Pit membrane remnants in perforation plates and other vessel details of Cornales

EDWARD L. SCHNEIDER[1] AND SHERWIN CARLQUIST

Schneider, E. L. & S. Carlquist (Santa Barbara Botanic Garden, 1212 Mission Canyon Rd., Santa Barbara, CA 93105, U.S.A.; e-mail for ELS: eschneider@sbbg.org; email for SC: s.carlquist@verizon.net). Pit membrane remnants in perforation plates and other vessel details of Cornales. Brittonia 56: 275–283. 2004.—Perforation plates and other vessel details as studied with scanning electron microscopy (SEM) have been reported for four species of Cornaceae (s.l.): similar features are shown by the four, suggesting that a more extensive sampling of the family might reveal similar phenomena. Perforation plates contain pit membrane remnants in the form of threads or, less commonly, laminar portions perforated by pores. When least well-represented, the pit membrane remnants are restricted to lateral ends of perforations and to the perforations transitional to lateral wall pitting. Perforations are all clearly bordered. Helical thickenings that do not form a continuous gyre are reported for the vessel walls of *Aucuba*. The presence of pit membrane remnants in vessel elements of Cornaceae correlates with the mesic habitats occupied by species in this family. The presence and type of pit membrane remnants reported by us in the three genera is very similar, although pit membrane remnants are doubtless a symplesiomorphy and thus not an indicator of relationships. The presence of pit membrane remnants in the three genera, however, does attest to the primitiveness of wood and other features of Cornaceae s.l.

Key words: Cornaceae, ecological wood anatomy, Garryales, perforation plates, phylogenetic wood anatomy, vessel evolution.

Introduction

The species studied here belong to Cornaceae and two families segregated from it by various authors: Aucubaceae and Griseliniaceae. Ohtani and Ishida (1978) reported pit membrane remnants in Japanese Cornaceae, although these structures were not reported by Meylan and Butterfield (1978) in the two New Zealand species of *Griselinia* that they studied with SEM. Whether pit membrane remnants occur more widely in Cornaceae has not been hitherto investigated, and the present paper is a contribution to knowledge of this distribution. Earlier papers on wood anatomy mostly have not used that technique (see Gregory, 1994). More recent studies of wood anatomy of Cornaceae (Li & Chao, 1954; Noshiro & Baas, 1998) were not concerned with fine details of perforation plate structure, although a few SEM photographs of wood details were offered in the Noshiro and Baas (1998) paper. Both Li and Chao (1954) and Noshiro and Baas (1998) reviewed the taxonomic problems associated with traditional alliances that include Cornaceae. Recent molecular data analyzed cladistically (e.g., Soltis et al., 2000) showed that Cornales (from which they studied *Cornus*) and Garryales (in which they in-

location within perforation plates are aspects that have remained unexamined. Because our reports are based on viewing large areas of radial sections, we believe we can address these questions at least in a preliminary way. As these aspects of pit membrane presence become better known, the physiological and evolutionary significance of these structures can be assessed. In addition, studies such as the present one provide a basis for selection of species for experimental work on pit membrane remnants. The way in which these form and the conditions under which they persist need to be elucidated. Cornaceae (s.l.) are a woody family ideal as subjects for examining such questions, since they grow in a range of habitats from frost-free to markedly temperate, and thus the relationship between nature of perforation plates and formation of ice within the conductive system could be examined. Because Cornaceae characteristically occupy moist habitats, the effect of drought on the conductive stream should not be a complicating factor.

Although our sampling of Cornaceae and allied families is small, the fact that all three genera examined do possess pit membrane remnants is of potential significance in resolution of familial definitions and relationships in this complex (for information on various phylogenetic treatments, see Goldberg, 1986; Noshiro & Baas, 1998; Thorne, 2000).

