

Origin and Nature of Vessels in Monocotyledons. 6. *Hanguana* (Hanguanaceae)¹

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Abstract: Vessel elements from macerations of roots and stems of *Hanguana malayana* were studied with scanning electron microscopy (SEM). Vessel elements are present in both stems and roots. The vessel elements of roots are slightly more specialized than those of stems in having greater differentiation of perforation plates from lateral wall areas. Long areas of transition between lateral wall areas and perforation plates, consisting of 10 or more pits (arguably perforations) with porose membranes or threadlike pit membrane remnants, characterize vessel elements of both stems and roots of *Hanguana*. Tracheids may be present, but cannot be identified with certainty. The vessel elements of *Hanguana* are like those of *Acorus* (Acoraceae) in primitiveness and are among the most primitive recorded for monocotyledons. These facts are consistent with placement of *Hanguana* in a monogeneric family, rather than in Flagellariaceae or any other family of monocotyledons.

THE GENUS *Hanguana*, earlier assigned to Flagellariaceae, was assigned to the monogeneric family Hanguanaceae by Airy Shaw (1965). The family has recently been placed in Commelinales (APG 2003). This placement is similar to the idea of Rudall et al. (1999) that Hanguanaceae are in Zingiberales and form a sister group to the remainder of Zingiberales. Attention has been focused recently on anatomy of Hanguanaceae (Rudall et al. 1999, Tillich and Sill 1999). These papers add interesting new aspects, especially with regard to pollen and floral anatomy, to the earlier account of Tomlinson (1969) on vegetative anatomy.

Availability of suitable material for study of xylem of *Hanguana malayana* (Jack) Merrill has led to our investigation of vessels using scanning electron microscopy (SEM). In the account of Tomlinson (1969), vessels are absent from stems and leaves in *Hanguana*, and

no vessels were reported for roots of the genus. This suggested to us the possibility that either tracheids or very primitive tracheidlike vessels were present. In the investigation reported here, as in earlier studies (Carlquist and Schneider 1997, 1998a,b, Schneider and Carlquist 1997, 1998), we have attempted to study what appear to be more primitive vessels of monocotyledons and to show tracheidlike features in them with SEM. Many of the monocotyledons with more-primitive vessels seem to be associated with permanently moist habitats; roots of these monocotyledons are often submersed in water (Acoraceae, Juncaginaceae, Scheuchzeriaceae) or embedded in mud that rarely if ever dries (Araceae, Lowiaceae). Hanguanaceae occupy the latter type of habitat: sunny but wet. *Hanguana* forms prostrate rhizomes (stolons) at or beneath the surface of mud, and, from these, upright rosettes branch. In season, tall paniculate inflorescences grow from the rosettes.

According to the data of Cheadle (1942), vessels originated in roots of monocotyledons and phylogenetically proceeded acropetally into the plant body. The degree of specialization of these vessels has followed the organographic sequence, so that more-specialized vessels are to be expected in roots, least-specialized vessels in leaves. Cheadle (1942) designated some families of monocotyledons

¹ Manuscript accepted 21 June 2004.

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as having vessels in roots only (see also Carlquist 1975:106). In five families so reported by Cheadle that we have studied (Acoraceae, Araceae, Juncaginaceae, Lowiaceae, Scheuchzeriaceae), we have observed vessels in both stems and roots. Moreover, in these families, the stem vessels were very similar to root vessels when viewed with SEM. With these findings as a precedent, we thought that there might be vessels, albeit of a primitive type, in *Hanguana* roots and stems. In fact, the primitive nature of vessel elements of *Hanguana* invites study because they are so pertinent to understanding the transition between tracheids and vessel elements in monocotyledons.

MATERIALS AND METHODS

Portions of stolons and roots of *Hanguana malayana* were pickled in 50% aqueous ethanol from plants cultivated in the National Tropical Botanical Garden (NTBG), Kōloa, Kaua'i, Hawai'i. The collection data for the plant studied are: NTBG accession no. 980152, from material collected in Palau (Belau) by Tim Flynn and David Lorence in 1998. Macerations were prepared with Jeffrey's Fluid kept at 60°C; the maceration process was carefully monitored because the period during which maceration has begun to occur yet tissues have not dissociated excessively is brief.

Macerated tissues were stored in 50% aqueous ethanol. Portions were dispersed onto aluminum stubs, air-dried, sputter coated with gold, and examined with a scanning electron microscope (Hitachi S2600N). The degree of maceration chosen was not difficult to select, because only a little more maceration would have resulted in destruction of cell details.

