

ORIGINS AND NATURE OF VESSELS IN MONOCOTYLEDONS. I. ACORUS

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Vessels are demonstrated in metaxylem of both roots and rhizomes of both species of *Acorus* (Acoraceae) by scanning electron microscopy (SEM). The end walls of vessel elements are characterized by perforations that retain porose pit membranes; the pores sometimes coalesce into larger openings. Lateral walls lack pores in primary walls as detectable by our SEM. Striations in pit membranes, as in Nymphaeaceae, were observed. Although vessel elements, as defined by porose pit membranes in end walls, occur in both roots and rhizomes, the long scalariform nature of end walls of vessel elements and the porose nature of the membranes are interpreted as primitive. Thus, the nature of vessels in *Acorus* is compatible with the recent idea that *Acorus* (Acoraceae) is the sister group to the remainder of the monocotyledons. Cheadle's (1942) view that presence of vessels only in roots is primitive in monocotyledons was based on light microscopy. The addition of SEM data has revealed new vessel distributions. The pores in pit membranes in end walls of vessels of *Acorus* are larger than pores in pit membranes in end walls of tracheids in vessel-less dicotyledons. The difference between vessel elements and tracheids in groups such as *Acorus* may be not the absence of pit membranes in perforation plates but the size of pores in the membranes of the perforation plates. The conductive capabilities and air bubble transmission capacities of pores of various sizes in pit membranes need physiological analysis in view of SEM findings on porosity in pit membranes of vessel perforation plates.

Introduction

In recent years, DNA-based phylogenies have given enhanced significance to the relationships of *Acorus*, often included in Araceae but now more often placed in its own family, Acoraceae. The results of Duvall et al. (1993) on *rbcL* sequence data, Nadot et al. (1995) on *rps4* sequence data, and Davis (1995) on chloroplast DNA restriction site variation place *Acorus* (and, in the case of Davis [1995], a second genus, *Gymnostachys*) in a basal position in monocotyledons. Davis (1995) opts for "the placement of either *Acorus* or *Acorus* + *Gymnostachys* as the earliest-diverging lineage within the monocots" (p. 521). French and Kessler (1989) find that *Gymnostachys*, which is less well known than *Acorus*, is also isolated; Duvall et al. (1993) offer strong support that *Gymnostachys* is an aroid, and the data of Davis (1995), who studied few genera, offered only weak support for a clade consisting of *Acorus* and *Gymnostachys*. We did not have material of *Gymnostachys*. Regardless of the ultimate placement of *Gymnostachys*, there is unanimous agreement that *Acorus* is the outgroup to the remainder of the monocotyledons. Thus, the nature of conductive tissue in *Acorus* is of prime importance in our understanding of evolution of xylem in monocotyledons.

Acoraceae was recognized as a family as long ago as 1822 by C. A. Agardh (see Takhtajan 1987). Until the molecular studies cited above added reasons for recognition of Acoraceae, the two genera *Acorus* and *Gymnostachys* had often been treated as a tribe, Acoreae, in the subfamily Pothoideae of Araceae (see, e.g., Dahlgren et al. 1985). Grayum (1987, 1990) favored removal of *Acorus* as Acoraceae. According to most authors, *Acorus* includes two species, *A. calamus* L., from eastern North America and eastern temperate

Asia (Siberia and Manchuria), and *A. gramineus* Ait., from Japan and Southeast Asia (Ohwi 1965). There are numerous synonyms for both species. *Acorus calamus* has become naturalized in Europe (Ohwi 1965).