Materials and Methods

Samples of stems were taken from living plants cultivated in Washington Park Arboretum, Seattle (*Aucuba japonica* Thunb.#215-76-A, *Cornus stolonifera* Mich. #638-41, *Griselinia littoralis* Raoul) and Santa Barbara Botanic Garden (*Cornus glabrata* Benth., # 75-147). The stems used were between three and five years old, and thus a mature wood pattern is represented. No observations are based on vessel elements that might be immature by virtue of being near the cambium.

Stem segments were cut into portions suitable for sectioning and fixed in 50%

able for that procedure. Only radial sections were prepared; thick sections (>30 μm) were employed because of ease of handling and the chance that in thick sections, larger portions of perforation plates would be intact. The sections were dried between clean slides, mounted on aluminum stubs, sputter-coated with gold, and examined with a Hitachi S2600N scanning electron microscope.

Descriptions of species are arranged in alphabetical order. The pit membranes figured are believed to represent natural appearances, such as would be present in the living plant (see Schneider & Carlquist, 2003, for a discussion of identification of artifacts). There are obvious artifacts (e.g., the transverse tear in pit membrane of the top perforation, Fig. 2A), but other than such clear structural disruptions, the pit membranes represented are considered reliable representations. Prolonged exposure to the electron beam, especially at accelerating voltages above 20 kv, can result in tearing, and therefore we have adjusted voltage strength and exposure carefully so as to avoid any such artifact formation. We would point, as one evidence of the validity of our observations, to the fact that entirely intact and pore-free pit membranes occur in lateral wall pits of vessels, forming a kind of control to what is observed in perforation plates. For additional criteria for separation of artifacts from valid pit membrane remnants, see Carlquist and Schneider (2001). The steps in formation of pit membrane remnants in vessels would be worthy of study, but at present, no literature on this exists to the best of our knowledge.

Results

Aucuba japonica (Fig. 1). More than half of the perforation plates examined lack appreciable pit membrane remnants. The remainder range from perforation plates in which pit membrane remnants are abundant (Fig. 1A) to present only at lateral ends of the perforations (Fig. 1C). The appearance of these remnants is mostly intermediate
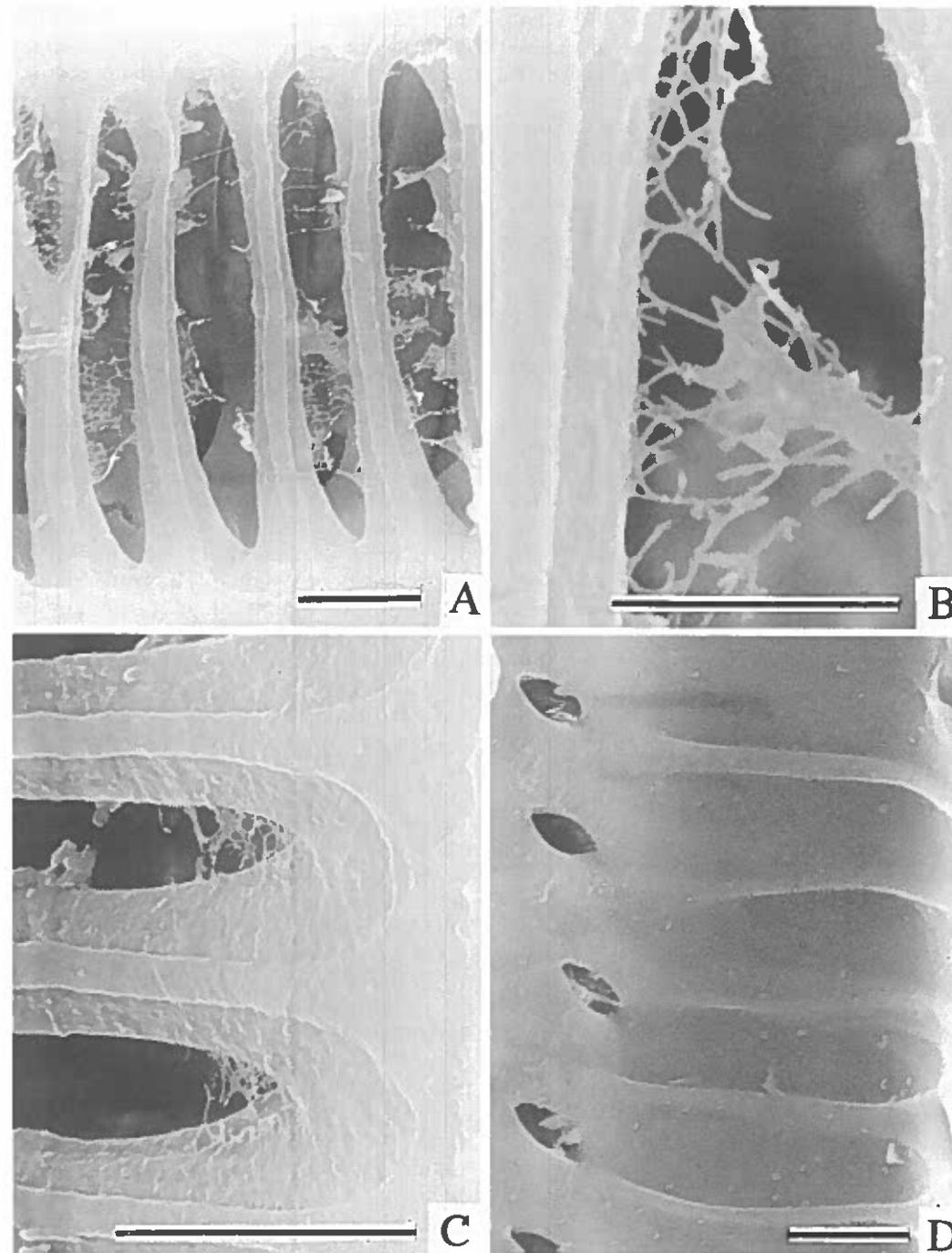
FIG. 1.   *Aucuba japonica*. Portions of radial sections of wood. A–C. Portions of perforation plates. A. Several