Care was taken in interpretation of images. Rips or tears in pit membranes can be readily identified (Figure 2, upper right; Figure 4, below). Complete absence of pit membranes was not by itself considered evidence that perforations were present. In Araceae, distinction between end walls and perforation plates is very clear: lateral wall pits always contain intact pit membranes. However, we are reporting our observations conservatively.

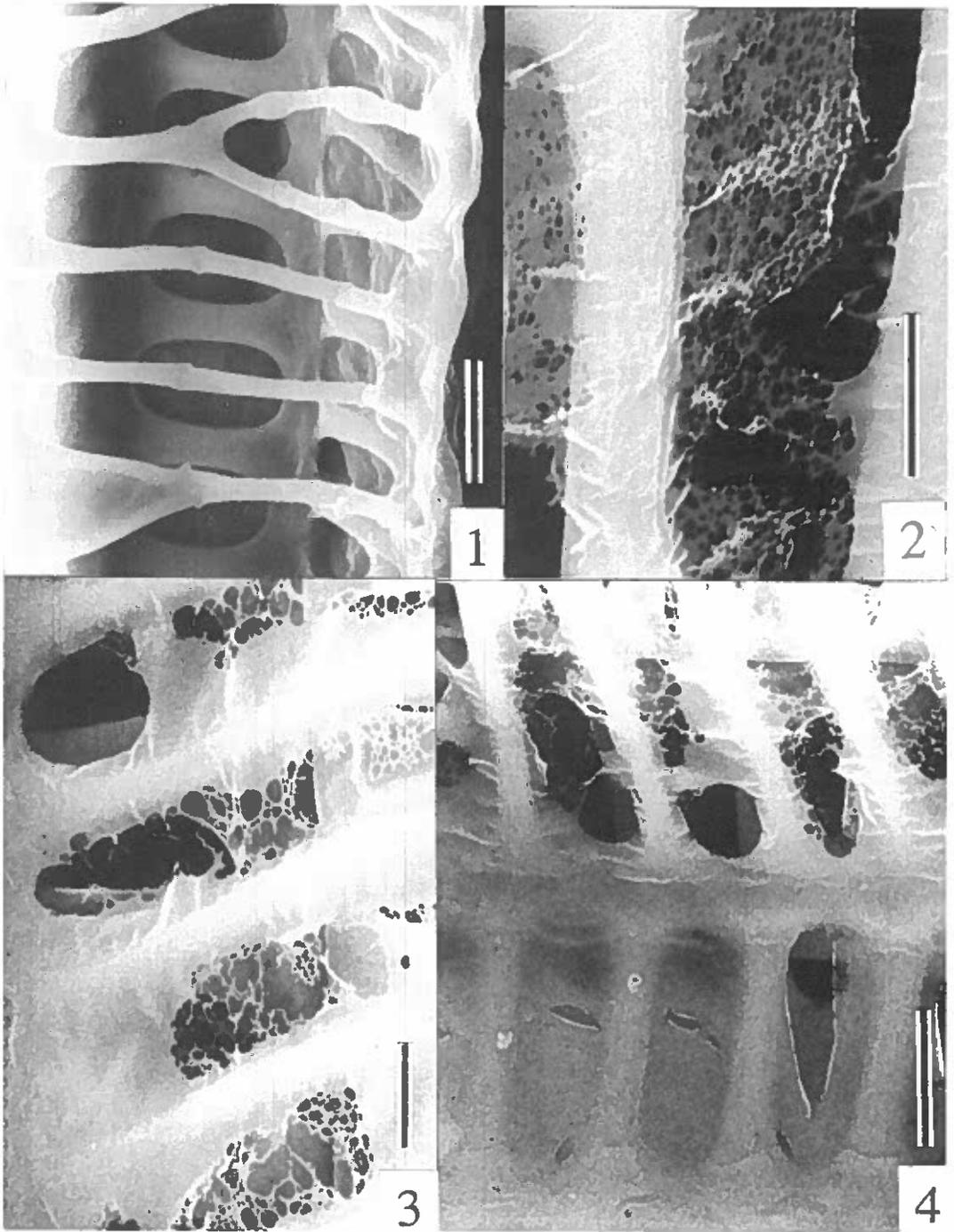
Presence of a pit membrane that contains obvious and numerous pores (Figures 5–7) or threads (Figure 8) or intermediate pit membrane remnant manifestations (Figures 2–4) is considered to be indicative either of perforation plate presence or of pits adjacent to perforation plates and transitional to the perforations. The word "porose" is used here to indicate a lamina pit membrane that contains circular holes much larger than plasmodesmata (the latter cannot, in fact, be resolved with our equipment). The contrast between porose and nonporose membranes is shown in Figure 5 and demonstrates that porose membranes are not merely random artifacts. The location of porose membranes within tracheary elements, as mentioned in Results, also is basic to our interpretations. These criteria are not assumed for the purposes of this study but have been the basis for interpretations of earlier papers in the series (Carlquist and Schneider 1997, 1998a,b, Schneider and Carlquist 1997, 1998).