Cheadle (1942) hypothesized that vessels in monocotyledons appeared first in roots, then phylogenetically spread into stems, leaves, and inflorescence axes. He further hypothesized that phylogenetic specialization of vessels, as judged from perforation plate morphology, has followed the same sequence. The data suggesting these conclusions have been updated by Wagner (1977). Until now, our knowledge of vessels and their perforation plates in monocotyledons has been based on light microscopy studies. Light microscopy is undoubtedly useful in defining the nature of perforation plates in monocotyledons that have marked differences in morphology between end walls and lateral walls (e.g., simple perforation plates). However, in tracheary elements of monocotyledons with long scalariform end walls, light microscopy cannot reliably give information about the presence or absence of pit membranes in the end walls. In some monocotyledons with long end walls, the morphology of the perforation plate differs from that of the lateral walls (perforations wider than lateral wall pits), leading to the assumption that pit membranes are lacking in what appears to be a perforation plate. This assumption may be based on the fact that this is frequently true in dicotyledons. However, in many "primitive" woody dicotyledons with a wide range of plesiomorphic features, scanning electron microscopy (SEM) reveals remnants of pit membranes in the perforations, ranging from extensive to vestigial (Carlquist 1992). Such SEM data, based on secondary xylem, are not applicable to monocotyledons, where one is concerned with primary xylem. However, our studies of two dicotyledon families that lack secondary xylem, Nymphaeaceae (Schneider and Carlquist 1995a, 1995b; Schneider et al. 1995) and Cabombaceae (Schneider and Carlquist 1996a, 1996b), have shown relatively little difference between

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end walls and lateral walls in secondary wall configurations using light microscopy. However, SEM shows that the end walls bear porose pit membranes, with porosities of varied sizes, whereas the lateral wall pits lack porosities, at least any resolvable with our equipment. Thus, the patterns seen in primitive woody dicotyledons with pit membrane remnants in perforation plates are similar to findings in Nymphaeaceae and Cabombaceae.

These tracheary elements with pit membrane remnants in perforation plates or with porose end walls form a series of morphological patterns, the significance of which has not yet been appreciated or widely discussed. Scalariform perforation plates devoid of pit membranes likely offer more resistance to water flow than do simple perforation plates, but the porose pit membranes on scalariform perforation plates with pit membrane remnants must offer considerably more friction. The physiological significance of such end walls needs to be investigated.

Reports of vessel distribution and morphology in monocotyledons (Cheadle 1942; Wagner 1977) are based on light microscopy. Because SEM reveals porosities in end walls that cannot be demonstrated by light microscopy, there is the possibility that vessel occurrence, as revealed with SEM, is more extensive than the distributions reported on the basis of light microscopy. Our study reports that vessel occurrence in *Acorus* is more extensive within the plant than hitherto has been described. Vessel distribution in a number of monocotyledons where the differences between vessel elements and tracheids are not pronounced should be reinvestigated. These are the monocotyledon families considered more primitive, if one compares the data of Cheadle (1942) and Wagner (1977) with the cladograms of Duvall et al. (1993) and Davis (1995). These are also monocotyledon families that are chiefly from more mesic habitats (Carlquist 1975). *Acorus* occurs in perpetually wet marshy areas (Ohwi 1965). As our knowledge of vessel morphology and distribution in monocotyledons increases, we will likely be able to achieve a better synthesis between vessel morphology and ecology.

We initiated SEM studies of xylem in monocotyledons with Acoraceae because its position in the cladograms of Duvall et al. (1993) and Davis (1995) makes it a likely candidate for the retention of numerous plesiomorphies. Thus, unless autapomorphies are quite common in the evolution of monocotyledon xylem, the Acoraceae are a monocotyledon family likely to have retained primitive xylary expressions. Acoraceae, along with other monocotyledon families reported by Cheadle

(1942) and Wagner (1977) to have vessels only in roots, and to have vessels with scalariform perforation plates, are likely to provide information on unspecialized character states in monocotyledon xylem and are therefore worthy of study with SEM. By study of such families, we are likely to find whether pit membrane remnants are common in perforation plates of vessels, as they appear to be in woody dicotyledons rich in plesiomorphic character states (Carlquist 1992).

