SEM STUDIES ON VESSELS IN FERNS. 2. *PTERIDIUM*[1]

SHERWIN CARLQUIST[2] AND EDWARD L. SCHNEIDER

Santa Barbara Botanic Garden, 1212 Mission Canyon Road,
Santa Barbara, California 93105

Xylem from roots and rhizomes of two infraspecific taxa of *Pteridium aquilinum* was studied by means of scanning electron microscopy (SEM). All tracheary elements proved to be vessels. End wall perforation plates were all scalariform, lacked pit membrane remnants in at least the central part of the perforation plate, and varied with respect to width of bars, from wide to tenuous, and with respect to presence of pit membrane remnants. In addition, porose pit membranes on walls that are likely all lateral vessel-to-vessel walls must be considered to be perforations also, although different from those on end walls. Lateral wall perforation plates, hypothesized by one worker on the basis of tylosis presence but denied by another on the basis of light microscopy, were confirmed by demonstration of pores with SEM. In addition, lateral walls of *Pteridium* vessels bear some grooves interconnecting pit apertures; this feature is newly figured by SEM for ferns. Lateral wall pitting that is not porose may either have striate thickenings of the primary wall or be smooth. Vessel presence and degree of specialization in *Pteridium* vessels may bear a relationship to the wide ecological tolerances of the genus.

Key words: bracken; ferns; perforation plates; *Pteridium*; vascular tissue; vessels; xylem.

By means of SEM studies of primary xylem, we have been able to see details of perforation plate morphology and other aspects of vessel structure not visible with the light microscope. Our work began in Nymphaeales (Schneider and Carlquist, 1995a, b, 1996a, b, in press a; Schneider et al., 1995); the monocotyledon family Acoraceae (Carlquist and Schneider, 1997); and ferns (Carlquist and Schneider, in press). In the present paper, we have turned our attention to the fern genus *Pteridium* (Pteridaceae). *Pteridium* is the fern in which vessels were first claimed to be present (Russow, 1873). As noted by White (1962), claims and counterclaims of vessel presence in various ferns were made by workers such as Gwynne-Vaughan (1908), Bancroft (1911), Bliss (1939), and Duerden (1940). All of these workers, however, agreed that vessels were present in *Pteridium,* and that vessels occur in roots, rhizomes, and petioles.

In our re-examination of *Pteridium,* we were interested in looking for diversity in perforation plate morphology and other vessel features, diversity not reported on the basis of light microscope studies. For example, we wished to know whether porose pit membrane remnants, which are not visible with light microscopy, are present: SEM work has shown that pit membranes are present in perforations of some primitive dicotyledons (Carlquist, 1992) and monocotyledons (Carlquist and Schneider, 1997), whereas other genera lack pit membrane remnants. The nature of the perforation plate in *Pteridium* has been reported in diverse ways. Bliss (1939) figured strictly scalariform perforation plates in *Pteridium* on the basis of sectioned material. White (1962), who worked with macerations, illustrated either end walls with bars that incompletely traverse the end wall, or a simple perforation plate, for vessels of *Pteridium.* Presence of lateral perforation plates in *Pteridium,* hypothesized by Gwynne-Vaughan (1908) because of the extensive presence of tyloses, was discounted by Bierhorst (1960), who observed

pit membranes with light microscopy. Tyloses in which parenchyma protoplasts invade deactivated vessels by breaking through pit membranes are not indications of lateral wall perforation plates in dicotyledons, and thus are not valid evidence of perforation plate presence in *Pteridium.* Use of SEM can demonstrate presence and nature of perforations not visible with light microscopy.

Although some workers recognized several species of *Pteridium* (Copeland, 1947; Pichi-Sermolli, 1959), more recent workers have grouped these as a single polymorphic species, *P. aquilinum* (L.) Kuhn, divisible into six to eight infraspecific taxa (Christensen, 1938; Tryon, 1941; Page, 1976, 1990). Study of phenols and flavonoids reveals no diversity within *P. aquilinum* (Cooper-Driver, 1976), although isozymes (Wolfe, Sheffield, and Haufler (1990), chloroplast DNA (Tan and Thomson, 1990a) and genome size (Tan and Thomson, 1990b) vary significantly with taxonomy and biogeography. The present study samples two of the infraspecific taxa, *P. aquilinum* var. *latiusculum* Underw. (eastern North America and eastern Asia) and *P. aquilinum* var. *pubescens* Underw. (central to western North America), in order to see whether plants from diverse geographical sources differ in vessel structure as seen with SEM. According to data from DNA (Tan and Thomson, 1990a) significant taxonomic and biogeographic differentiation between northern and southern hemisphere populations has occurred.

