PERFORATION PLATE PIT MEMBRANE REMNANTS AND OTHER VESSEL DETAILS OF CLETHRACEAE: PRIMITIVE FEATURES IN WOOD OF ERICALES

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Wood of 12 species of *Clethra* was studied with SEM; wood features are illustrated here for eight of these. Pit membrane remnants were observed in perforations of all species, although in some, the remnants occur only in the form of small webs at the lateral ends of perforations. Pit membranes take the form of threads or porose sheers; the former are more common. Extensive pit membrane remnants are uncommon in tropical species and are more characteristic of temperate species, perhaps because of water flow characteristics. As with other dicotyledons, occurrence of pit membrane remnants in perforations is considered a primitive feature in Clethraceae. Pit membrane remnants also occur in families of Ericales placed in primitive positions in the order and close to Clethraceae: Cyrillaceae and Sarraceniaceae. Bars of perforation plates are numerous and are prominently bordered. Grooves interconnecting pit apertures occur in distal portions of vessel elements and are accompanied by pairs of ridges in some species; this feature has not been reported so far either in Clethraceae or in Ericales. Grooves associated with pit apertures are very short or absent in central portions of vessel elements. The significance of groove occurrence on vessel walls is discussed. Presence of pit membrane remnants is clearly correlated with mesic habitats in which moisture availability is constant and may indicate, for the phylad, uninterrupted occupancy of such habitats.

Keywords: coalescent pit apertures, Ericales, grooves interconnecting pit apertures, perforation plates, primitive wood structure, Theales, vessel evolution, wood evolution, xylem.

Introduction

Clethraceae consist of a single genus, *Clethra*, containing 64 shrubby to arborescent species (Sleumer 1967; Thorne 2001). *Clethra* ranges from Japan, Korea, and China southward through Indomalesia to New Guinea; in the New World, it extends from the eastern United States through Mexico, Central America, and the Windward Islands and into South America as far south as Argentina; a single species occurs on Madeira (Sleumer 1967; Thorne 2001). Although placed in Theales until recently (Thorne 2001; historical review in Giebel and Dickison 1976), molecular data have shown that the concept of Theales should be included within Ericales (Soltis et al. 2000). According to that study, the closest relatives of Clethraceae are Actinidiaceae, Cyrillaceae, Ericaceae, and Sarraceniaceae. Although Ericales contain families with wood showing various degrees of phylogenetic advancement according to widely accepted criteria of wood evolution (Carlquist 2001), the woods of Clethraceae and the four families closely related to it qualify as primitive on most grounds. Pit membrane remnants in perforation plates have been reported so far in two species of *Clethra*, *Clethra arborea* Ait. and *Clethra barbinervis* from Japan (Carlquist 1992). Because pit membrane remnants have been found in the perforations of scalariform perforation plates of Cyrillaceae

(Carlquist 1992; Schneider and Carlquist 2003*b*), Ericaceae (Ohtani and Ishida 1978), and *Heliamphora* of the Sarraceniaceae (Carlquist 1992), the woods of these families may be expected to contain other features that are primitive and represent symplesiomorphies within Ericales. The relatively few reports of pit membrane remnants in vessel perforations in the families motivate one to search these families more intensively for this phenomenon, and this article is one contribution in this regard.

Pit membrane remnants are not a single phenomenon but take a wide range of forms, and the distribution of these forms within genera can be of considerable interest (Carlquist and Schneider 2002; Schneider and Carlquist 2003*a*). The ecological significance of retention of pit membrane remnants is also of potential interest, and evidence may be developed from the distribution of this phenomenon within the families that exhibit these structures. Until relatively recently, the use of scanning electron microscopy (SEM) was not applied to many woods, so that investigation of this feature with SEM in wood of families well surveyed with light microscopy is a timely enterprise.

The presence of pit membrane remnants in perforations of scalariform perforation plates in families proposed as closely related and relatively primitive within their orders, as indicated by molecular studies, offers other opportunities. The presence of pit membranes, if correlated with other putatively primitive features, adds to our understanding of symplesiomorphic features in wood evolution. In addition, the ecological distribution of species with these wood features becomes a concern: are primitive wood features retained in

phylads with an unbroken history of occupation of mesic sites? The ecological distribution of scalariform perforation plates indicates that this is a likely interpretation (Carlquist 1975). As each group with primitive wood features is more carefully examined in the light of molecular phylogenies, our understanding of the systematic and ecological distribution of wood anatomical character states becomes increasingly clear.

