

Origins and nature of vessels in monocotyledons. 10. Boryaceae: xeromorphic xylem structure in a resurrection plant

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Abstract

Boryaceae are unusual in having xylem with an abundance of thick-walled tracheids, combined with relatively few vessel elements with scalariform perforation plates that have very slender bars. These features are relevant to the resurrection plant habit of *Borya*. The abundance of thick-walled tracheids provide sites for maintenance of water columns during hot dry months, whereas the perforation plates offer minimal impedance to flow during wet periods. This combination is unusual in monocotyledons. Root xylem is like stem xylem in the two species studied. *Borya sphaerocephala* occurs in the Mediterranean-type climate of south west Western Australia, whereas *Borya subulata* grows in a tropical summer-monsoon area, the Kimberley plateau of northern Western Australia. Despite differences in climatic regimes of the two species, survival of the dry season appears to be the primary factor in design of the xylem of both, and explains their similarity. Boryaceae are now placed in Asparagales, near Asteliaceae, Blandfordiaceae, Hypoxidaceae, and Orchidaceae. Xylem structure does not parallel the phylogenetic interconnections among these families, in which moist habitats, succulent habits, and other modifications seem to mitigate the effect of ecology in patterning xylem structure.

Keywords: Asteliaceae, Blandfordiaceae, Hypoxidaceae, Orchidaceae, Origin of vessels, scalariform perforation plates, xylem.

Introduction

Borya Labill. (16 described species) and *Alania* Endl. (2 species) form a family of resurrection plants, Boryaceae, confined to Australia. Earlier recognized within Liliaceae (tribe Johnsonieae), or a segregate family, Anthericaceae, the distinctiveness of Boryaceae has been amply confirmed (Chase *et al.*, 1997). *Borya* plants resemble large mosses in form: they have narrow leaves borne on upright stems. Prop roots attach them to the substrate (sandstone or granite). Little soil is present on the “pavement” areas where they grow, and because of bright sunlight, the habitats are essentially dry for perhaps half of the year. *Borya* is commonly considered a resurrection plant, the leaves of which become green soon after commencement of the rainy season. A number of studies relate to this habit (Gaff, 1981, 1989; Gaff and Churchill, 1976; Gaff *et al.*, 1976; Hetherington and Smillie, 1982; Hetherington *et al.*, 1982; Kauff *et al.*, 2000; Keighery, 1984).

The groups of monocotyledons the xylem of which has been studied with SEM in earlier papers of this series, such as Acoraceae (Carlquist and Schneider, 1997) and Araceae (Carlquist and Schneider 1998; Schneider and Carlquist, 1998), represent plants that live in mesic situations (Carlquist, 1975) and may have had an unbroken history in such habitats. They therefore

represent stages in xylem evolution generally regarded as primitive (*e.g.*, Cheadle, 1942). The xylem of Orchidaceae (Carlquist and Schneider, 2006) and *Sansevieria* (Carlquist and Schneider, 2007) represents primitive conditions that may have persisted by virtue of succulence and other modifications. Boryaceae clearly are not mesomorphic and represent a potential contrast with those families.

Various characters of Boryaceae invite comparison to those of related families. Boryaceae have been considered to belong to a clade with the order Asparagales that includes Blandfordiaceae (*Blandfordia*, 3 species, eastern Australia), Asteliaceae, Hypoxidaceae, and Orchidaceae (Fay *et al.*, 2000; Soltis *et al.*, 2000. Cheadle and Kosakai (1971) included *Borya nitida* Labill. in a monograph of xylem of Liliaceae. They also list *Borya* among seven species, representing as many genera, of the tribe Johnsonieae. *Blandfordia*, which would no longer be included in that tribe, is illustrated as having long scalariform perforation plates in vessels of roots. Cheadle and Kosakai (1971) do not illustrate the xylem of *Borya*, and only include merged data for Johnsonieae as a whole in a table. The xylem of *Borya* offers excellent material for examination of a question: to what extent are tracheids and vessels in monocotyledons indicators of shared heritage (symplesiomorphies) and to what extent are they templates that reflect adaptation to ecology? What factors hasten or slow changes (or possibly even reverse them) in adaptation to ecological conditions? Cheadle (1942) has offered a scheme for understanding

of evolution of monocotyledon xylem based on morphology. However, the picture he produced expresses constraints and pathways.

