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WOOD, BARK, AND STEM ANATOMY OF GNETALES: A SUMMARY

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Data from a series of eight papers, representing a survey of Gnetales at the species level, are summarized in order to develop concepts on the relationship between the anatomical data and ecology, phylogeny, and systematics. Most of the characters and character states newly reported have phylogenetic and ecological correlations for Gnetales as a whole rather than systematic significance within the genera. Wood features are sensitively related to habit, although strategies in lianoid species of *Ephedra* differ from those in lianoid *Gnetum* species. Data also bear close relationships to organography. Vessel details are given special attention because workers have questioned whether vessels originated in Gnetales independently of those in angiosperms, and thus whether or not vessel origin in the two groups is a synapomorphy. The evidence cited here favors origin of vessels in Gnetales independent of vessel origins in angiosperms. Hitherto unappreciated contrasts between vessels of Gnetales and those of angiosperms are detailed: the torus-margo structure of pits in Gnetales (both *Ephedra* and *Gnetum*), and the aspiration ability of gnetalean pits represent modes of conduction and promotion of conductive safety different from those in angiosperms, and the shape difference (circular vs. scalariform), cited in data matrices of cladograms, is secondary to the different functional syndromes. The intercalation of circular bordered pits into helical secondary wall thickenings of primary xylem tracheary elements of *Ephedra* and *Gnetum* (a feature found also in conifers and *Ginkgo* but not in angiosperms) is given new functional interpretations. *Ephedra* and *Gnetum* contain both tracheids and fiber-tracheids that co-occur in a mode different from that in angiosperms which contain both cell types together. Ray structure like that of Gnetales occurs widely within seed plants and likely offers little conclusive information about relationships. Wood data on *Gnetales* are compatible with the hypothesis that a vesselless group of gymnosperms, such as Pentoxylales or Bennettitales, is the closest sister group of angiosperms. Formation of successive cambia in *Gnetum* differs from that in *Welwitschia*: the latter has phellem but no other bark. Because *Welwitschia* has only phellem, there are more numerous bark features in common between *Ephedra* and *Gnetum* than one might have expected. The three genera appear monophyletic on the basis of wood and bark. Infrageneric anatomical features of systematic importance are few, although the arboreal *Gnetum gnemon* differs from the lianoid *Gnetum* species in significant ways.

Introduction

This article summarizes eight papers that attempt to survey wood, bark, and stem anatomy of Gnetales at the species level, and thereby concludes the series. With respect to anatomy of wood and bark, earlier literature (e.g., Martens 1971) is based on study of a very small number of species. Therefore, a new survey, representing many more species than had been studied before, was initiated. This point is made because earlier conclusions about the nature of wood and bark of Gnetales are based on a small base of information, and many character states summarized here do not appear, therefore, in recent papers on relationships of Gnetales. The fact that much new information has been acquired requires integration of that information in various ways: (1) simple descriptions of features, (2) alternations of earlier descriptions, (3) integration of the new information into the patterns provided by earlier workers, (4) analysis of the phylogenetic significance of the data now available, and (5) consideration of the ecological implications of wood and bark data. The task at hand in analyzing these data sets, therefore, is much more than citation of how features of wood impinge on hypotheses of seed plant evolution. The organization of this article cannot, therefore, be a linear examination of past hypotheses in terms of current evi-

dence: the new wood and bark information must be analyzed and summarized in diverse contexts.

The studies on wood and bark of Gnetales at the species level was begun with a study of the New World species of *Ephedra* (Carlquist 1989), the Old World species of *Ephedra* (Carlquist 1992), and the phenomenon of near-vessellessness in some high-alpine species of *Ephedra* (Carlquist 1988b). The initiation of the survey with study of the New World species of *Ephedra* was related to the fact that material of those species was more readily available to me. Although *Welwitschia* has been studied for many decades, important new structural data proved worthy of reporting (Carlquist and Gowans 1995). One tree species of *Gnetum*, *Gnetum gnemon*, is widespread (Markgraf 1930) and was considered in a separate paper (Carlquist 1994) because this species differed so much from the lianoid species of the genus. Both African lianoid species of *Gnetum* were available (Carlquist and Robinson 1995); all New World species except a recently named one were studied (Carlquist 1996b). The series concluded with a study of Indomallesian and Asiatic lianoid species (Carlquist 1996a). Representation at the species level was 92% in *Ephedra* and 66% in *Gnetum*. Although less than optimal in coverage of the Indomallesian and Asiatic species of *Gnetum*, the survey provides a baseline to which information on species not included can readily be added as material becomes available.

Previous studies on wood, bark, and axis anatomy of *Ephedra*, *Gnetum*, and *Welwitschia* were summarized by Pearson (1929) and Martens (1971). The de-

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scription of *G. gnemon* wood by Greguss (1955) has not been cited widely. Information on wood anatomy was assembled on a floristic basis for *Ephedra* by Fahn et al. (1985) and for *Gnetum* by ter Welle and Detienne (1991). Muhammad and Sattler (1982) presented some observations on certain *Gnetum* vessels as a way of promoting particular phylogenetic concepts.

By surveying Gnetales at the species level, much new information was uncovered. The following features were newly reported for Gnetales as a whole: (1) helical thickenings in secondary xylem vessels and tracheids of *Ephedra* (Carlquist 1989, 1992); (2) near-vessellessness in *Ephedra* (Carlquist 1988b); (3) presence and nature of fiber-tracheids as well as tracheids in wood, and distribution of these cell types with relation to each other, in *Ephedra* (Carlquist 1989, 1992) and *Gnetum* (Carlquist 1994, 1996a, 1996b); (4) presence and nature of axial parenchyma in *Ephedra* (Carlquist 1989, 1992), *Gnetum* (Carlquist 1994, 1996a, 1996b; Carlquist and Robinson 1995), and *Welwitschia* (Carlquist and Gowans 1995); (5) diversity in axial parenchyma distribution types in *Ephedra* (Carlquist 1989, 1992) and *Gnetum* (Carlquist 1994, 1996a, 1996b; Carlquist and Robinson 1995); (6) minute calcium oxalate crystals lining intercellular spaces in *Ephedra* (Carlquist 1989, 1992) and *Welwitschia* (Carlquist and Gowans 1995); (7) tyloses in vessels of *Gnetum* (Carlquist 1996b); (8) tyloses in laticifers of *Gnetum* (Carlquist 1996b); (9) storied structure of wood in *Ephedra* (Carlquist 1989, 1992); (10) phelloderm origin of lateral meristem activity in *Welwitschia* (Carlquist and Gowans 1995); and (11) presence of various types of sclerenchyma and crystals in bark of *Ephedra* (Carlquist 1989, 1992) and *Gnetum* (Carlquist 1994, 1996a, 1996b; Carlquist and Robinson 1995).

In addition, some features were newly reported for particular genera of Gnetales, although they were already reported for one or both genera not listed: (1) tori in pits of tracheary elements in *Gnetum* (Carlquist 1996a, 1996b; Carlquist and Robinson 1995); (2) wood plan as in other plants with secondary growth, rather than vascular strands organized on some other basis, in *Welwitschia* (Carlquist and Gowans 1995); (3) growth rings in *Gnetum* (Carlquist 1994); (4) presence of uniseriate as well as multiseriate rays in *Ephedra* (Carlquist 1989, 1992); (5) branching in laticifers in *Gnetum* (Carlquist 1996b); (6) secretory canals, in addition to laticifers, in *Gnetum* (Carlquist 1996a); and (7) nature of phloem parenchyma, with respect to secondary xylem cells, in *Gnetum* (Carlquist 1996a).

Some of these features are of major importance phylogenetically. For example, the demonstration that lateral meristem activity originates from cortex in *Gnetum* (La Rivière 1916; Carlquist 1994, 1996a, 1996b; Carlquist and Robinson 1995) but from phelloderm in *Welwitschia* (Carlquist and Gowans 1995) means that lateral meristems have had different origins in the two genera and that the presence of successive cambia