Material and Methods

Clethra, the basis for this study, contains the following species: *Clethra acuminata* Michx., North Carolina, USw-32368; *Clethra barbinervis* Sieb. & Zucc., Japan, USw-23745; *Clethra bicolor* HBK., Venezuela, USw-25371; *Clethra brasiliensis* Cham. and Schlect., Brazil, USw-31594; *Clethra hondurensis* Britton, Belize, Usw-31941; *Clethra mexicana* DC., Mexico, USw-32261; *Clethra ovalifolia* Turcz., Cerro Negro, Bolivar, Ecuador, USw-21297; *Clethra pubifolia* Merrill, Indonesia, USw-28957; *Clethra salvadorensis* Britton, El Volcan, Salvador, USw-20142; *Clethra scabra* Pers., Brazil, USw-25561.

All wood samples were available in dried form. They were boiled in water, stored in 50% aqueous ethanol, and sectioned with a sliding microtome. Only radial sections were prepared. The sections were dried between clean slides, mounted on stubs, sputter-coated, and examined with a Hitachi S2600N scanning electron microscope with an accelerating voltage of 25 kV. The use of sections prepared with these methods does not, in our opinion, result in formation of artifacts other than obvious rips or distortions in the pit membranes of perforations. Comparison of several methods of preparation for a species characteristically known to have pit membrane remnants (Schneider and Carlquist 2003a) was used as a test for potential artifact formation, but differences among the methods of preparation were not evident. Also arguing for the natural appearance of the membrane remnants that we have illustrated is the fact that the pit membranes at the upper and lower ends of perforation plates show transitions (via intact membranes with pores in pitlike perforations or perforation-like pits) between perforations and lateral wall pits (fig. 1C, *bottom*; fig. 2A). Lateral-wall pitting does not show porose pit membranes. If pit membrane remnant presence is minimal, the membranes are restricted to the lateral ends of perforations (fig. 3A, 3E). Ripping and other obvious artifacts are readily discerned (fig. 1A, 1B; fig. 3F, *bottom*). Accumulations of stray materials on pit membrane remnants such as starch grains can be readily identified (figs. 1E, 3F, *top*). Also obvious are accretions of secondary plant compounds on membrane surfaces: they produce rugose surfaces and evidently prevent enzymatic dissolution of the pit membrane. Other workers have not questioned the presence of pit membrane remnants as a valid phenomenon (Meylan and Butterfield 1978; Ohtani and Ishida 1978), and even when all possible artifacts are taken into account, a genuine anatomical phenomenon remains. Resolution of objects in concave contexts with SEM is more difficult than that for convex objects (e.g., pollen grains), but the scanning electron microscope used produces sharp images up to ca. 15,000 ×.

Anatomical terms follow, in general, the IAWA Committee on Nomenclature (1964). These terms as well as additional ones used here are presented and defined in Carlquist (2001). Illustrations of vessel portions of the species of *Clethra* are arranged here in alphabetical order.

Results

Pit Membrane Remnants in Perforations of Vessel Elements

The mean number of bars in *Clethra* is high; in *Clethra brasiliensis*, we observed some perforation plates with more than 100 bars, but the mean for the species was well below that. A perforation plate, typical for *Clethra*, demonstrates not only the large number of bars typical for the genus but also the narrow spaces between the bars (fig. 3C). Narrowness of perforations seems frequently correlated with presence of pit membrane remnants. However, in the perforation plate of *Clethra luzonica* (fig. 3C), only the most minimal pit membrane remnants (in lateral ends of perforations) were observed. Perforation plates with a reticulate morphology (fig. 3D) occur occasionally in Clethraceae.

In the species of *Clethra* examined, pit membrane presence ranged from characteristically present as threads or porose membranes to quite rare or minimal in size. The species for which no SEM illustrations are offered fall into the latter category. Our illustrations are intended to represent not a typical picture of this phenomenon in the genus but rather instances of presence of pit membrane remnants and the diverse morphological appearance of pit membrane remnants in these species.