Materials and Methods

The specimen of *Borya sphaerocephala* was collected by K. F. Kenneally and K. G. Varnavides on 31 January 2007 (PERTH) along a granite apron surrounding the Darling Scarp, 15 km ENE of Perth (32°01'S 116°01'E), adjacent to Bickley Brook Reservoir. The mean rainfall is ca. 1000 mm in this region, but hot weather (to 37°C) renders the shallow sandy soil dry during the summer months. *B. sphaerocephala* flowers during the spring months (August to October). Plants are often in tufts, less than 10 cm in height.

The material of *B. subulata* was collected by M. D. and R. L. Barrett on 24 January 2007 (PERTH) on a sandstone pavement at Bachstein Creek in the Kimberley Region of northern Western Australia. At this locality, it grows with *Calytrix*, *Drosera*, *Eriachne*, *Eriocaulon*, *Goodenia*, *Micraira*, *Triodia*, and *Utricularia*. During the summer rainfall season, the shallow seepage areas where *B. subulata* grows are covered with 2–3 cm of trickling water, although the pavement areas are dry during the winter. Flowering occurs during December to April. The plants are up to 50 cm tall, with stilt roots up to 30 cm long; longer stilt roots (often carbonaceous) occur on plants that grow on bare rock.

Material of both species was collected in the field, then preserved in 70% aqueous ethanol. Freehand razorblade longisections of roots were washed with distilled water, air dried between glass slides, and mounted on aluminium stubs. Macerations were prepared with Jeffrey's Fluid, washed in distilled water, and air dried on aluminium stubs. In the case of both sections and macerations, material on aluminium stubs was sputter coated with gold and examined with a Hitachi S-2600N scanning electron microscope (SEM).

Results

Macerations proved more useful than sections for the majority of observations because tracheids much outnumber vessel elements in roots and stems of both species, and vessel elements are thus insufficiently exposed in sections. Macerations are illustrated in Figs. 1–3, Fig. 4A–B here, while sections were used to show pitting of tracheids (Fig. 4C–F). Both vessel elements and tracheids have very thick walls that are apparently not highly lignified, and thus the oxidative treatment of the macerating fluid, followed by washing and drying, caused curvature in tracheid shapes, presumably because of the helical distribution of soluble compounds. When such compounds (very likely hemicelluloses and pectins) were in part dissolved from walls by the macerating fluid and the tracheids subsequently dried, coiling of tracheids resulted. Such coiling was not common in sections. We cannot exclude the possibility that some vessel elements in stems might be those of adventitious roots embedded in the stem, although we attempted to minimize that possibility.

Borya sphaerocephala roots (Figs. 1A–1C)

Vessel elements have perforation plates that range from near-transverse (Fig. 1A) to markedly oblique (Figs. 1B, 1C). In most vessel elements, perforation plates have very slender bars that stretch across the plate. Although the bars are so tenuous that in macerations they often break (Figs. 1B, 1C) or collapse to the margins of the plates (Fig. 1A), we believe that bars traversing the entire width of a perforation plate are the rule, and that bars that extend partly across the plate are infrequent. The bars taper abruptly from the lateral edges of the perforation plate. Perforation plates qualify as scalariform, with 10–20 bars (possibly some of them partial bars).

Lateral wall pitting is alternate (Figs. 1A, 1C). Where pitting is not evident on lateral walls (Fig. 1B, right), the wall may represent an interface with fibers rather than with tracheids.

Borya sphaerocephala stems (Figs. 1D, 2A–2F)

Variation in the width of bars on perforation plates is evident: the bars shown in Fig. 1D are somewhat thicker than the norm (Fig. 2A), but the range is not very great. Vessel elements mostly have oblique perforation plates (Figs. 1D, 2A); a portion near the end of a fibriform vessel element is shown in Fig. 2D.

Pit membrane remnants may be found at the top and bottom of a given perforation plate, where transitions to lateral wall pitting occurs. The pit membrane remnant in Fig. 2B represents an enlargement of the perforation plate portion shown in Fig. 2A (bottom). Note that an intact pit membrane (wrinkled) occurs at the pit at the bottom of Fig. 2B. The perforation plate portion in Fig. 2E represents the top end of the perforation plate shown in Fig. 2D; the enlarged portion focuses on a pit membrane remnant in the transitional zone of a perforation plate. Pit membrane remnants contain holes of various sizes. There are some inconspicuous borders present on the perforations shown in Fig. 2E.