Material and methods

Roots and stems of *Acorus calamus* and *Acorus gramineus*, stored in dilute aqueous ethyl alcohol, were obtained from the Vernon I. Cheadle Collection at the University of California, Santa Barbara. Because segments of stems and roots were preserved rather than entire plants, we do not know how many individual plants were present in these collections; we studied several stems and root segments of each species. Specimens from this collection were provided through the courtesy of Mary Cheadle, Jennifer Thorsch, and Wayne Ferren. Root and stem portions were embedded in paraffin and sectioned according to the commonly employed methods (Johansen 1940). Both transverse and radial longitudinal sections of roots and stems (rhizomes) were prepared. Some sections were mounted on aluminum stubs, cleansed of paraffin, sputter-coated, and examined with a Bausch and Lomb Nanolab SEM. Other sections to be used for light microscopy were mounted on glass slides and stained with a safranin-fast green combination corresponding to Northen's modification of Foster's tannic acid-ferric chloride method (Johansen 1940).

Material of *A. calamus* (Cheadle M-77) was collected near Kingston, Rhode Island. The specimen of *A. gramineus* (Cheadle 1252) was cultivated in Florida by Monroe Birdsey and sent via P. B. Tomlinson to Vernon I. Cheadle.

Results

Radial longisections of roots of *Acorus gramineus* (figs. 1-4) show very clearly the porose nature of pit membranes on the end walls of vessel elements. About two-thirds of an end wall is shown (fig. 1). Between the bars of the scalariform perforation plate (fig. 1), all degrees of porosity may be seen, ranging from relatively large holes occupying up to half of a perforation area to minute circular holes. In ca. five or six of the pit membranes at the top of the photograph (fig. 1), pores are absent or minimal. The pits lacking pores should not be considered part of the perforation plate, although there is not a sharp demarcation between pits that lack pores in pit membranes and those in which a few pores may be found. The perforation plate portion (fig. 2) illustrates numerous small circular pores in pit membrane remnants, some of which have coalesced in groups into larger holes. In the perforation

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Figs. 1-4 Portions of vessels from roots of *Acorus gramineus* from SEM photographs. Fig. 1, About two-thirds of a perforation plate; pores ranging from minute to large may be seen in most of the perforations. Fig. 2, Portion of a perforation plate, showing numerous small circular pores, some of which have coalesced into larger holes, in the pit membranes. Fig. 3, Portion of a perforation plate in which pores are large, separated from each other mostly by threadlike remnants of the pit membranes. Fig. 4, Lateral wall of vessel element; pores are absent in pit membranes. Bars in all figures = 5 μ m.

