

WOOD ANATOMY, STEM ANATOMY, AND CAMBIAL ACTIVITY OF *BARBEUIA* (CARYOPHYLLALES)

by

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SUMMARY

Liquid-preserved material of mature stems of *Barbeuia madagascariensis* Steud. permitted analysis of meristematic activities. The species has successive cambia, each producing secondary xylem and phloem; outside of these vascular strands is a nondiffuse lateral meristem, probably functionally a single cell in thickness, which produces radial files of secondary cortex to the outside and conjunctive tissue and vascular cambia to the inside. The secondary xylem of *Barbeuia* has dimorphism in vessel diameter, reminiscent of vessel dimorphism in other lianas, such as *Agdestis*. Diffuse parenchyma and tracheids, found in *Barbeuia*, also occur in *Stegnosperra* and *Agdestis* but not other Phytolaccaceae s.l., and are traditionally considered primitive features. Wood anatomy and lateral meristem activity of *Barbeuia* are distinctive enough to support segregation of the genus from Phytolaccaceae s. s.

Key words: Fibriform vessel elements, Phytolaccaceae, successive cambia, tracheids, vessel dimorphism.

INTRODUCTION

Barbeuia madagascariensis Steud. has been segregated from its traditional position in Phytolaccaceae as Barbeuiaceae by those who recognize such segregate families as Achatocarpaceae, Agdestidaceae, and Stegnospermataceae (Heimerl 1934; Rodman et al. 1984; Takhtajan 1987; Thorne in Cronquist & Thorne 1994). Additional families have been considered segregates and satellites of Phytolaccaceae: Rivinaceae (= Petiveriaceae) and Sarcobataceae (Behnke 1997). Close to these families and with them forming the suborder Phytolaccineae of Caryophyllales are Aizoaceae and Nyctaginaceae (Thorne in Cronquist & Thorne 1994; Behnke 1997). The families of Phytolaccineae listed above show evolution with respect to gynoecium configuration and fruit type (Heimerl 1934). These features are commonly used as familial criteria in other orders of angiosperms, so the recognition of families segregated from Phytolaccaceae is understandable. However, features of the Phytolaccineae other than those can be used to stress the closeness of these families, whether or not they are recognized as segregates or included under Phytolaccaceae s.l. Unfortunately, molecular data are not yet available to aid in defining the systematic position of *Barbeuia*. The schemes produced by the phenetic and cladistic work of Rodman et al. (1984)

and Rodman (1994) are not uniform in their results, but open the possibility that *Barbeuia* does not lie in suborder Phytolaccineae, close to Nyctaginaceae and Phytolaccaceae, as traditionally treated (e.g., Thorne in Cronquist & Thorne 1994), but rather may be allied with Amaranthaceae and Chenopodiaceae. Obviously, data from DNA are very much needed to resolve this question.

Those who were involved in the search for the material studied in the present paper discovered that *Barbeuia madagascariensis*, a 'lianoïd small tree' once reported in coastal forests near Fort Dauphin, Vatomandry, and Tampina, Madagascar (Heimerl 1934), is now quite scarce (Mark Olson, personal comm.). Hopefully, molecular data will be obtained while this species is still extant. Material from liquid-preserved stems of moderate but mature diameter (2 cm) were available for the present study. Such material was essential to determine the nature of cambial activity in stems of this species. Successive cambia, and the meristematic processes that are basic to these cambia are much in need of study and improved understanding in Caryophyllales and in other orders in which these phenomena occur. Successive cambia, although characteristic of diverse families of angiosperms and Gnetales, are particularly common in Caryophyllales, and have been reported in at least some species of all families of this order (as presently constituted: Thorne in Cronquist & Thorne 1994) with the exception of Achatocarpaceae, Cactaceae, Didieriaceae, Hectorellaceae, and Portulacaceae (stems of Molluginaceae are probably too small for successive cambia to be expected). Wood and stem features that may lead to familial, ordinal, and subordinal criteria as well as to a better understanding of cambial activity have been the subject of a series of recent papers on Caryophyllales (Carlquist 1995, 1997, 1998, 1999a, b, c). Cactaceae and Didieriaceae will not be included in this series because they have been well covered by Gibson (e.g., 1973, 1978) and Rauh & Dittmar (1970), respectively. A survey of wood anatomy of Caryophyllales, offered earlier by Gibson (1994), was not able to examine wood and stem features at length because of space limitations.