MATERIALS AND METHODS

Material of *Pteridium* was preserved in 50% aqueous ethanol. The liquid-preserved specimen of *P. aquilinum* var. *latiusculum* was available from the Vernon I. Cheadle Collection through the courtesy of the Museum of Systematics and Ecology of the University of California, Santa Barbara. The plant of *P. aquilinum* var. *pubescens* was cultivated in the Santa Barbara Botanic Garden.

Portions of the liquid-preserved specimens were infiltrated with the tertiary butyl alcohol series and embedded in paraffin according to the usual techniques (Johansen, 1940). Longisections of rhizomes and roots were mounted on aluminum stubs just as they would be on glass slides, cleansed of paraffin with xylene, sputter coated, and examined with a Bausch and Lomb Nanolab SEM. Longisections of roots and rhizomes

provide more numerous tracheary element surfaces likely to yield information on structure, but preparation of longisections was difficult because of the hardness of walls of tracheary elements. Some areas on these sections were informative, but we realized that sectioning would not be a satisfactory way of determining the nature of perforation plates for SEM study, especially if the bars were tenuous. Displacement and breakage proved excessive. Consequently, we have, for most of our data, relied upon macerations of xylem of roots and stems. Macerations were prepared by removal of as much fibrous tissue and parenchyma as possible prior to immersion of the vascular tissue in Jeffrey's Fluid (Johansen, 1940). When degree of maceration was judged to be sufficient, the treated portions were transferred to 50% aqueous ethanol. After several hours, the macerated material was placed on SEM aluminum stubs to which electrically conductive adhesive discs had been affixed. Using drops of 50% alcohol to aid in spreading cells and with minimal use of forceps to avoid cell breakage, cells were spread over the surface of the adhesive disc. This preparation was then slowly dried, sputter coated, and examined with SEM. We believe that maceration alters primary walls of tracheary elements or, for that matter, parenchyma cells to a negligible extent, judging by our comparisons with sectioned material. Therefore, macerated tracheary elements are judged to be free from artifacts except where torsion due to handling is concerned. The wider facets of vessel elements and especially the perforation plates (Figs. 3, 4, 7) of maceration preparations often are slightly sunken. This conformation may be caused by the evaporation of liquid within the cell as the maceration dries.

RESULTS

Our observations showed that all tracheary elements of *Pteridium* are vessel elements. We did not observe any tracheary elements with end walls devoid of perforations. Perforation plates, however, do show a range in structure (Figs. 1–7). End walls similar to lateral walls in width of pits and bars were observed in some vessel elements (Fig. 1). In these, the central portions of perforation plates are clear of primary wall material or mostly so (Fig. 1, lower third), although pits in upper and lower ends of the perforation plates retain pit membranes to various degrees (Fig. 1, upper two-thirds of photograph). If perforation plates such as these were studied with light microscopy, they might be interpreted as end walls of tracheids.

The majority of perforation plates of *Pteridium* rhizomes and roots are clearly differentiated from lateral walls: the perforations are larger and more elongate than are lateral wall pits, and the intervening wall portions (= bars of the perforation plate) are more slender than the wall portions separating a lateral wall pit from another above or below it. These perforation plates are like those illustrated by Bliss (1939) for *Pteridium*. Some of these plates have relatively wide bordered bars (Fig. 2), but there is a continuum between wide bars and very slender bars, and Figs. 3–5 are presented to show progressively more tenuous bars. Borders are apparently not present in the central portions of these bars, although borders do occur at the ends of the perforations. The number of bars per perforation plate varies greatly; although a range is shown in Figs. 2–5, we have not attempted to figure some perforation plates that had ≈ 50% more bars than any we have illustrated.

In addition to perforation plates that occupy long oblique end walls, we observed some perforation plates on near-transverse end walls (Figs. 6, 7). The bars on these plates are commonly slender and unbranched (Fig.

6). In a few plates, wider, fused bars were observed (Fig. 7).

In roots of *Pteridium*, White (1962) figured a simple perforation plate as well as several that have only a few bars, and many of these bars were shown not to traverse the perforation plate but to extend only partially across it. We were unable to find any such plates in our root material of *P. aquilinum* var. *pubescens* (Figs. 14, 15). Although some vessel elements in the root showed a great number of bars (e.g., Fig. 14: only about two-thirds of the perforation plate are shown), other plates had only a few bars (Fig. 15).