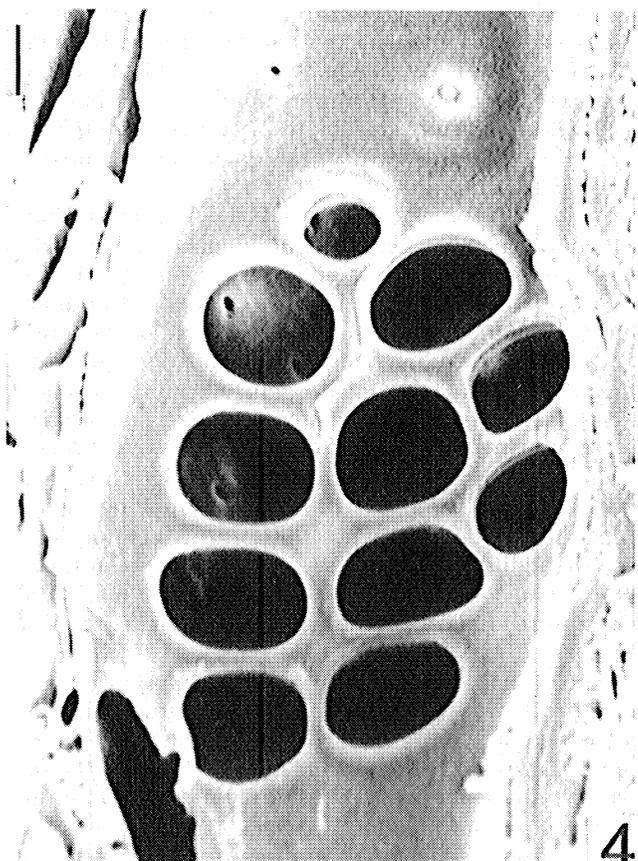
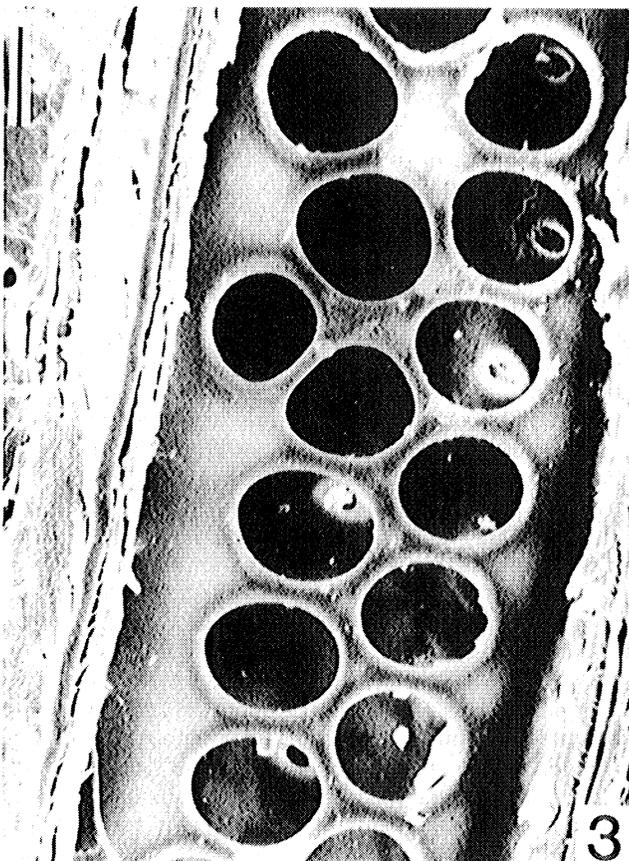
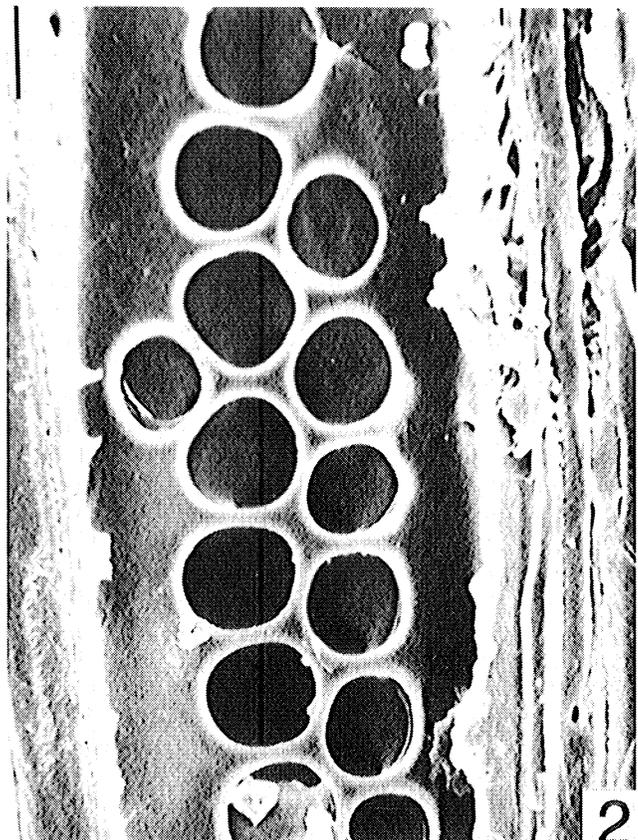
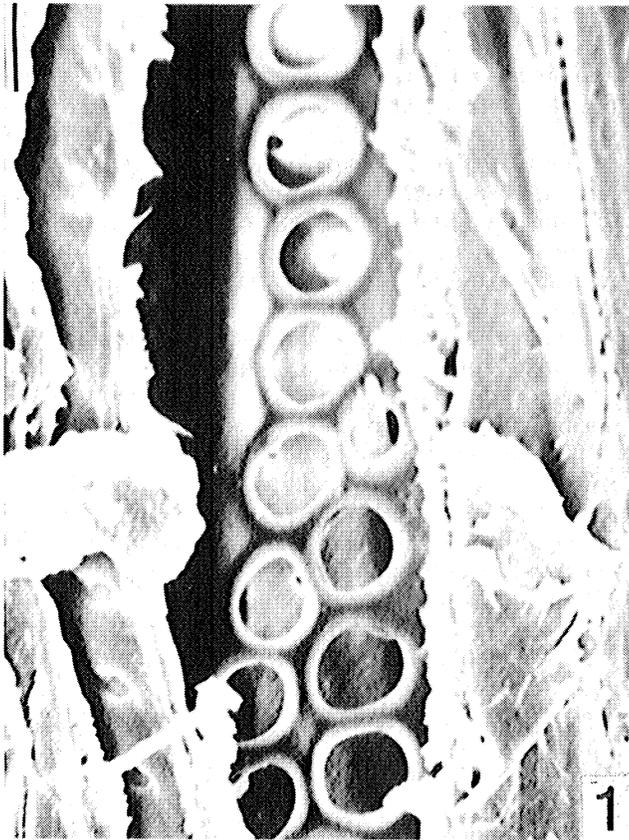
(“anomalous secondary growth”) in the two genera cannot be regarded as a synapomorphy but rather as two autapomorphies. On the contrary, the presence of minute intercellular calcium oxalate crystals in *Ephedra* and *Welwitschia* (a feature otherwise not reported in vascular plants, to my knowledge), seems best interpreted as a symplesiomorphy. Perhaps phylogenetically the most important new report is that of tori in pits of tracheary elements of *Gnetum*, because this feature represents a clearly gymnospermous feature rather than one transitional to angiosperms. These examples show that the new reports of structural features in Gnetales take on significance when integrated with our previous knowledge of wood, bark, and stems of Gnetales. Thus, before conclusions with regard to ecology and phylogeny are attempted below, a summary at the generic level of axial anatomy is presented. Infrageneric diversity is cited in terms of exceptions to common conditions within the genera. Although the salient features of wood and bark of Gnetales are summarized below, many details presented in the earlier papers of this series are not repeated here. Likewise, details on methods, techniques, material, and terminology are not repeated here.

Wood anatomy

EPHEDRA

Stems and roots of *Ephedra* feature a single cambium with secondary phloem formed externally and secondary xylem, internally. Rays, chiefly multiseriate, separate broad fascicular areas. Particular fascicular areas may experience addition of secondary xylem more rapidly than others; this lack of synchronization becomes evident in diagonal orientation of ray cells that interconnect equivalent portions of adjacent fascicular areas (Carlquist 1989, figs. 8, 12; Carlquist 1992, figs. 36–38; Lev-Yadun and Aloni 1993, figs. 3, 4). In rather old *Ephedra* stems (diameter depends on species) distortion in orientation of fascicular and ray portions can occur (Lev-Yadun and Aloni 1993). This phenomenon should not be equated with lateral meristem activity leading to the production of successive cambia, features characteristic of *Gnetum* and *Welwitschia*.

Vessels of *Ephedra* are essentially solitary; if contacts between vessels occur, they appear attributable to random vessel placement in wood in which vessel density is great. There is no tendency toward vessel grouping as there is in dicotyledons that have fiber-tracheids or libriform fibers as the imperforate tracheary element type (Carlquist 1984a). *Ephedra*, like other Gnetales, exemplifies the hypothesis that when tracheids are present as the imperforate tracheary element type, grouping of vessels does not occur to any appreciable extent (Carlquist 1984a). The shrubby species of *Ephedra* show strongly marked growth rings. Not only are vessels larger in earlywood than in latewood but vessels may be absent in latewood in some species (Carlquist 1989, 1992). In a few species from high elevations, vessels are extremely few in number, re-



sulting in a near-vesselless condition (Carlquist 1988b). The lack of vessels in latewood in dicotyledons that have tracheids as an imperforate tracheary element type has been interpreted as representing maximal abundance of tracheids, which are more resistant to formation of air embolisms than are vessel elements (Carlquist 1988a). Woods are mostly ring porous.