RESULTS

Roots

In roots (Figures 1–4), secondary wall configurations clearly show the difference between pits of lateral walls and perforations in the tracheary elements of *Hanguana* (Figure 1). The bars of the perforation plate (forward in photo) are much narrower than the strips of secondary wall between pits (farther back in photo). Whether there are any perforations entirely free from pit membrane remnants is difficult to determine, but in our preparations, perforations in vessel elements of the roots showed fewer remnants than did those of the stolons.

Quite conspicuous are the transitional areas of perforations (which may also be considered pits) between areas clearly defined as lateral wall pitting and areas that qualify as perforation plates. These transitional areas are definable by presence of pit membranes that contain pores of various sizes (Figures 2, 3) or tend to be a series of threads. In these descriptions, we take into account ripped areas (e.g., Figure 2, upper right; lower half



FIGURES 1-4. SEM photographs of vessel portions from macerations of roots of *Hanguana malayana*. 1, Portion of perforation plate, to show comparison between width of bars and wall configuration of lateral wall pitting, seen behind the perforation plate. 2, Portions of two pits from transition between perforation plate and lateral wall pitting; pit membranes contain numerous pores of various sizes. 3, Portions of pits from transition between lateral wall pitting and perforation plate, or, possibly, perforations from a perforation plate; pit membrane remnants are intermediate between porose and threadlike. 4, Portion of perforations or pits adjacent to perforations (*above*) and lateral wall pits (*below*), to show contrast between membranes in and adjacent to a perforation plate and those of lateral wall pits. Figures 1, 4, scale bars = 5 μm ; Figures 2, 3, scale bars = 2 μm .

of photo), which may be assumed to have had patterns like those of intact areas before formation of the tears. Pit membranes with abundant pores or composed of threadlike structures do not occur at random but may be seen in zones transitional between lateral wall pitting and perforation plates. Pit membranes of lateral wall pitting may show artifact formation, such as rips (Figure 4, below), but pores are not detectable in the membranes. We have designated as "transitional areas" zones intermediate between perforation plates in secondary wall and pit membrane characteristics. Transitional areas are located distal and proximal to the perforations. More than 100 bars were counted in perforation plates of a few vessel elements. The lengths of perforation plates and of the vessel elements themselves are difficult to establish because vessel elements are rarely isolated in the preparations but rather are almost always partially covered by parenchyma cells of the ground tissue or fibers of the bundle sheath.

Stolons

The tracheary elements of stolons (Figures 5–8) are like those of roots but with less-pronounced differences between perforation plates and lateral wall pitting. In Figure 5, a comparison between lateral wall pits (far right) and a perforation plate (remainder of photograph) can be made. Strips of secondary wall between the lateral wall pits are wider than the bars of the perforation plate, but the difference is not as pronounced as in roots (Figure 1). The nonporose nature of lateral wall pitting is also evident in Figure 5. The numerous perforations covered by porose pit membranes in Figure 5 are striking—this area of transition is unusually extensive and homogeneous. Variety in distribution and size of the pores in pits of the transitional area is evident in Figure 6. Figures 7 and 8 show a contrast between the pit membranes of transitional areas (Figure 7) and central areas of the perforation plate (Figure 8). The pit membrane of Figure 7 has a meshworklike configuration. The pit membrane of Figure 8 consists of slender threads that, understand-

ably, show breakage. Lengths of perforation plates and of vessel elements themselves in stems are like those of roots (see Roots).