Many lateral walls of vessels consist of scalariform pits with pit membranes that are smooth (Fig. 8). In occasional lateral walls, one can see pit membranes that are striate because of thickenings (Fig. 9). Such striations have been reported in SEM studies of tracheary elements of Nymphaeaceae (e.g., Schneider and Carlquist, 1995a), monocots (Carlquist and Schneider, 1997), and ferns (Carlquist and Schneider, in press). Another feature we observed on walls of a few vessels is the occurrence of grooves interconnecting pit apertures (Fig. 10). Such grooves are common in woods of dicotyledons (Metcalfe and Chalk, 1950), and occur in *Triglochin* of the Juncaginaceae (Schneider and Carlquist, in press b). Grooves interconnecting pit apertures have been figured for *Pteridium* and a few other fern genera by Bierhorst (1960), who refers to this formation as "pits in valleys."

Lateral walls of *Pteridium* vessels, however, characteristically exhibited a feature hitherto unreported in vascular plants, unless as an anomaly. Many of the lateral walls show pores (Figs. 11–13). These range from circular to elongate, and are relatively large (Figs. 11, 12) or small (Fig. 13). These pores occur on vessels that also have a well-demarcated perforation plate (e.g., the bars at right, Fig. 13, deliberately included to show that such a perforation plate occurs in a vessel with porose lateral wall pitting). Thus, vessels in *Pteridium* can have well-marked end wall perforation plates combined with porose lateral walls that also qualify as perforation plates of a second kind, lateral perforation plates.