The vessel elements of *Ephedra* have perforation plates composed of foraminate (circular) perforations similar to the outlines of pit cavities of lateral wall pits on vessels on tracheid-to-vessel or tracheid-to-tracheid pits (figs. 1–4). There is little variation in these foraminate perforations (sometimes termed “ephedroid perforations”). The number of series of perforations ranges from one, in the most slender vessels, to two (fig. 1) or three (figs. 2–4), but rarely more than three, series. There are borders on perforations in some species (fig. 1), but most borders are minimal (figs. 2–4). Both prominently bordered and minimally bordered perforations can characterize a single species (figs. 1, 2), although perforations tend to be more prominently bordered in *Ephedra americana* Humb. & Bonpl. and *Ephedra andina* Poepp. ex Mey. (Carlquist 1989). No fusion of perforations is evident, although in a few species, such as *Ephedra kokanica* Regel, relatively large perforations placed closely tend to be somewhat polygonal in outline rather than circular (fig. 4). Pits on lateral walls of vessels, like tracheid-to-tracheid pits, are circular and fully bordered, and have prominent tori connected to margo threads (fig. 9). Helical thickenings occur in inner surfaces of secondary xylem vessels of the majority of New World species (Carlquist 1989) and some Old World species (Carlquist 1992); in species with helical sculpturing in vessels, such sculpturing also occurs in tracheids. Imperforate tracheary elements in *Ephedra* are clearly differentiated into tracheids, with large bordered pits and without cellular contents, and fiber-tracheids, which have pits bordered on contacts with vessels or tracheids, but pits simple or quite vestigially bordered on contacts with other fiber-tracheids. Fiber-tracheids of *Ephedra* are nucleate but not septate. The reason why fiber-tracheids of *Ephedra* are not termed “axial parenchyma” is that they are not strands of cells separated by lignified walls; such strands do occur in a few species of *Ephedra* (*Ephedra foliata* Boiss., *E. kokanica* Regel; Carlquist 1992, fig. 31). By contrast, *Gnetum* characteristically has both fiber-tracheids and axial parenchyma. Other arguments for use of this terminology, not used throughout Gnetales prior to the current series of papers, have been presented (Carlquist 1989, p. 421). Fiber-tracheids in *Ephedra* are arranged in diffuse or diffuse-in-aggregates fashion (the

latter illustrated in Carlquist 1992, fig. 24); occasional large fiber-tracheid aggregations are present.

Rays are mostly multiseriate, but uniseriate occur in a few species, in which they are much less common than multiseriate. Rays are composed of upright, square, and procumbent cells; upright cells predominate in rays of smaller-diameter samples, whereas procumbent cells increase in abundance as a stem increases in diameter (Carlquist 1989). Starch grains are common in ray cells, as they are also in fiber-tracheids.

Minute calcium oxalate crystals occur among tracheary elements and ray cells. Although uncommon in a few species of *Ephedra*, these minute crystals are abundant in the vast majority of *Ephedra* woods.

Storying has been reported in *Ephedra coryi* Reed var. *viscida* Cutler (Carlquist 1989), *Ephedra equisetina* Bunge, and *E. foliata* (Carlquist 1992). Storying is more common in dicotyledons with short fusiform cambial initials, so the occurrence of storying in *Ephedra*, which has shorter fusiform cambial initials than do other gymnosperms (with the exception of *Welwitschia*), is not surprising (Carlquist 1992).

Wood of lianoid or scandent species of *Ephedra* has some distinctive features. Lianoid species include *Ephedra pedunculata* Engelman ex Watson in the New World, and in the Old World, species of section *Pseudobaccatae*, tribe *Scandentes*: *Ephedra altissima* Desf., *Ephedra aphylla* Forssk., *Ephedra campylopoda* C. A. Mey., *E. foliata*, *Ephedra fragilis* Desf., and *E. kokanica*. Compared to nonlianoid species, these species have a much greater density of vessels per mm² of transection, longer vessel elements, and greater perforation diameter (table 1), as well as less thick walls on imperforate tracheary elements. Differences between lianoid and nonlianoid *Ephedra* species with respect to vessel diameter and in wall thickness of vessels are negligible.

WELWITSCHIA

The axis of *Welwitschia* consists of a long taproot and a relatively short, wide stem. From investigations to date, there is every reason to believe that the same histology (except for organization of primary xylem) is found in both roots and stems. The vascular strands of stems are so contorted that secondary growth and histology in them cannot be readily demonstrated. Therefore, descriptions here are based primarily upon root material. In both stems and roots, there is a first cambium that produces a limited amount of secondary xylem and secondary phloem, not unlike the secondary growth in seedlings of various vascular plants with secondary growth. This stage has been illustrated by Bower (1881), Sykes (1910), Pearson (1929), and But-

←

Figs. 1–4 SEM photographs of perforation plates of *Ephedra*, from radial sections. Figs. 1, 2, *Ephedra major* Host, Stainton 18280. Fig. 1, Perforation plate with clearly bordered perforations. Fig. 2, Perforation plates with minimal borders. Fig. 3, *Ephedra intermedia* Schrenk, Elias, July 21, 1983; perforations of nearly circular outline, minimally bordered. Fig. 4, *E. kokanica*, Elias 9742; relatively large perforations somewhat polygonal in outline. Bars at upper left in each photomicrograph = 10 μm.

Table 1
WOOD CHARACTERISTICS OF GNETALES

| Group | VD (1) | VM (2) | VL (3) | VW (4) | CA (5) | TL (6) | TW (7) |
|--|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| <i>Ephedra</i> , Old World nonlianooid | 32 | 65 | 585 | 2.1 | .05 | 669 | 3.1 |
| <i>Ephedra</i> , Old World lianooid | 38 | 143 | 723 | 2.4 | .16 | 808 | 2.2 |
| <i>Welwitschia</i> | 15 | 249 | 508 | 5.0 | .04 | 592 | 3.8 |
| <i>Gnetum gnemon</i> (trees, shrubs)..... | 82 | 21 | 1235 | 3.5 | .11 | 1691 | 4.8 |
| <i>Gnetum</i> , African lianas | 82 | 31 | 1062 | 4.9 | .16 | 1200 | 2.9 |
| <i>Gnetum</i> , New World lianas | 155 | 10 | 1057 | 5.7 | .19 | 1360 | 4.4 |
| <i>Gnetum</i> , Asiatic lianas..... | 134 | 19 | 1250 | 4.6 | .27 | 1532 | 3.5 |
| <i>Gnetum</i> , all lianas averaged..... | 124 | 20 | 1123 | 5.1 | .21 | 1364 | 3.6 |

Sources. Data from Carlquist (1992, 1994, 1996a, 1996b); and Carlquist and Robinson (1995).

Key to columns: 1, VD = mean lumen diameter of vessels, μm ; 2, VM = mean number of vessels per mm^2 transection; 3, VL = mean length of vessel elements, μm ; 4, VW = mean vessel wall thickness, μm ; 5, CA = mean conductive area per mm^2 transection, mm^2 ; 6, TL = mean length of tracheids, μm ; 7, TWT = mean wall thickness of tracheids, μm . Data on *Welwitschia* is original. Asiatic *Gnetum* species include Indomalaysian species.

ler et al. (1973). Subsequent stages have been less figured, although all authors agree that vascular tissue outside of the first cylinder is formed from successive cambia (Martens 1971). Although the first cambia outside of the original cylinder arise in cortical parenchyma, cortical parenchyma sites for origin of these cambia are exhausted before long. Subsequent cambia originate within radial files of parenchyma formed from a lateral meristem. These radial files are intercontinuous with phelloderm, and thus have a mode of origin different from that of the radial files of parenchyma involved in lateral meristems in stems and roots of lianooid species of *Gnetum* (Carlquist and Gowans 1995). All of the parenchyma tissues produced by the lateral meristem, other than parenchyma cells formed in the vascular strands, are termed "conjunctive tissue." Conjunctive tissues consist of starchy parenchyma, secretory cavities, and large fibrosclereids with gelatinous walls near the outer surface of which are embedded relatively large calcium oxalate crystals. Many intercellular spaces in the conjunctive tissue are lined with minute calcium oxalate crystals (Carlquist and Gowans 1995) much like those of *Ephedra* (Carlquist 1989).

The successive cambia of *Welwitschia* produce relatively large amounts of secondary phloem, as indicated by abundance of gelatinous phloem fibers, compared with the secondary xylem the cambia produce (Carlquist and Gowans 1995). Nevertheless, the secondary xylem, despite the use of the vague term "vascular strand" above, has a secondary xylem plan much like that of other vascular plants with secondary xylem: fascicular and ray areas are present (Carlquist and Gowans 1995). Some rays begin as uniseriate but rapidly widen to multiseriate. All ray cells are upright. Minute calcium oxalate crystals line intercellular spaces among some ray cells. Axial parenchyma interrupts some of the bands of fascicular xylem, which are one to three tracheary elements wide. The vessel elements are only 30%–35% as numerous as tracheids (original data). Vessel elements and tracheids have grooves that

represent lateral extensions of inner elliptical pit cavities (Bierhorst 1960; Carlquist and Gowans 1995) rather than thickenings, as in *Ephedra*, although Sykes (1910) shows thickenings in *Welwitschia*. Perforation plates of vessel elements consist of one, rarely two, bordered circular perforations (Bierhorst 1960). Tori are absent in pits of tracheary elements, although figures by Bierhorst (1960) suggest pit membranes elliptical in sectional view; these figures were copied by Martens (1971). Careful examination with both light microscopy and SEM has thus far revealed only membranes that are flat in sectional view, with no evidence of tori. Tracheids are the only type of imperforate tracheary elements present; no fiber-tracheids were observed.

GNETUM GNEMON

Gnetum gnemon has a single cambium and a single woody cylinder at least in trees of moderate age; in bases of older trees, additional xylem segments may be found (Rao and Keng 1975). These segments originate from cambia formed in bark (Carlquist 1994), although in some way the new vascular tissue formed from these cambia must be interconnected with xylem and phloem of the main cylinder. This is a topic in need of further investigation.