Sheetlike pit membranes perforated by holes or holes of various sizes proved common in perforations of our material of *Clethra hondurensis* (fig. 4C) and *Clethra ovalifolia* (fig. 4F, 4G). Sizes, shapes, and groupings of pores or holes in the pit membranes appear random. Perforations that are transitional between the perforation plate and lateral wall pitting typically bear membranes perforated by small pores (fig. 1C, *top upper right, bottom*).

Threadlike pit membrane remnants cover less area of the perforation than do the porose sheetlike pit membranes but are quite conspicuous. Threadlike remnants can be regarded as forming network-like patterns (fig. 1B, 1D). These threads are not always discrete and independent but often tend to form weblike fusions (fig. 1A, *upper right*; various places in fig. 1E, 1F).

A third category is formed by the presence of pit membrane remnants only in the lateral ends of perforations (fig. 3A, 3E; fig. 4A, 4B). An instance of minimal presence of such a remnant is illustrated in figure 3E. More abundant pit membrane presence, transitional to a sheetlike disposition, occurs in the upper perforation of figure 4B.

Sculpturing on Lateral Walls of Vessels

The lateral wall pits of vessels are scalariform or opposite. In central portions of vessel elements, we consistently observed grooves interconnecting pit membranes (figs. 3B, 4E). If pits are isolated, the groove may extend for a short
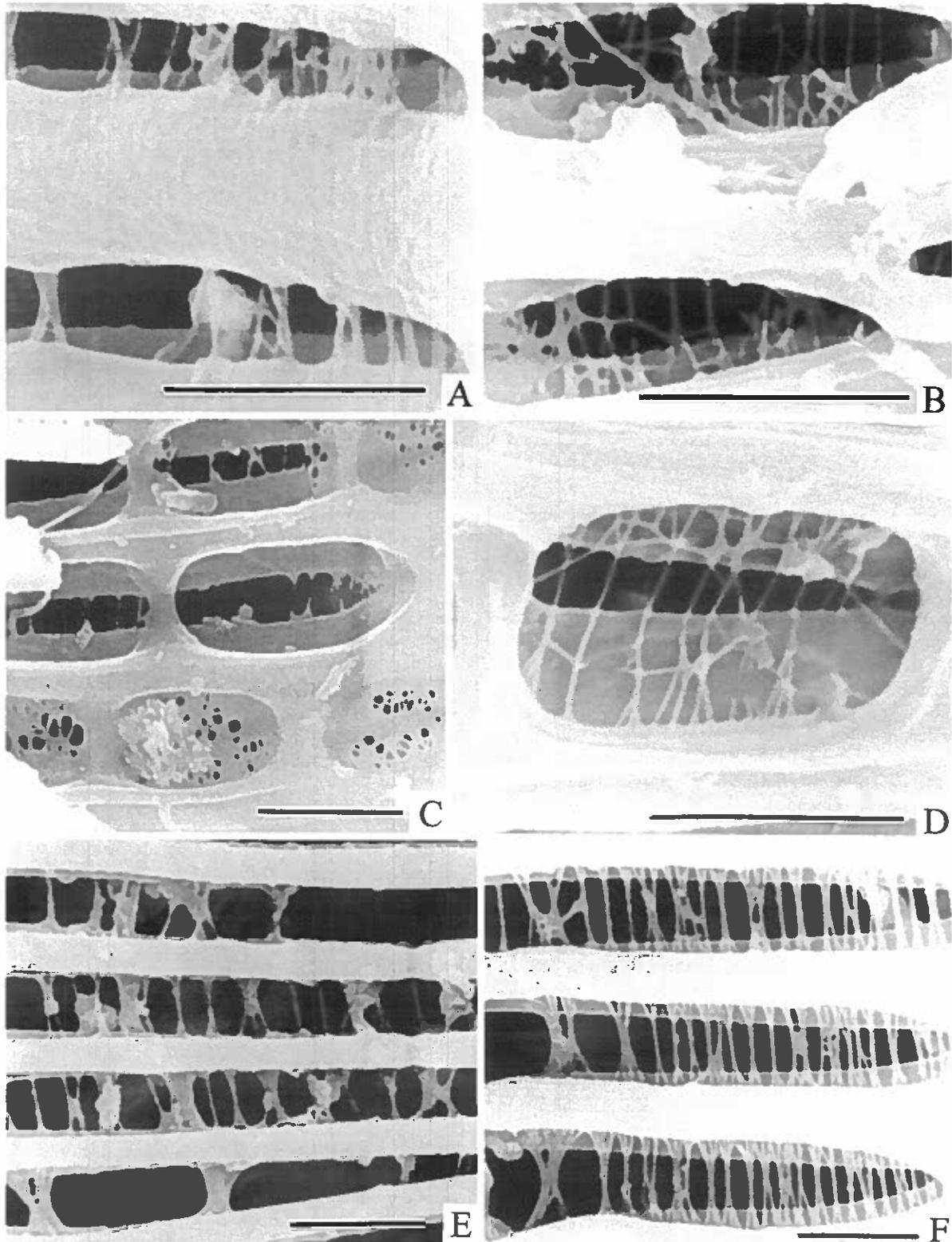
Fig. 1 SEM photographs of perforation plate portions of *Clethra acuminata* (A–D) and *Clethra barbinervis* (E, F); bars oriented horizontally. A, Threadlike remnants fused together in places to form porose sheets. B, Threadlike remnants that form a reticulate pattern. C, Perforations at tip of perforation plate; wall of one vessel element present. D, Short perforation with reticulate pattern of delicate threads. E, Threads and small sheetlike remnants interconnecting the bars of a perforation plate. F, Numerous threadlike remnants tending to be fused adjacent threads. Scale bars = 3 μm.
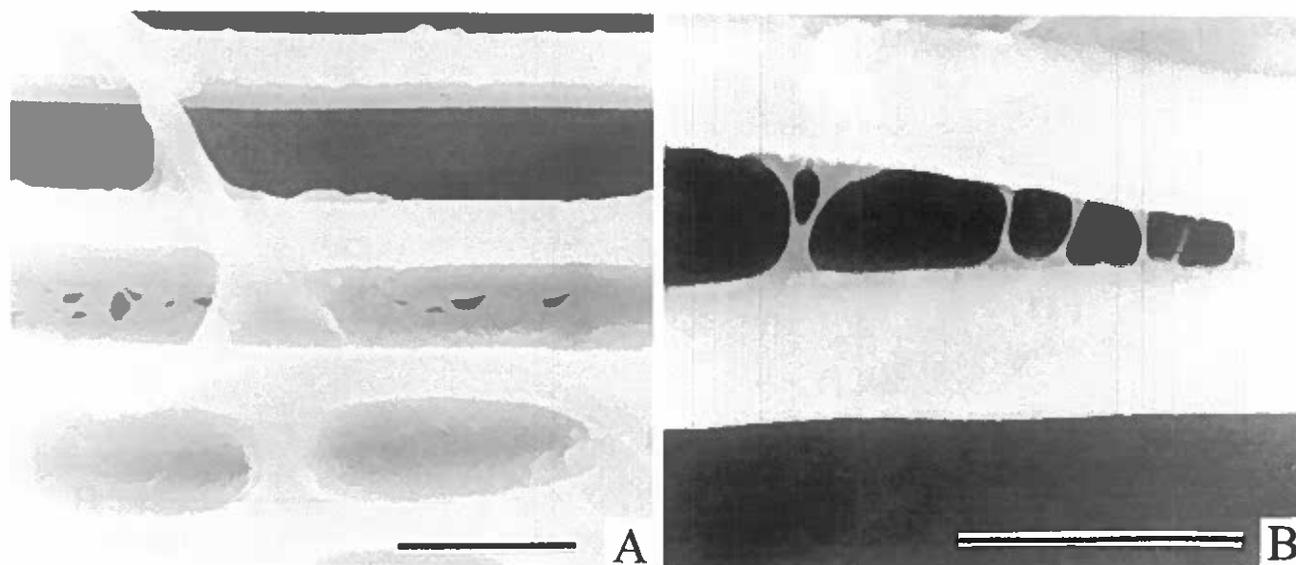
Fig. 2 SEM photographs of perforation plate portions of *Clethra pubifolia* (A) and *Clethra scabra* (B); bars oriented horizontally. A, Transition between perforation plate (*top*, perforation clear of pit membrane) and lateral wall pitting (*bottom*, intact pit membranes). Pit in center contains pores in pit membrane; a diagonal contaminant is present, left of center. B, Portions of two perforations; the upper contains a few threadlike membrane remnants. Scale bars = 3 µm.