MATERIAL AND METHODS

Stems of *Barbeuia madagascariensis* about 2 cm in diameter were collected and preserved in 50% aqueous ethanol. The voucher collection is R.N. Franck 39 (MO, TAN). The stem material of this species has xylem and, in the bark, sclerenchyma that are quite hard, although the phloem, conjunctive tissue, and meristematic zones are soft. Plant structures with such a configuration cannot be sectioned successfully on a sliding microtome; the best sections of such material when cut on a sliding microtome are quite thick, obscuring details of the meristematic zones. A method in which material is softened with ethylene diamine, embedded in paraffin, and sectioned on a rotary microtome (Carlquist 1982) provided satisfactory results. Sections for study with light microscopy were stained with safranin and fast green according to Northen's modification of Foster's tannic acid-ferric chloride method (Johansen 1940). Other sections were mounted on aluminum stubs, cleansed of paraffin with xylene, dried, sputter coated, and observed with a Bausch and Lomb Nanolab SEM. Macerations of secondary xylem were prepared by means of Jeffrey's Fluid (Johansen 1940) and stained with safranin.

The terminology used corresponds to the IAWA Committee on Nomenclature (1964). The term 'successive cambia' derives from Schenck (1893) and Pfeiffer (1926). The term 'lateral meristem', which corresponds to 'Folgermeristem' of Pfeiffer (1926), was used by Carlquist to refer to the meristem that forms in 'secondary cortex' (Carlquist 1999c) and gives rise to vascular cambia.

Quantitative data are means derived from 25 measurements (diameter of wider vessels, length of fibriform vessel elements, length of wider vessel elements, length of tracheids) or measurements derived from conditions judged to be typical (e.g., wall thicknesses). In transections, one cannot discriminate between tracheids and fibriform vessels, which are about the same as tracheids in diameter. Because of variation in conformation and size of rays, no quantitative data on ray tissue were attempted.

RESULTS

Secondary xylem

The plan of the outer stem (excluding phellem and phelloderm) is shown in Figure 1, and will be discussed more in detail in relation to lateral meristem activity, below. Figures 1 and 2 show that secondary xylem and phloem are formed in short tangential bands (Fig. 1, recently formed secondary xylem to the right of and just below 'vc') and strands surrounded by conjunctive tissue (Fig. 2, strand containing three large vessels just above center of photograph). Between secondary xylem and secondary phloem of each vascular band or strand, a vascular cambium producing tracheids and vessel elements as well as rays can be observed (Fig. 12). No growth rings were observed. Vessels are dimorphic in diameter; the wider vessels are easily identified (Fig. 1 & 2, 11 & 12). In macerations, small perforations in cells that otherwise appear to be tracheids are visible. These are fibriform vessel elements. Perforation plates of fibriform vessel elements can also be seen in sections viewed with SEM (Fig. 5–7). The wider vessel elements are solitary. Wider vessel elements average 96 μm in lumen diameter (this includes vessels that are only a little wider than fibriform vessel elements or tracheids but can be identified as vessels in transection). Wider vessel elements average 359 μm in length; vessel-to-vessel pits are alternate, circular to oval, about 7 μm in diameter parallel to axis of stem. Grooves interconnect some pit apertures (Fig. 4). The mean length of fibriform vessel elements is 542 μm . Wall thickness of wider vessel elements is 7.2 μm ; wall thickness of fibriform vessel elements is 2.3 μm . Wider vessel elements have nonbordered simple perforation plates (Fig. 5, 6); fibriform vessel elements with simple perforation plates that have vestigial borders were seen with SEM (Fig. 5). All imperforate tracheary elements are tracheids with a pit diameter about 5 μm . The mean length of tracheids is 615 μm ; mean tracheid wall thickness is 2.3 μm . Axial parenchyma is vasicentric, scanty (less commonly diffuse), consisting mostly of 2 or 3 cells per strand. Axial parenchyma is conspicuous in longisections at junctures of vessel elements (Fig. 6), where axial parenchyma cells are isodiametric in shape rather than elongate parallel to vessel elements. Rays are multiseriate (Fig. 1, 3). Rays and conjunctive tissue are composed of square to upright cells with lignified walls about 1.1 μm thick. Starch is common in rays and in conjunctive tissue. Wood cells are nonstoried (Fig. 3).