Vessels are solitary or nearly so—mean number of vessels per group, 1.09—a number likely close to what random placement of vessels would dictate. Growth rings of an inconspicuous sort have been observed in a few collections (Carlquist 1994). Perforation plates are predominantly simple (fig. 8, portion shown). In narrow vessels near primary xylem, foraminate perforation plates occur occasionally (figs. 5–7). The narrowest of these vessels have perforation plates that consist of a row of well-bordered perforations (Carlquist 1994, fig. 15). Perforation plates in somewhat wider vessels, such as those illustrated here (figs. 5–7), can be characterized as foraminate-coalescent; the perforation shapes and sizes suggest enlargement and merging of foraminate perforations in response to se-

lective pressure for greater conductive area within the perforation plate.

Lateral wall pits of vessels have circular bordered pits with a cavity diameter much smaller than the diameter of perforations. These pits bear vestures (fig. 8) that occur at or near the mouth of the pit aperture, and vesturing is lacking within the pit cavity. Imperforate tracheary elements consist of both fiber-tracheids and tracheids. The fiber-tracheids are relatively thin walled and usually septate; they have vestigially but clearly bordered pits that are relatively small and are distributed randomly with respect to tracheids (fig. 13). Tracheids have thicker walls than fiber-tracheids, lack contents and septa, and have large circular bordered pits very close in cavity diameter to those on lateral walls of vessels. No tori have thus far been observed on pit membranes of *G. gnemon* tracheary elements.

Paratracheal axial parenchyma is vasicentric and relatively abundant in *G. gnemon*; apotracheal parenchyma is scarce and is diffuse. Strands consist of four to 10 cells, have thin but lignified walls, and contain starch and sometimes calcium oxalate crystals (fig. 13).

Rays are both uniseriate and multiseriate, with almost all cells procumbent, except at tips of multiseriate rays and in uniseriate rays. The ray cells have lignified walls. Bordered pits are common on tangential walls of ray cells; simple pits predominate elsewhere on ray cells. Calcium oxalate crystals of various sizes and starch grains are common within ray cells.

LIANOID SPECIES OF GNETUM

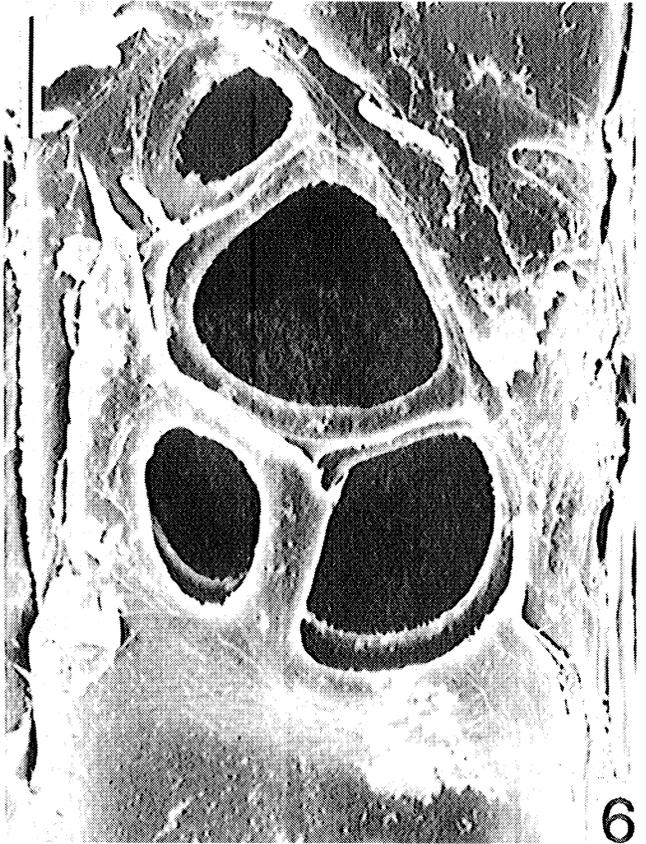
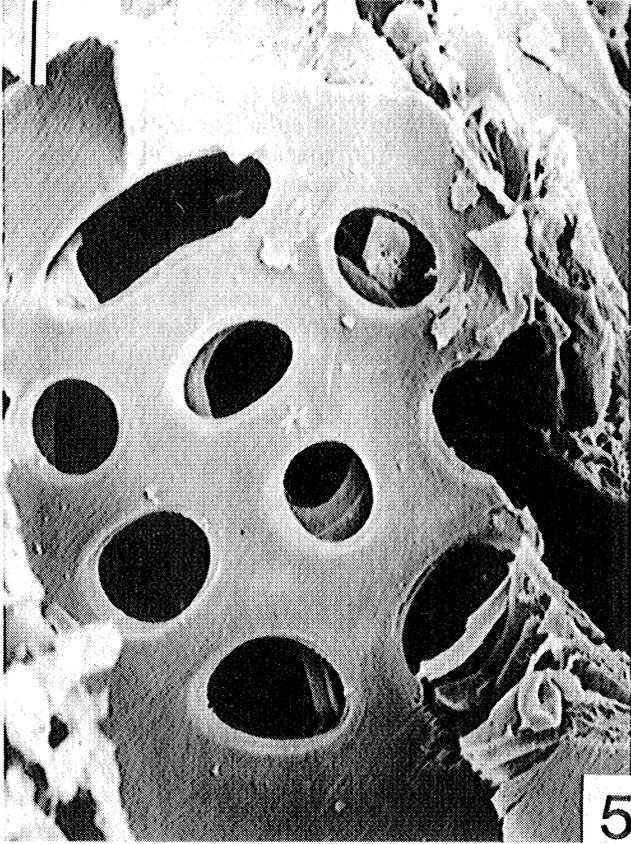
The lianoid *Gnetum* species have a first cambium that forms a cylinder of secondary xylem—with a pith in the stem, without a pith in the root. Subsequent vascular cylinders arise from successive cambia, and parenchyma between these vascular cylinders is termed “conjunctive tissue” here. Conjunctive tissue is composed of parenchyma that contains starch and, near rays, calcium oxalate crystals. The conjunctive tissue may contain laticifers (fig. 17) in some species. Secretory cavities were observed in conjunctive tissue external to strands of phloem in *Gnetum gnemonoides* Brongn. The second and perhaps several more of the successive cambia originate in cortical parenchyma. Cortical parenchyma would be used up in the formation of these cambia eventually, but what I am terming a lateral meristem here forms additional parenchyma in the cortical region through production of radial files of cells (figs. 17, 18). Pearson (1929) hypothesized that more than one site of origin for lateral meristem activity might occur. However, a cortical site of origin for origin of lateral meristem activity was demonstrated in *Gnetum* by La Rivière (1916), and this has been confirmed in each of three main taxonomic and geographical groupings of lianoid *Gnetum* species (Carlquist and Robinson 1995; Carlquist 1996a, 1996b). Cambia originate within the radial files formed by periclinal divisions I am terming “lateral meristem.” The origin of these cambia and the first secondary phloem

and secondary xylem they yield are most clearly visible in *Gnetum africanum* Welw. (Carlquist and Robinson 1995).

Cambial action is sluggish in future ray areas but much more active in fascicular zones. Wide multiseriate rays originate from the successive cambia. Within the fascicular areas, biseriate or uniseriate rays also originate (fig. 16). The interrelationships between these narrower rays, tracheids, and fiber-tracheids in the lianoid species have not been stated clearly in earlier literature. An attempt to clarify this situation can be found in Carlquist (1996a). The vessel elements of the lianoid species of *Gnetum* are much like those of *G. gnemon*; as in that species, only a few narrower vessels have foraminate perforation plates, and the majority of perforation plates are simple. Vesturing is variously represented on lateral wall pits of vessels, but in no species is vesturing wholly absent in these pits.

In addition to vessel elements, the fascicular secondary xylem of lianoid *Gnetum* species consists of tracheids, fiber-tracheids, and axial parenchyma (figs. 14, 15). The features by which these three cell types differ from each other are as in *G. gnemon*, except that diffuse and diffuse-in-aggregates axial parenchyma are more abundant than in *G. gnemon*. The large circular bordered pits of vessel elements and of tracheids have pit membranes that bear tori, in at least some species. Tori were observed in *G. africanum*, *Gnetum buchholzianum* Engler (Carlquist and Robinson 1995), *Gnetum leyboldii* Tul. (Carlquist 1996b), *Gnetum cuspidatum* Blume, and *Gnetum microcarpum* Blume (Carlquist 1996a). Liquid-preserved material of all of these species was available. Lack of tori might bear a relationship to the mode of sample preservation. Tori were demonstrated by both light microscopy and SEM, although in some SEM photomicrographs, differentiation between torus and margo is less marked than in *Ephedra* or conifers.