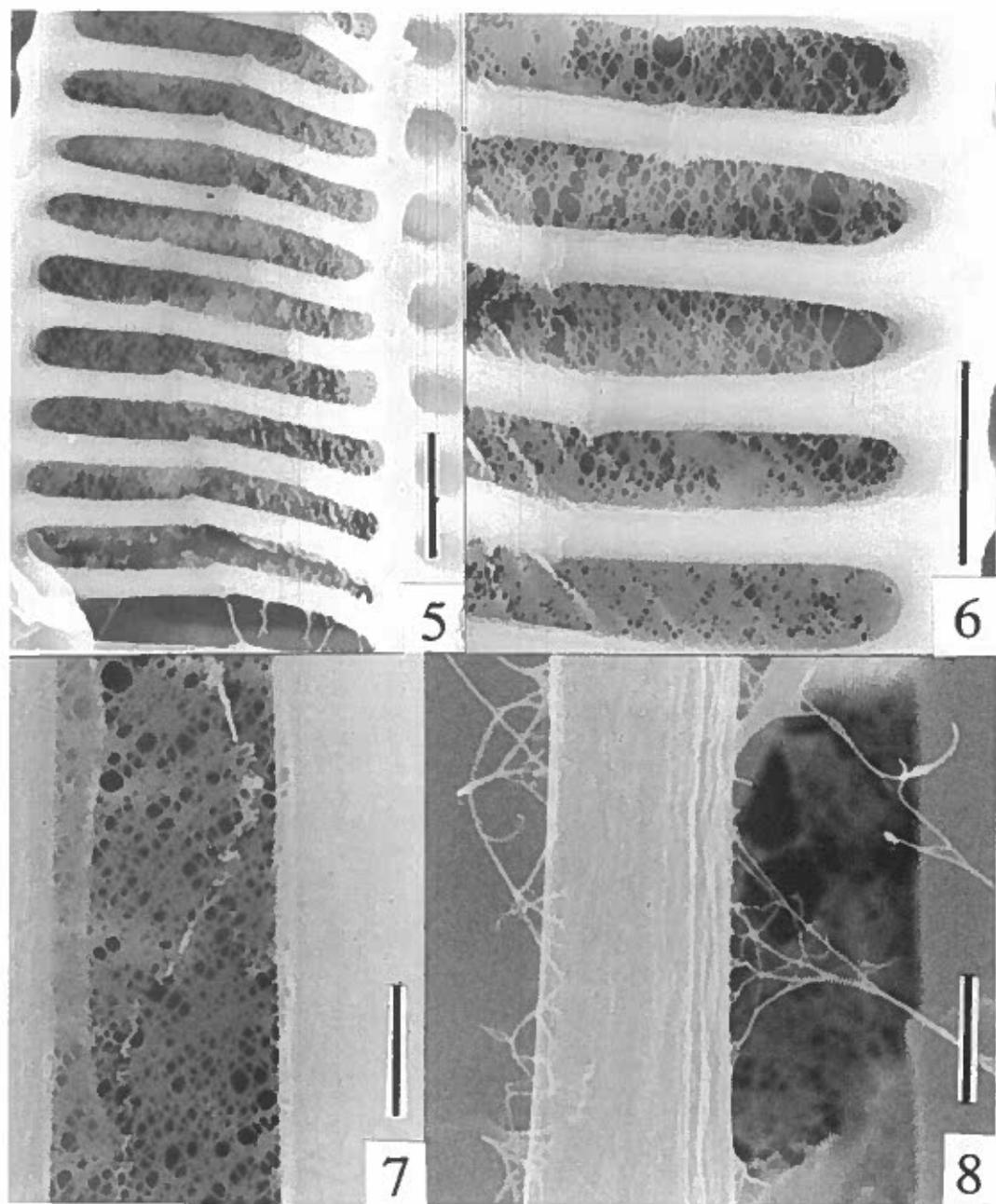
DISCUSSION AND CONCLUSIONS

The metaxylem vessel elements of *Hanguana* are like those of other monocotyledons with presumptively primitive xylem, such as *Acorus* (Carlquist and Schneider 1997). However, the vessel elements of *Hanguana* are notable in showing features that indicate minimal differentiation from tracheid modes of structure. Perforation plates can be distinguished from areas of lateral wall pits in *Hanguana*, more clearly in the roots than in the stems. The long zones of transitional pits (perforations) with porose or threadlike pit membrane remnants in *Hanguana* vessels are notable. Such transitional areas tend to consist of one to several pits in *Acorus* vessel elements but may consist of more than 10 pits in *Hanguana* vessel elements.

Although bars in perforation plates of vessel elements in *Hanguana* tend to be more slender than the bands of secondary wall between lateral wall pits, the difference is not great, and there is variability within each of the two categories.

Although lateral wall pitting bearing pit membranes can be easily differentiated from perforation plates lacking pit membranes in such monocotyledons as Araceae (Carlquist and Schneider 1998b), the difference is not so pronounced in *Hanguana*. In vessel elements of the stems of *Hanguana*, the central portions of perforation plates may consist entirely of perforations (pits) traversed by threadlike pit membranes. This might be true in vessel elements of the roots also, although we tend to think that in roots, some perforations clear of any pit membranes exist. Because threadlike pit membrane remnants are so delicate, they might be entirely destroyed by preparation methods in some perforations. Clearly, there are vessel elements in both stems and roots of *Hanguana*.