DISCUSSION

Although earlier accounts (Bliss, 1939, Duerden, 1940, White, 1962; Mehra and Soni, 1997) mention vessels in *Pteridium*, they do not clearly indicate whether tracheids are also to be found in xylem. Typical of this vagueness is the statement by Bliss (1939) that variation in end wall angle "may be regarded as transitional between the tracheid and the vessel in this genus." White's (1962) table of "tracheid lengths" includes *Pteridium*, although this suggestion that *Pteridium* has tracheids may have been unintentional. Bierhorst (1960) noncommitally refers to "tracheary elements" in the genus. Our SEM studies revealed tracheary elements we could clearly define as vessel elements. There are some end wall perforation plates that are more tracheidlike in having pits like those of lateral wall pits, but these pits lack pit membranes except at the upper and lower end of the perforation plates. Most end wall perforation plates in *Pteridium* consist of wide to very slender bars traversing the width of the plate, and thus these perforation plates differ markedly in morphol-
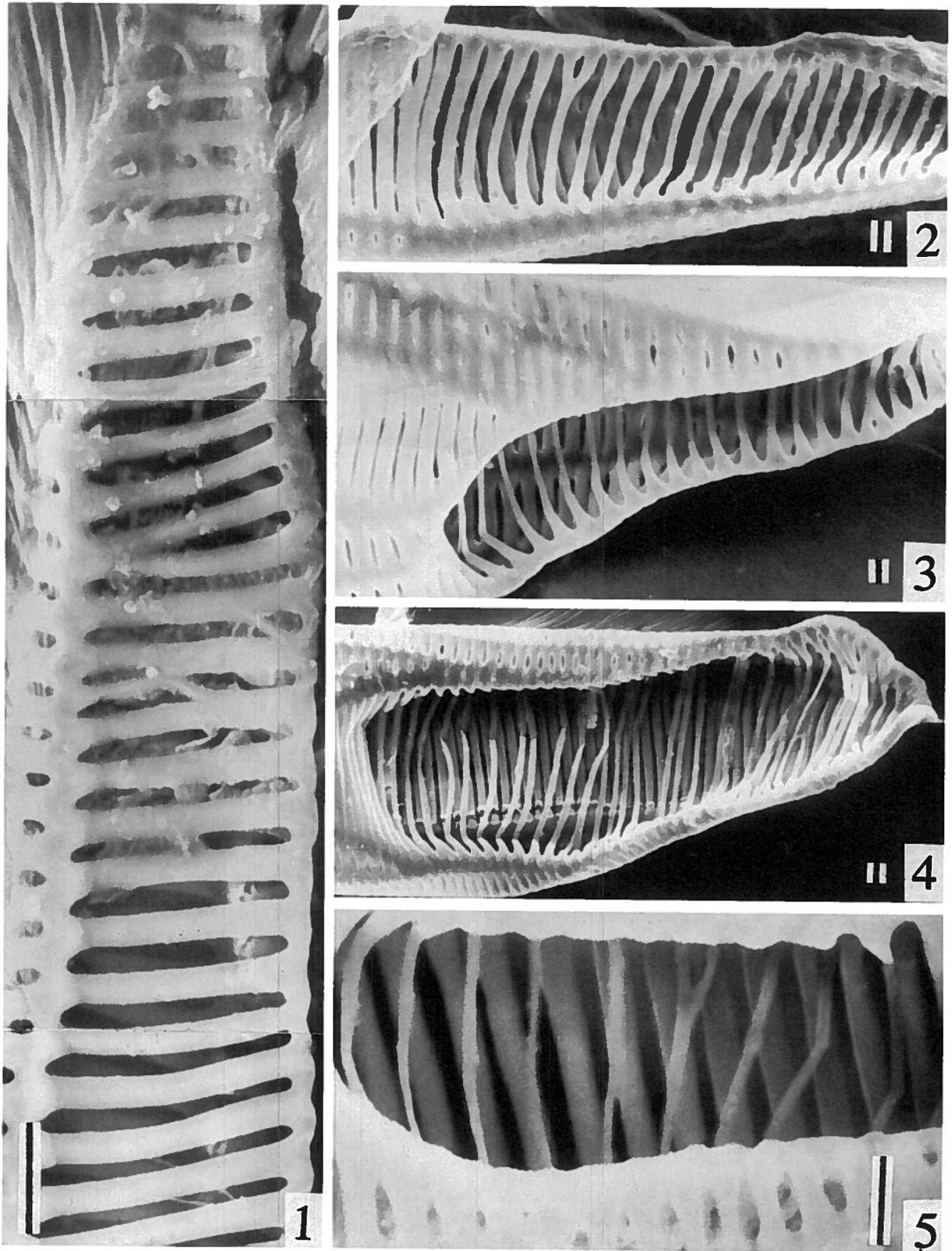
Figs. 1–5.   SEM photographs of portions of rhizome vessel elements from *Pteridium aquilinum* var. *latiusculum* to show perforation plates. **1.** Perforation plate with pitting similar to lateral wall pitting; pit membranes are present in the upper two-thirds of the perforation plate. **2.** Perforation plate with moderately wide bars. **3.** Perforation plate with slender, well-spaced bars. **4.** Bars are slender, closely spaced. **5.** Bars are few, well spaced. Scale bars = 10 μm.
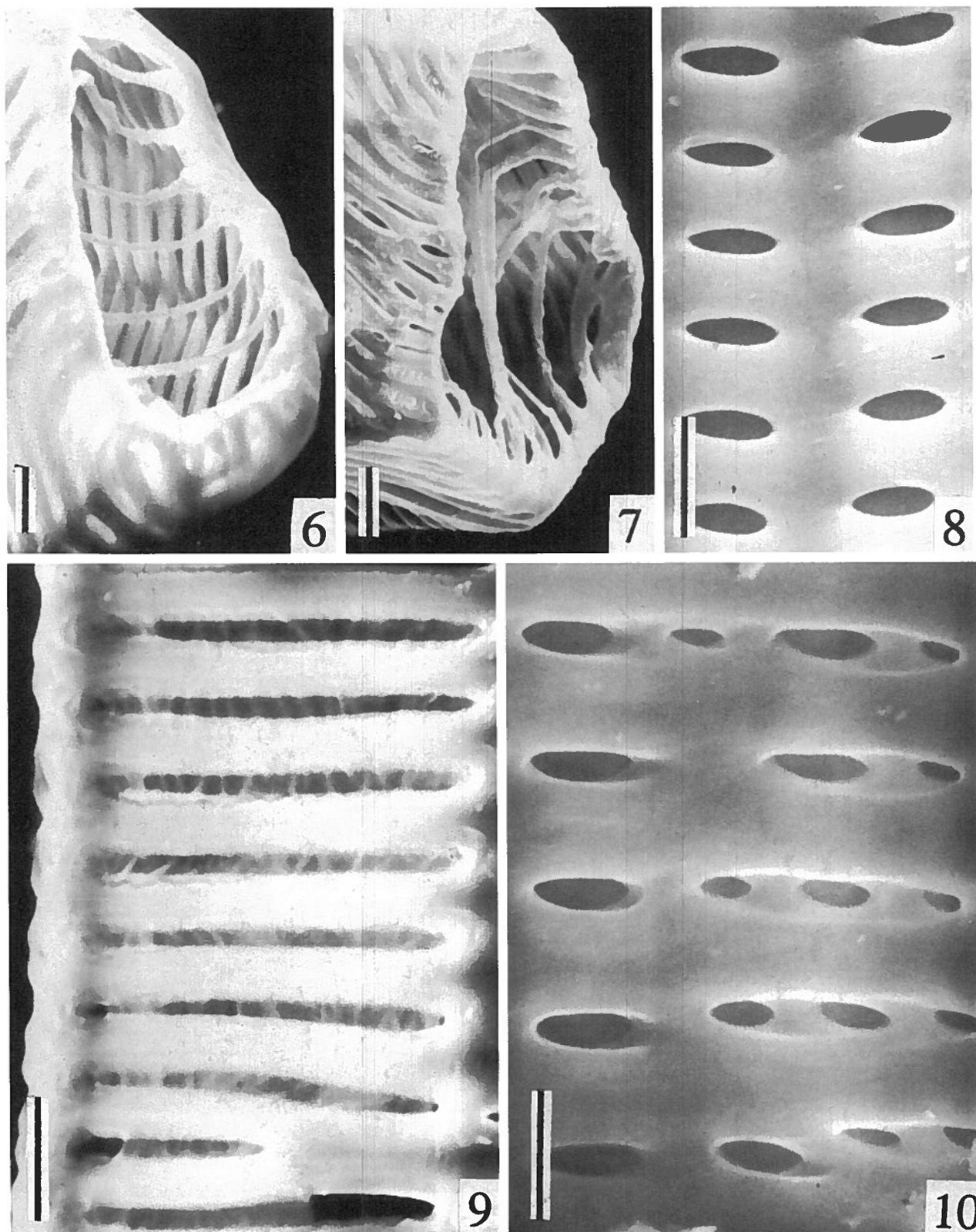
Figs. 6–10. SEM photographs of portions of vessels of *Pteridium aquilinum* var. *latiusculum* (Figs. 6–8, 10) and *P. aquilinum* var. *pubescens* (Fig. 9). 6, 7. Nearly transverse perforation plates from vessel macerations. **6.** Bars are relatively few and very slender. **7.** Bars are few but thicker, some fused. **8.