxinea* Sm. (Marattiaceae) [Carlquist and Schneider, 1999]; *Nephrolepis exaltata* (L.) Schott (Davalliaceae) [Schneider and Carlquist, 1999b]; *Ceratopteris thalictroides* (L.) Brongn. (Parkeriaceae or Pteridaceae) [Carlquist and Schneider, 2000a]; *Asplenium nidus* L. (Aspleniaceae), *Elaphoglossum hirtum* (Sw.) C. Christens. (Elaphoglossaceae), and *Vittaria lineata* L. (Vittariaceae) [Schneider and Carlquist, 1999c]; *Alsophila australis* R. Br. (Cyatheaceae), *Cibotium splendens* (Gaud.) Kraj. ex Skottsb. and *Dicksonia antarctica* Labill. (Dicksoniaceae), and *Sadleria cyatheoides* Kaulf. (Blechnaceae) [Carlquist, and Schneider, 2000b]; *Psilotum nudum* (L.) Griseb. and *Tmesipteris obliqua* Chinnock (Psilotaceae) [Schneider and Carlquist, 2000a]; *Bommeria hispida* (Mett.) Underw., *Cheilanthes californica* Hook., *C. tomentosa* Link, and *Pellaea mucronata* (D. C. Eaton) D. C. Eaton (Pteridaceae or Cheilantheaceae) [Carlquist and Schneider, 2000c]; *Marsilea drummondii* Braun, *M. quadrifolia* L., and *M. vestita* Hook. & Grev. (Marsileaceae) [Schneider and Carlquist, 2000b]; *Callistopteris baldwinii* (Eaton) Copel., *Mecodium recurvum* (Gaud.) Copel., and *Vandenboschia davallioides* (Gaud.) Copel. (Hymenophyllaceae) [Carlquist, Schneider, and Lamoureux, 2000]. In listings of genera below, the above sequence is followed.

INTERPRETIVE CRITERIA

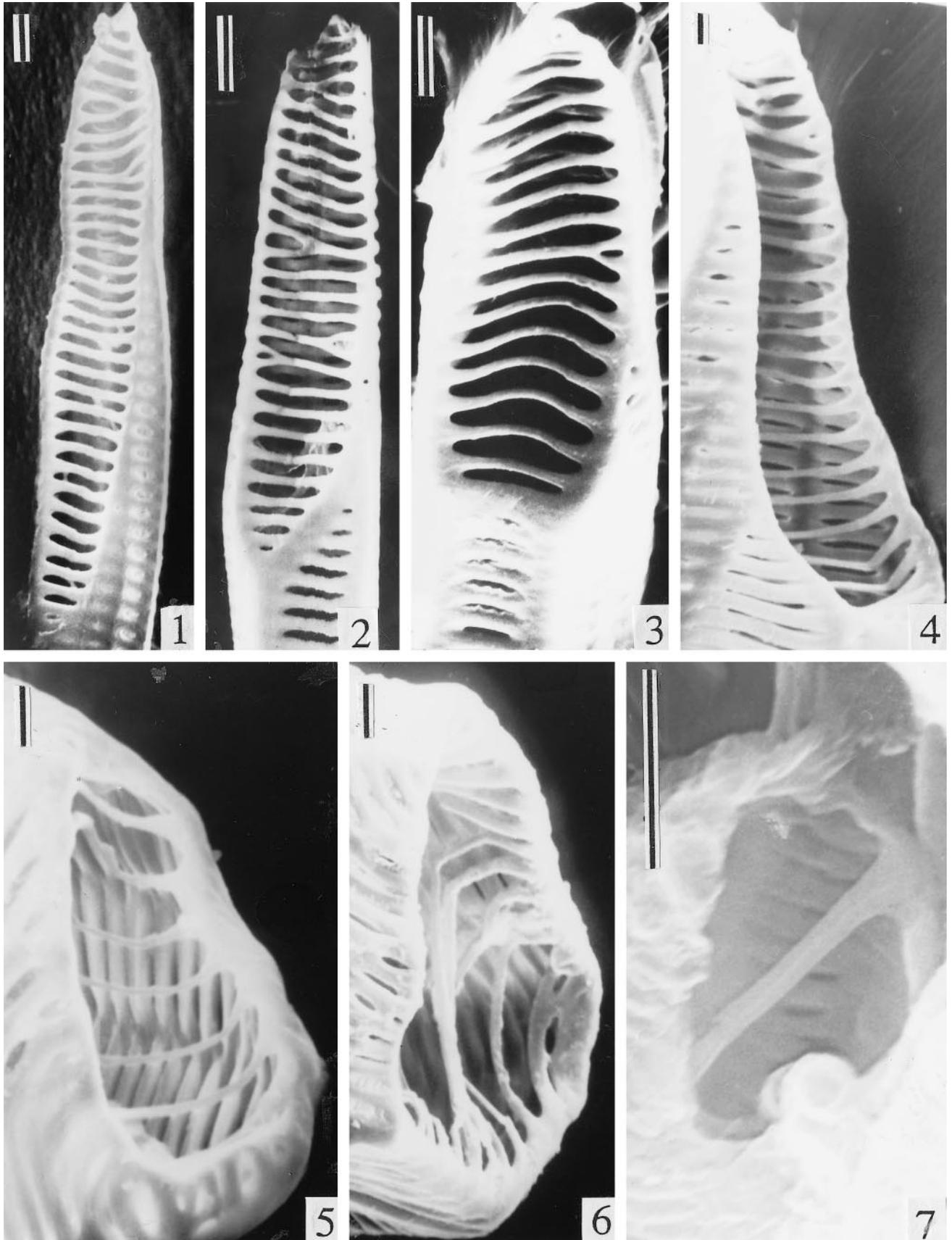
Our studies are based mostly on macerations, but in the first two papers of the series, sections were also employed. Our methods are summarized in those two papers (Carlquist, Schneider, and Yatskievych, 1997; Schneider and Carlquist, 1997a). Because pit membranes are sometimes thought to be very delicate with relation to reagents and preparation methods, we have assembled a series of reasons, with citations of earlier papers, that document why we believe our results are reliable.

1) As observed by a wide variety of methods, pores develop as a natural form of cell wall hydrolysis (achieved by pectinase and cellulase) in particular pit membranes of particular species (Butterfield and Meylan, 1982). The end product of this action can be seen as perforations in vessels of dicotyledons and margins of pit membranes in coniferous tracheids. The result of this lysis is a mesh of threads or, if the action of cellulase is sufficient, removal of the entire pit membrane.

2) Different species of gymnosperms and angiosperms characteristically have pores of particular sizes in pit membranes of tracheary elements; pores of these characteristic sizes are observed despite the use of a variety of different methods of preparation (Shane, McCully, and Canny, in press, Tables 2 and 3).

3) Although the data cited above are derived from gymnosperms and angiosperms, no worker has suggested that the

Figs. 1–7. Perforation plates of fern vessels with distinct end-wall perforation plates. Figs. 1–3. *Woodsia ilvensis*. 1. Perforation plate very similar in perforation dimensions to lateral-wall pits. 2. Perforation plate with perforations wider axially and horizontally than lateral-wall pits. 3. Perforation plate markedly different from lateral-wall pitting. Figs. 4–6. *Pteridium aquilinum*, perforation plates with relatively few bars. 4. Oblique perforation plate with slender bars, wide perforations. 5. Near-transverse plate with slender bars. 6. Transverse plate with bars that fuse. 7. *Marsilea quadrifolia*, perforation plate with one bar. Scales = 10 μ m in all figures.



same kinds of porose pit membranes observed in ferns and *Selaginella* (Schneider and Carlquist, in press a, b) are artifacts of reagents or preparation methods in ferns but not in the dicotyledons and monocotyledons where they have been reported (Carlquist, 1992, 1997; Carlquist and Schneider, 1998a; Meylan and Butterfield, 1978; Schneider and Carlquist, 1998a).