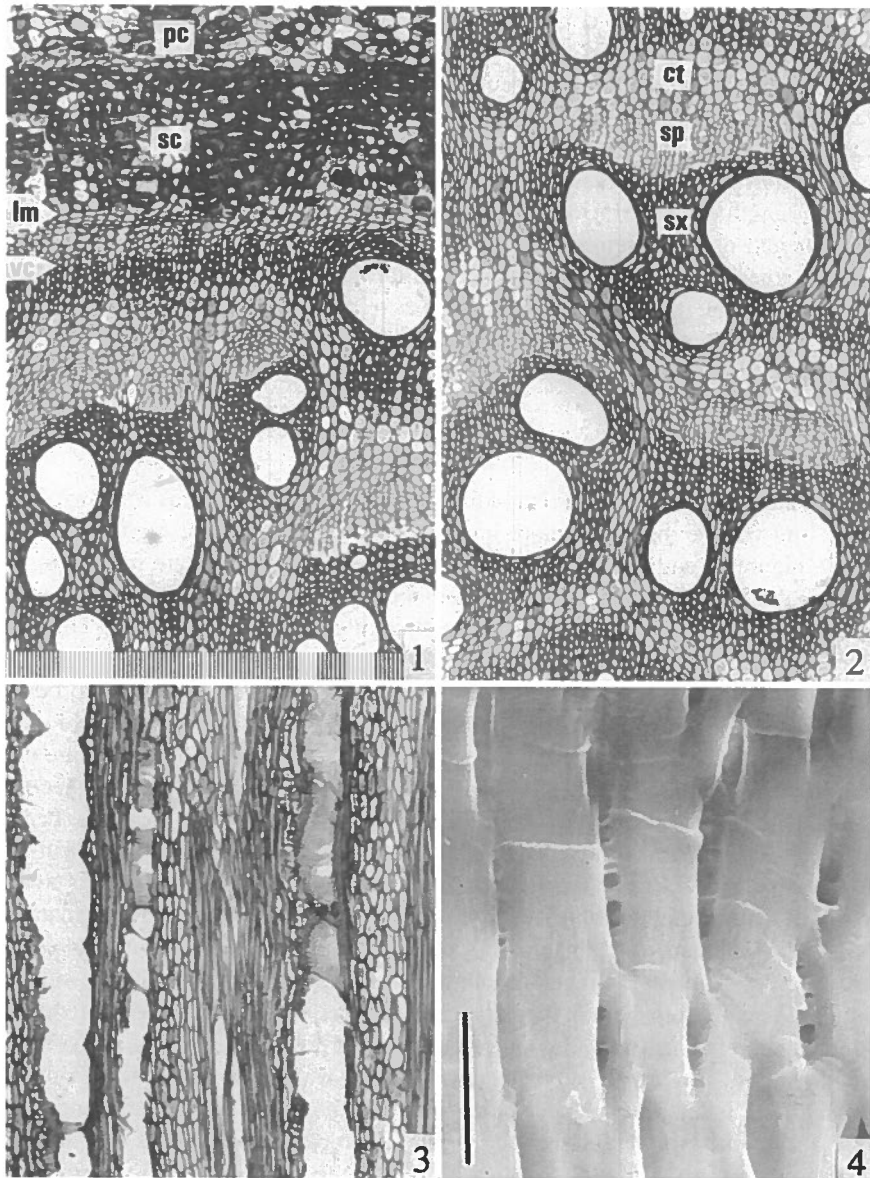


Fig. 1–4. Wood sections of *Barbeuia madagascariensis* stem. – 1: Transection of inner bark and adjacent vascular tissues (lm = lateral meristem, pc = primary cortex, sc = secondary cortex, vc = vascular cambium). – 2: Transection of secondary vascular tissues (ct = conjunctive tissue, sp = secondary phloem, sx = secondary xylem). – 3: Tangential section of secondary xylem, to show rays and vessel elements; slender cells, center middle to bottom, are a portion of secondary phloem. – 4: SEM photograph of wall of wider vessel (longitudinal axis oriented horizontally) to show grooves interconnecting pit apertures (left, above) and elongate apertures on other pits (right, below). — Fig. 1–3, magnification scale below Fig. 1 (divisions = 10 μ m); Fig. 4, scale at left = 10 μ m.

