Unusual pit membrane remnants in perforation plates of Cyrillaceae[1]

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SCHNEIDER, E. L AND S. CARLQUIST (Santa Barbara Botanic Garden, 1212 Mission Canyon Road, Santa Barbara, CA 93105). Unusual pit membrane remnants in perforation plates of Cyrillaceae. J. Torrey Bot Soc. 130: 225–230. 2003—Pit membrane remnants occur in all collections of Cyrillaceae studied: they are minimal in *Cliftonia monophylla*, *C. parviflora*, and *Purdiaea antillana*, whereas in the specimen of *Cyrilla racemiflora*, many perforations possess intact pit membranes or membranes with small pores or, in a few cases, large holes. Some perforation pit membranes in *C. racemiflora* appear to possess holes only in one of the two pit membranes of the two adjacent cells. Retention of pit membrane remnants in *C. racemiflora* may represent a tendency to lose conductive capability related to the habitat (understory, marshy ground) This could be considered a stage in vessel loss except for the fact that three other criteria for vessel loss are not met. Presence of pit membrane remnants in Cyrillaceae is consistent with such presence in the sister family Clethraceae as well as in other families of Ericales (Theales of some authors) as defined on the basis of recent molecular data.

Key words: Clethraceae, ecological wood anatomy, Ericales, primitive angiosperms, Theales, vessel loss, vessel origin, xylem.

Scalariform perforation plates with numerous bars have long been regarded as typical of vessels of primitive dicotyledons (Frost 1930). Investigation of these with SEM (scanning electron microscopy) has led to discovery of pit membrane remnants in some of these primitive phylads (Meylan and Butterfield 1978, Ohtani and Ishida 1978, Carlquist 1992, Carlquist and Schneider 2002a, b). The pit membrane remnants are primary walls that are dissolved to various extents, despite various methods of preservation and handling (Schneider and Carlquist 2003). Variation within species and within genera occurs, although characteristic patterns are present in some species (Carlquist and Schneider 2002b).

Cyrillaceae consist of 14 species, according to most authors. *Cliftonia monophylla* and *Cyrilla racemiflora* are from the Atlantic states of the southeastern U.S.A.: Virginia and southwards (Melchior 1964). These two genera are commonly considered monotypic, although segregates of *Cyrilla racemiflora* (e.g., *C. parvifolia*) are sometimes recognized. *Purdiaea* (= *Costaea*) consists of 11 species, which range from southern Mexico to northern Brazil and Peru, and are present on some of the larger West Indies, especially Cuba (Melchior 1964). Cyrillaceae tend to grow in wet forest or even marshy lowlands (Melchior 1964). Recent molecular data show that Cyrillaceae lies close to Clethraceae in a large group now known as Ericales (Soltis et al. 2000, Thorne 2001). Many of these families were formerly recognized as Theales (Thorne 1992).

Pit membrane remnants of a distinctive type (threads, oriented axially in the perforations) were reported previously in wood of one species of Cyrillaceae, *Cliftonia monophylla* (Carlquist 1992). In the present paper, more species were examined. Unfortunately, the present study represents only a third of the species of the family, although all three genera. No monograph of wood anatomy of Cyrillaceae has been offered to date, although Gregory (1994) lists eight publications in which some data on wood of the family can be found. Presumably, the incomplete knowledge of wood of Cyrillaceae is the result of its shrubby status (wood of shrubs is collected less often than that of trees) and the fact that the species occur on small, local populations in a number of countries. Focused field work would therefore be required to assemble woods of this family. Nevertheless, the present paper shows that all species of Cyrillaceae studied do have pit membrane remnants in perforation plates. These results suggest that study of perforation plates by means of SEM of more species in the family as well as species in nearby families would be of considerable interest in as-

sessing the taxonomic distribution of this phenomenon. In turn, the relationships between pit membrane retention in perforation plates and phylogeny and ecology of the species that show this condition are likely to be clarified as more instances are reported.

The extensive pit membrane remnants of *Cyrilla racemiflora* may be pertinent to the question as to how many times vessels have originated or been lost in angiosperms. This controversy, in which Bailey (1944) opted for vessellessness as primitive in angiosperms whereas Young (1981) claimed vessellessness as derived has recently been renewed by the positioning of Amborellaceae, a monotypic vesselless genus and monogeneric family, as the outgroup of the remainder of the angiosperms (see Soltis et al. 2000, for a fuller discussion). The extensive presence of pit membranes in perforations of *Cyrilla racemiflora* will appeal to some workers as showing that in boggy habitats, a phylad with scalariform perforation plates that has some pit membrane remnants might fail to dissolve these membranes. Discussion of this possibility is a focus of this study.