4) Where end wall perforation plates of fern tracheary elements are different from lateral walls (by virtue of different patterns in the underlying secondary wall framework), the end wall perforation plates lack pit membranes while the lateral walls possess them in *Woodsia* (e.g., Fig. 1–3, 17). If maceration techniques or drying techniques were responsible for removal of pit membranes in the end wall perforation plates of *Woodsia* spp., the pit membranes should be removed from lateral walls as well, and pit membranes should be removed in a random fashion in tracheary elements in ferns at large. Absence of pit membranes, except in instances we have been able to attribute to handling, is not at all random in pits (or perforations) of the fern tracheary elements we examined. The absence of random removal of pit membranes seems to us very strong evidence for believing that our methods have produced essentially accurate images.

5) Facets lacking pit membranes are adjacent to facets with intact pit membranes in many cases we have observed (Fig. 10). The marked difference in pit membrane conditions in facets adjacent to each other does not support interpretation of membrane absence as an artifact.

6) In the first two papers of our series (Carlquist, Schneider, and Yatskievych, 1997; Schneider and Carlquist, 1997) we used both macerated and sectioned materials, and obtained the same results with both techniques.

7) In vessel elements in which the size and morphology of the perforations are like the lateral wall pits, upper and lower ends of perforation plates (as well as incipient perforation plates) often bear porose pit membranes. The porose pit membranes indicate a transition between lateral wall pitting and perforations. In Fig. 16, porose pit membranes of such a transitional area are shown at left, adjacent to a facet with intact pit membranes at right. The distribution of porose pit membranes (in comparison to distribution of non-porose pit membranes) is not random in our preparations.

8) We generally have not illustrated torn or ripped pit membranes in our papers. However, torn pit membranes or areas of pit membranes were present in our preparations and were readily recognized as such. Torn membranes contrast clearly with the pores and holes in pit membranes because the pores have smooth outlines, whereas artifacts produced by tearing have irregular edges (Fig. 21).

9) Tracheids with intact pit membranes and a narrow shape (Fig. 15) coexist with vessel elements that are wider and have perforation plates on end walls in *Ceratopteris* (Fig. 14, 15) and *Alsophila* (Fig. 22). If preparation methods could artificially produce perforation plates, narrower tracheids covered

with unaltered pits as well as vessel elements (wider than the tracheids) with facets bearing intact pit membranes would not be expected in preparations such as ours.

10) Striate pit membranes (Figs. 16, 22) represent artifacts, in our opinion. We believe that the action of the SEM electron beam results in this appearance or at the least exaggerates it. We have considered all appearances related to striate nature of pit membranes as potential artifacts. Uneven thickness in pit membranes resulting in weblike appearances, however, is interpreted as natural.

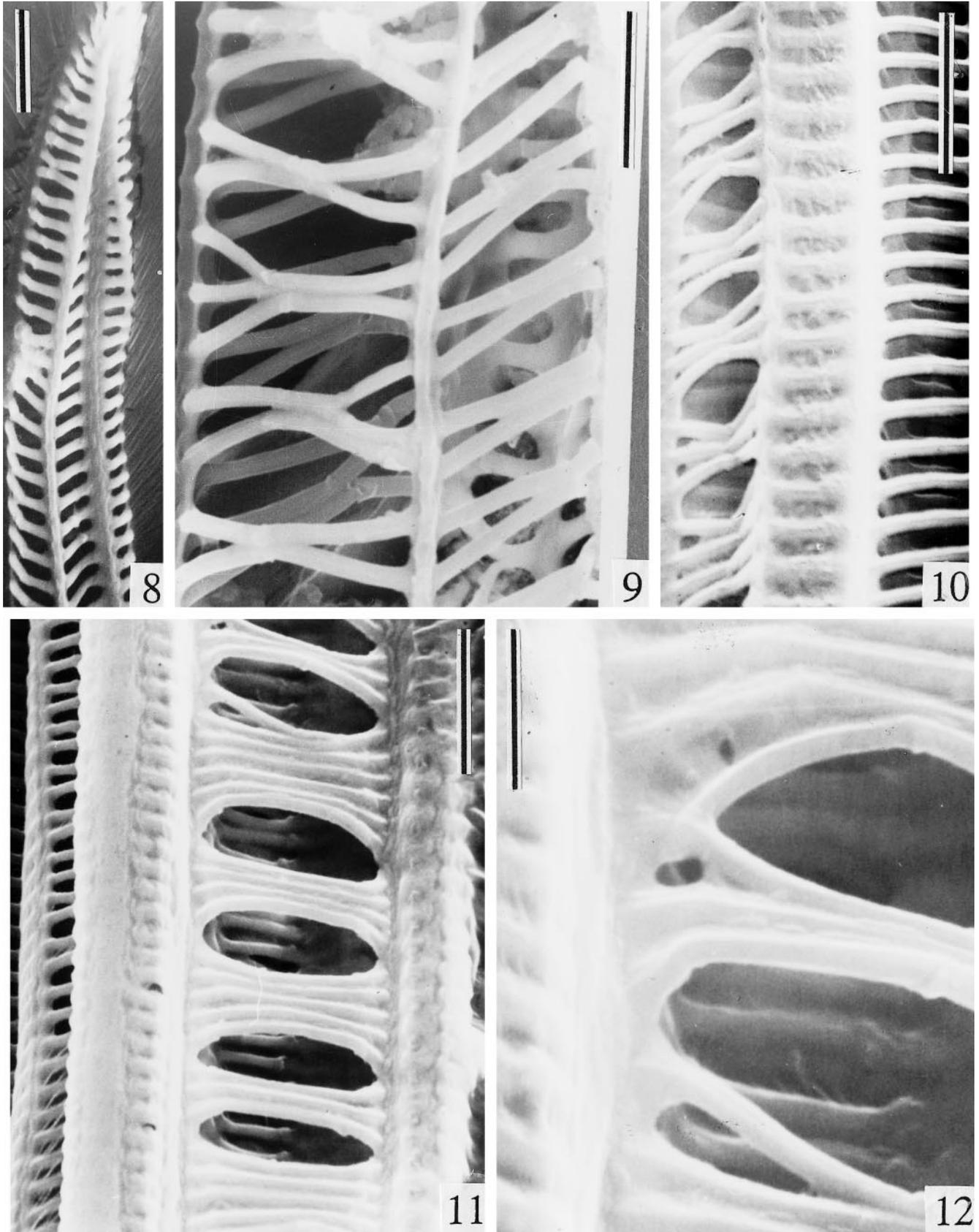
11) We believe that air drying of cells does not produce the porosities or weblike appearances of pit membranes. When a wide range of methods is used to demonstrate presence or absence of pores (including introduction of particles of known diameters into the conductive stream), porosity size is not related to preparation method, but rather characterizes particular species (Shane, McCully, and Canny, in press, Tables 2 and 3). More significantly, students of xylem have repeatedly reported cavitation of xylem cells in the living plant. Ferns have not been studied in this respect, but can be assumed to experience replacement of water by air in tracheary elements under stress conditions but inevitably by age also. Therefore, pit membranes of air-filled tracheary elements have been subjected to air drying *in the living plant*. To rehydrate such elements in a pickling solution and then apply critical point drying to such cells would be superfluous and illogical. If porose pit membranes were the result of air drying, the extensive areas of intact nonporose membranes on facets adjacent to membranes with porose membranes would be inexplicable.