distance on either side of the pit aperture. In the tapering distal portions of vessel elements, grooves interconnecting pit apertures (coalescent pit apertures) are present, but in addition, ridges occur (fig. 4F). These ridges are predominantly in pairs flanking the grooves. Helical sculpturing on vessel walls of dicotyledons is not at all uniform when studied with SEM, and in order to categorize these phenomena properly, SEM studies that reveal details not readily seen with light microscopy are to be recommended.

Discussion and Conclusions

The systematic occurrence of pit membrane remnants in sclariform perforation plates seems to indicate clearly a correlation with other primitive wood features (Carlquist 1992). In *Clethra*, these features include great length of vessel elements (averaging more than 1000 µm: Giebel and Dickison 1976, their table 2), long scalariform perforation plates with numerous bordered bars associated with narrow perforations, and scalariform to opposite lateral wall pitting (often scalariform on intervascular contacts, which are chiefly on vessel element endwalls adjacent to the perforation plates). Vessels are angular in transectional outline in Clethraceae (Giebel and Dickison 1976).

The imperforate tracheary elements on *Clethra* seem best designated as tracheids, in accord with the definitions of the IAWA Committee on Nomenclature (1964). Tracheid presence is important because it is related to other primitive features, such as the vessel features presented in this study. The illustrations of Giebel and Dickison (1976, their figs. 4, 5, 7) clearly show rather densely placed circular pits with wide borders on imperforate tracheary elements. This description is also present in the summary for the family by Metcalfe

and Chalk (1950). Moreover, there is a confirmatory piece of evidence. Giebel and Dickison (1976) state, "The pore distribution of most specimens ranges between 95 and 100 percent solitary, with only two samples ... having less than 90 percent solitary vessels." The presence of tracheids as the imperforate tracheary element type offers a subsidiary conductive system, as has been demonstrated by Braun (1970), and has been shown to be related to a lack of vessel grouping (Carlquist 1984). In terms of relationships of Clethraceae, one should note that tracheids occur in Sarraceniaceae (De Buhr 1977), Cyrillaceae (Metcalfe and Chalk 1950; Carlquist 2001), Actinidiaceae (Carlquist 2001), and Ericaceae (Carlquist 2001). The occurrence of tracheids in all of these families marks tracheid presence as a symplesiomorphy for the ericalean clade that includes all of these families (in the cladogram of Soltis et al. 2000).

Parenchyma conditions in Clethraceae are also primitive according to widely accepted criteria (2001). The axial parenchyma in the family is diffuse or diffuse in aggregates, and the rays qualify as heterogeneous type I. These parenchyma conditions occur in Actinidiaceae, Cyrillaceae, Ericaceae, and Sarraceniaceae (Metcalfe and Chalk 1950; De Buhr 1977).