Lateral wall pitting of vessel elements from *B. sphaerocephala* stems is composed entirely of circular bordered pits (Figs. 2C, 2D). These circular bordered pits are identical in size and shape to those of the tracheids (Fig. 2F). The pit borders are notably wide compared to the pit apertures (Fig. 2C).

Borya subulata roots (Fig. 3)

Roots of *B. subulata* have vessel elements with oblique perforation plates. The tapering of bars is sharp in some plates (Figs. 3A, 3B), suggesting that bars may not extend across the entire perforation plate. However, in other plates, one can find portions of very tenuous bars that have collapsed against the sides of the perforation plates, an inevitable result of the macerating process (Fig. 3C, left). Determining whether or not slender bars extend entirely across a plate is difficult because of the tendency of the plate bars to roll inwards because of the maceration process, obscuring the lateral ends of the bars.

In the short zones of transition between perforations and lateral wall pitting (Fig. 3D), pit membrane remnants are present. As with the pit membrane remnants in *B. sphaerocephala*, these remnants are porose. Lateral wall pitting of vessel elements consists of alternate circular

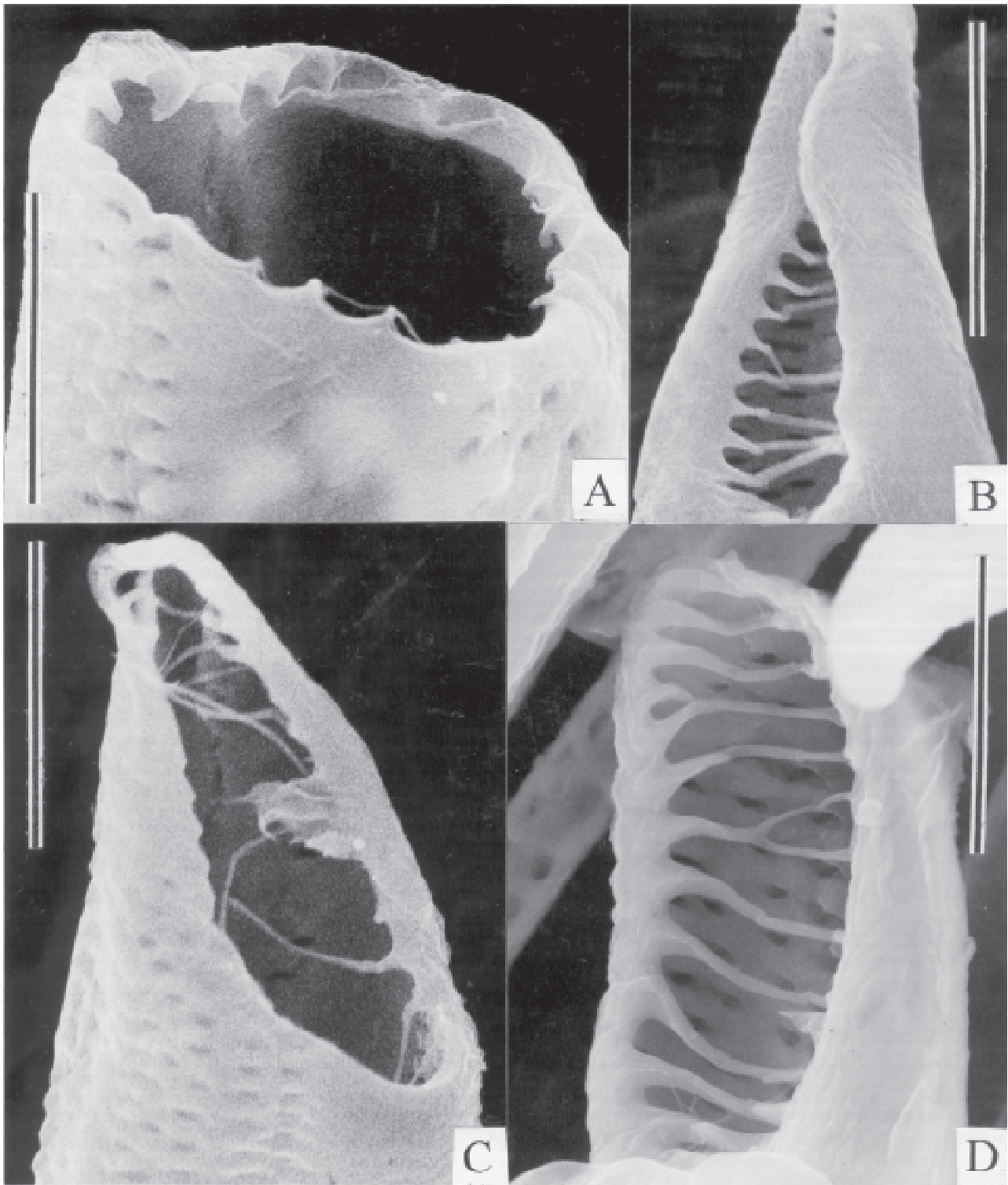


Figure 1. Vessel elements of *Borya sphaerocephala* from macerated roots (A–C) and stems (Fig. D). A. Near-transverse perforation plate; note fine threadlike portions of bars, displaced by the maceration process, at the apparent edges of the perforation plate. B. Highly oblique perforation plate; the margins are incurved, a result of the maceration process. C. Oblique perforation plate; displaced slender bars are present. 4D. Near-vertical perforation plate; borders are faintly visible at the lateral edge of the plate at left. Figs. 1A, 1B, scales = 10 μ m; Figs. 1B, 1C, scales = 20 μ m.