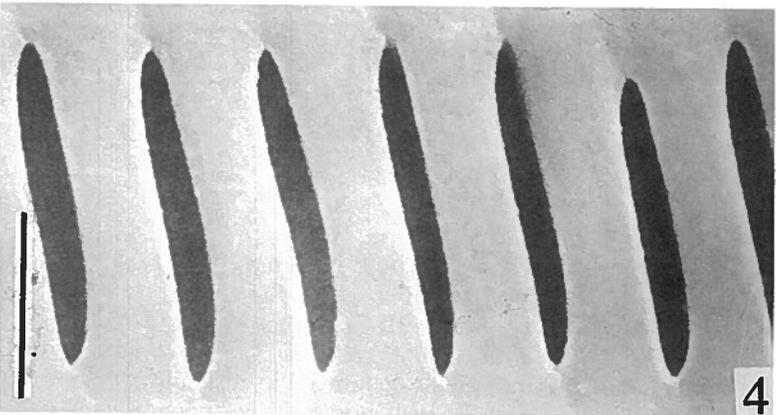
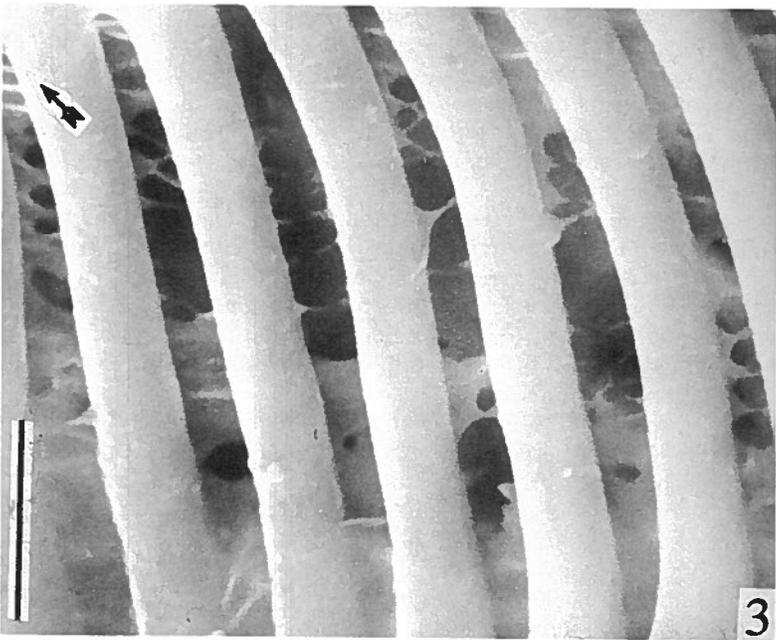
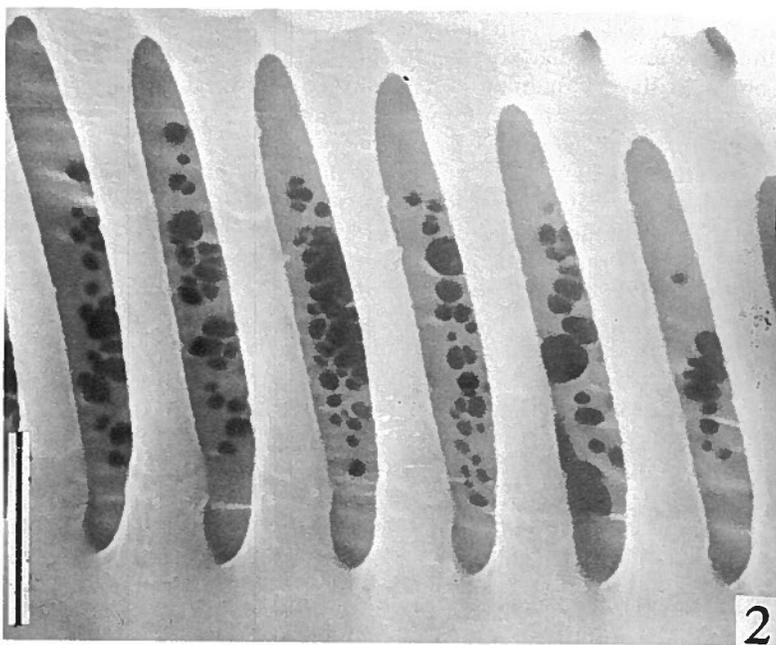
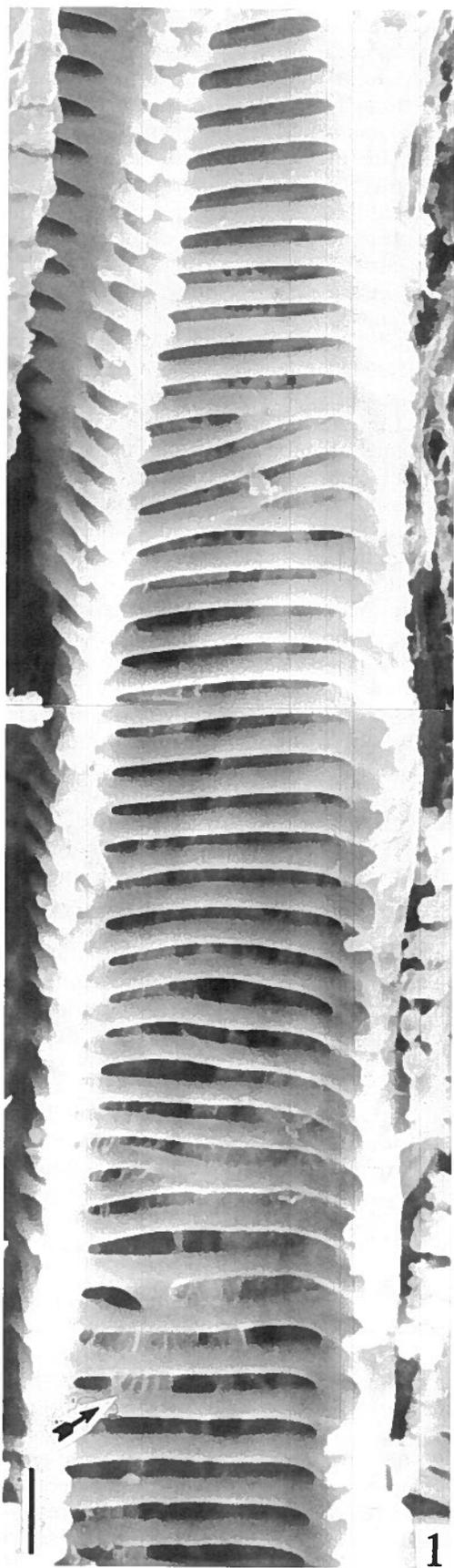