12) Our results are entirely compatible with results obtained thus far with other methods by other workers. For example, Morrow and Dute (1998, 1999) did not find pores in pit membranes in *Botrychium* using transmission electron microscopy, nor did we with SEM (unpublished data).

13) We have avoided use of stem or root portions that could contain meristematic or differentiating cells. Therefore, the presence or absence of pit membranes cannot be attributed, in our preparations, to different degrees of maturation of cells.

14) We did not attempt to control for age of tracheary elements (as indicated by distance from an apical meristem, for example), although we used liquid-preserved portions of living plants. Therefore, in any given preparation, some of the tracheary elements might have been inactive ones while others were actively conducting elements. We did not observe differences among tracheary elements, within a preparation. While conceivably bacterial or fungal action might remove pit membranes from inactive tracheary elements, this is unlikely to account for the patterns we observed, and no evidence for such deterioration of fern tracheary elements has been presented in studies we know. Duerden (1934) claimed vessels with simple perforation plates (but with pit membranes on lateral walls) in homophyllous species of *Selaginella*. Duerden's (1934) studies have never been questioned, despite the fact

Figs. 8–12. Perforations that are not arranged in a single scalariform perforation plate per end wall. Figs. 8–9. *Microgramma nitida*. 8. All facets at element tip are perforation plates. 9. Facets that are perforation plates, showing pit dimorphism (alternating wide and narrow perforations). 10. *Vandenboschia davalloides*. Facets, from left to right, that illustrate perforations formed in relation to pit dimorphism, a lateral wall of intact pit membranes, and a portion of a scalariform perforation plate. Figs. 11–12. *Bommeria hispida*. Facets that show aspects of pit dimorphism. 11. Five perforations alternating with groups of very narrow pits; other facets in the photograph show intact lateral-wall pitting. 12. Portion of facet with pit dimorphism; membranes of narrow pits, upper left, are porose. Scales = 10 μ m in Figs. 8–11; scale = 5 μ m in Fig. 12.



that they were based on herbarium species and the preparation method involved only maceration techniques.

CHARACTERISTICS OF FERN TRACHEARY ELEMENTS

Vessel elements with distinctive perforation plates—The perforation plates of Figs. 1–7 are arranged from those that show progressively greater degrees of simplification of the perforation plates. In all of these, however, the perforation plates are end walls that lack pit membranes, whereas the lateral wall pits contain pit membranes. The first three in this sequence (Figs. 1–3), from *Woodsia ilvensis*, show long perforation plates with perforations little if at all larger than lateral wall pits in the first (Fig. 1), but in Fig. 2, we see perforation size detectably different from lateral wall pit size. In Fig. 3, the perforations are markedly larger than the lateral wall pits. A continuation of this series is shown in Figs. 4–6, *Pteridium aquilinum*. The bars of the perforation plates in Fig. 4 are slender and probably lack borders; the plate is oblique. More nearly transverse plates are represented in Figs. 5 and 6. The perforation plate of Fig. 5 has few and slender bars; that of Fig. 6 has bars that fuse as they traverse the plate. A single bar across a perforation plate is shown for *Marsilea* in Fig. 7. The ultimate reduction would be represented by a simple perforation and some simple perforation plates are reported for *Marsilea* by White (1961, 1962).

A moderate degree of differentiation between perforation plates and lateral wall pitting was also observed in *Astrolepis sinuata*, *Polystichum acrostichoides* and *Woodsia scopulina*. Ferns not mentioned in this and the preceding paragraph were not observed to have secondary wall frameworks for perforation plates that differed from those of the lateral wall pitting.

Multiple end-wall perforation plates—Reduction in number of bars in perforation plates, described in the preceding section, has been considered an indicator of phylogenetic specialization in angiosperms. Most ferns, however, do not exemplify this trend and have vessel elements with end walls that resemble lateral walls (Fig. 13). However, multiple end-wall perforation plates, a feature rarely reported in other vascular plants, were observable in a number of fern tracheary elements.

Multiple perforation plates per vessel element tip are illustrated here by *Microgramma* (Figs. 8, 9). In the element tip of Fig. 8, the three facets shown lack pit membranes. The vessel element portion shown in Fig. 9 illustrates absence of pit membranes in all facets, both those of the facing facets and those on the back facets. We have figured the occurrence of several perforation plate facets per tracheary element tip in *Polystichum acrostichoides*, *Woodsia scopulina*, *Platyzoma microphyllum*, *Dicranopteris linearis*, *Todea barbara*, *Ophioglossum pendulum*, *O. vulgatum*, *Danaea wendlandii*, *Macroglossum smithii*, *Marattia fraxinea*, *Nephrolepis exal-*

tata, *Elaphoglossum hirtum*, *Sadleria splendens*, *Alsophila australis*, *Dicksonia antarctica*, *Cheilanthes californica*, *Marsilea* spp., *Callistopteris baldwinii*, and *Vandenboschia davallioides*. However, multiple perforation plates are probably more widespread in ferns than our reports presently indicate. The occurrence of multiple end-wall perforation plates on tracheary elements can be correlated with the arrangement (packing) of tracheary elements in ferns, as discussed later.