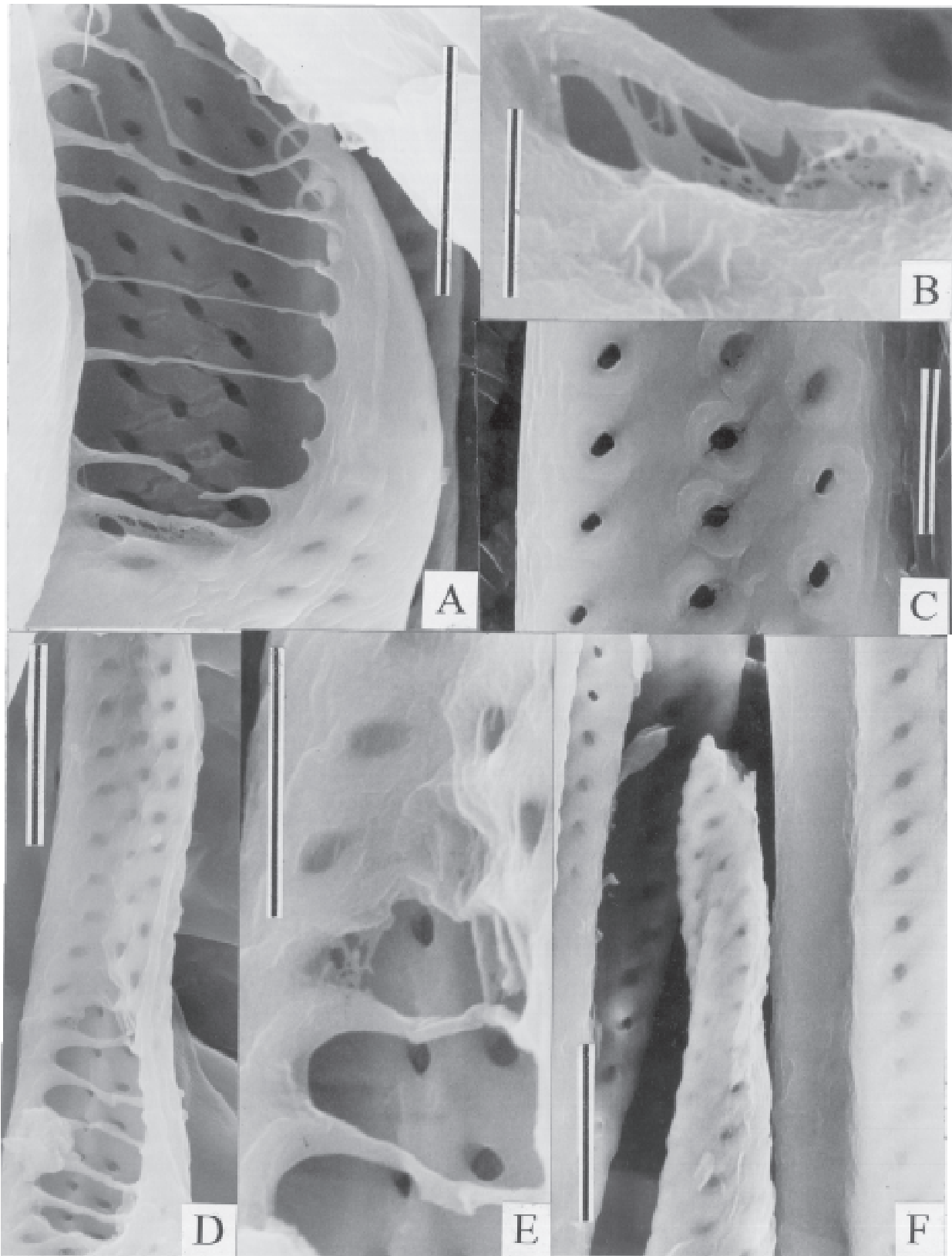


Figure 2. Portions of vessel elements (A–E) and tracheids (F) from macerations of stems of *Borya sphaerocephala*. A. Most of a perforation plate, to show attenuated nature of bars. B. Micrograph of the lower portion of the plates shown in A, to show the nature of pit membranes in the bottom two pits (or alternatively, perforations) of the plate. C. Outer surface of a vessel; apertures are oval, similar in shape to the rather wide borders. D. Subterminal perforation plate (below); the vessel element is fibriform. E. Portion of the perforation plate shown in D, to show the nature of the transition region between perforations (below) and lateral wall pitting (above). F. Portions of tracheids; the tracheids have been only partially separated by the macerating process. Figs. 2A, E, F, scales = 10 μm ; Fig. 2B, scale = 2 μm ; Fig. 2C, scale = 5 μm .