branes to form fine reticulate webs in lateral ends of perforations is illustrated. The wide borders of the perforations (Fig. 1C) are similar in tone to the pit membrane remnants, and therefore the patterns formed by the remnants are clearest in areas overlying the inner pit aperture of the vessel. Vessel wall areas that are free from pits tend to show widely spaced, shallow thickenings (Fig. 1D). These thickenings are transverse and not helical in the sense of forming a continuous gyre, but they would fall under the phenomenon of "helical thickenings" in the all-inclusive sense of the term as used by most comparative wood anatomists. Helical thickenings have been reported for Cornaceae previously in *Corokia* (Patel, 1973) but not, apparently, in *Aucuba*.

Cornus glabrata (Fig. 2). The majority of the perforations contain few or no pit membrane remnants. Perhaps 10% of the perforation plates have pit membrane remnants present throughout the perforations (e.g., Fig 2B). Pit membrane remnants are chiefly confined to ends of perforations (Fig. 2A, C). In Figure 2A, a relatively uncommon expression is shown: pit membranes present in laminar form perforated by pores of various sizes. The most common form taken by the pit membrane remnants is intermediate between threads and flakes, with a decided tendency for the threads to be oriented parallel to the long axis of the vessel (Fig. 2B–D). Where present, pit mebrane remnants are always more abundant in the lateral ends of the perforations rather than in the central portions (Fig. 2B–D). In *Cornus glabrata*, as in other Cornaceae, there is a sharp transition between lateral wall pitting and perforations. Pits of lateral walls have intact membranes that do not contain any pores (Fig. 2C, extreme left; Fig. 2D, extreme left). The first perforation adjacent to lateral wall pitting contains somewhat more abundant pit membrane remnants than do perforations in the main part of the perforation plate. This phenomenon is well illustrated in Figure 2D, where the lateral wall pit (left) contains

tion contains relatively abundant pit membrane remnants. In the instance of the perforation in Figure 2D, center, the central portion of the perforation contains a reticulum of threadlike remnants, whereas the lateral ends (Fig. 2D, center, extreme top and bottom) have laminar membranes perforated by small pores.