Thus, the clade of Ericales into which Clethraceae fall retains highly primitive wood conditions, a fact that likely indicates continued occupancy of mesic sites. Exceptions may be found in the woods of more specialized Ericaceae, such as *Arctostaphylos*, which have radiated into drier habitats (Carlquist and Hoekman 1985). A minor deviation from the primitive vessel conditions in *Clethra* that may be correlated with greater flow rates in the secondary xylem is the relative absence of pit membrane remnants in the perforation plates of the tropical species we studied (e.g., *Clethra brasiliensis*, *Clethra salvadorensis*). Another moderate deviation from
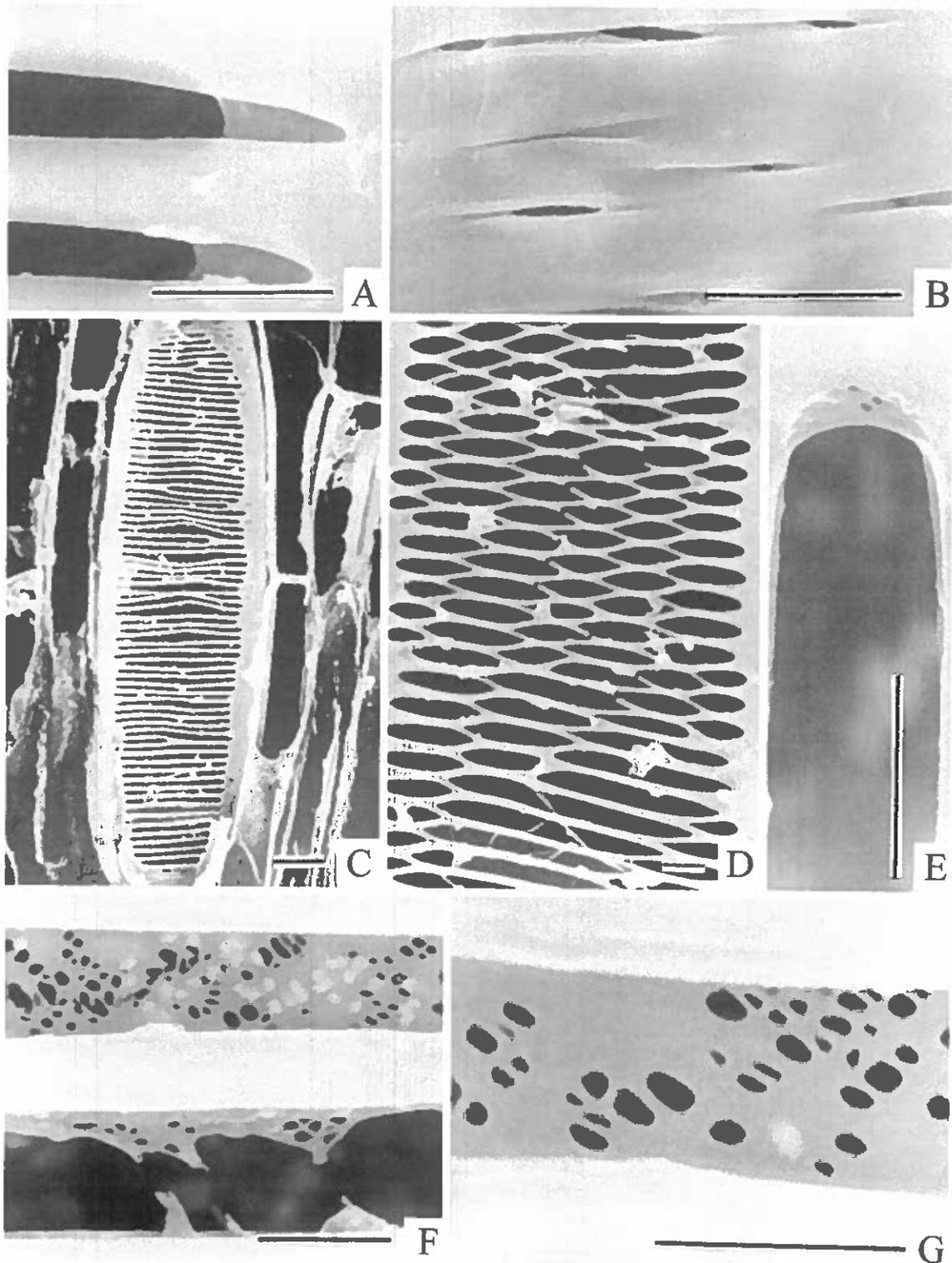
Fig. 3 SEM photographs of perforation plates and plate portions of *Clethra luzonica*. *A*, Two perforations to show presence of pit membrane remnants; no pores are evident in the pit membranes. *B*, Lateral wall from central portion of vessel to show grooves that mostly interconnect pit apertures or else extend laterally from a single pit aperture. *C*, Entirety of a perforation plate, which is almost perfectly scalariform (a few bars fork); number of bars is typical for most species of the genus. *D*, Portion of a reticulate perforation plate, with perforations of various lengths. *E*, Perforation with pit membrane