** Lateral wall pitting with smooth pit membranes, from section. **9.** Scalariform lateral wall pitting with striate membranes, from maceration. **10.** Scalariform lateral wall pitting with grooves interconnecting pit apertures, from section. Scale bars = 10 μm.
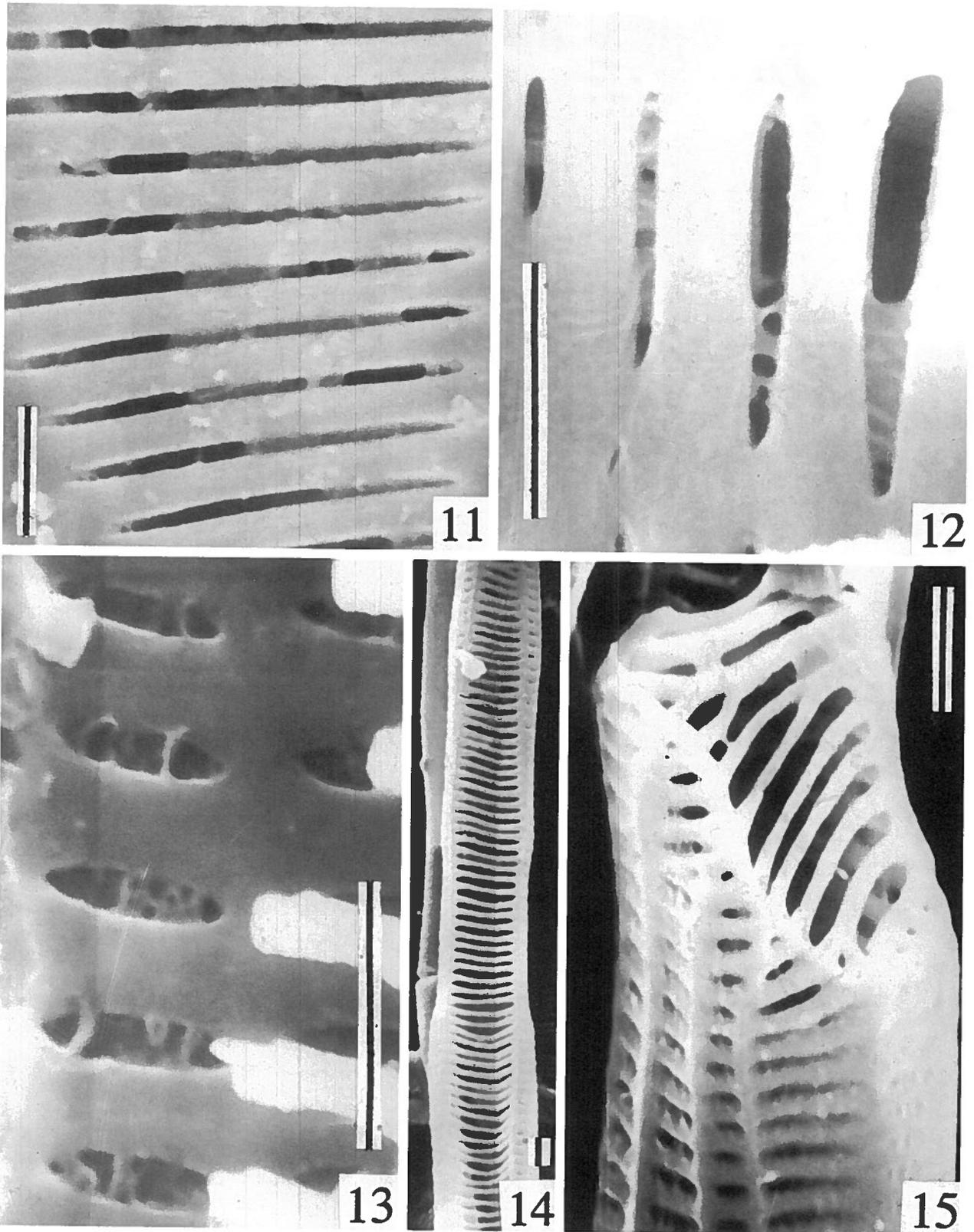
Figs. 11–15.   SEM photographs of vessel details from *Pteridium aquilinum* var. *latiusculum* (Figs. 11–13) and *P. aquilinum* var. *pubescens* (Figs. 14, 15); from macerations (Figs. 11, 12, 14, 15) and sections (Fig. 13). 11–13. Vessels from rhizomes. **11.** Lateral vessel wall, showing abundance of elongate pores in pit membranes. **12.** Portion of lateral vessel wall, to show pores of various sizes and shapes in pit membrane. **13.** Small pores in membranes of lateral wall pits; portions of bars from perforation plate at right. 14, 15. Perforation plates from vessels of roots. **14.** About two-thirds of a long perforation plate. **15.** Short perforation plate with few, wide bars. Scale bars = 10 μm.