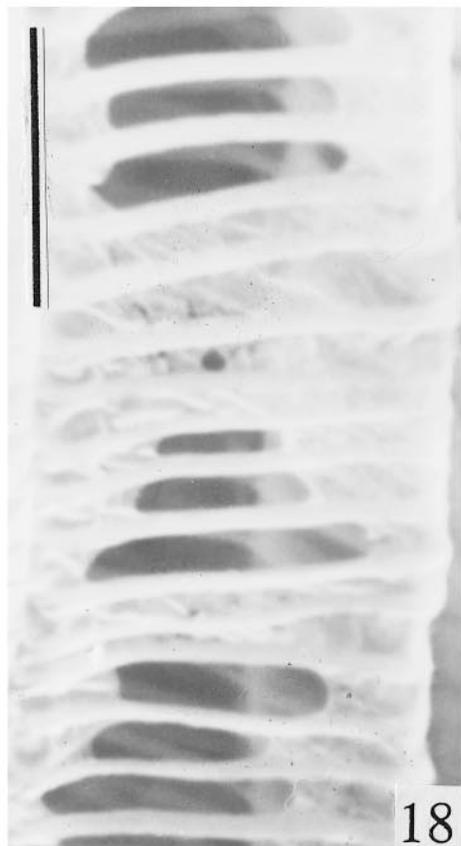
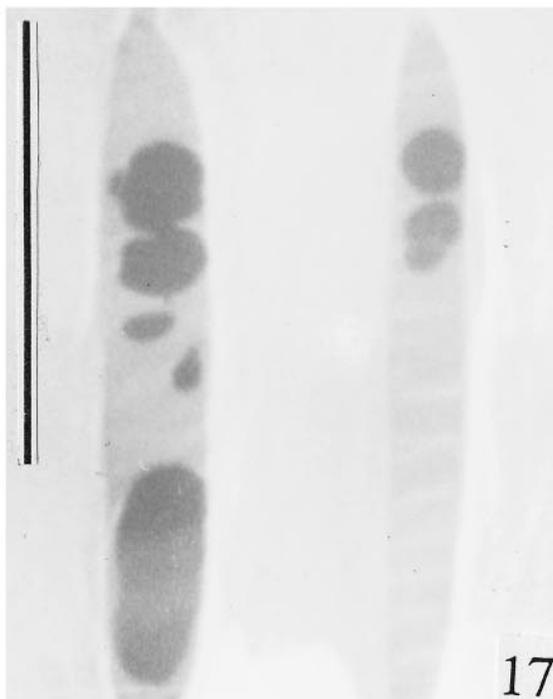
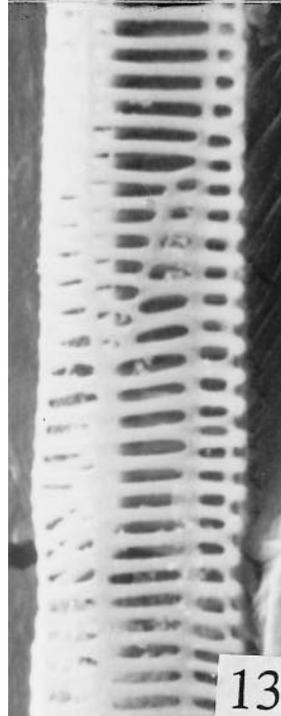
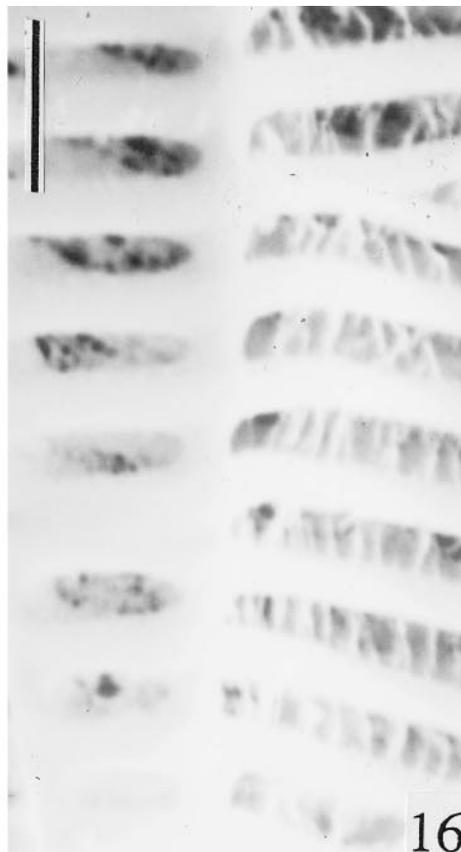
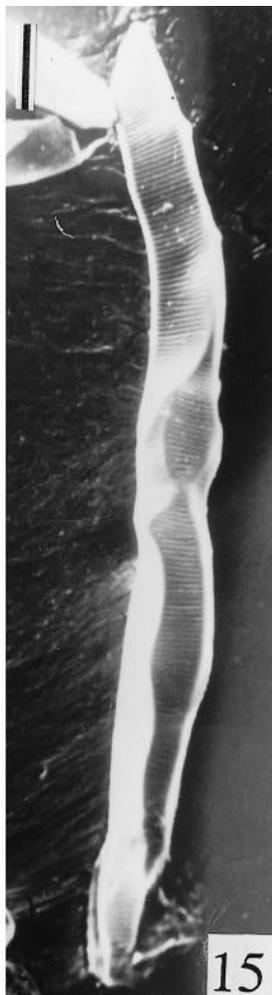
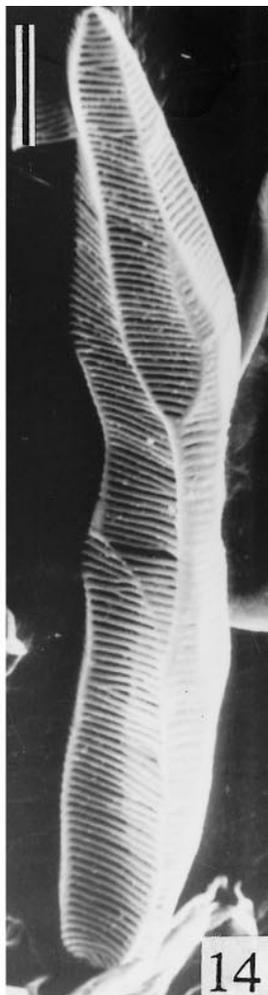
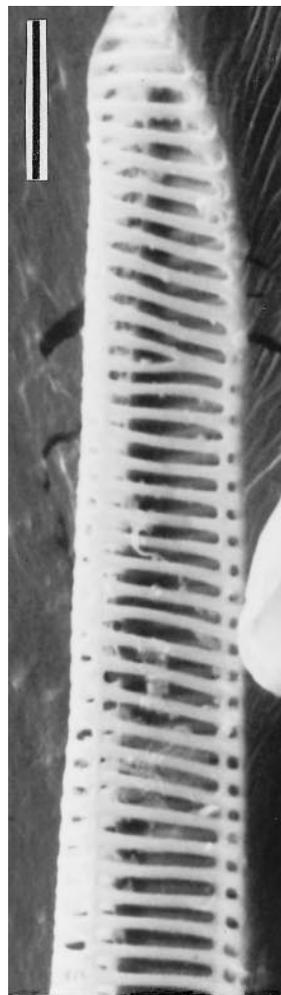
Pit dimorphism—Pit dimorphism that results in the formation of wide perforations alternating with one or more narrow intervening pits is illustrated here for *Microgramma nitida* (Fig. 9), *Vandenboschia davallioides* (Fig. 10), and *Bommeria hispida* (Figs. 11, 12). Similar pit dimorphism resulting in formation of perforations interspersed with pits on a wall was figured by us in *Phlebodium aureum*, *Polystichum acrostichoides*, *Platyzoma microphyllum*, *Dicranopteris linearis*, *Anemia phyllitidis*, *Ophioglossum pendulum*, *Danaea elliptica*, *D. wendlandii*, *Psilotum nudum*, and *Cheilanthes californica*. Wide pits (possibly perforations) alternating with narrow pits were figured by Bierhorst (1960) for *Angiopteris evecta* Hoffm., *Asplenium viride* Huds., and *Osmunda cinnamomea* L. Bierhorst's work was based on light microscopy, so he could not have readily observed absence of pit membranes in the wide pits, and thus his lack of comment on this phenomenon is understandable.

Lateral-wall perforation plates—In macerations of some ferns, lateral walls are not readily distinguished from end walls. This is not true in *Pteridium*, in which lateral walls are easily distinguished from the end wall perforation plates. In our *Pteridium* study (Carlquist and Schneider, 1997, Figs. 11–13), we showed areas of lateral wall pits in which pores are so large that one should likely say that the pits have become converted into perforations, and therefore, lateral-wall perforation plates are present. These lateral wall perforation plates are readily distinguished from the end-wall perforation plates of *Pteridium*, which have fewer, narrower bars intervening between wide perforations, and no pit membrane remnants in the perforations. In the present paper, lateral-wall perforation plates are illustrated for *Woodsia obtusa* (Fig. 7), in which porose pit membranes are present, and *Ceratopteris thalictroides* (Fig. 18), in which relatively large holes are present.

Intermittent perforation plates—The lateral-wall perforation plate figured for *Ceratopteris thalictroides* (Fig. 18) is subdivided by intact pits, and is thus an intermittent perforation plate. We observed such intermittent perforation plates in other ferns as well. By definition, pit dimorphism in which the wide pits are perforations results in intermittent perforation plates.

At left in Fig. 16 is a lateral-wall facet with porose pit membranes; this facet therefore constitutes an incipient lateral wall perforation plate.