Radially oriented files of parenchyma cells in secondary phloem of lianoid species of *Gnetum* (fig. 16) have been likened to companion cells (Maheshwari and Vasil 1961), but they are not sister cells to sieve cells and were accurately designated as parenchyma cells by Esau (1969). What has not been clarified is the relationship between these parenchyma cell files and the extensions of these files into the secondary xylem (fig. 16, bottom). The radial orientation of xylem cells intercontinuous with the phloem parenchyma files suggests that they might be xylem rays, and in fact some of them are rays, as confirmed by presence of uniseriate and biseriate rays as seen in tangential and radial sections of these fascicular zones. However, study of these tangential and radial sections reveals relatively few such rays, too few to account for the numerous radial files of narrow cells in secondary xylem as seen in transections (fig. 16). The radially oriented rows of cells that lie between radial files of tracheids prove mostly to be fiber-tracheids or possibly a few axial parenchyma strands. Although fiber-tracheids were not mentioned for African species of *Gnetum*



(Carlquist and Robinson 1995), careful study of slides of those species revealed fiber-tracheids, albeit in small numbers. Therefore, all of the lianoid species of *Gnetum*, as well as *G. gnemon*, are now claimed to possess fiber-tracheids as defined for *Gnetum* by Carlquist (1994).

Bark

Bark of *Ephedra* (Carlquist 1989, 1992) has been shown to contain the following cell types: (1) sieve cells, phloem parenchyma, phloem ray parenchyma (obliterated to various extents depending on age of bark); (2) sclereids derived from axial parenchyma cells; (3) suberized phellem; (4) phelloderm (thin-walled or sometimes sclerenchymatous), often associated with minute intercellular calcium oxalate crystals; (5) sclereids derived from phloem ray cells; (6) gelatinous phloem fibers; in addition, younger stems reveal the presence of (7) gelatinous fibers diffusely distributed in the cortex (fig. 20). The diffuse cortical fibers were not mentioned in earlier accounts of bark because they were not evident once the cortex was lost. The diffuse fibers are noteworthy because similar fibers occur in cortex and bark of lianoid *Gnetum* species. Not all of the above seven features may be present in all *Ephedra* species, and accurate data on systematic distribution of the features within the genus are not available.

In *Gnetum gnemon* bark, the following cell types occur (Carlquist 1994): (1) thin-walled (nonsclerenchymatous) phellem; (2) starch-rich parenchyma; (3) nests of brachysclereids, some of which contain large calcium oxalate crystals; and (4) gelatinous phloem fibers. In the lianoid *Gnetum* species, all of these cell types occur, but in addition, one may find (5) occasional sclereids in phloem (Carlquist 1996b); (6) gelatinous fibers scattered in the cortex (fig. 19); (7) a cylinder of brachysclereids, outside of the parenchymatous inner cortex but inside of the nests of brachysclereids (fig. 17, top; fig. 18, top); (8) laticifers (easily visible in some species, such as *Gnetum schwackeanum* Taub. (fig. 17); and (9) sclerenchymatous phelloderm (Carlquist 1996a). The sclerenchymatous cylinder and sclerenchymatous phelloderm are absent in underground roots and stems of lianoid species (fig. 19; Carlquist 1996a).

There is no bark in the ordinary sense in *Welwitschia*, only phellem, which contains a few of the giant gelatinous fibrosclereids in the walls of which large crystals are embedded. Secondary phloem in *Welwitschia* vascular strands does contain large numbers of the gelatinous phloem fibers of the sort seen in *Ephedra* and *Gnetum* bark, however.

The roster of features in the bark of *Gnetum*—especially the diffuse gelatinous fibers and the presence of sclerenchymatous phelloderm—is remarkably similar to that for *Ephedra*. At least some of these features seem likely to be symplesiomorphies. Rather than concluding that the similarities between the two genera mark them as more closely related to each other than either is to *Welwitschia*, one should consider the possibility that in its anatomical structures, as well as in its bizarre gross morphology, *Welwitschia* has departed from a hypothetical common ancestor and thereby contains a number of autapomorphies the other two genera lack.

Conclusions on organography, habit, and ecology

ORGANOGRAPHY

In *Ephedra*, relationships of vessel features to organography were detailed (Carlquist 1989, p. 447) because collections of particular portions were made in the field. These data, in sum, show that vessels are wider in roots and underground stems than in upright aerial stems, thereby agreeing with those in dicotyledons (Patel 1965). However, the reverse trends occur in *Gnetum*, admittedly on the basis of a small sampling of lianoid species (Carlquist 1996a). Vessel diameter in roots is less than it is in stems not only in lianoid *Gnetum* species but in *Gnetum gnemon* as well (Carlquist 1994), perhaps because the roots studied are smaller than stems (although not dramatically so; see data in Carlquist 1994). In dicotyledons, vessels ordinarily increase in diameter with increase in stem diameter (Carlquist 1984b). Increase in vessel diameter with increase in stem diameter was evident in *Ephedra* (*Ephedra boelckii* Roig, *Ephedra trifurca* Torr., and *Ephedra viridis* Coville). Relationships between quantitative vessel features and organography deserve detailed further study in *Gnetum*.

HABIT

In *Ephedra*, habit ranges from small shrubs to large shrubs to small trees and to lianas or sprawling shrubs. To be sure, the lianas and sprawling species of *Ephedra* are not a uniform group with respect to habit. The lianas in *Gnetum* vary somewhat but seem amply distinct in habit from the tree species *Gnetum gnemon* and *Gnetum costatum* K. Schum., judging from the descriptions of Markgraf (1930). However, Markgraf (1930, p. 436) claims that the species one usually sees as a tree, *G. gnemon*, can be a shrub or a liana as well. The nature of lianoid individuals of *G. gnemon* is in need of investigation, because such lianas have not

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Figs. 5–8 Perforation plates of *Gnetum gnemon*, Carlquist 8088; from radial sections (figs. 5, 6, 8) illustrated with SEM and from a maceration (fig. 7) illustrated with light microscopy. Fig. 5, Perforation plate showing little alteration from a foraminant condition. Fig. 6, Perforation plate with fewer, larger perforations. Fig. 7, Perforation plate with three perforations, one partially traversed by wall material. Fig. 8, Simple perforation plate (vesturing visible in apertures of lateral wall pits of vessels). Fig. 7, Magnification scale above photograph (divisions = 10 μm). Figs. 5, 6, 8, Bars, upper left, = 10 μm.

received comment or study since Markgraf's brief mention.

When one compares the wood of lianas in *Ephedra* to the wood of shrubby species (table 1), one finds that there is little difference in vessel diameter (col. 1) but much more difference in vessel density (col. 2): the lianas have a vessel density more than twice that of the shrubs. Old World species were used exclusively in table 1 for the liana sample because there were 11 collections of seven species available in tribe *Scandentes* (Carlquist 1992). Perforation diameter of these lianas (13.9 μm) is appreciably greater than that of the nonlianoïd Old World species (10.4 μm). The conductive area per mm^2 transection (mean perforation area times mean number of perforations times mean number of vessels per mm^2 ; table 1, col. 5), of the lianoïd species is more than three times as large as that of the nonlianoïd species. The numerous and relatively large (for *Ephedra*) latewood vessels in the lianoïd species account for most of this difference. Vessels are few to absent and rather narrow in latewood of shrubby species. The perforation area, although a subsidiary factor differentiating lianas and nonlianas, is statistically significant. The difference in conductive area between lianas and nonlianas in *Ephedra* is interesting because the stem surfaces of *Ephedra* are so limited, and about the same in lianas and nonlianas. Possible correlations between habit and ecology and the vessel characteristics of lianas and shrubs need to be investigated. The greater length of vessel elements in lianoïd versus nonlianoïd Old World species (table 1, col. 3) is also statistically significant. This trend would be expected if shrubby species are more xeromorphic, since shorter vessel elements characterize xeric species in dicotyledons (Carlquist 1975).

When comparing lianoïd to nonlianoïd *Gnetum* species (table 1; figures based on all *Gnetum* collections studied in the present series of papers), differences in quantitative vessel features are much more dramatic than in *Ephedra*. Perforation plates of *Ephedra* are always foraminiate, whereas only a few perforation plates in any stem or root of *Gnetum* are foraminiate; by far the majority are simple. The mean number of perforations per perforation plate is thus only a fraction above 1.0 in either the arboreal or lianoïd *Gnetum* collections. Evidently, the selective pressure for simple perforation plates is great both in a broad-leaved tree and in a broad-leaved liana. In *Ephedra*, it is very likely that microphyllly reduces transpiration so considerably that greater vessel density and a slightly larger mean perforation diameter suffice to accommodate increased flow rates in lianas, and there is no selective pressure for modification of the morphology of the foraminiate perforation plate. Reinforcing the interpretation that the lianoïd habit relates to simplification of the perforation plate in *Gnetum* is the observation that in dicotyledonous families with scalariform perforation plates that have both shrubby/arboreal and lianoïd broad-leaved species, the number of bars per perforation plate is much lower in the lianoïd species. This is

shown by *Piptocalyx* in Trimeniaceae (Carlquist 1984b) and by *Hibbertia* and *Tetracera* in Dilleniaceae (Metcalfe and Chalk 1950; Dickison et al. 1978). Wider vessels also have accelerated simplification of perforation plates in dicotyledons (Carlquist 1975). That trend in the arboreal *Gnetum* collections as well as in the lianoïd ones is, therefore, understandable.