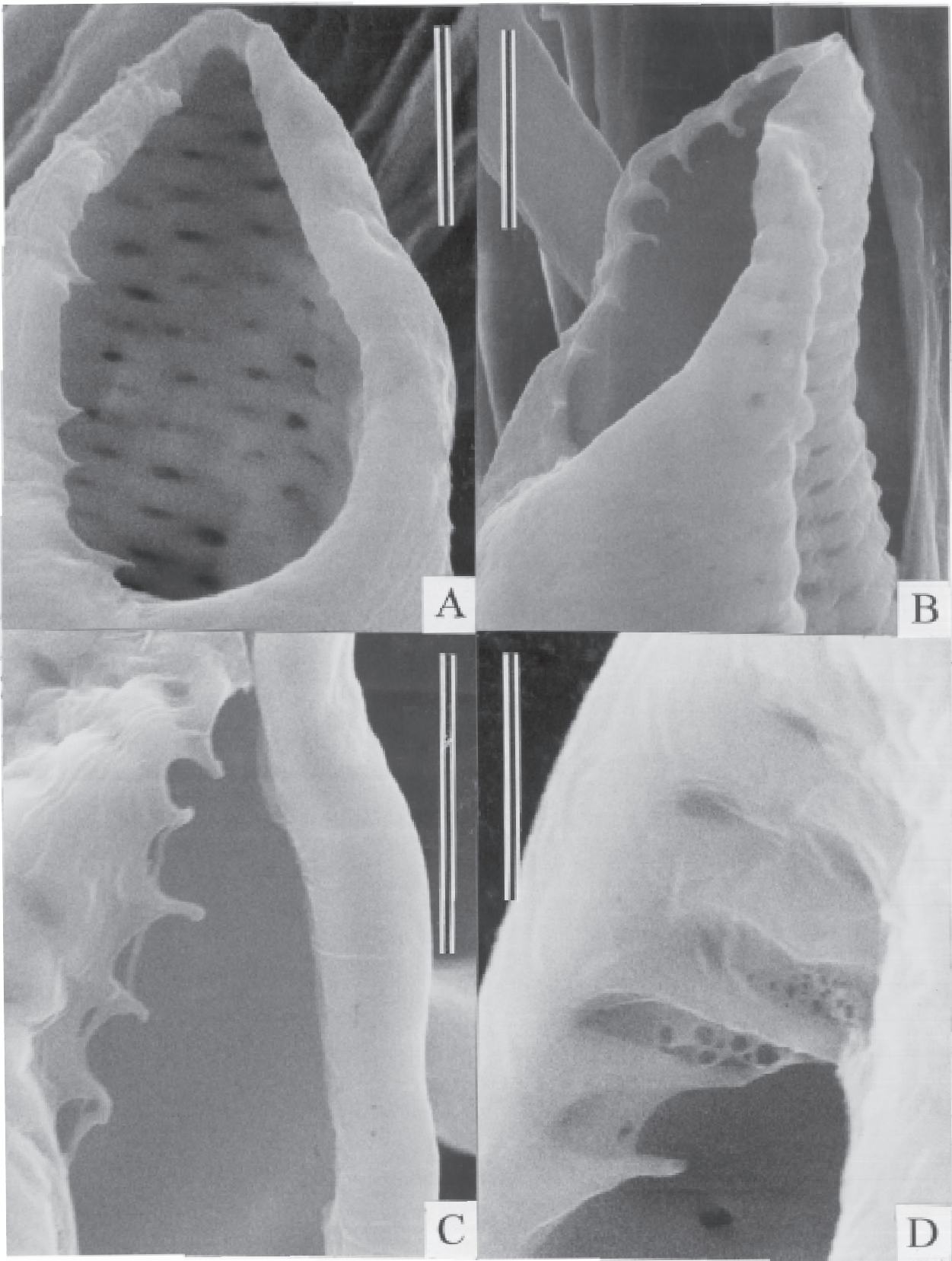


Figure 3. Portions of vessel elements from macerations of roots of *Borya subulata*. A. A perforation plate, margins (especially at right) incurved as a result of the maceration process; bars may be broken from the attenuated tips or else bars may be absent. B. A perforation plate, viewed laterally. C. A portion of a perforation plate, showing that bars are present, but slender portions of them have collapsed against the edges of the perforation plate due to handling. D. A portion of a perforation plate, showing the transition between perforations (below) and pits at the end of the perforation plate; porosities are present in the pit membranes of the three pits (or alternatively, perforations). Figures 3A–3C, scales = 10 μm ; Fig. 3D, scale = 5 μm .