plate (fig. 3), lysis of pit membranes is more extensive. Although some circular pores are evident (fig. 3), larger holes in the pit membranes, separated from each other by threadlike pit membrane remnants, are present.

The lateral wall pitting of *A. gramineus* (fig. 4) shows narrower pit areas and wider wall bands between them as compared to the perforation plates (figs. 1–3). No pores could be detected in the pit membranes of lateral wall pits of vessels. A portion of a lateral wall of a root tracheary element of *Acorus calamus* (fig. 5) shows scalariform and transitional pitting. Striations are evident in the pit membrane of the lateral wall pitting shown in figure 5 (arrow). Striations in pit membranes are evident in the perforation plates of *A. gramineus* (fig. 1, near bottom, arrow; fig. 3, arrow at upper left).

The perforation plate portion of an *A. calamus* root (fig. 6) illustrates perforations as seen from the lumen side of the cell; absence of a pit membrane in the center perforation may be an artifact, but the circular pores in the lower perforation are undoubtedly natural. The perforation plate portion of an *A. calamus* root (fig. 7) illustrates perforations as seen from the outside of a tracheary element; the primary wall is intact despite separation of this vessel element from a neighboring vessel element. Striations are prominent in the pit membrane remnants, and circular pores are conspicuous in the membranes.

End walls are represented in the rhizome tracheary element portions shown (figs. 8 and 9). In both of these photographs, end walls are represented. The narrowness of the perforations, however, is more like the narrow lateral wall pits of tracheary elements than is true of roots: there is less differentiation of end walls from lateral walls in rhizomes than in roots of *Acorus*. A perforation plate of *A. calamus* (fig. 8) viewed from the lumen side of the cell shows an irregular surface on the secondary wall. In most of the perforations shown in figure 8, several circular to oval pores can be observed. The rhizome vessel element of *A. gramineus* (fig. 9) is viewed from the outside of the wall; the adjacent vessel element has been sectioned away. Two perforations shown (fig. 9) lack any wall remnants; this absence may be an artifact due to the sectioning process. The remainder of the perforations (fig. 9) contain intact pit membranes with one or more pores. We have not figured lateral wall pits for the rhizomes of the two *Acorus* species; they are much like the perforations except that, as in the roots, the lateral wall pits lack any perceptible pores.