The lianoïd *Gnetum* species have appreciably greater vessel diameter (124 μm) than do the arboreal (*G. gnemon*) collections (82 μm ; table 1, col. 1). This difference is similar to values shown for vessel diameter in a range of dicotyledonous growth forms (Carlquist 1975, p. 206). The apparent similarity in vessel density (table 1, col. 2) between lianoïd and nonlianoïd *Gnetum* collections (both close to 20 vessels per mm^2) may be in part an artifact of the conventions adopted for measurement: conjunctive tissue and the wide rays were excluded in obtaining figures for vessel density in the lianas. Still, the conductive area per mm^2 transection of lianas compared with nonlianas in *Gnetum* is similar to the difference between lianas and mesic woody samples of dicotyledons (Carlquist 1975, p. 206). The African species of *Gnetum* do not have quantitative features comparable to those of the other groups of lianas probably because the size of the plants available for the African species is much smaller than for the other lianas.

One noteworthy feature that emerges from lianoïd-nonlianoïd comparisons in *Gnetum* is that vessel walls are appreciably thicker in the *Gnetum* lianas than in *G. gnemon* (table 1, col. 4). This, too, is in accord with the tendency for lianoïd species of dicotyledons to have thicker-walled vessels (Carlquist 1975, p. 181). Tracheids in the lianoïd species of *Gnetum* have thinner walls (3.6 μm) than do the *G. gnemon* collections (4.8 μm), as shown in table 1, column 7. This would be in accord with the concept that there is greater selection for wall thickness to promote mechanical strength in a tree than in a related liana. Data on this point are infrequently collected in dicotyledons and would be desirable. Tracheid walls in the lianoïd *Ephedra* species (table 1, col. 7) are thinner (2.2 μm) than those of the shrubby species (3.1 μm), an interesting confirmation of the trend in *Gnetum*.

XEROMORPHY

Quantitative vessel and tracheid data have not hitherto been presented for secondary xylem of *Welwitschia*. Analyzing the significance of these data is difficult, because the wood of *Welwitschia* is not really comparable to that of a desert shrub, a stem succulent, or any of various other xerophytes. Although collections undoubtedly differ, a typical root transection was selected to develop the data for *Welwitschia* in table 1. The figure for vessel density (mean number of vessels per mm^2 , col. 2) is essentially an estimate, because transectional areas of xylem are so small and scattered in such large areas of conjunctive tissue that finding comparable areas by the random scanning methods usually used is inapplicable. Nevertheless, the quanti-

tative wood data for *Welwitschia* show it to have a remarkably xeromorphic wood. The mean vessel diameter for a sampling of dicotyledonous desert shrubs is 29 μm (Carlquist 1975, p. 206), whereas the figure for *Welwitschia* is 15 μm . To be sure, the former sample was obtained measuring outside diameter of vessels, so that for that sample the lumen diameter (which was what was measured for the Gnetales in table 1) would be ca. 24 μm , but that is still significantly wider than the figure for *Welwitschia*. The conductive area per mm^2 transection (based on number of vessels per mm^2) is low for *Welwitschia* (.04) but close to figures for dicotyledonous desert shrubs (0.18), stem succulents (.09), and woody species with successive cambia (.06) (Carlquist 1975, p. 206; because vessel wall thickness was included in vessel diameter in these dicotyledon figures, figures based on lumen area as in the Gnetales data would be smaller by perhaps 10%). The conductive area for liana stems is, by comparison, 0.36 mm^2 per mm^2 transection (Carlquist, 1975, p. 206). However, tracheids are likely quite effective in conduction in *Welwitschia* and are ca. three times as numerous as vessel elements in *Welwitschia* secondary xylem (tracheids were not included in the estimate of conductive area per unit transection, only vessels).

The vessel element length of *Welwitschia* (508 μm ; table 1, col. 3) does not seem short by comparison to that of dicotyledonous desert shrubs (218 μm ; Carlquist 1975, p. 206). That length is shorter than the vessel element length for shrubby species of *Ephedra* (585 μm ; table 1, col. 3) and is less than half the vessel element length of any *Gnetum* species. The mean vessel lumen diameter of dicotyledonous desert shrubs is ca. 24 μm ; the vessel lumen diameter of *Ephedra* is slightly larger (nonlianooid species, 32 μm ; lianooid species, 38 μm), but the order of magnitude is comparable: few mesic dicotyledonous trees have vessel diameter less than 100 μm (Metcalf and Chalk 1950). As in *Welwitschia*, we cannot estimate the likely conductive capabilities of tracheids as compared to those of vessels in *Ephedra* or *Gnetum*. Comparisons with conductive areas per unit transection of dicotyledon woods with tracheids, dicotyledon woods with libriform fibers or fiber-tracheids, or with conifer woods where conductive areas are concerned are premature until we know the role tracheids play relative to vessels in conduction in Gnetales.

Welwitschia and many species of *Ephedra* possess a feature that is considered indicative of xeromorphy: helical sculpturing on the inner surfaces of vessels and tracheids. In *Ephedra*, this takes the form of helical thickenings on the inner surfaces of vessels and of tracheids of most of the New World species (Carlquist 1989) and a scattering of the Old World species (Carlquist 1992). In *Welwitschia*, grooves that represent lateral extensions of pit apertures around the cell occur in vessels and tracheids, providing texturally a grooved wall surface similar topographically, even if different in phylogenetic origin, to the helical sculpturing present in vessels and tracheids of *Ephedra*. In dicotyle-

dons, sculpturing in vessels and tracheids characterizes species of areas drier and colder than those occupied by congeners that lack the helical sculpturing (Carlquist 1975). Although there is uncertainty about the physiological significance of helical sculpturing in secondary xylem vessels and tracheids of dicotyledons, there is little doubt that it represents a xeromorphic adaptation because of the ecological and systematic distribution of this phenomenon. Helical sculpturing occurs in tracheids of some conifers, notably Taxaceae and some Cupressaceae (Greguss 1955).

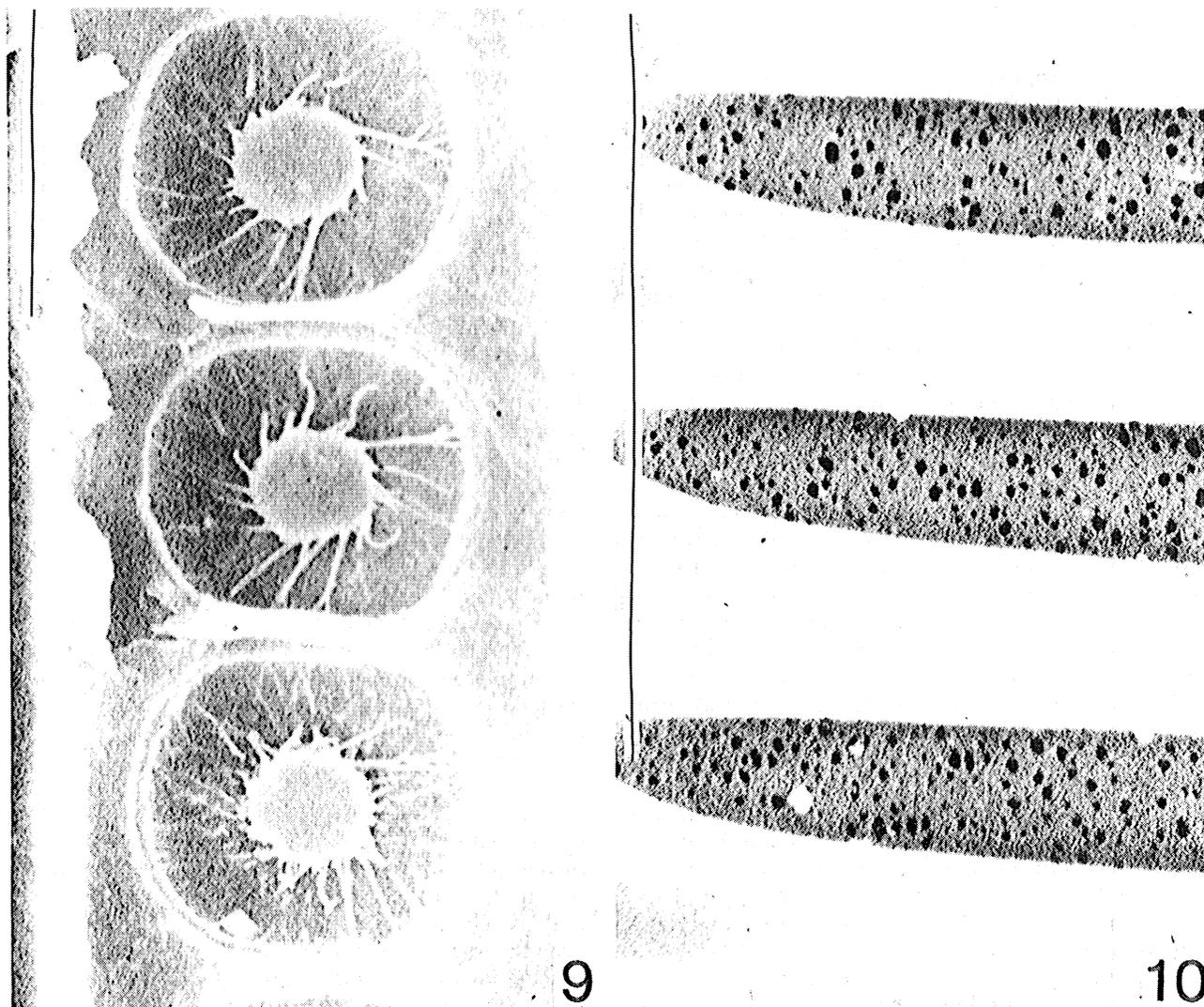
The mesic ecology of *Gnetum* is evident in low vessel density: the vessel density in lianooid *Gnetum* species is almost the same as for a sampling of dicotyledonous lianas (Carlquist 1975, p. 206). The mean diameter of vessels in *G. gnemon* (82 μm) does not fall much short of that for a sampling of mesic dicotyledon woody species (108 μm), and the discrepancy can be explained by the inclusion of a young stem sample and a shrub sample in the *G. gnemon* survey. The vessel diameter of *Gnetum* lianas (124 μm) is less than outside vessel diameter for dicotyledon lianas (157 μm , with lumen diameter probably as much as 10 μm less than 157 μm). However, again taking into account some immature liana samples—notably the African *Gnetum* species—*G. gnemon* seems to fit the templates for mesic woody dicotyledons.