Cornus stolonifera (Fig. 3). The features illustrated are similar to those of *C. glabrata*, but with somewhat different texture in the pit membrane remnants. Pits transitional between the lateral wall pitting and the perforation plate of vessel elements are shown in Figure 3A–B, left. In Figure 3D, the entire transition from lateral wall pit with intact membrane (left) to perforation with no perceptible pit membrane remnants in the central portion is shown. The perforation to the left of the one at far right in Figure 3D does show, bottom, a pit membrane remnant at the lateral end, reinforcing what is a typical pattern for the family. One can see a similar instance in Figure 3B, above right, in the form of a few threads. Threadlike pit membrane remnants in lateral ends of perforations are illustrated in Figure 3C, left. The similarity of the transitional pit in Figure 3A to the transitional pit in Figure 3D is striking: in both cases, the perforated portion is more abundant on one side (left in Fig. 3A, right in Fig. 3D).

Griselinia littoralis (Fig. 4) shows pit membrane features very similar to those of *Cornus*. Pit membrane remnants are chiefly threadlike and confined to the lateral ends of perforations (Fig. 4A), although more than half of the perforation plates examined do not show perceptible pit membrane remnants. A degree of intermediacy between laminar pit membrane and pit membrane in the form of slender threads may be seen in Figure 4A–C. The pit