ogy from the lateral wall pitting on the vessels. Our data concur with Bliss's (1939) figures of scalariform perforation plates, based on sectioned material. White's (1962) figures of perforation plates in *Pteridium* show some plates traversed by a few bars (with the remainder of the plate lacking bars), some plates partially traversed by bars (which do not reach from one side of the plate to the other), and even a simple perforation plate. We did not find any of these conditions in our materials. Apparent absence of bars and portions of bars might be explained by the difficulty of staining and focusing on tenuous bars of perforation plates with light microscopy.

The presence of lateral wall pitting that has intact membranes, either striate or smooth, is not unexpected, but the presence in *Pteridium* of lateral walls in which the pits are porose and qualify as a kind of lateral perforation plate is a feature that has not, as a characteristic feature, been reported in vascular plants. Because our best views were obtained from macerations, in which the cell contact represented by a facet on a vessel wall is unknown, we cannot be positive, but we are of the opinion, based on our observations, that the porose lateral wall pits, or perforations, represent vessel-to-vessel contacts rather than vessel-to-parenchyma or other types of contacts. Lateral-wall perforation plates are absent in *Woodsia* (Carlquist and Schneider, in press) so this feature is not universally present in ferns that have vessels.

Gwynne-Vaughan (1908) hypothesized that lateral wall perforations must occur in *Pteridium* because tyloses are abundant in *Pteridium* vessels. Tyloses are indeed abundant in *Pteridium* vessels, as figured by Bliss (1939) and Bierhorst (1960), but do not necessarily indicate presence of perforations: tyloses burst through pit membranes when they enter vessels in dicotyledons (Zimmermann, 1983, and papers cited therein). Bierhorst (1960) did not find lateral perforation plates in *Pteridium* because he observed pit membranes by means of light microscopy. As noted above, pit membranes are present, but because of their porose nature, perforations are present, a fact clearly demonstrable with SEM.

The occurrence of vessels in roots, rhizomes, and petioles of *Pteridium* is interesting, and one notes at the same time that none of the perforation plates (at least in our material) is simple or close to simple in morphology. This is in contrast with *Marsilea,* where vessels with simple perforation plates occur in roots, but not elsewhere in the plant (White, 1961). In *Marsilea,* one could correlate the presence of simple perforation plates in roots with rapid uptake of water by roots in habitats such as ephemeral ponds. In *Pteridium,* on the contrary, water uptake is probably never rapid: *Pteridium* has an exceptionally high diffusive resistance, a measure of diminished transpiration according to the data of Gates (1968). If lowered transpiration is assured, a relatively slow rate of transpiration would be correlated with the scalariform perforation plates and thus their lack of simplification would be understandable. Acclerated loss of bars in perforation plates in response to probable rapid flow rates can be shown in lianas such as *Tetracera.* Alternatively, scalariform perforation plates might be retained by *Pteridium* because they might be able to sieve out small bubbles if water freezes and then thaws in the vessels, a function hypothesized by Zimmermann (1983). The presence of pores of various sizes in the lateral pitting of *Pteridium* vessels would enhance lateral transport of water without markedly increasing the vulnerability of vessels to transfer of air embolisms from one vessel element to another laterally. Experimental work to show the nature of conduction in *Pteridium* not only under normal conditions but under conditions of drought and frost is needed. Further study may confirm our impression that porose membranes on lateral wall pits represent vessel-to-vessel contacts, whereas nonporous membranes on lateral wall pits would represent contacts between vessels and other cell types.

The presence of striations on primary walls of pit membranes in lateral wall pits in *Pteridium* is not unprecedented (see introduction), but the ease of resolution of these structures with SEM shows the value of this method of study of tracheary elements. Likewise, the grooves interconnecting pit apertures in *Pteridium* are clearly shown by means of SEM. Bierhorst (1960) figured these for vessels of *Pteridium* and several other ferns; he describes this conformation as "pits that lie in valleys."

Our SEM studies of tracheary elements in ferns (Carlquist and Schneider, in press; unpublished data) indicate that vessels occur much more widely in ferns than hitherto supposed. In ferns, as well as dicotyledons and monocotyledons, some of these vessels are characterized not by large perforations, but by porose pit membranes in perforation plates. Obviously, our concepts of morphology of vessel elements and of their systematic occurrence will have to be widened considerably. Because studies in progress are contributing to this picture, an accurate summary of vessel distribution in vascular plants cannot be offered. The significance of different vessel types, especially those in which the perforation plates retain porose membranes, remains to be elucidated. Do such pores aid in localization of air embolisms within a vessel element, or are they just incipient lysis of the membrane without such physiological significance? As the distribution of vessels and various types of perforation plates in vascular plants becomes better known, we will be able to obtain a much better idea of the physiological, ecological, and phylogenetic significance of vessel occurrence.

LITERATURE CITED

BANCROFT, N. 1911. On the xylem elements of the Pteridophyta. *Annals of Botany* 25: 746–759.

BIERHORST, D. W. 1960. Observations on tracheary elements. *Phytomorphology* 10: 249–305.

BLISS, M. C. 1939. The tracheal elements in the ferns. *American Journal of Botany* 26: 620–624.

CARLQUIST, S. 1992. Pit membrane remnants in perforation plates of primitive dicotyledons and their significance. *American Journal of Botany* 79: 660–672.

———, AND E. L. SCHNEIDER. 1997. Origins and nature of vessels in monocotyledons. 1. *Acorus* (Acoraceae). *International Journal of Plant Science* 158: 51–56.

———, AND ———. In press. SEM studies on vessels in Ferns. 1. *Woodsia obtusa* (Sprengel) Torrey (Dryopteridaceae). *American Fern Journal.*

CHRISTENSEN, C. 1938. Filicineae. *In* F. Verdoorn [ed.], Manual of pteridology, 522–550. M. Nijhoff, The Hague.

COOPER-DRIVER, G. A. 1976. Chemotaxonomy and phytochemical

ecology of bracken. *Botanical Journal of the Linnean Society* 73: 35–46.