Phylogenetic conclusions

The uncertain nature of relationships of Gnetales to other seed plants remains a central interest. The value of data from morphology and anatomy in this respect has not lessened but has increased because at least several extinct groups of seed plants may be the sister groups closest to Gnetales. We can continue to accumulate anatomical information both on these sister groups and on Gnetales, but DNA data for the extinct groups are not available. The importance of this circumstance cannot be overemphasized, because examples of the effect on phylogeny of inclusion versus exclusion of DNA data in cladograms based on several data sets can be cited. Such examples are shown in the cladograms of Albert et al. (1994), Doyle et al. (1994), and Nixon et al. (1994) that exclude fossils. In cladograms based on DNA evidence alone, Gnetales invariably appear closer to angiosperms, sometimes showing angiosperms as the sister group closest to Gnetales. When DNA evidence and macromorphology are combined (Doyle and Donoghue 1992; Albert et al. 1994, pp. 549–551; Doyle et al. 1994, p. 426), Pentoxylales and Bennettitales are shown to be the closest sister groups of Gnetales, with Glossopteridales and Caytoniales separated from Gnetales by relatively small quantitative cladistic values.

VESSELS

The most important item from vegetative anatomy in attempting to decipher the relationships of Gnetales is the nature of the gnetalean vessel. Thompson (1918) and Bailey (1944, 1953) claimed that the angiosperm



Figs. 9, 10 SEM photomicrographs of pits from tracheids as seen on radial sections. Fig. 9, *Ephedra gerardiana* Wallich, Freitag 1425; three pits showing conspicuous torus in center of each connected with margo threads, at least some of which are intact. Fig. 10, *Tetracentron sinense* Oliver, AW, s. n.; three pits from end wall of tracheid to show small size of pores in pit membranes. Bars at upper left in each = 10 μ m.

vessel and the gnetalean vessel had separate origins. This concept has been questioned by Muhammad and Sattler (1982). Vessel presence in the two major groups as a symplesiomorphy would be a significant piece of evidence for closeness of the two groups, whereas homoplastic origin of vessels in Gnetales and angiosperms would favor the idea that Gnetales arose from a vesselless group of gymnosperms.

The central reasons why Thompson (1918) and Bailey (1944, 1953) considered gnetalean vessels to have originated separately from those of angiosperms lie in the nature of the perforation plate. In turn, the nature of the perforation plates is considered to represent an extension of, or a modification of, lateral wall pitting, so that lateral wall pitting is not excluded from the argument. Thus, the foraminiate perforation plate of *Ephedra* (figs. 1-4) is regarded, according to the

Thompson-Bailey hypothesis, as derived from an assemblage of circular bordered pits like those on the lateral walls (fig. 9). The condition in *Ephedra* is regarded in this concept as little modified from primitive vessels of Gnetales, in accordance with the virtually universal cladistic placement of *Ephedra* as basal to *Welwitschia* and *Gnetum* (see cladistic papers cited above). The modifications of the ephedroid perforation plate would include increase in size of the perforations as compared to lateral wall pits and loss of pit membranes. *Gnetum* vessels would represent further modifications, notably in enlargement and coalescence of the perforations so as to result in a simple perforation plate (figs. 5-8), but the ephedroid condition can still be seen in *Gnetum*, most clearly in narrow secondary xylem vessels close to primary xylem. The accelerated modification of the *Gnetum* perforation plate, evident

in coalescent-foraminate conditions (figs. 5, 6) as well as in simple and near-simple (figs. 7, 8) conditions, is easily explained in terms of flow rate. With minute foliar surface, selective pressure for simplification of the perforation plate of *Ephedra* is minimal, whereas the greater probable transpirational rates of the broad leaves of *Gnetum* favor simplification of the perforation plate to accommodate peak flows in vessels.

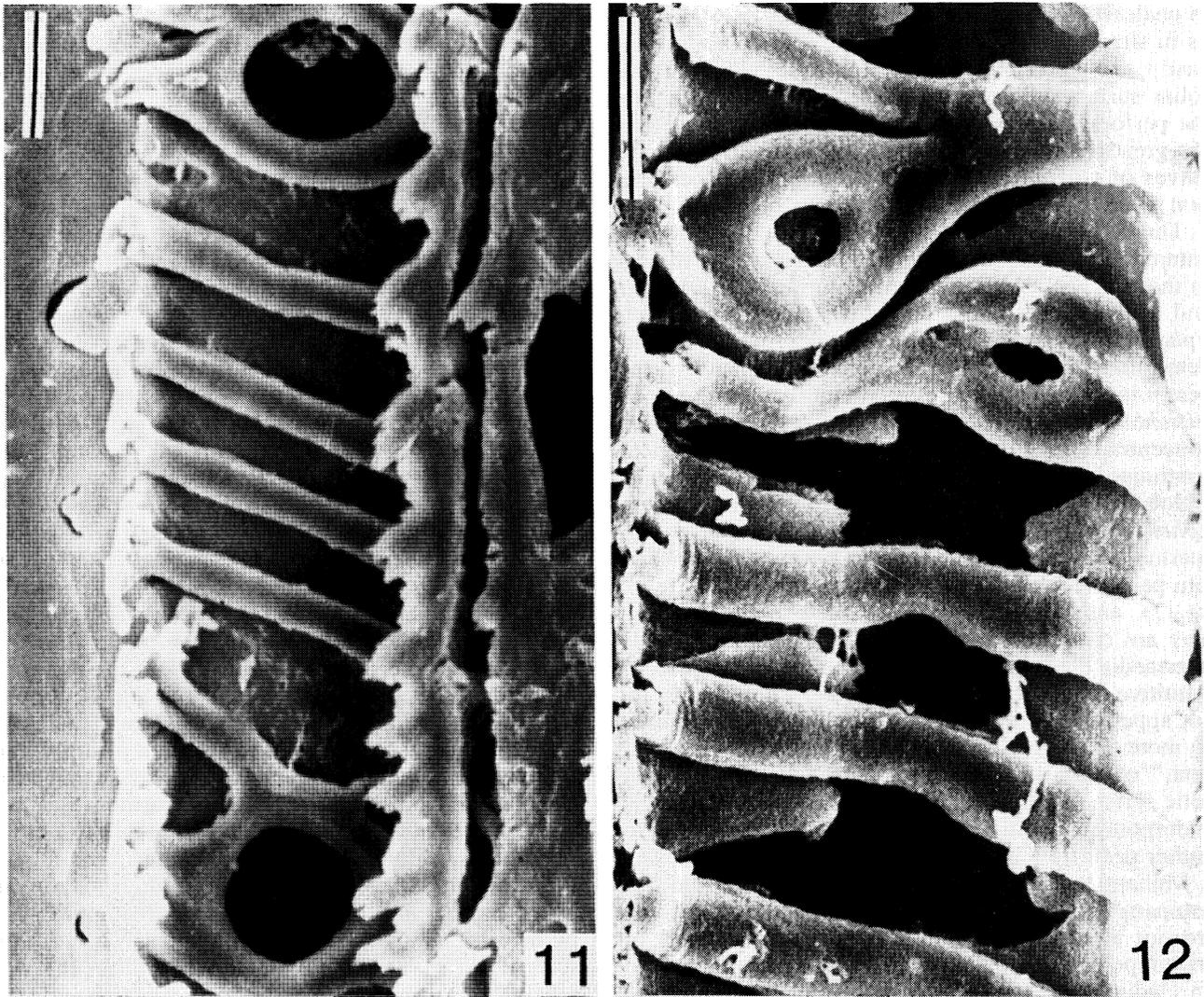
The perforation plate of *Welwitschia* is not generally entered into this sequence, presumably because vessels in that species are so different from those in *Ephedra* and *Gnetum*. Also, the consequence of considering *Ephedra* the basal genus of Gnetales means that in determining relationships of Gnetales, the nature of the vessel in *Ephedra* is more important than that of *Welwitschia*. Muhammad and Sattler (1982), however, concentrate on attempting to show a morphological continuum between vessels of selected angiosperms and those of *Gnetum* rather than a continuum between *Ephedra* or *Welwitschia* vessels and those of angiosperms. Muhammad and Sattler (1982) designate certain perforation plates of *Gnetum* as "scalaroid" (e.g., fig. 7), and apparently present these plates because they are considered to be suggestive of what vessels intermediate between those of Gnetales and those of primitive angiosperms might be like. These plates do not appear to me to be scalariformlike but, rather, can be more accurately categorized as "foraminate coalescent," exactly what would be expected in a phylogenetic series in which an ephedroid perforation plate is undergoing simplification in response to adaptation to higher peak flow rates.