The vessel elements of *Hanguana* are more nearly tracheidlike than any hitherto described for monocotyledons. We cannot be sure that no tracheids exist in the xylem of



FIGURES 5-8. SEM photographs of portions of vessel elements from macerations of stolons of *Hanguana malayana*. 5, Transition area between perforations and lateral wall pits, showing the nature of pitting in this area; presence of threads rather than laminae membrane in the bottom perforation may denote a perforation rather than a pit. 6, Enlarged portion of pits in transition area shown in Figure 5, to illustrate the porous nature of the laminae pit membranes. 7, Portion of a pit from a transition between a perforation pit and lateral wall pitting, to illustrate a meshwork-like presence of pit membrane material. 8, Portions of two perforations, to show the vestigial threads of primary wall material that traverse the perforation; threads are broken and otherwise distorted but are believed to represent the threadlike appearance of pit membrane remnants. Figures 5, 6, scale bars = 5 μ m; Figures 7, 8, scale bars = 1 μ m.

Hanguana because entire, isolated tracheary elements are infrequent, and even if one sees large portions of a tracheary element that apparently lack perforations, there might be perforations on cell faces not exposed to view.

The occurrence of such primitive vessel elements in *Hanguana* may be related to the habitat in which it occurs: permanently moist mud in the open areas of rain forest. Under such conditions, vessel elements that experience relatively slow rates of flow would not be under negative selective pressure. In the same habitat, doubtless there are angiosperms with more specialized vessels because vessels adapted to relatively rapid flow rates are not under any disadvantage in such a situation. However, one could conclude that vessels as primitive as those of *Hanguana* are relictual in nature and might be cited as evidence of unbroken occupancy of a mesic habitat.

The vessels of Flagellariaceae (Tomlinson 1969) are much more specialized than those of *Hanguana*. Removal of the genus from Flagellariaceae (Airy Shaw 1965) and recognition of the family within Commelinales (APG 2003) or Zingiberales (Rudall et al. 1999) are consistent with our observations.

ACKNOWLEDGMENTS

We thank Paul Cox of the National Tropical Botanical Garden for drawing our attention to plants of *Hanguana* cultivated in the Allerton Garden. Special thanks are extended to the following at the National Tropical Botanical Garden: Chipper Wichman, for his enthusiastic support in making accessioned material available for research; David Larence and Cristina Salvador, for packaging material of *Hanguana* from the living collections; and Diane Ragone, who transported the material to E.L.S.

Literature Cited

- Airy Shaw, H. K. 1965. Diagnoses of new families, new names, etc., for the seventh edition of Willis's 'Dictionary.' Kew Bull. 18:261.
- APG (Angiosperm Phylogeny Group). 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Bot. J. Linn. Soc. 141:399–436.
- Carlquist, S. 1975. Ecological strategies of xylem evolution. University of California Press, Berkeley.
- Carlquist, S., and E. L. Schneider. 1997. Origins and nature of vessels in monocotyledons. 1. *Acorus*. Int. J. Plant Sci. 158:51–56.
- . 1998a. Origins and nature of vessels in monocotyledons. 3. Lowiaceae, with comments on rhizome anatomy. Blumea 41:219–224.
- . 1998b. Origin and nature of vessels in monocotyledons. 5. Araceae subfamily Colocasioideae. Bot. J. Linn. Soc. 128:71–86.
- Cheadle, V. I. 1942. The occurrence and types of vessels in the various organs of the monocotyledons. Am. J. Bot. 29:441–450.
- Rudall, P. J., D. W. Stevenson, and H. P. Linder. 1999. Structure and systematics of *Hanguana*, a monocotyledon of uncertain affinity. Aust. Syst. Bot. 12:311–330.
- Schneider, E. L., and S. Carlquist. 1997. Origins and nature of vessels in monocotyledons. 2. Juncaginaceae and Scheuchzeriaceae. Nord. J. Bot. 17:397–401.
- . 1998. Origin and nature of vessels in monocotyledons. 4. Araceae subfamily Philodendroideae. J. Torrey Bot. Soc. 125:253–260.
- Tillich, H. J., and E. Sill. 1999. Systematisch Studien zur Morphologie und Anatomie von *Hanguana* Blume (Hanguanaceae) und *Flagellaria* L. (Flagellariaceae) mit der Beschreibung einer neuen Art, *Hanguana bogneri* spec. nov. Sendtnera 6:215–238.
- Tomlinson, P. B. 1969. Flagellariaceae. Pages 70–82 in Anatomy of monocotyledons III. Commelinales—Zingiberales. Clarendon Press, Oxford.