Figs. 13–18. Tracheary elements of *Ceratopteris thalictroides* (Figs. 13–16, 18) and *Woodsia obtusa* (Fig. 17). **13.** End-wall perforation plate that extends about two-thirds of the length of the photograph, showing similarity between perforation plate and lateral-wall pitting. **14.** Typical short vessel element of *C. thalictroides*, with a distinct end-wall perforation plate (above). **15.** Slender tracheary element on which no perforations are visible, and which therefore is interpreted as a tracheid. **16.** Adjacent facets from a single tracheary element; facet at left contains pits with porose membranes, whereas facet at right contains pits with nonporose membranes. **17.** Portion of lateral-wall perforation plate, to show pores of various sizes in the pit membranes. **18.** Lateral-wall perforation plate in which perforations take the form of elongated ellipses; this perforation plate is intermittent, with intact pits between groups of perforations. Scales = 10 μ m in Figs. 13–15 scales = 5 μ m in Figs. 16–18.



We could identify perforations as present on lateral walls in some tracheary elements. However, in our preparations, wall facets are very long and few elements are isolated well enough and intact enough to designate all facets either as end walls or as lateral walls. Thus, certain identification of lateral wall perforation plates in all cases was not possible.

Vessel elements and tracheids—In *Ceratopteris thalictroides*, the shortness of tracheary elements and the success of macerations permitted us to see isolated tracheary elements well. Some of these elements were wider and shorter and had perforation plates on end walls and thus were clearly vessel elements (Fig. 14). Other tracheary elements in this species were narrower, and end walls were revealed with sufficient clarity, so that we could reasonably identify them as tracheids (Fig. 15). In other ferns, we were rarely able to make this distinction. Because the SEM method can only reveal the surfaces facing away from the mount, there is always some degree of doubt about whether a facet hidden from view contains perforations or not. Nevertheless, we can generalize, based on the abundance of perforation plates observed in our preparations, that in the xylem of ferns we studied, tracheids were few or none. Prior to our studies, vessel elements were assumed to be absent in most ferns. In the ferns in which vessels had been reported (e.g., *Pteridium*), earlier authors did not present analyses of whether tracheids were also present (e.g., Bliss, 1939). Thus, no study prior to our work on *Ceratopteris* presented the likelihood that vessel elements could coexist with tracheids in fern xylem. Further work, perhaps with particles of various sizes introduced into actively conducting xylem, needs to be done to confirm the idea that fern xylem can consist mostly or wholly of vessel elements.

Porose pit membranes and incipient perforation plates—Porose pit membranes (Figs. 19, 20) were observed in all of our studies, cited above. The modes of occurrence of these pores are as follows: (1) throughout incipient perforation plates (usually lateral wall perforation plates, Fig. 16, left); (2) at upper and lower ends of perforation plates, where the porose pits form a transition between perforations and intact pits; (3) in narrow pits, where accompanied by wide pits in instances of pit dimorphism (Fig. 12, upper left).

Modifications of porose pit membranes in the direction of enlargement of pores are common. These modifications include: (1) occurrence of one to several large holes as well as porose membranes, forming an incipient perforation (Figs. 17, 20, 22) or else presence of large holes without porose pit membranes (Fig. 18); (2) threadlike or weblike pit membrane remnants (Fig. 21), which may or may not include porose segments.

Most of the facets we examined, however, possess intact pit membranes (Fig. 23) in which pores, if present, were so minute that they were beyond the resolving ability of our equipment and which probably would be referable to the category of plasmodesmata rather than pores.

Other newly reported structural details—During the course of our studies to determine presence of vessel elements in the ferns we studied, we encountered a number of noteworthy structures.

1) Tracheary elements that are short and have numerous short facets (Fig. 23). These were observed only in Cyathea-ceae.

2) Grooves interconnecting pit apertures (Fig. 24). These were observed only on the inner surfaces of vessels of *Pteridium aquilinum*. Such grooves may be more widespread in ferns, but we viewed the inner surfaces of vessels only in our first two fern studies in which sections of tracheary elements were prepared and observed. Such grooves (also termed “coalescent pit apertures”) are widespread in dicotyledons, but our report is apparently the first in ferns.

3) Cushions of wall material present at facet edges of tracheary elements. We observed these in *Danaea elliptica* (Figs. 25, 26) and *Elaphoglossum hirtum* (Fig. 27), two ferns that are not at all closely related. We know of no previous report of such structures prior to ours.

4) In *Vandenboschia davallioides* (Fig. 28), *Dicranopteris linearis*, and *Anemia phyllitidis* we observed that at facet edges, the horizontal secondary wall framework segments terminate. Thus, the secondary wall framework consists of transverse bars with widened ends, and the facet angle therefore consists of primary wall separating the ends of these barlike structures. Ordinarily, a secondary wall framework that forms a continuous skeleton for the tracheary element is illustrated for fern metaxylem, and a vertical (axial) ridge of secondary wall material, rather than an absence of secondary wall material, is characteristic of tracheary elements of most ferns.

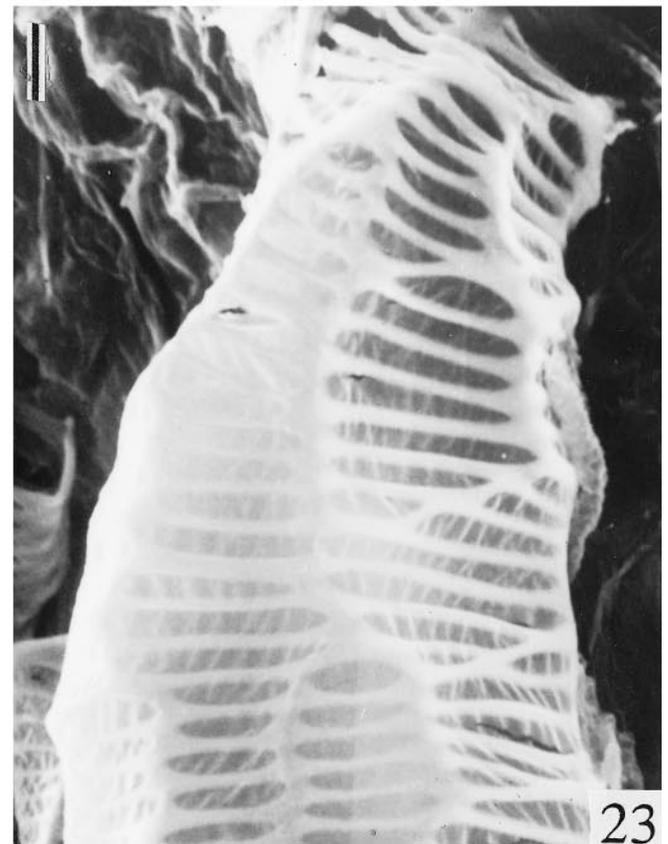
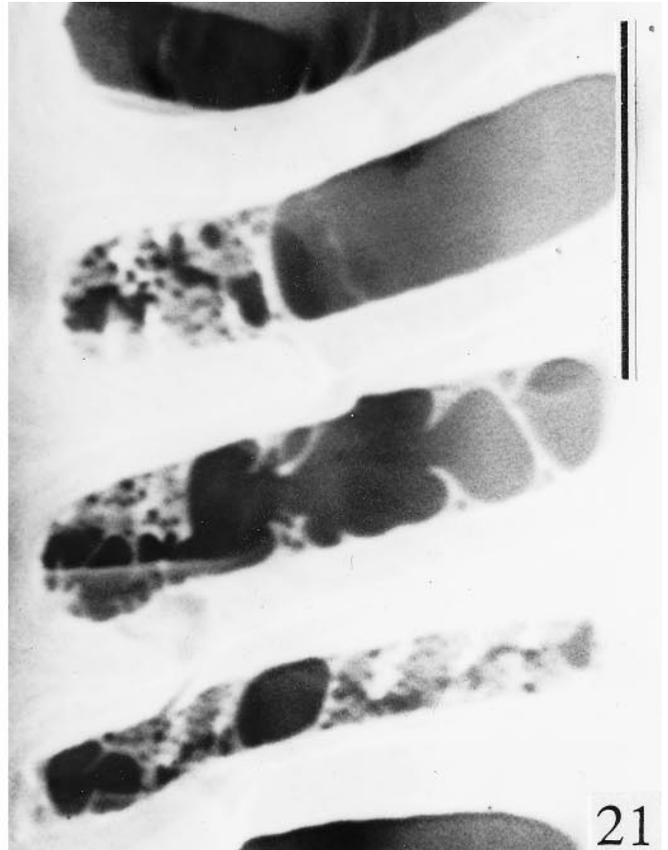
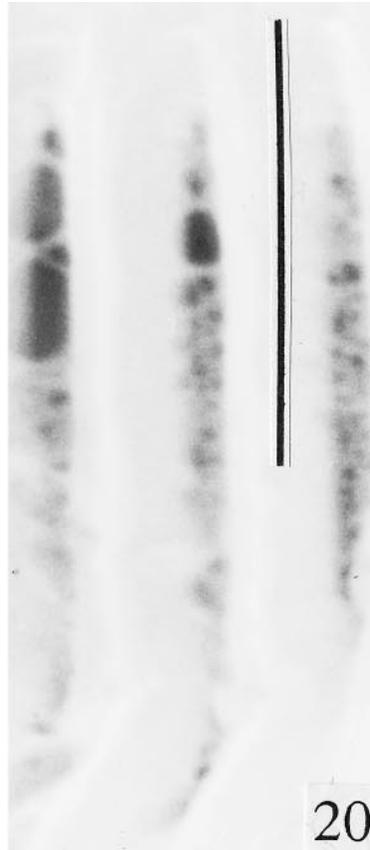
5) We examined roots and stems (rhizomes) of all ferns we studied (the axis of Psilotaceae is not differentiated into roots and stems, however). In all species for which we had both root and stem material that yielded good results, vessel elements were observed in both roots and stems.