membrane of Figure 4D, laminar but perforated by small pores, is unusual, and others like it were not observed in our material. The three pits/perforations of Figure 4B show the transition region from lateral wall pitting (top) to perforation plate (bottom), and illustrate the difficulty in defining the terms "pit" and
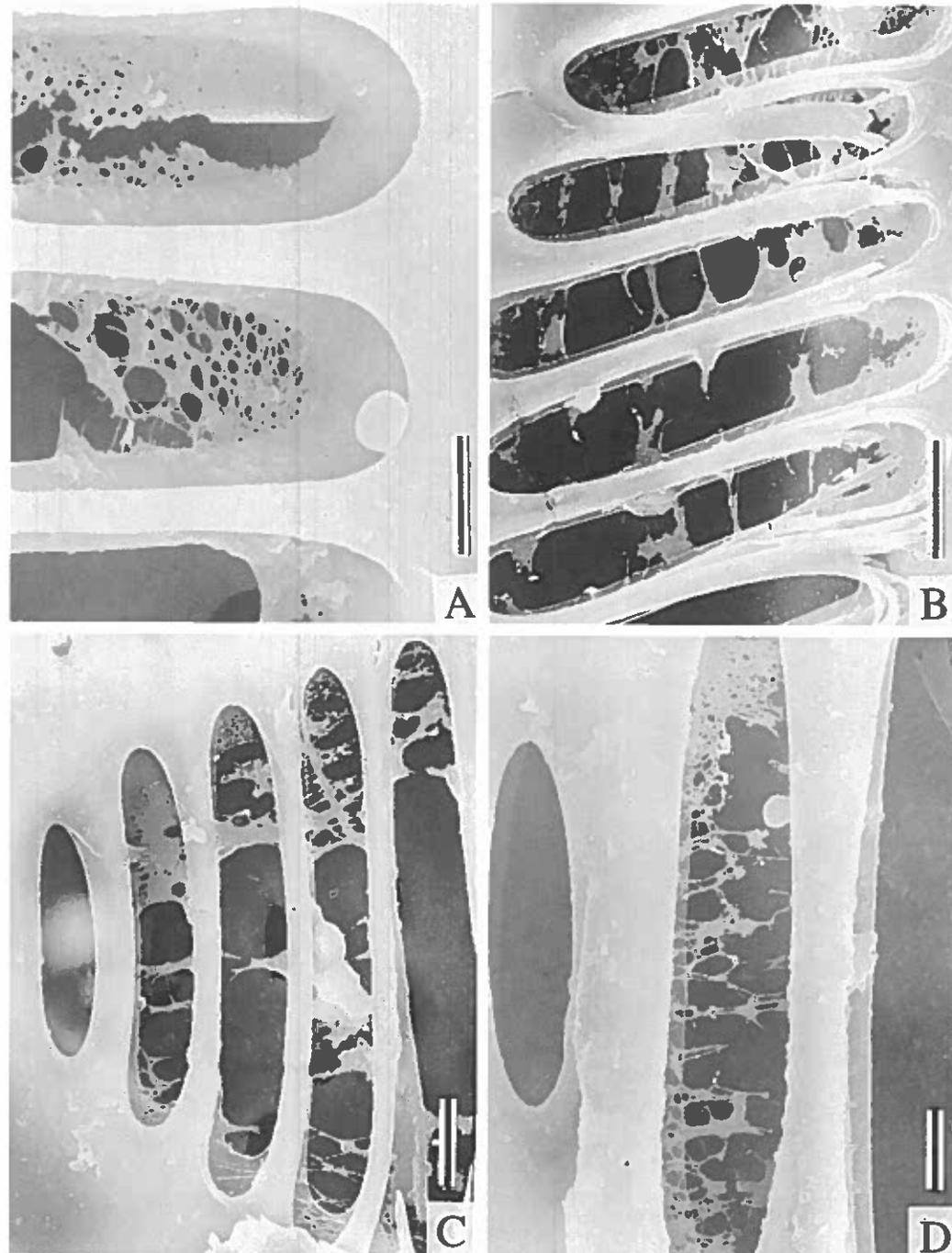
FIG. 2. *Cornus glabrata*. Portions of perforation plates from radial sections of wood. A. Ends of perforations, to show laminar pit membrane remnants perforated by pores; tear in top membrane is an artifact. B. Several perforations to show remnants which form similar threadlike and flakelike conformations. C, D. Transition
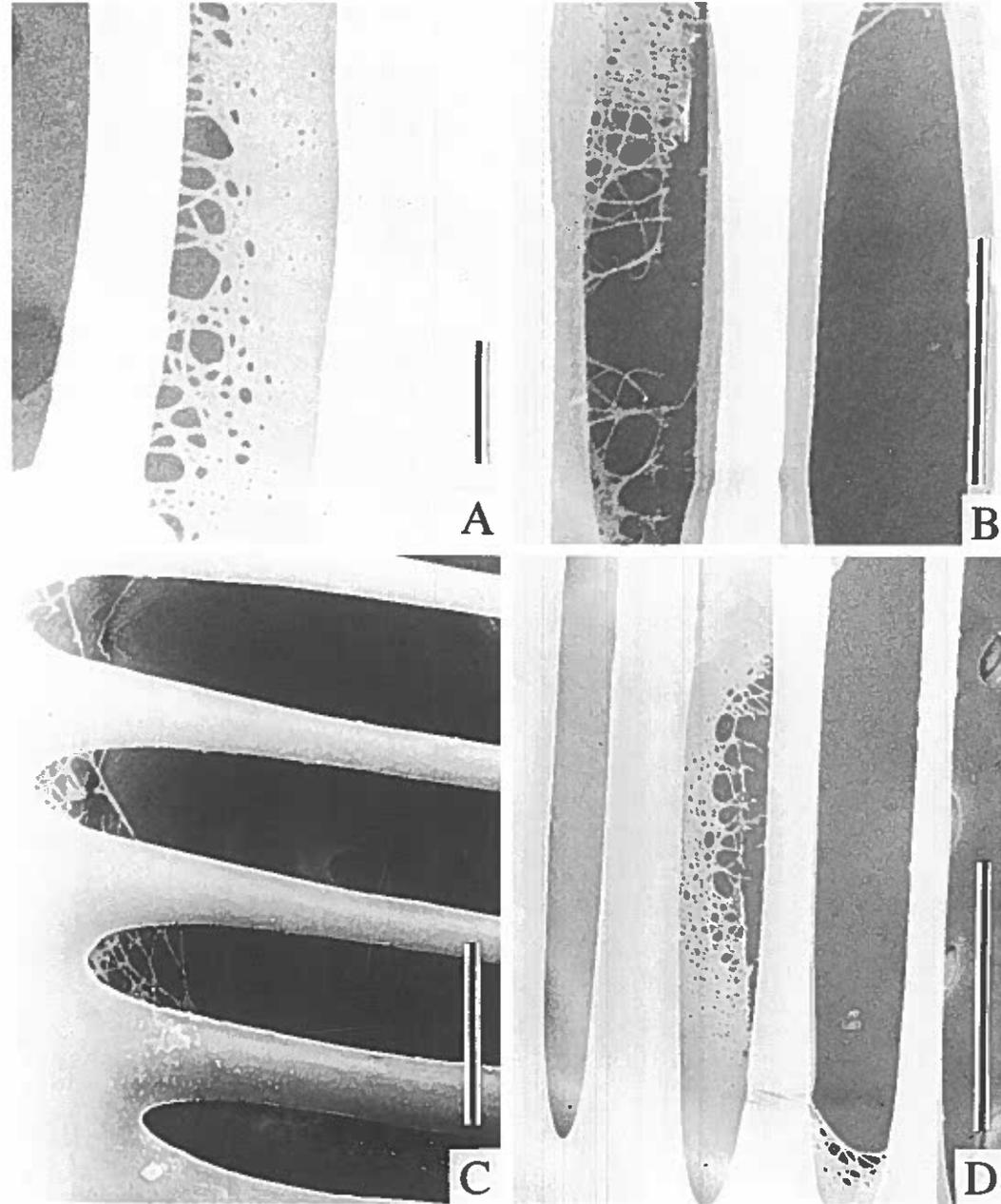