Materials and Methods. Dried wood samples suitable for sectioning were obtained from the xylarium of the Department of Botany, U.S. National Museum of Natural History, were the basis for this study. The collections studied were *Cliftonia monophylla* (Lam.) Sarg., Archie F. Wilson 739, USw-19574, Appalachicola, Florida, U.S.A.; *Cyrilla parvifolia* Raf., J. L. Stearns 20, USw-9827, southeastern U.S.A.; *C. racemiflora* L., Maguire 28207, USw-24769, Venezuela; and *Purdiaea nipensis* Marie-Vict. and Leon, Ducke 160, USw-31440, Brazil. Samples were boiled in water and stored in 50% aqueous ethanol. Radial sections about 30 μm in thickness were cut on a sliding microtome, dried between clean glass slides, sputter coated with gold, and examined with SEM. The sections were studied either with a Bausch and Lomb Nanolab or a Hitachi S2600N SEM. Observations of sections of *Purdiaea antillana* were not as extensive as could be wished. Observations with light microscopy of sections of wood of this species revealed that the tawny color of the wood sample is the result of extensive deposits of gumlike or resinlike compounds throughout the wood, and many of these deposits obscure perforation plates. The illustrations (Fig. 1–8), however, cover the range of pit membrane presence observed in the specimens studied. Except for Fig.

3, all photomicrographs of perforation plates are oriented with the long axis of the vessel horizontal in order to show various portions of the pit membrane remnants more effectively. Unfortunately, neither of the scanning electron microscopes we used (one an old analogue instrument, the other a new digital machine) were able to offer truly sharp resolution. When studying concave items or surfaces surrounded by raised areas (e.g., the membranes in perforations), electron return is relatively low, and increasing the magnification so as to make the nature of the pit membranes more easily visible demonstrates loss of resolution; in our experience, sharp resolution of details of pit membranes above 5,000× is rarely achieved. Standard techniques (coating with gold, 15kv accelerating voltage, etc., were used). Our prior experience in study of pit membranes, together with the previous studies by Ohtani and Ishida (1978) and Meylan and Butterfield (1978) show clearly that in the perforations, we are dealing with pit membrane remnants, not deposits of secondary metabolites (although we recognize and identify as such the presence of contaminating material, such as starch grains, etc). The term "pit membrane remnant" in analyzing a perforation is the only reasonable way of referring to the phenomena we are describing: to call the perforations of a perforation plate "pits" when they contain pit membrane remnants and are morphologically and in size like other perforations would be a misnomer. The phenomena with which we are dealing have nothing to do with fracturing in pit membranes as seen in pits of fibers and other cells in woods: such fracturing is the result of drying or handling methods. We are dealing with an entirely natural phenomenon (although handling may result in some tearing, which we recognize and term as such).