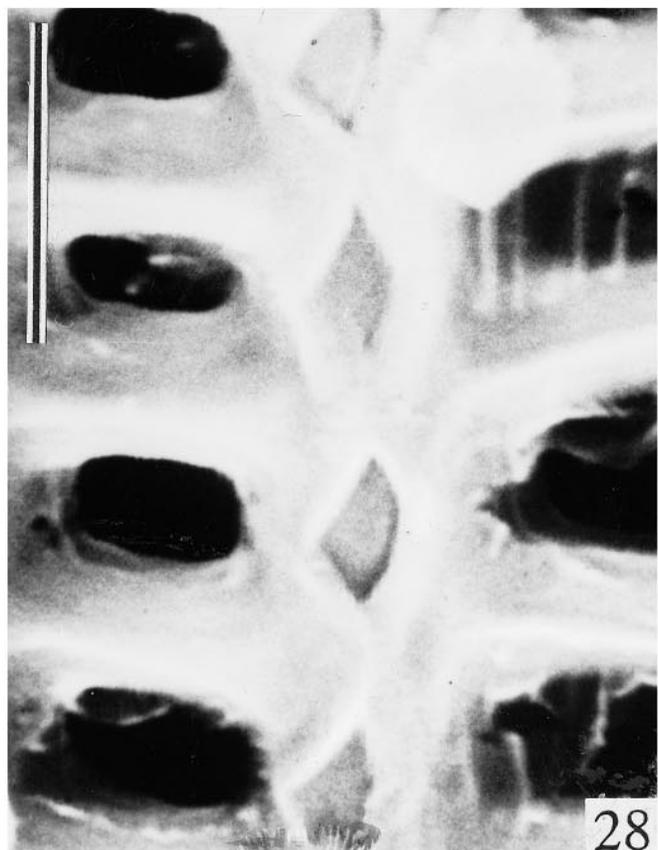
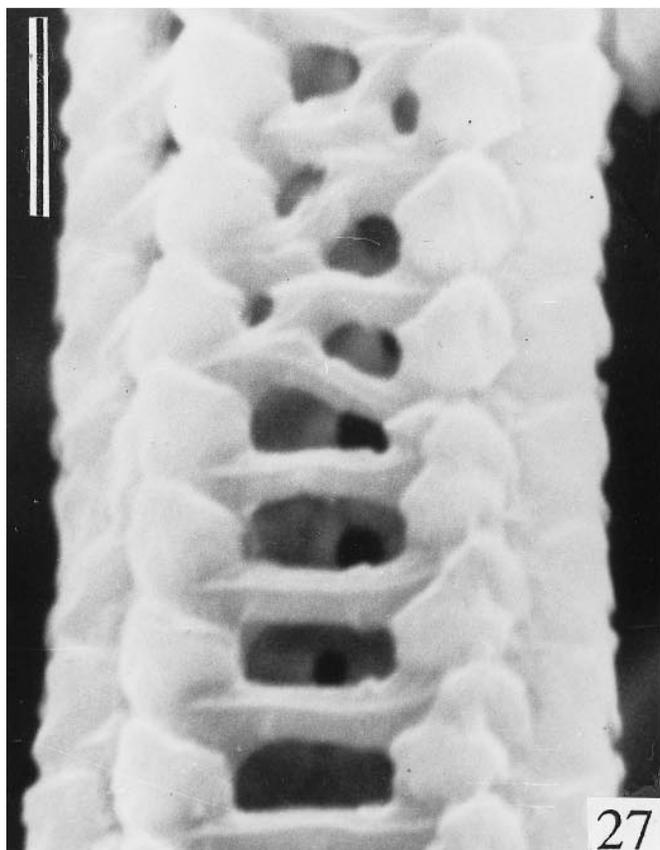
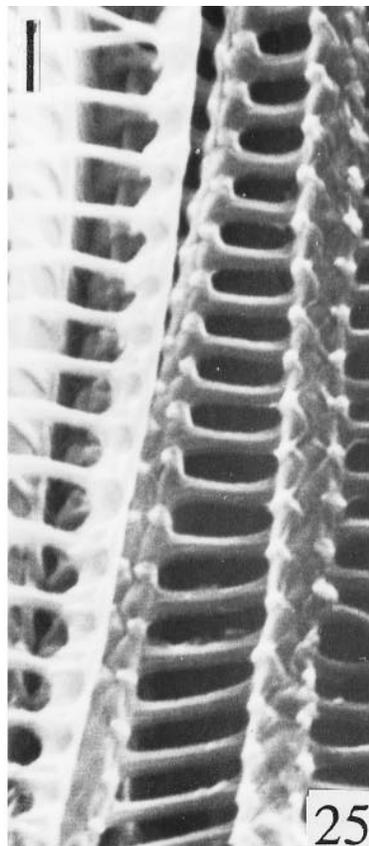
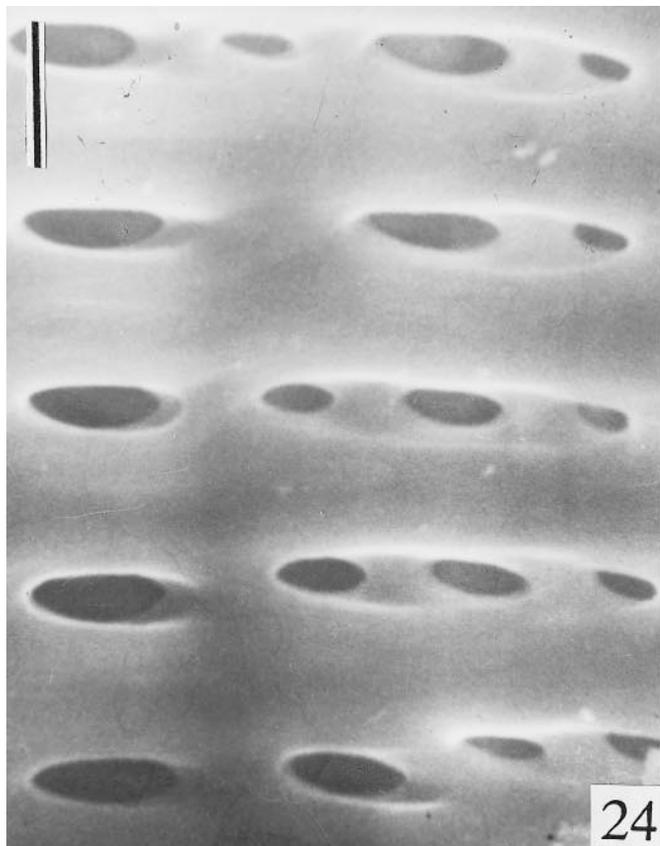
FERN TRACHEARY ELEMENTS: NEW INTERPRETATIONS