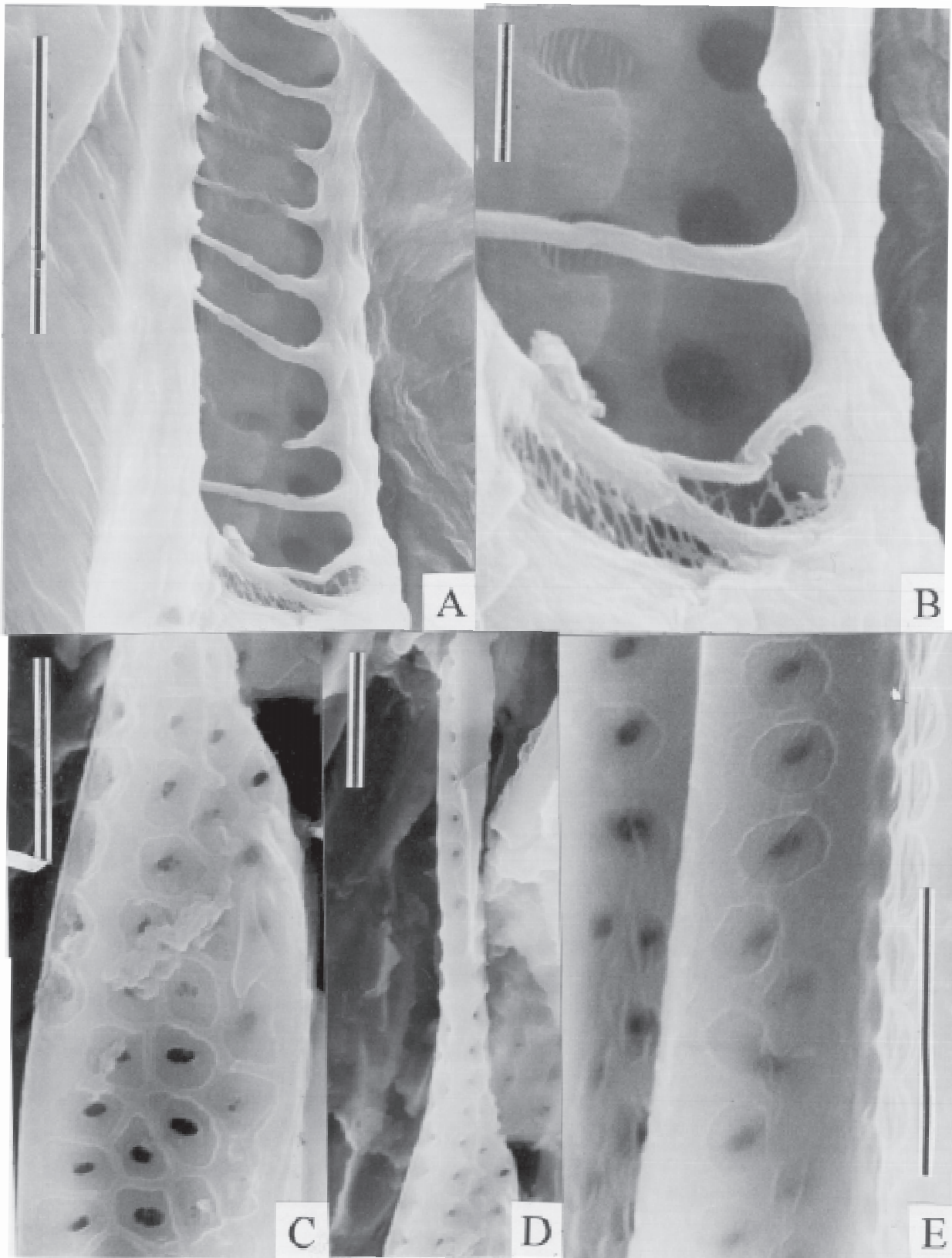


Figure 4. Portions of vessel elements (A–C) and tracheids (D, E) from macerations (A, B) and sections (C–E) of stems of *Borya subulata*. A. Perforation plate portion; some bars are intact, others are broken. B. Portion of perforation plate corresponding to the bottom of A, to show the pit membrane remnants in the pits (alternatively, perforations) of the transition area between perforation plate and lateral wall pitting. C. Area below the tip of a vessel element; pits mostly lack pit membranes because of handling; some pits are polygonal in shape. D. Tip of a tracheid, to show the attenuated shape. E. Portions of surfaces of several adjacent tracheids, to show pitting; pit membranes are present. Figs. 4A, 4C–4E, scales = 10 μm ; Fig. B, scale = 2 μm .

bordered pits (Fig. 3A). Tracheids are abundantly present in roots of *B. subulata*.

***Borya subulata* stems (Fig. 4)**

The vessel elements observed in *B. subulata* stems have highly oblique perforation plates with 10 or more bars (Fig. 4A). The transitions between lateral wall pitting and ends of perforation plates (Fig. 4A, bottom; shown enlarged in Fig. 4B) features pit membrane remnants. As in the pit membrane remnants shown elsewhere in this paper (Figs. 2D, 2E, 3S.), porosities of various sizes penetrate the pit membrane in these areas. Pit membrane remnants are confined to transition areas of perforation plates and do not occur in most of the perforations.