FIG. 3. *Cornus stolonifera*. Portions of perforation plates from radial sections of wood. **A.** Perforation in zone intermediate between lateral wall pitting and perforation plate, to show porose pit membrane. **B.** Two perforations, one at left (nearer to lateral wall pits, which are not shown) contains more abundant pit membrane remnants. **C.** Several adjacent perforations, each with threadlike membrane remnants at lateral ends of perforations. **D.** Transition between lateral wall pitting (extreme left) and perforation plate (remainder of photograph). Scale bars: A = 2 μm; B–D = 5 μm.
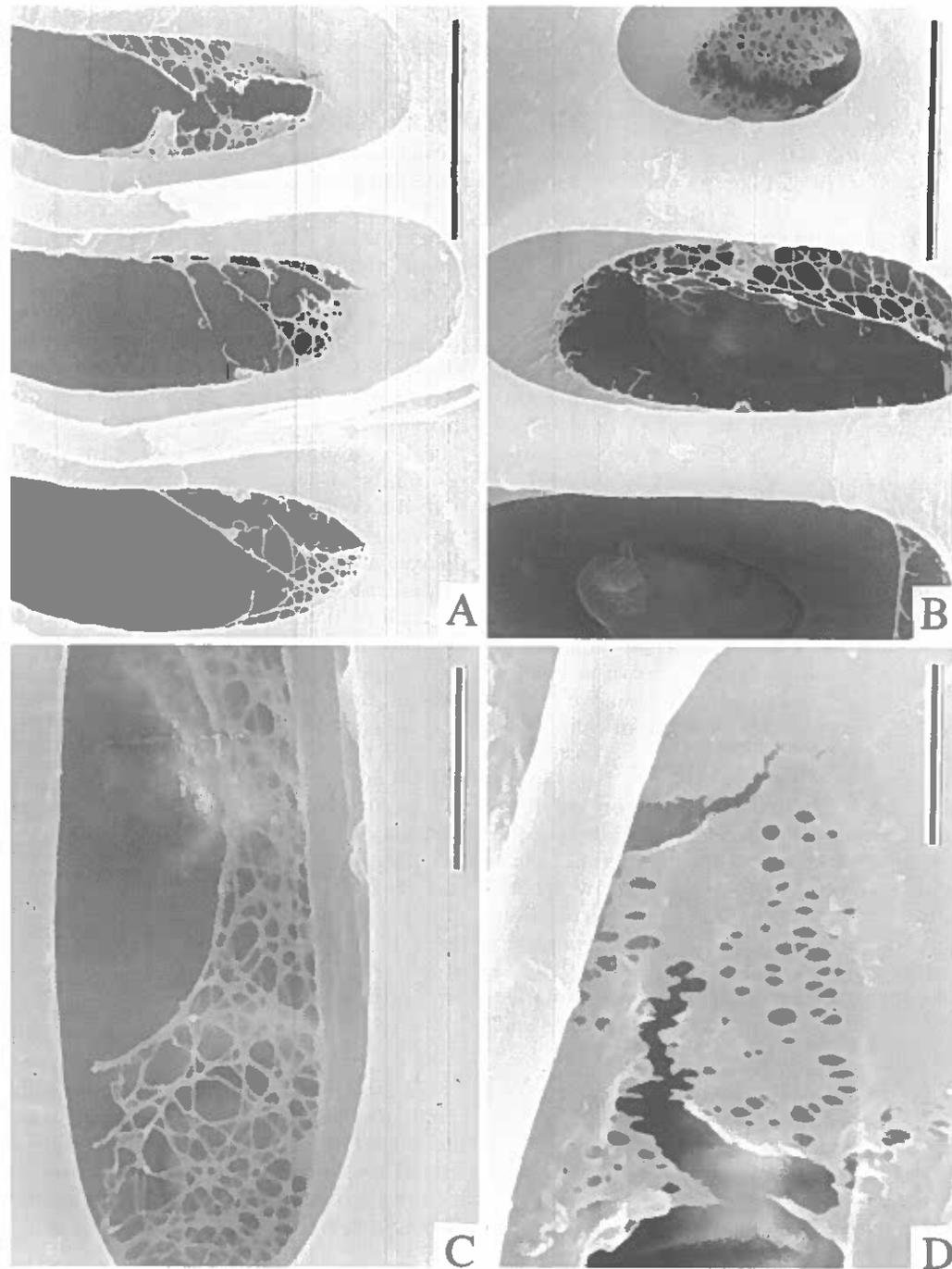
FIG. 4. *Griselinia littoralis*. Portions of perforation plates from radial sections of wood. A. Three perforation