at lateral end of perforation (*top*); the membrane contains a few inconspicuous pores. *F*, Portions of adjacent perforations to show intact (*top*) and ruptured (*bottom*) pit membranes; white spots on upper membrane are an unidentified contaminant. *G*, Portion of a pit membrane from a perforation to show scattered groups of pores. Scale bars = 3 μm except for C (20 μm) and D (5 μm).
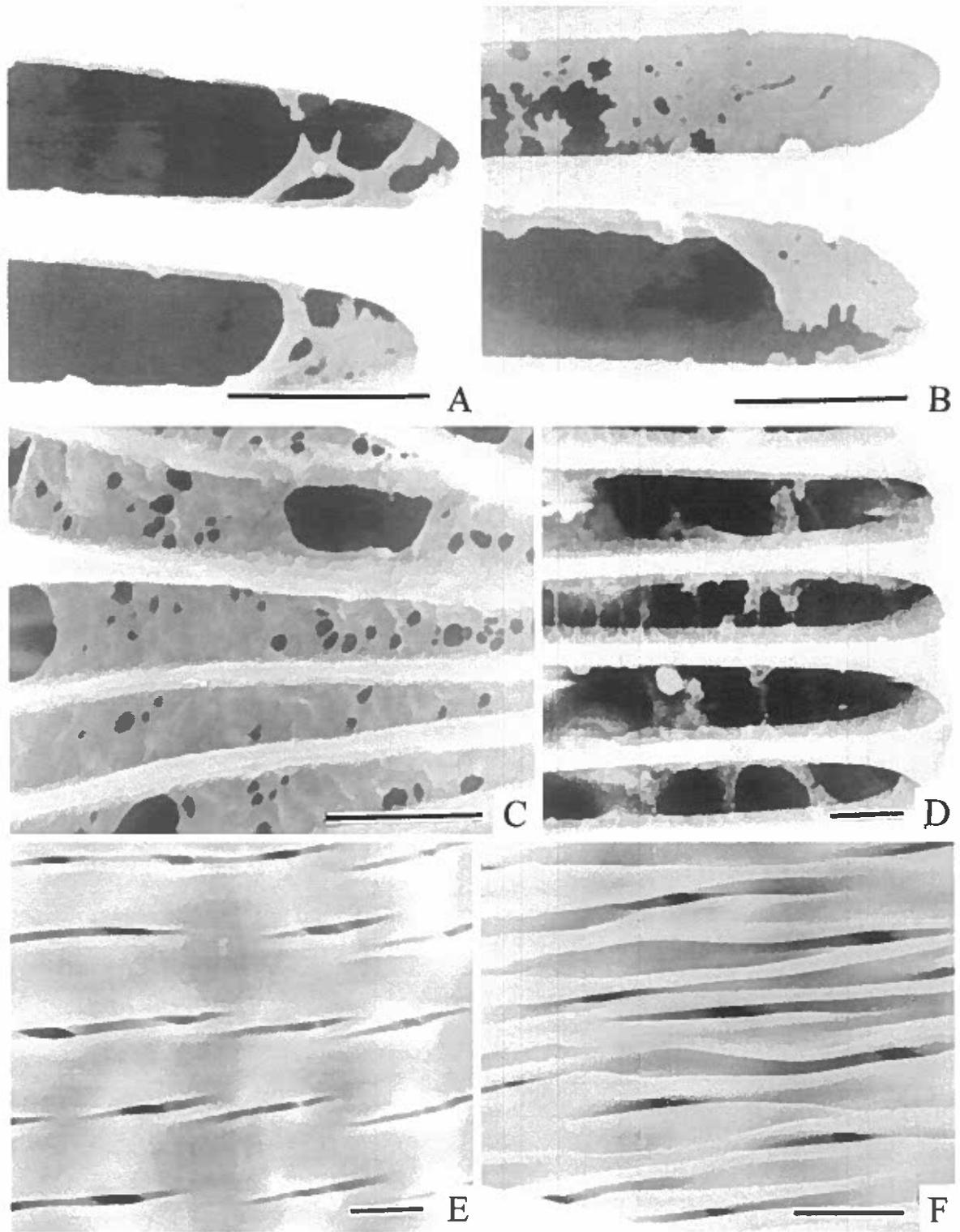
Fig. 4 SEM photographs of perforation plate portions of *Clethra bicolor* (*A*, *B*) and *Clethra hondurensis* (*C–F*); bars oriented horizontally. *A*, Pit membrane remnants restricted to lateral ends of perforations. *B*, Pit membrane remnants restricted to lateral ends of perforations but more extensive and bearing pores of various sizes; rips evident in both of the pit membranes are artifacts. *C*, Portion of a perforation plate with membranes containing numerous near circular holes as well as a few larger openings. *D*, Portion of a pit membrane with threadlike membrane remnants. *E*, Lateral wall portion from central portion of vessel showing grooves interconnecting pit apertures. *F*, Portion of a vessel lateral wall opposite a perforation plate in the distal portion of a vessel element, showing ridges accompanying the grooves interconnecting the pit apertures. Scale bars = 3 μm.