1) Vessel elements with a distinctive end wall perforation plate at either end characterize angiosperms. Angiosperm vessels, with few exceptions, are thought to be like superposed pipe segments, and lateral contacts between vessels bear pits rather than perforations. This type of end-wall perforation plate does occur in ferns that have end walls different from lateral walls (wide perforations between narrow bars in the perforation plates), such as *Pteridium* or *Woodsia* (Figs. 1–6). However, even in these fern genera, lateral wall perforation plates, less specialized than those on end walls, occur. This may relate to the packing of tracheary elements in ferns (Figs. 29, 30). Because tracheary elements occur in fascicles in fern steles, there are numerous lateral-wall contacts on which perforation plates can develop (Fig. 29). In *Pteridium* (Fig. 30), parenchyma intrudes into the xylem and subdivides it into segments of fewer vessels each. This situation resembles the pri-

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Figs. 19–22. Tracheary elements of Hymenophyllaceae (Figs. 19, 21), Dicksoniaceae (Fig. 20), and Cyathea-ceae (Figs. 22, 23). Figs. 19–20. Porose pit membranes of incipient perforation plates. **19.** *Callistopteris baldwinii*, two pits. **20.** *Dicksonia antarctica*, three pits. **21.** Perforations in which pit membrane remnants form threadlike or weblike configurations. Figs. 22–23. *Alsophila australis*. **22.** Perforation-plate facet in which each pit has a porose membrane with a single, large central hole. **23.** Tip of tracheary element with short facets with intact pits. Scales = 5 μ m in Figs. 19–22; scale = 1 cm in Fig. 23.





mary xylem of angiosperms, in which rows of vessels are separated from each other and therefore lateral-vessel contacts are relatively few. Ferns with little lateral contact among tracheary elements might be expected to show prominent end-wall perforation plates, as is the case in *Pteridium* and *Woodsia*.

2) Most ferns, in contrast with *Pteridium* and *Woodsia*, have perforation plates that are not different from lateral-wall pitting with respect to the secondary-wall framework of the end wall. The only difference is in the absence of pit membranes in the perforations. Although angiosperms show specialization of end-wall perforation plates in most genera, no such modifications are present in most fern genera. Perhaps the reason why ferns have perforation plates yet retain unspecialized structure in them is that there is a multiplicity of perforation plates on fern tracheary elements and that most ferns do not have the selective pressure for rapid rates of conduction that angiosperms experience.

3) Most ferns have multiple end-wall facets that are converted into perforation plates by lysis of pit membranes (Fig. 8), rather than single perforation plates per end wall. These multiple perforation-plate facets are likely related to packing of metaxylem elements (Fig. 28). The end of a given tracheary element is not in contact with the end wall of a single superposed element at either end, but with side walls of a multiplicity of elements. The multiple contacts among tracheary elements in most ferns may predispose the tracheary elements to develop both lateral-wall and end-wall perforation plates, as suggested under (1) above. Tracheary elements in ferns are often very long, and the differentiation into end-wall and lateral-wall facets is minimal.

4) The occurrence in ferns of both end-wall and lateral-wall perforation plates has the effect of converting a fascicle of tracheary elements into a kind of aggregate vessel. No part of such a fascicle of elements is isolated from any other part in terms of conduction. Fascicles of vessel elements interconnected with each other by lateral perforation plates as well as end-wall perforation plates have not been reported in other groups of vascular plants except *Selaginella*, depending on definition of lateral-wall perforation plate (Schneider and Carlquist, in press a, b).

5) A series of wide perforations alternating with narrow pits, a condition that we are terming "pit dimorphism," has a potential effect on the conductive stream of a scalariform perforation plate. Evidently ferns have been able to develop these vicarious perforation plates by means of pit dimorphism more successfully than have other vascular plants, because we know of no reports of pit dimorphism resulting in perforation-plate equivalents in groups other than ferns except for *Selaginella* (Schneider and Carlquist, in press a, b).

6) Porose pit membranes that range from incipient perforation plates (where pores are small) to perforation plates in which the pores in pit membranes open large portions of a pit membrane to the conductive stream, are common in ferns. The existence of this range of perforation plates has not, hitherto, been reported in other groups of vascular plants, except for

recent instances such as our studies of vessels of monocotyledons (e.g., Carlquist and Schneider, 1998a; Schneider and Carlquist, 1998a) and *Selaginella* (Schneider and Carlquist, in press a, b).