term that indicates intermediacy between the two categories. Certainly the perforation at the bottom in Figure 4B shows the restriction of threadlike pit membrane remnants to lateral ends of perforations, typical in perforation plates of Cornaceae (s.l.) that retain pit membranes in perforations. The apparent shrinkage of a pit membrane remnant to one side of the perforation, shown in Figure 4B, center, and Figure 4C, appeals to us as probably an artifact. However, we have no way of knowing whether such deformation occurs in the xylem of a living plant or is the result of the drying required for examination of sections with SEM.

Conclusions

Our sampling of Cornaceae (s.l.) is admittedly very small, and was influenced by the desire to find whether pit membrane remnants could be found in several genera of the family as broadly defined. We therefore selected species with more numerous bars per perforation plate. In the cornalean alliance, some species have few bars, some none, and species with few or no bars would, in our experience, probably lack pit membrane remnants. Pit membrane remnants have been found in only a few genera with relatively few bars per perforation plate (e.g., Bruniaceae & Sarraceniaceae; Carlquist, 1992).

Despite the small sampling, the similarity of the three genera with respect to types and distribution of pit membrane remnants is considerable. This similarity is not necessarily indicative of close relationship, however.

More significantly, though, the patterns reported here are not unique, but occur in families and orders in which pit membrane remnants are relatively vestigial. The similarity to occurrence of pit membrane remnants in Ericaceae (Carlquist & Schneider, in press), a family that has not been proposed to be related to the cornalean families in any phylogenetic scheme, is striking. In both Cornaceae s.l. and Ericaceae s.l., pit membrane remnants are present in fewer

the remnants are mostly confined to lateral ends of the perforation plates. An exception occurs in the transitional zone between lateral wall pitting and the perforation plate. In this area, one or two pits (perforations) contain more extensive pit membranes, but the pit membranes are abundantly perforated or threadlike. The occurrence of such a transitional pit/perforation shows that at least in families with pit membrane remnants of this sort, designating any particular structure as a pit or a perforation may be difficult.

The vestigial nature of the pit membrane remnants suggests possible correlations with the conductive stream. If the remnants are considered an impedance to the conductive stream, the remnants present in the Cornaceae studied represent a minimal interruption to that stream. Remnants occur in fewer than half of the perforation plates. In the perforation plates where they are present, most perforations contain remnants only at lateral ends of the perforations, and most of the length and width of the perforations are free from the remnants. One or two perforations intermediate between true perforations and lateral wall pits occur in the transition between lateral wall pitting and perforations. These constitute only a minor impedence to conduction and, indeed, if they were viewed as modifications of pits rather than of perforations, would represent an enhancement of conduction.

Acknowledgments

Literature Cited

Adams, J. E. 1949. Studies in the comparative anatomy of the Cornaceae. J. Elisha Mitchell Soc. 65: 218–244.

Carlquist, S. 1992. Pit membrane remnants in perforation plates of primitive dicotyledons and their significance. Amer. J. Bot. 79: 660–672.

———— & E. L Schneider. 2001. Vesels in ferns: structural, ecological, and evolutionary significance. Amer. J. Bot. 88: 1–13.

———— & ————. In press. Vestigial pit membrane remnants in perforation plates and helical thickenings in vessels of Ericaceae. Nord. J. Bot.

Goldberg, A. 1986. Classification, evolution, and phylogeny of the families of dicotyledons. Smithsonian Contrib. Bot. 58: 1–314.

Gregory, M. 1994. Bibliography of systematic wood anatomy of dicotyledons. IAWA J., Suppl. 1: 1–265.

Li, H.-L. & C.-Y. Chao. 1954. Comparative anatomy of the woods of the Cornaceae and allies. Quart. J. Taiwan Mus. 7: 19–136.

Meylan, B. A. & B. G. Butterfield. 1978. The structure of New Zealand woods. DSIR Bulletin 222. New Zealand Department of Scientific and Industrial Research, Wellington.

Noshlro, S. & P. Baas. 1998. Systematic wood anatomy of Cornaceae and allies. IAWA J. 19: 43–97.

Ohtani, J. & S. Ishida. 1978. An observation on the perforation plants in Japanese dicotyledonous woods using scanning electron microscopy. Res. Bull. Coll. Exp. Forests Hokkaido Univ. 33: 407–435.

Patel, R. N. 1973. Wood anatomy of the dicotyledons indigenous to New Zealand. 1. Cornaceae. New Zealand J. Bot. 11: 3–22.

Schnelder, E. L. & S. Carlquist. 2003. Perforation plate diversity in *Illicium floridanum* (Illiciaceae) with respect to organs, provenance, and microtechnical methods. Sida 20: 1047–1057.

Soltis, D., P. S. Soltis, M. W. Chase, M. E. Mort, D. C. Albach, M. Zanls, V. Savolainen, W. H. Hahn, S. B. Hoot, M. F. Fay, M. Axtell, S. M. Swensen, L. M. Prlnce, W. J. Kress, K. C. Nixon & J. S. Farris. 2000. Angiosperm phylogeny inferred from 18s rDNA, *rbc*L, and *atp*B sequences. Bot. J. Linn. Soc. 133: 381–461.

Thorne, R. F. 2000. The classification and geography of the flowering plants: dicotyledons of the class Angiospermae (subclasses Magnoliidae, Ranunculidae, Caryophyllidae, Rosidae, Asteridae, and Lamiidae). Bot. Rev. 66: 441–647.