Results. The material studied here of *Cliftonia monophylla* (Fig. 1) may include a few artifacts clinging to bars of the perforation plate. However, we believe that most of the irregular surfaces observable on the margins of the perforation plate bars is the result of pit membrane remnants that have mostly been dissolved, with remnants clinging to the bar surface in irregular clotlike form. This impression is reinforced by the oblique view of bars of *Purdiaea nipensis* (Fig. 2). In both of these species, occasional sheets of pit membrane that had not been dissolved were observable, but characteristically, perforations were open with only irregular fring-
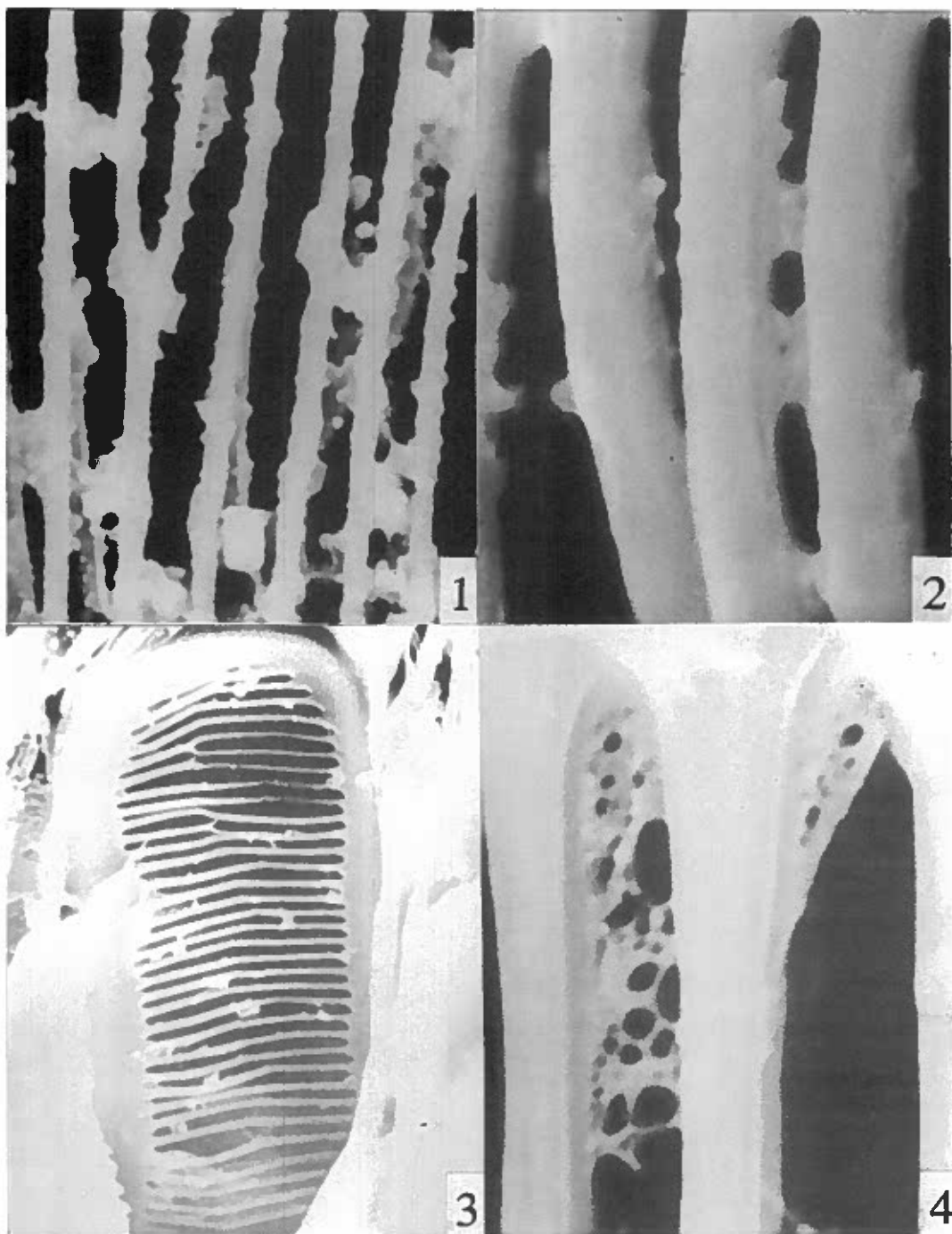
Figs. 1–4.   Portions from radial sections of vessels of stem wood of Cyrillaceae. Figs. 1, 2, 4. Portions of perforation plates (long axis of vessel oriented horizontally) to show pit membrane remnants. Fig. 1. *Cliftonia monophylla;* pit membrane remnants, minimal in extent and irregular in shape, clinging to bars; some artifacts present. Fig. 2. *Purdiaea nipensis,* sheet-like and wart-like pit membrane remnants on bars (bars seen somewhat obliquely). Fig. 3, 4. *Cyrilla parvifolia.