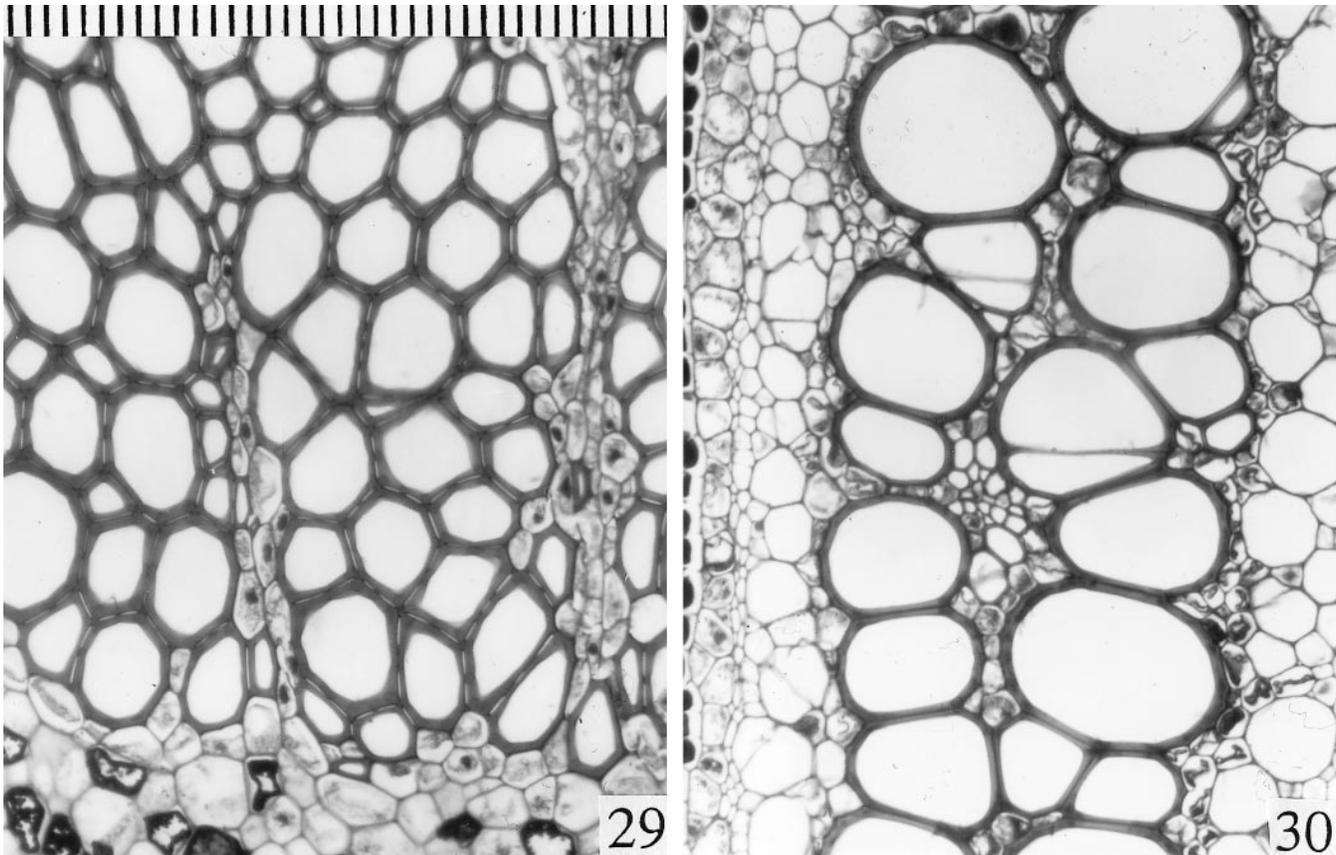
7) The occurrence in ferns of numerous incipient perforation plates or of perforation plates with appreciable areas of pit membrane remnants in perforations raises the question of terminology with respect to vessel elements. When are pit membranes provided with pores of such size and abundance that one must say perforations (and therefore vessel elements) are present? By using the term incipient perforation plate, we recognize a transition, even if we do not define a distinction between tracheids and vessel elements in such transitional instances. One might use quantitative criteria, such as the ability to transmit microspheres of known diameters from one tracheary element to another. Even this definition would likely be arbitrary. One notes the capability of margo pores in conifer tracheids to allow passage of relatively large particles in comparison to porosities in pit membranes of tracheary elements of other vascular plants (Shane, McCully, and Canny, in press), yet undoubtedly this information would not lead anatomists to question the presence of tracheids in conifers. As a working definition, we have tended to regard presence of porosities totalling $\approx 50\%$ of the area of a pit membrane or more as constituting a perforation. However, such criteria as passage of air bubbles of minimal size or other physiological criteria are needed: terminology of tracheary elements should reflect functional distinctions, if possible.

8) Porosities in pit membranes offer a potential method of interconnection between tracheary elements that are in contact but which have no intercontinuous ontogenetic relationship with each other. A prime example of this is the interconnection of vessels in adventitious roots with the vessels of the stem on which the adventitious roots form. The possible function of porosities in pit membranes to form a pathway for the conductive stream is important, because if vessels occur in both stems and roots of ferns (see above) and monocotyledons (Carlquist and Schneider, 1998a, and earlier papers in that series), such a mechanism for facilitating conduction between root and stem vessels is of selective value. Such a possible function has been mentioned in maize by Shane, McCully, and Canny (in press), and is equally applicable to ferns.

9) In those ferns with end-wall perforation plates that differ in framework from lateral-wall pitting (or lateral-wall perforation plates), the simplification of the perforation plate is related to ecology. *Marsilea*, *Pteridium*, and *Woodsia*, which exemplify this, live in situations with marked fluctuation in water availability and show trends to fewer bars associated with wider perforations. However, other ferns occur in ecological regimes in which drying or freezing of soil offers extremes as severe as those experienced by those three genera, yet these other ferns (e. g., *Cheilanthes*, *Pellaea*, *Platyzoma*) do not have such modifications of the secondary-wall framework of perforation plates. These latter genera do, however, have other means other than simplified perforation plates by

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Figs. 24–27. Unusual structural details of fern tracheary elements from sections (Fig. 24) and macerations (Figs. 25–27). 24. *Pteridium aquilinum*, inner surface of vessel from section, showing elliptical grooves interconnecting two or three pits (at right). Figs. 25–26. *Danaea elliptica*, portions of facet edge viewed obliquely. 25. Cushionlike structures at facet edges of adjacent tracheary elements. 26. Cushionlike structures adjacent to pits, in face view. 27. *Elaphoglossum hirtum*. Facet of tracheary element (center) on either side of which cushionlike structures are present. 28. *Vandenboschia davallioides*. Edge between two facets. The secondary wall is absent from elliptical portions of the facet edge. Scales = 10 μm in Figs. 24–25; scales = 5 μm in Figs. 26–28.



Figs. 29–30. Transsections of tracheary elements from rhizomes, as seen with light microscopy. **29.** *Osmunda cinnamomea*. Tracheary elements not intermixed with parenchyma cells. **30.** *Pteridium aquilinum*. Bands of parenchyma separate groups of two to several tracheary elements. Scale for both figures above Fig. 29 (divisions = 10 μm).

which the conductive stream potentially can be facilitated during brief periods of water availability. Such means include multiple end wall perforation plates, lateral-wall perforation plates, and perforations that result from pit dimorphism. In angiosperms, degree of simplification of perforation plate is closely keyed to degree of fluctuation in water availability (Carlquist, 1975). Enhancement of the conductive capabilities of a vessel of an angiosperm must be accomplished by modification of the end wall, because the variety of perforation plates and perforation-plate substitutes present in ferns are not available in angiosperms.

10) Vessels are much more common in ferns than hitherto supposed. Because many ferns that have perforation plates occur in habitats with relatively uniformly moist environments (e.g., Marattiaceae), strong fluctuation in moisture availability cannot be hypothesized as a requirement to explain vessel origin in ferns. Lysis of pit membranes by pectinase and cellulase is apparently not a complicated process (Butterfield and Meylan, 1982). If this is true, vessels could be expected to develop independently in a number of clades of vascular plants. Once large porosities are formed, removal of most or all of the pit membrane remnants is all but inevitable. Special mechanisms (e.g., fibrils separating large pores in margins of pits of conifer tracheids) that do not follow this pattern but represent alternative mechanisms for enhancement of conduction exist. Perforation plates (or equivalent structural enhancements of conductive capability) are not known to be of any

detriment in mesic situations. For example, dicotyledons of bogs show no fewer simple perforation plates than do plants of noninundated areas (Carlquist, unpublished data). The potential role of imperforate tracheary elements in deterring spread of air embolisms has often been cited, and undoubtedly does occur. However, mechanisms for control of transpiration may override the potential advantages of tracheids in increasing safety and integrity of the water columns of a plant from spread of air bubbles. For example, Gates (1968) measured a diffusive resistance in *Pteridium* higher than that of any other vascular plant he studied.

In summarizing our data, we wish to emphasize that our survey, although it covers all major families of ferns, is based on limited material when one considers the large number of fern species in existence. The opportunities for research in structure of fern tracheary elements by means of electron microscopy are extensive. Moreover, we hope that plant physiologists will become interested in studying vascular plants such as ferns that offer a range of conductive pathways not present in angiosperms or gymnosperms.

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