Lateral wall pitting of vessels may be so dense that pits are crowded and polygonal in shape (Fig. 4C) rather than circular. Tracheids (Figs. 4D, 4E) are much more abundant than vessel elements in *B. subulata* stems.

Conclusions

Study of two species of *Borya* (from a family of 14 or more species) may seem a small sample size, but the essential identity of xylem of the two species, despite marked differences between these species with respect to habitats and habits, suggests that the patterns described may extend throughout the family as a whole. Certainly *B. sphaerocephala* and *B. subulata* represent extremes where habitat and growth form are concerned (see Material and Methods).

Borya is a resurrection plant, a strategy that involves drying of root, stem, and leaf portions without actual death of all cells involved (Gaff, 1981, 1989; Hetherington and Smillie, 1982, Hetherington *et al.*, 1982). The presence of relatively large quantities of tracheids in xylem of *Borya* suggests that some water columns remain intact in the dry season in tracheid tissue, whereas vessels may embolize. The pit membranes of tracheids do not permit air bubbles to pass from one tracheid to another (Tyree and Zimmermann, 2002). Thick tracheid walls and alternate patterns of circular bordered pits are wall configurations of maximal strength that would resist the development of high negative pressures that are to be expected during periods of heat and drought (Carlquist, 1975). Vessel elements in *Borya*, because of their near-simple perforation plates, would convey water rapidly to new leaves and to flowering portions of the plant during brief wet seasons.