* Fig. 3. Intact perforation plate (a small portion at bottom cropped) to show absence of pit membrane remnants; some artifacts clinging to bars. Fig. 4. Lateral ends of two perforations (membrane remnant at right folded due to handling); pores of various sizes in the sheetlike remnant at left. Figs. 1, 2, 4, scale bars = 5 μm; Fig. 3, scale bar = 30 μm.
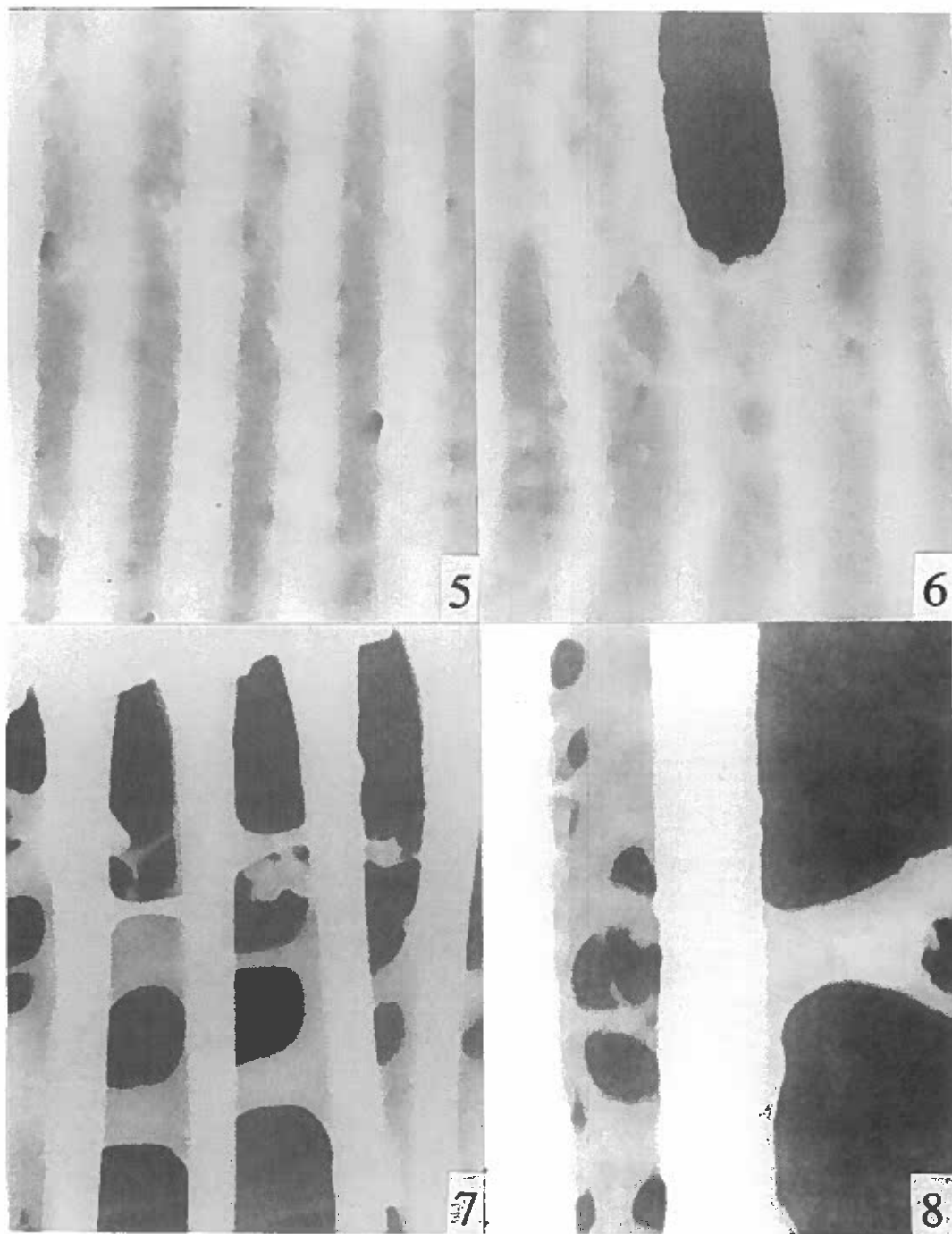
Figs. 5–8.   Portions of perforation plates (long axis of perforation plate oriented horizontally) from radial sections of wood of *Cyrilla racemiflora* to show presence of various types of pit membrane remnants. Fig. 5. Pit membranes intact, although a few small holes or depressions are present. Fig. 6. Pit membranes intact except for a large hole at top, center. Fig. 7. Bandlike pit membrane remnants separated by large pores. Fig. 8. Bandlike pit membrane remnant at right; at left, holes in sheetlike pit membrane remnant. Figs. 5–8, scale bars = 5 μm.