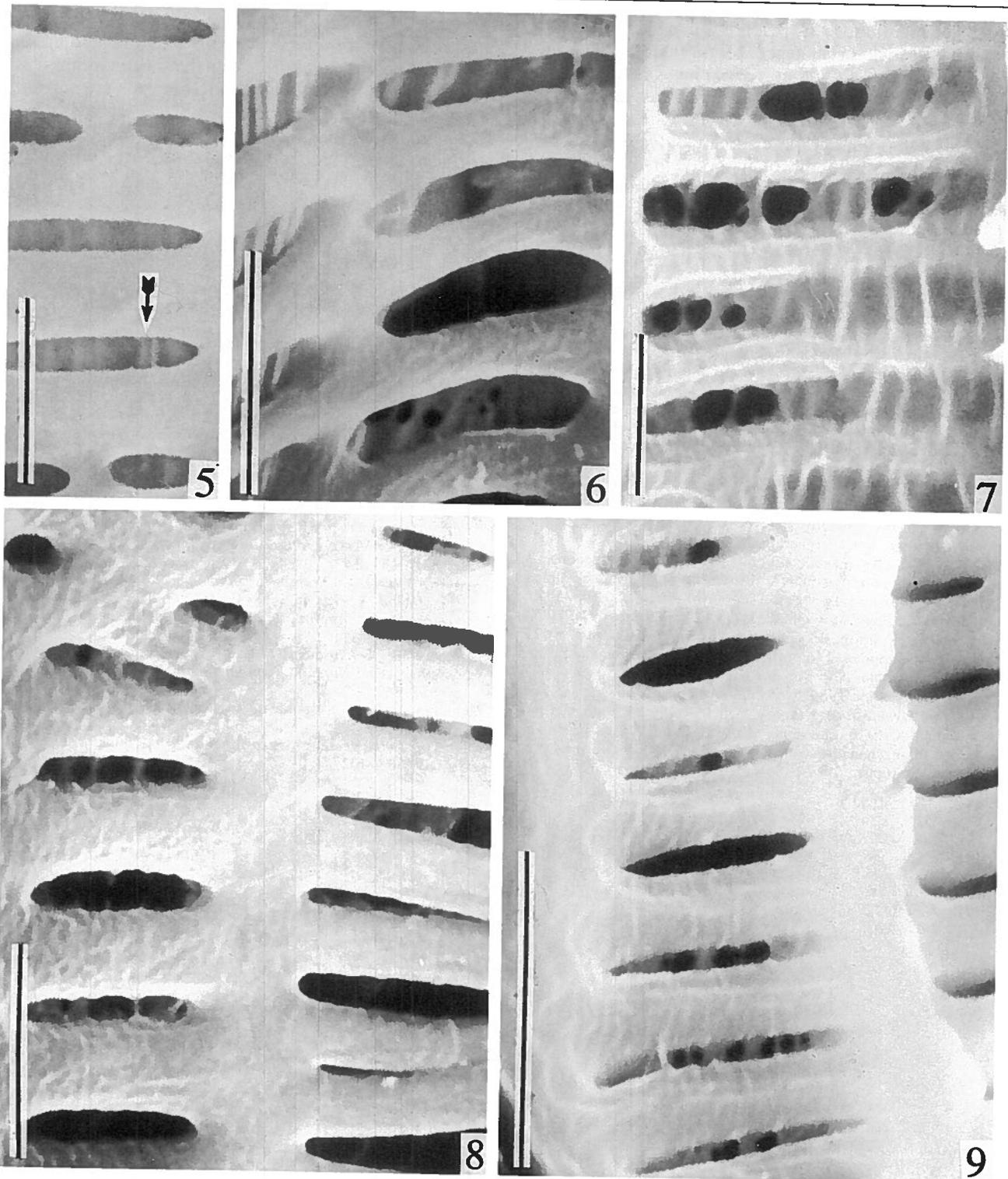
Discussion

Cheadle (1942) and Wagner (1977) reported that in Araceae (in which they included *Acorus*) vessels are restricted to roots. Cheadle (1942) states that in Araceae, "the stems of several (including *Acorus calamus* L.) were injected with India ink to confirm the absence of vessels" (p. 444). Using SEM, however, we conclude that porose pit membranes are present in end