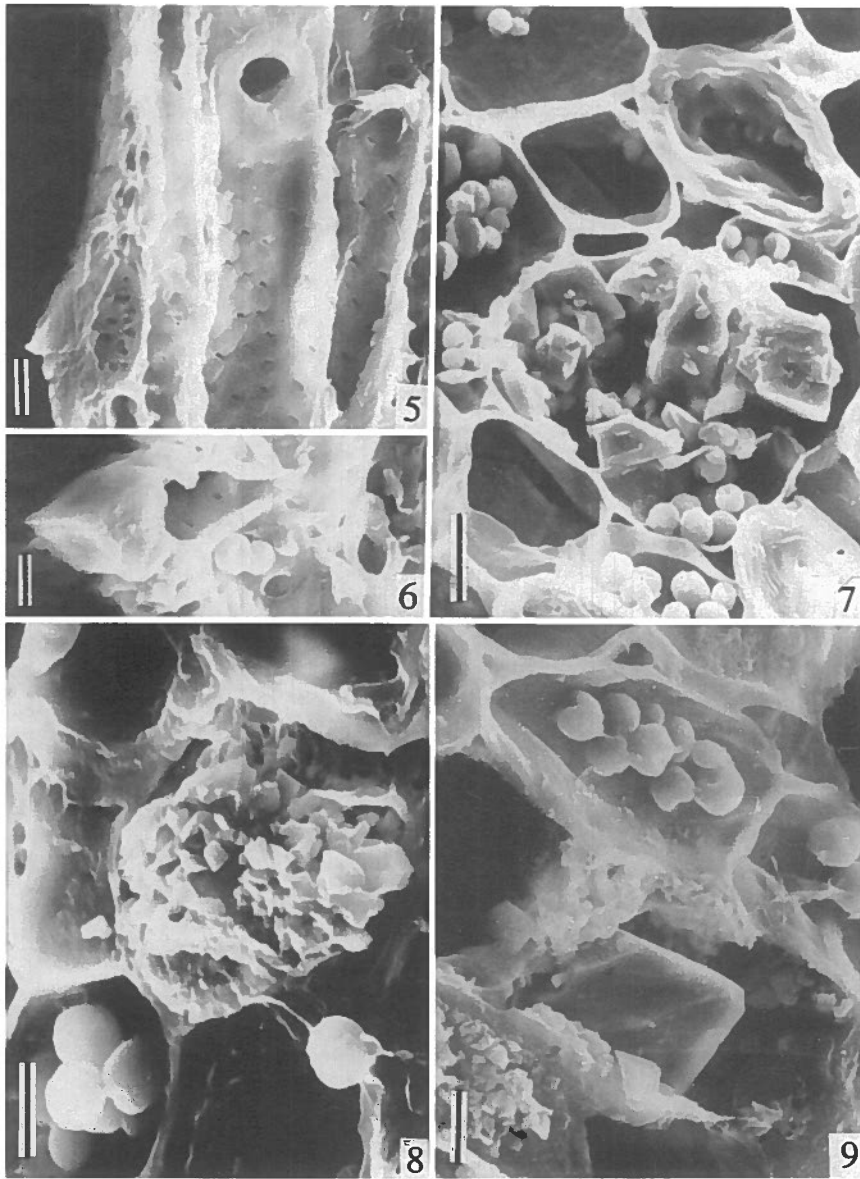


Fig. 5–9. SEM photographs of sections of *Barbeuia madagascariensis* stem. — 5: Circular perforation plate in fibriform vessel element from tangential section of secondary xylem; lumen of large vessel, left, with nonbordered perforation plate, lower left. — 6: Nonbordered perforation plate, left, from tangential section of secondary xylem (lumen of vessel at extreme left); a few starch grains are present in parenchyma cell adjacent to the perforation plate. — 7–9: Crystal-containing cells (and adjacent starch-containing cells) from secondary cortex of stem transection. — 7: Druse, the component crystals of which have been separated by sectioning. — 8: Aggregation of small rhomboidal crystals. — 9: Large solitary rhomboidal crystal. — Magnification scales at lower left in all figures = 10 μm .