es of pit membrane remnants clinging to the bars.

In *Cyrilla parvifolia* (Fig. 3, 4), perforations are mostly clear of pit membranes. This is suggested by Fig. 3, in which no pit membranes are clearly evident, although some fragments representing displaced pit membranes may be observed clinging to the plate surface, as though these fragments were too large to pass through the perforation and were caught on bars of the plate. At higher magnifications (Fig. 4), portions of pit membranes may be observed, restricted to the lateral tips of perforations. These pit membrane remnants, as with so many pit membrane remnants (Carlquist 1992), are porous. The pit membrane at right in Fig. 4 is probably folded over, and would otherwise be similar to the membrane remnant at left. Often, in our studies of perforation plates of *Cyrilla parvifolia*, small pit membrane portions in the lateral ends could be seen in adjacent perforations of a given perforation plate. No laminar portions of pit membranes were observed in a few of the perforation plates.

A distinctive series of patterns of pit membrane presence in perforations was revealed in our material of *Cyrilla racemiflora* (Fig. 5–8). Intact pit membranes, bearing only a few small and indistinct pores (Fig. 5) were much more common than perforation plates, such as those of Fig. 7–8, in which larger holes were present. The single large hole in the pit membrane, center above, of Fig. 6, might be an artifact, although perforation plates bearing a few large holes and seemingly transitional to the types seen in Fig. 7 and 8, were common enough so that this might be a natural appearance.

Where numerous holes were characteristically present in perforation plates of *C. racemiflora* (Fig. 7–8), they were usually separated by membrane remnants in the shape of coarse threads or bands, as in *Illicium* (Carlquist 1992, Carlquist and Schneider 2002b, Schneider and Carlquist 2003).

One phenomenon we were unable to photograph adequately but suspect to be a valid one is the presence of a hole in a pit membrane not matched by and therefore not intercontinuous with one on the adjacent pit membrane on the other side of the perforation plate. Pit membranes, when present in perforations, are theoretically two-ply structures, representing the primary wall surrounding each of the two adjacent cells in a perforation plate. We are unaware, either in our own studies, or those of others, of any instances in which lysis succeeds in a pit membrane of one vessel element but does not penetrate through to the adjacent pit membrane, which is, in fact, intimately appressed to the first pit membrane. Vague circular or oblong depressions, sometimes slightly grayer in tone, superimposed on an intact pit membrane lead us to think that such a phenomenon may be operating. However, our report is tentative and needs confirmation.

Conclusions. There is a wide range in degree of presence of pit membrane remnants in perforation plates of Cyrillaceae, although pit membrane remnants could be detected with SEM in all species studied. Earlier studies of *Cliftonia monophylla* (Carlquist 1992) differed from the present work in that thread-like pit membrane strands were observed at lateral ends of perforations earlier, whereas our new material of this species showed only minimal, clotlike remnants of pit membranes clinging to the edges of bars of the plate. *Cyrilla parviflora* (if recognized as a segregate species or population) is distinctive in having porose membrane remnants only in the lateral ends of perforations, a condition figured elsewhere to our knowledge only in several species of *Ascarina* (Carlquist 1992, Carlquist and Schneider 2002a) and in *Illicium majus* Hook. f. and Thoms (Carlquist and Schneider 2002b). Relatively few laminar portions of pit membrane remnants were observed in *Purdiaea nipensis*.

In contrast, extensive pit membrane remnants were observed on perforation plates of *Cyrilla racemiflora* (s. s.). This wood sample was from a large stem, and the section was from a site intermediate between cambium and pith, so the mature condition of perforation plates was represented. The majority of perforation plates had pit membrane remnants completely covering the perforations, or nearly so. This condition may relate to the perpetually wet, boggy habitats in which this species is found: vessels with more open perforation plates are of little advantage for rapid conduction under these conditions, because seasonally rapid conduction of water is unlikely: relatively constant, slow rates of conduction are more probable. In an understory locality in such a habitat, one has conditions where theoretically, perforation plates cleared of pit membranes would be of minimal selective value. One could argue that vessellessness would originate under such conditions, providing a scenario that would be required for the hypothesis of Young (1981) and others that ves-

sellessness is secondary in at least some woody angiosperms. However, only the failure of pit membrane remnants to dissolve in many perforation plates could be cited as an indication of a tendency toward vessel loss. There are other features that would have to be reversed before a vesseless condition could be achieved: identity in diameter between vessels and tracheids; vessels the same length as tracheids; vessels and tracheids both shorter than tracheids in a vesseless species (Carlquist and Schneider 2002a). Thus, the wood sample we studied for *Cyrilla racemiflora* (other wood samples may differ) represents only partial fulfillment of one criterion for the loss of vessels. Failure to dissolve perforation plate pit membranes in *Cyrilla racemiflora*, by not being accompanied by any other changes that could be interpreted as progressions to loss of vessels, validates the concept that there are multiple, independent features involved in the tracheid–vessel element transition (Carlquist and Schneider 2002a). Vessel loss, although theoretically a phyletic possibility, has not been demonstrated to be occurring in any woody phylad to any appreciable extent except for a few species of *Ephedra* and some cacti (Carlquist 1988), and these instances represent reduction in vessel number, not complete loss, and represent adaptation to extreme dryness.

The presence of pit membranes in Clethraceae (Carlquist 1992) is consistent with the concept that Cyrillaceae and Clethraceae represent sister families (Soltis et al. 2000). Pit membranes have been reported in other families of Ericales (sensu Soltis et al. 2000), such as Epacridaceae, Ericaceae, Sarraceniaceae, and Theaceae (see Carlquist 1992), so further exploration in this order for occurrence of pit membrane remnants in perforations is likely to yield many more examples.

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