walls of vessel elements of both roots and stems of both species of *Acorus*, and we designate these cells as vessels. The lateral walls of tracheary elements in both roots and stems of both species lack any perceptible pores. The pores in the pit membranes of the vessel elements are often small, and their small diameter doubtless likely confers higher friction for water flow than would be the case if no pit membrane remnants were present. The pores in pit membranes of perforation plates of *Acorus* are generally larger than the pores found in the margo regions of conifer tracheid pit membranes (Frey-Wyssling 1976). The *Acorus* pores are also larger than pores illustrated in end wall pits of tracheids of the vessel-less dicotyledons *Bubbia* (Carlquist 1983) and *Tetracentron* (Carlquist 1988). While noting the larger size of pores in the end wall pit membranes of *Acorus*, we note that the definition of a vessel element is changing as a result of SEM studies: vessels can no longer be defined in terms of absence of pit membranes on end walls, but we must take into account the size of pores in membranes of end walls in those angiosperms in which extensive pit membrane remnants are present in perforations. Coalescence of neighboring pores into larger holes can be considered one possible criterion of a vessel. The pit membrane remnants in perforations, and the pores of moderate size in these remnants can be considered primitive expressions of vessel formation, indicating incomplete lysis of the pit membrane at vessel element maturation. These primitive structural conditions in *Acorus* agree with the basal position accorded Acoraceae by Duvall et al. (1993), Davis (1995), and Nadot et al. (1995) and is in agreement with the designation of pit membrane remnants in perforation plates of dicotyledons as a plesiomorphic character state (Carlquist 1992). The long scalariform perforation plates of the primitive woody dicotyledons in which pit remnants occur and the presence of such remnants are features that could provide friction for water flow, in comparison to the minimal friction offered by vessels with simple perforation plates. The relatively greater friction of the primitive perforation plates is likely not of much negative selective value in plants of wet or marshy places where water is perpetually available to roots and where transpiration is moderate because leaf surfaces are relatively restricted. *Acorus* ecology and leaf morphology (Ohwi 1965) accord with these conditions.

The striate nature of pit membranes in end walls and also in some lateral walls of vessels of *Acorus* is noteworthy, because similar striations occur in primary xylem of dicotyledons. We reported this condition for Nymphaeaceae in *Barclaya* (Schneider and Carlquist 1995b) and *Euryale* and *Victoria* (Schneider and Carlquist 1995a). Such striations may be much more widely distributed in vascular plants—we found them in the fern *Woodsia* (Carlquist and Schneider, in press)—but primary xylem has not, as yet, been sampled extensively with SEM.

SEM offers an ideal tool with which to examine



Figs. 5-9 SEM photographs of vessel elements from roots and rhizomes of *Acorus*. Figs. 5-7, Vessel portions from roots of *Acorus calamus*. Fig. 5, Portion of lateral wall; pits are scalariform and transitional and have somewhat striate membranes. Fig. 6, Portion of perforation plate, seen from inside of vessel; perforation at bottom contains small pores; pits along left edge of photograph have striate membranes. Fig. 7, Portion of perforation plate, seen from outside of vessel; pit membranes are markedly striate; relatively large circular and oval pores in membranes are evident. Figs. 8, 9, Portions of perforation plates from rhizomes of *Acorus*. Fig. 8, Perforations from *A. calamus*, seen from inside of vessel; perforations at left contain several large pores each. Fig. 9, Perforations from *Acorus gramineus* seen from outside of vessel; although two wider perforations lack pit membranes (likely an artifact), the others contain membranes in which one to several circular pores are evident. Bars in all figures = 5 μ m.

porose pit membranes in perforation plates and demonstrate presence or absence of pit membrane remnants. Our results show that *Acorus* has vessels in both roots and stems, rather than just in roots as claimed by Cheadle (1942). According to the Cheadle criteria of progressive organographic spread and specialization of vessels throughout the plant body in monocotyledons, this would represent a moderate advance in vessel phyletic over presence of vessels in roots only.

However, the morphology of vessels in *Acorus* is of a markedly primitive type. Moreover, SEM studies may reveal vessels to be more extensive organographically in other monocotyledons in which vessels are currently reported in roots. In any case, our data are not in conflict with the basal position hypothesized for *Acorus* within monocotyledons by Duvall et al. (1993), Davis (1995), and Nadot et al. (1995).

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