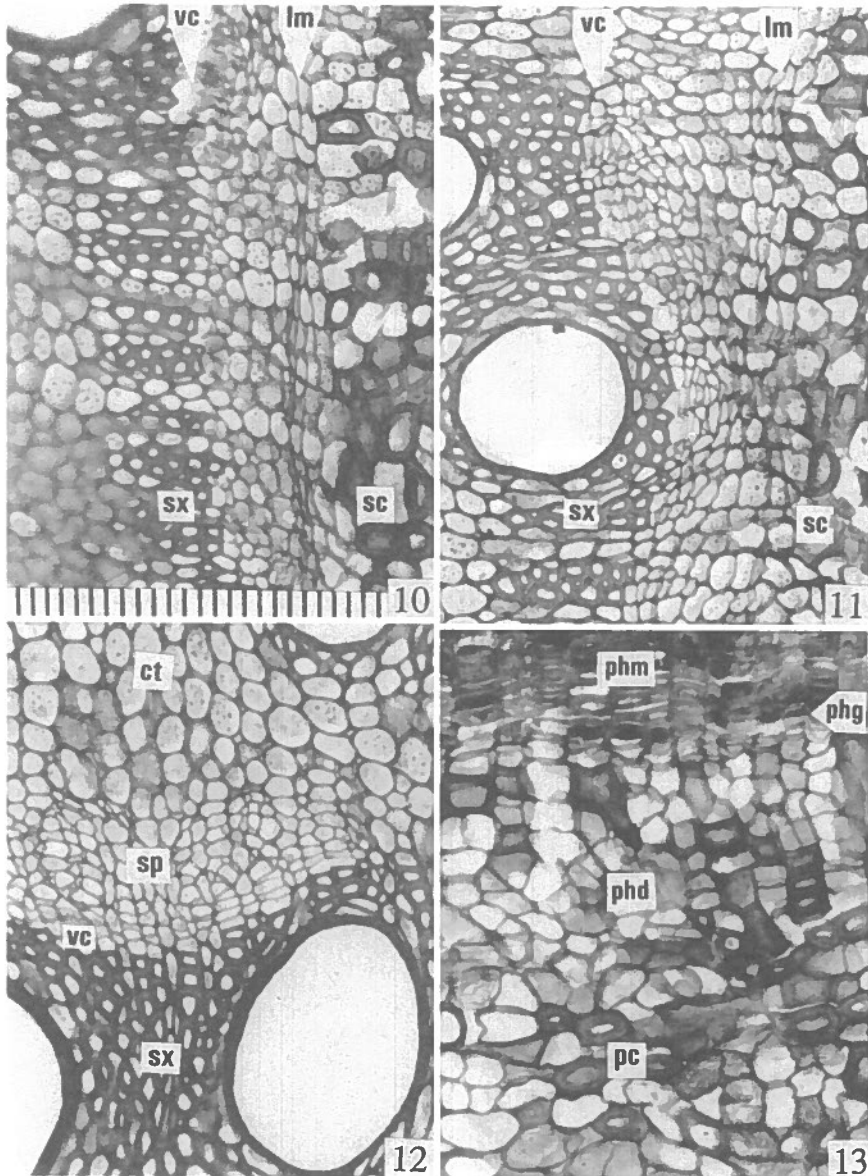


Fig. 10–13. Stem transections of *Barbeuia madagascariensis*. – 10 & 11: Juncture between bark and secondary vascular tissues (lm = lateral meristem, sc = secondary cortex, sx = secondary xylem, vc = vascular cambium). – 10: Secondary xylem comprised mostly of tracheids. – 11: Wider vessel (and portion of a second, above left) in secondary xylem; secondary phloem to the right of the secondary xylem. – 12: Histology of vascular tissues and associated cells (ct = conjunctive tissue, sp = secondary phloem; sx = secondary xylem; vc = vascular cambium). – 13: Portion of periderm (only inner phellem shown) and adjacent primary cortex (pc = primary cortex; phd = phellderm; phg = phellogen; phm = phellem). — Magnification scale for all figures below Fig. 13 (divisions = 10 μm).