The xylem reported here for *Borya* is uncommon in monocotyledons. Grasses, for example, have xylem that consists of vessels, with few or no tracheids; all of the vessels in grasses have simple perforation plates (Metcalf, 1960). The xylem of roots of Orchidaceae consists either of vessels (with scalariform perforations plates) in roots, or else tracheids that have some tendencies towards being vessel-like. The stems and inflorescence axes of orchids have tracheids with some tendencies toward being vessel-like (Carlquist and Schneider, 2006). Orchid xylem represents a conformation suited for relatively slow rates of water conduction, a condition related to the succulence, thick cuticles, and other factors likely to promote slow translocation. In addition, however, the presence of

tracheids in orchid xylem promotes potential conductive safety because of the ability of tracheids to confine embolisms to individual cells. Grasses have minimal conductive safety, but they have the ability to restore water columns rapidly if air embolisms have occurred (Stiller *et al.*, 2005). The adaptations of grass xylem and of orchid xylem represent modalities quite different from those of *Borya*, a fact that underlines the distinctiveness of *Borya* xylem as representing how the water economy of a resurrection plant can be achieved.

The slender bars on perforation plates of *Borya* are distinctive. Based on the morphology of vessels in monocotyledons at large (Cheadle, 1942), one might expect such a vestigial version of the perforation plate not to be represented so consistently in a genus. Mixtures of simple and scalariform perforation plates are reported in some genera and families of monocotyledons (Cheadle, 1942). That suggests that the transition from scalariform to completely simple plates are most commonly abrupt, with preservation of vestigial bars on perforation plates a rare condition. Many drawings of perforation plates of monocotyledons have been offered by Cheadle and Kosakai (e.g., Cheadle and Kosakai, 1971), but none of their drawings seems to suggest the presence of such tenuous bars on perforation plates. This may be an artifact of the drawing method used by Kosakai, who employed rather coarse lines and emphasized simplicity in representation.

No study of monocotyledon xylem known to us has involved determining the relative proportions of tracheids and vessel elements in xylem of particular species. In view of the preponderance of tracheids over vessel elements in *Borya*, such analysis becomes highly desirable. The assumption, perhaps aided by textbook drawings of xylem transactions of grass or palm bundles (usually labeled as composed of vessels only) or those of *Yucca* stems (usually labeled as composed of tracheids only), is that one cell type or the other is present. There is an interesting parallel in wood of dicotyledons. Vasicentric tracheids occur in various dicotyledon woods: they may be few in number, or they may (as in Fagaceae and Myrtaceae) be relatively abundant. The entire background of a wood may be composed of tracheids. These variations have considerable ecophysiological significance, particularly where xeric habitats are concerned (Carlquist, 1985). The xylem of *Borya*, therefore, opens a fascinating new avenue for investigation of xylem of monocotyledons.

One of the traditional objectives in the study of xylem anatomy has been the application of findings to the classification system. Indeed, the data in surveys of monocotyledon xylem have been organized in terms of familial units. Now that Boryaceae is recognized as a family (Chase *et al.*, 1997) belonging to Asparagales, the most closely related families are considered to be Asteliaceae, Blandfordiaceae, Hypoxidaceae, and Orchidaceae (Fay *et al.*, 2000; Soltis *et al.*, 2000). Indeed, these four families plus Boryaceae might be considered a suborder of Asparagales according to recent phylogenetic constructions. The xylem of the four families other than Boryaceae contains vessels with very primitive perforation plates in roots, and tracheids only in stems (Cheadle and Kosakai, 1971; Carlquist and Schneider, unpublished data). Cheadle and Kosakai (1971) actually

do not give illustrations for *Borya* xylem, and give data only in a merged tabular form for the tribe Johnsonieae (of Liliaceae) as a whole, so that our illustrations and descriptions are apparently the first. The difference between xylem of Boryaceae and that of the four other families with respect to xylem is conspicuous. This difference corresponds to the unusual nature of Boryaceae as a resurrection plant, and should not be interpreted as evidence of a lack of relationship. DNA-based phylogenies have supplanted comparative anatomy as a prime source of evidence for natural relationships. If we analyze clades of vascular plants in the light of DNA-based phylogeny, we find unappreciated patterns of diversity in anatomical features (Carlquist, 2006). These patterns can be traced to the operation of ecology in evolution of tracheary elements and other anatomical features that relate to water economy.

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