COPELAND, E. B. 1947. Genera filicum. Ronald Press, New York, NY.

DUERDEN, H. 1940. On the xylem elements of certain ferns. *Annals of Botany* 4: 523–531.

GATES, D. M. 1968. Transpiration and leaf temperature. *Annual Review of Plant Physiology* 19: 211–238.

GWYNNE-VAUGHAN, D. T. 1908. On the real nature of the tracheae in the ferns. *Annals of Botany* 22: 517–523.

JOHANSEN, D. A. 1940. Plant microtechnique. McGraw Hill, New York, NY.

MEHRA, P. N., AND S. L. SONI. 1971. Morphology of tracheary elements in *Marsilea* and *Pteridium*. *Phytomorphology* 21: 68–71.

METCALFE, C. R., AND L. CHALK. 1950. Anatomy of the dicotyledons. Clarendon Press, Oxford.

PAGE, C. N. 1976. The taxonomy and phytogeography of bracken—a review. *Botanical Journal of the Linnean Society* 73: 1–34.

———. 1990. Taxonomic evaluation of the genus *Pteridium* and its active evolutionary state. *In* J. A. Thomson and R. T. Smith [eds.], Bracken biology and management, 23–34. Australian Institute of Agricultural Science, Sydney.

PICHI-SERMOLLI, R. E. G. 1959. Pteridophyta. *In* W. B. Turrill [ed.], Vistas in Botany, 421–493. Pergamon Press, London.

RUSSOW, E. 1873. Vergleichende Untersuchungen betreffend die Histologie (Histographie und Histiogenie) der vegetativen und sporenbildenden Organe und die Entwickelung der Sporen der Leitbündel-Kryptogamen, mit Berücksichtigung der Histologie der Phanerogamen, ausgend von der Betrachtung der Marsiliaceen. St. Petersburg Academy of Sciences Memoirs, Ser. 7, 19(1): 1–207.

SCHNEIDER, E. L., AND S. CARLQUIST. 1995a. Vessels in the roots of *Barclaya rotundifolia* (Nymphaeaceae). *American Journal of Botany* 82: 1343–1349.

———, AND ———. 1995b. Vessel origins in Nymphaeaceae: *Euryale*

and *Victoria*. *Botanical Journal of the Linnean Society* 119: 185–193.

———, AND ———. 1996a. Vessels in *Nelumbo* (Nelumbonaceae), *American Journal of Botany* 83: 1101–1106.

———, AND ———. 1996b. Vessels in *Brasenia* (Cabombaceae); new perspectives on vessel origin in primary xylem of angiosperms. *American Journal of Botany* 83: 1236–1240.

———, AND ———. In press a. Vessel origin in *Cabomba*. *Nordic Journal of Botany*.

———, AND ———. In press b. Origins and nature of vessels in monocotyledons. Z. Juncaginaceae and Scheuchzeriaceae. *Nordic Journal of Botany* 17(4).

———, ———, K. BEAMER, AND A. KOHN. 1995. Vessels in Nymphaeaceae: *Nuphar, Nymphaea*, and *Ondinea. International Journal of Plant Science* 156: 857–862.

TAN, M. K., AND J. A. THOMSON. 1990a. Evolutionary studies of the chloroplast genome in *Pteridium. In* J. A. Thomson and R. T. Smith [eds.], Bracken biology and management 95–104. Australian Institute of Agricultural Science, Sydney.

———, AND ———. 1990b. Variation of genome size in *Pteridium. In* J. A. Thomson and R. T. Smith [eds.], Bracken biology and management 87–94. Australian Institute of Agricultural Science, Sydney.

TRYON, R. M. 1941. A revision of the genus *Pteridium. Rhodora* 43: 1–31.

WHITE, R. A. 1961. Vessels in roots of *Marsilea. Science* 133: 1073–1074.

———. 1962. A comparative study of the tracheary elements of the ferns. Dissertation, University of Michigan, Ann Arbor, MI.

WOLF, P. G., E. SHEFFIELD, AND C. H. HAUFLER. 1990. Genetic attributes of bracken as revealed by enzyme electrophoresis. *In* J. A. Thomson and R. T. Smith [eds.], Bracken biology and management, 71–78. Australian Institute of Agricultural Science, Sydney.

ZIMMERMANN, M. H. 1983. Xylem structure and the ascent of sap. Springer Verlag, Berlin.