Bark

Bark of *Barbeuia* can be subdivided into three main concentric components: secondary cortex, secondary phloem, primary cortex, and periderm. Secondary cortex (sc) is illustrated in Figures 1, 10, and 11 (sc); primary cortex (pc) is seen in Figures 1 (top) and 13 (bottom); and periderm is incompletely shown in Figure 13 (phm = phellem, phg = phellogen, phd = phelloderm).

Primary cortex consists of sclereids and parenchyma. The parenchyma is rich in starch and contains groups of cells with dark contents. Parenchyma cells are somewhat more abundant than brachysclereids in the primary cortex.

Along the inner margins of the primary cortex, one can find strands of phloem fibers. Between the strands of phloem fibers are dilated phloem rays that have been converted into brachysclereids. These phloem rays belong to the secondary phloem, as do some sclereids adjacent to the strands of primary phloem fibers. Secondary phloem cells of the first of the vascular tissue formed by the stem do not comprise a definable zone.

The tissue designated as secondary cortex here consists of radial files of sclereids, among which are some parenchyma cells. These radial files are clearly products of the lateral meristem, not of a vascular cambium (Fig. 1, 10, 11). The parenchyma of the secondary cortex is rich in starch (Fig. 10, 11) and includes idioblastic cells that contain druses or crystals of various sizes (Fig. 7–9). A fractured druse is illustrated in Figure 10, a mass of small (apparently not aggregated) rhomboidal crystals in Figure 8, and a single large rhomboidal crystal in Figure 9. The secondary cortex is formed after cessation of formation of secondary phloem from the outer band of vascular tissue. Secondary cortex formation is continuous, not in successive increments. There is only one lateral meristem (lm in Fig. 1, 10, 11) in a stem, and therefore there is only one accumulation of secondary cortex cells outside that meristem.

The outer portion of the periderm consists of a thick layer of phellem, only the inner part of which is illustrated in Figure 13. Phellem cells that have lignified thick secondary walls and are therefore sclereids occur singly and in groups. Phellem cells are filled with dark contents that account for the black color of the bark as seen in gross aspect. In the stem studied, about ten layers of phelloderm were present. The phelloderm, like the secondary cortex, consists of radial rows of sclereids intermixed into a parenchyma background.

Successive cambia and lateral meristem activity

A critical review of origin and products of successive cambia is needed. Prior to appearance of such a review, I am using a simple descriptive terminology. The term 'vascular cambium' is used here for a cambium that produces secondary phloem externally and secondary xylem internally in stems and roots, and is illustrated in Figure 12. Multiple vascular cambia in a stem such as that of *Barbeuia* were termed successive cambia by Schenck (1893) and Pfeiffer (1926). A meristem that gives rise to the successive cambia, and therefore to the tissue that the successive (vascular) cambia produce is present in *Barbeuia* and other Caryophyllales that do not have 'normal' cambia (= a single vascular cambium for the life of the stem or root). The

meristem that gives rise to successive cambia and the tissue the successive cambia produce is termed 'lateral meristem' here, as it was for *Stegnosperma* (Carlquist 1999c).

The material of *Barbeuia* was suitable for study of the lateral meristem. Two photographs (Fig. 10 & 11) illustrate the lateral meristem. The lateral meristem produces secondary cortex externally in an uninterrupted fashion (there is only one band of secondary cortex, not several of them), and thus the lateral meristem is not replaced by new lateral meristems but lasts as long as growth in the stem occurs. The cells produced on the inner face of the lateral meristem are conjunctive tissue and vascular cambia. The lateral meristem in *Barbeuia*, like a vascular cambium, probably consists functionally of a single layer that can be identified in transections (Fig. 10, 11). The layer so identified in Figures 10 and 11 cannot be a vascular cambium because there is continuity between it and the files of secondary cortex external to it. If the layer indicated were a vascular cambium, phloem would be produced external to the cambium, not secondary cortex.