uniformly primitive wood conditions may be found in *Clethra barbinervis* (Japan), which has well-marked growth rings, vessels circular in outline, and vessels with relatively great diameter. The contrast between freezing conditions in winter and warm, humid conditions in summer in localities where *C. barbinervis* occurs is doubtless related to these character states. The distribution of woods with primitive characteristics, suited for ecological conditions that feature relatively uniform temperatures and relatively constant moisture availability, should be examined with relation to the relatively precise molecular phylogenies now available. The inherent interest of such comparisons is whether particular phylads can retain primitive wood conditions regardless of change in other characters and the degree to which reversibility in evolution of wood characters occurs.

Acknowledgments

Literature Cited

Braun HJ 1970 Funktionelle Histologie der sekundären Sprossachse. I. Das Holz Handbuch der Pflanzenanatomie. Spezieller Teil, Band IV, Teil 1. Gebrüder Borntraeger, Berlin.

Carlquist S 1975 Ecological strategies of xylem evolution. University of California Press, Berkeley.

——— 1984 Vessel grouping in dicotyledons: significance and relationship to imperforate tracheary elements. Aliso 10:505–525.

——— 1992 Pit membrane remnants in perforation plates of primitive dicotyledons and their significance. Am J Bot 79:660–672.

——— 2001 Comparative wood anatomy. 2d ed. Springer, Berlin.

Carlquist S, DA Hoekman 1985 Ecological wood anatomy of the woody southern California flora. IAWA (Int Assoc Wood Anat) Bull, NS, 6:329–347.

Carlquist S, EL Schneider 2002 Vessels of *Illicium* (Illiciaceae): range of pit membrane remnant presence perforations and other vessel details. Int J Plant Sci 163:755–763.

De Buhr L 1977 Wood anatomy of Sarraceniaceae: ecological and evolutionary implications. Plant Syst Evol 128:159–169.

Giebel KF, WC Dickison 1976 Wood anatomy of Clethraceae. J Elisha Mitchell Sci Soc 92:17–26.

IAWA Committee on Nomenclature 1964 Multilingual glossary of terms used in wood anatomy. Verlagsbuchanstalt Buchdruckerei Konkordia, Winterthur.

Metcalfe CR, L Chalk 1950 Anatomy of the dicotyledons. Clarendon, Oxford.

Meylan BA, BG Butterfield 1978 The structure of New Zealand woods. New Zealand Department of Scientific and Industrial Research, Wellington.

Ohtani J, S Ishida 1978 An observation of the perforation plates in Japanese dicotyledonous woods using scanning electron microscopy. Res Bull Coll Exp For Coll Agric Hokkaido Univ 35:65–98.

Schneider EL, S Carlquist 2003a Perforation plate diversity in *Illicium floridanum* with respect to organs, provenance, and microtechnical methods. Sida Contrib Bot 30:1047–1057.

——— 2003b Unusual pit membrane remnants in perforation plates of Cyrillaceae. J Torrey Bot Soc 130:225–230.

Sleumer H 1967 Monographie Clethracearum. Bot Jahrb Syst 87: 36–175.

Soltis DE, PS Soltis, MW Chase, ME Mort, DC Albach, M Zanis, V Savolainen, et al 2000 Angiosperm phylogeny inferred from 19S rDNA, *rbcL*, and *atpB* sequences. Bot J Linn Soc 133: 381–461.

Thorne RF 2001 The classification and geography of flowering plants: dicotyledons of the class Angiospermae (subclasses Magnoliidae, Ranunculidae, Dilleniidae, Rosidae, Asteridae, and Lamiidae). Bot Rev 66:441–647.