DISCUSSION AND CONCLUSIONS

The secondary xylem of *Barbeuia* is similar to that of *Stegnosperma*, except that uniseriate rays are lacking and axial parenchyma is primarily paratracheal. The occurrence of tracheids in both *Stegnosperma* and *Barbeuia* is noteworthy, because tracheids have not been reported in other genera of Phytolaccaceae s.l., except for the vasicentric tracheids of *Agdestis* (Carlquist 1999b). Presence of tracheids, as well as presence of both multiseriate and uniseriate rays and presence of diffuse axial parenchyma are features that have been considered primitive according to traditional criteria (Metcalfe & Chalk 1950: xlv, "fibres with distinctly bordered pits"; Kribs 1935, 1937).

Nonbordered perforation plates have been reported in segregate families of Phytolaccaceae: Rivinaceae (Carlquist 1998) and Agdestidaceae (Carlquist 1999b). Although bordered perforation plates do occur in some families such as Cactaceae (Gibson 1973; Carlquist, unpublished data), nonbordered perforation plates may occur more widely in Caryophyllales than present reports indicate.

In *Stegnosperma*, the lateral meristem does not appear as a single layer. Rather, divisions in radial files, producing secondary cortex externally (but not as much of it as in *Barbeuia*) and conjunctive tissue and vascular cambia internally, were not synchronized in time or space. Consequently, the lateral meristem in *Stegnosperma* was termed a diffuse lateral meristem (Carlquist 1999c). The lateral meristem of *Barbeuia* may be considered a nondiffuse lateral meristem. Attention will be paid in future studies to analyzing the nature of lateral meristems in Phytolaccaceae s.s. and Nyctaginaceae, both of suborder Phytolaccineae, and of families that have been given positions somewhat removed from Phytolaccineae in cladograms of Caryophyllales, e.g., Amaranthaceae and Chenopodiaceae. Possibly there have been evolutionary changes (but likely not major ones) in the nature of lateral meristems within Caryophyllales. The distinctive secondary cortex production by the lateral meristem in *Barbeuia* may be an apomorphy.

The wood features of *Barbeuia* mark it as similar in relatively primitive phylogenetic level to *Stegnosperma*, which has been placed in a near-basal position in Caryophyllales by some workers (Rodman 1994; Manhart & Rettig 1994) but not by others (Downie & Palmer 1994), although these authors do not claim a high degree of certainty for their results. The wood of *Barbeuia* does not appear similar to that of Amaranthaceae and Chenopodiaceae on the basis of information presently at hand (Metcalf & Chalk 1950; Fahn et al. 1986; Gibson 1994). Further studies of wood of Phytolaccaceae s. s. and of the Amaranthaceae–Chenopodiaceae alliance will doubtless offer some clarification, although molecular data on *Barbeuia* and on more numerous genera of Caryophyllales may prove to be more decisive. Attention should be paid to the distribution of nonbordered perforation plates in Caryophyllales; they occur in *Petiveria* and *Rivina* (Carlquist 1998b); *Agdestis* (Carlquist 1999b), and *Stegnosperma* (Carlquist 1999c). Nowicke (1994) finds the pollen of *Barbeuia* similar to that of *Stegnosperma*.

The presence of marked dimorphism in vessel diameter, a characteristic of lianas (Carlquist 1985), is shared by *Agdestis* and *Barbeuia*. The distribution of conjunctive tissue in both genera may act as sheaths to protect the integrity of vascular tissue when twisting of stems occurs. The presence of crushed secondary phloem as well as recently formed secondary phloem in vascular strands of *Barbeuia* suggests that phloem conduction persists, and that therefore older secondary xylem may also be functional (tyloses are notably lacking). The preservation of conductive ability in older vascular tissue may be related, in *Barbeuia*, to the lianoid habit and provide a relatively great amount of functional secondary xylem and phloem. Species with successive cambia have a notably low conductive area per mm² of transection of stem (Carlquist 1975: 206), but prolonged conductive capability of secondary xylem and phloem in these species may compensate for that low conductive area. Certainly the occurrence of apparently viable secondary phloem, produced over indefinite periods of time by vascular cambia in older vascular strands of *Barbeuia*, is an indication of this, and signs of such prolonged conductive activity should be sought in other species with successive cambia.

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