Muhammad and Sattler (1982) have chosen for comparisons with *Gnetum* a series of angiosperms that are not at all basal within angiosperms in terms of contemporary phylogenies, either those that are based on cladistics (e.g., Qiu et al. 1993) or those that use more traditional methods (e.g., Thorne 1992). Vessels of Juglandaceae, Myricaceae, and Salicaceae are used by Muhammad and Sattler (1982) for purposes of comparison, although those families seem, on the basis of our current ideas of their phylogenetic placement, unlikely to retain perforation plates like those of ancestral angiosperms.

However, additional features in vessel phyletic with regard to Gnetales are worthy of consideration and have not been discussed to date to any appreciable extent at all. The circular bordered pits on lateral walls of *Ephedra*, *Gnetum*, or *Welwitschia* vessels are markedly unlike those on lateral walls of angiosperm vessels considered to be primitive. In turn, the primitive gnetalean tracheid from which gnetalean vessels are to be derived bears little resemblance to tracheids widely considered to be primitive in angiosperms. This contrast is stressed by the juxtaposition of figs. 9 and 10 but needs additional explanation. A difference in the shape of perforations or the shape of pits of lateral walls between Gnetales and angiosperms has been considered by many authors, especially recent seed plant cladists (e.g., Doyle et al. 1994; Nixon et al.

1994). However, should shape be the central issue? The circular shape of gnetalean pits is contrasted by these authors with the scalariform type of pitting widely thought to be primitive in angiosperms. However, if examined in terms of adaptations to function that underlie phyletic, shape may not be the primary consideration. The gymnospermous tracheary element pit, as exemplified by those of *Ephedra* (fig. 9), contains a thick central torus, suspended by margo threads. The function of the torus (Frey-Wyssling 1976) is closure of the pit aperture during aspiration, when a tracheid that has filled with air is sealed off, by displacement of tori so as to close pit apertures, from tracheary elements that are still functioning. Moreover, the margo strands feature wide spaces, spaces wide enough to accommodate passage of colloidal gold particles (Frey-Wyssling 1976) or other objects about 0.2 μm in diameter (Panshin and De Zeeuw 1980). These spaces are much larger than the micropores in pit membranes of angiosperms (fig. 10; pits from an end wall of a *Tetracentron* tracheid are shown there, but lateral wall pits would be quite similar in this respect). The margo strands, by their slender, flexible strandlike nature and the way they suspend the torus, allow for movement of the torus and thus closure of the gymnospermous pit during aspiration. The angiospermous pit (fig. 10), on the other hand, lacks a torus and is, in any case, generally too rigid to function in aspiration. Exclusion of potential air entry from a deactivated tracheid of this type into a functioning water-filled tracheid is prevented by the excessively small size of the micropores, through which air bubbles cannot pass (Carlquist 1988a, p. 108; Jarbeau et al. 1995). A circular shape is irrelevant to the primitive angiosperm pit because a movable torus is not present; instead, the scalariform shape covers the lateral width of the wall efficiently, maximizing pit area on a tracheid and contact with adjacent cells. Thus, water handling and conductive safety mechanisms of the circular gymnospermous pit and the scalariform angiospermous pit represent two alternative functional modes, and the nature of this difference should not be described primarily in terms of pit shape. The different shape is, as we have seen, a by-product of differences in physiological mechanisms, not a cause for them or a fundamental phylogenetic feature in its own right.

The discovery of tori in tracheary elements of *Gnetum* (Carlquist and Robinson 1995; Carlquist 1996a, 1996b) is of significance because thus the pits of *Gnetum* tracheids are not transitional from those of cycads to those of conifers, as thought by Frey-Wyssling (1976, p. 61), and the simplified perforation plates of *Gnetum* that allegedly look transitional ("scalaroid") to scalariform angiosperm plates must be viewed as modifications of lateral wall pitting that, in *Gnetum*, is not at all like that of angiosperms. The fact that tracheary elements of *Gnetum* are not fundamentally different from those of *Ephedra* can be considered evidence for monophyly of Gnetales. Thus, a transitional position for *Gnetum* between more primitive Gnetales



Figs. 11, 12 SEM photographs of walls of primary xylem tracheary elements from radial sections of stems of *Gnetum leyboldii*, McPherson 10003. Fig. 11, Pit membranes lacking in the circular pitlike structures, top and bottom, which therefore are perforations. Fig. 12, Smaller circular bordered pits intercalated into the helical thickenings; a pit membrane is clearly present in the pit at left, and neither of the pitlike structures is likely a perforation. Bars at upper left = 10 μm .

and angiosperms is not supported. There is similar evidence in phloem: according to Ray F. Evert (personal communication), ultrastructure of sieve elements of Gnetales is like that of gymnosperms rather than that of angiosperms.

A highly significant formation in the primary xylem of Gnetales also offers evidence about the phylogenetic position of Gnetales. In metaxylem tracheids and vessel elements of *Gnetum* (fig. 11, 12) and *Ephedra*, bordered helical thickenings, composed of lignified secondary wall material, are attached to the thin primary walls, just as in many vascular plants. As has been described extensively, the gyres of helical thickenings in such metaxylem elements can stretch out, accommodating increase in length by the tracheary elements. A special feature of the helical bands of secondary wall material in the metaxylem tracheids of

conifers, *Ginkgo*, and Gnetales is that circular bordered pits are intercalated into these bands. This situation has been noticed for many years (Bierhorst 1960). Such intercalated bordered pits are not present in the primary xylem of angiosperms. Thus, those who visualize Gnetales as transitional to angiosperms are faced with explaining the presence of this neglected feature in Gnetales.

I hypothesize that the presence of circular bordered pits intercalated into the helical secondary wall bands of primary xylem tracheary elements of conifers, *Ginkgo*, and Gnetales is related to functions that have not yet been considered, largely because emphasis has been placed on this feature from a descriptive or taxonomic point of view. One functional possibility for these circular pits is suggested by their structure in figure 11, in which the two circular pits are, in fact,

not pits, but perforations. Lack of pit membranes is well shown for these two circular areas, and earlier studies by means of light microscopy were not able to demonstrate lack of pit membranes with any certainty. Presumably, perforations of this sort would occur in primary xylem of *Gnetum* and *Ephedra* but not in *Ginkgo* or conifers, which are vesselless.

A second likely functional significance of the intercalated circular bordered pits of primary xylem elements of Gnetales has to do with the torus-margo organization of secondary xylem tracheary element pits. By forming the circular bordered pits, the conductive capabilities of the large spaces that characteristically occur among margo strands are available. Also, the pit aspiration mechanism, by virtue of the presence of a torus, is also potentially present. The primary xylem elements evidently have negligible microporosities in the lateral primary wall between the helical gyres of secondary wall material, according to my SEM photographs (figs. 11, 12). Thus, conduction capabilities of the primary xylem tracheary elements with the intercalated circular pits are greatly enhanced by the availability in these pits of the spaces between the margo strands. This potential mechanism for enhancement of conduction is available to *Ginkgo* and conifers, as well as Gnetales, and has not received comment as a functionally valuable feature.

OTHER XYLARY FEATURES

Ephedra, *Gnetum*, and *Welwitschia* were claimed, in almost all earlier literature, to have only two types of tracheary elements: tracheids and vessel elements. Axial parenchyma was claimed to be present in *Ephedra* and *Gnetum* (Martens 1971), although drawings by Greguss (1955) claim fiber-tracheids for *Gnetum gnemon*. Fiber-tracheids are not present in *Welwitschia*, but fiber-tracheids are present in all species of *Ephedra* and *Gnetum* (fig. 13) studied thus far. The phylogenetic significance of this is interesting in several respects. Dimorphic tracheary elements—tracheids co-

existing with fiber-tracheids in a wood in this instance—do not appear to be basal within angiosperms. Among dicotyledons that appear at all close to being basal in recent phylogenetic treatments, dimorphism of this kind has been identified only in Austrobaileyaceae (Carlquist 1988c), and such dimorphism has not been identified in any gymnosperms other than *Ephedra* and *Gnetum*. Axial parenchyma is present in numerous gymnosperms and in primitive dicotyledons, and it occurs in *Welwitschia* (Carlquist and Gowans 1995) and in all species of *Gnetum* (figs. 13–15) and in a few species of *Ephedra* (Carlquist 1992). In addition to differing from wood of other gymnosperms and from woody dicotyledons other than *Austrobaileya* and some more specialized phylads in having tracheid dimorphism, *Gnetum* and *Ephedra* woods are further distinct in the distribution of fiber-tracheids with respect to tracheids. In dicotyledons that have tracheid dimorphism, the tracheids occur in a vascentric pattern, whereas fiber-tracheids are distal to the vessels (Carlquist 1985). In *Gnetum gnemon*, fiber-tracheids appear distributed randomly with relation to tracheids; in the lianoid *Gnetum* species, fiber-tracheids tend to be in radial files alternating with tracheids (fig. 16). In *Ephedra*, fiber-tracheids are arranged diffusely or in tangential bands (“diffuse-in-aggregates” is the term applied to that distribution, but this term is normally applied to axial parenchyma, not to fiber-tracheids). In no species of *Ephedra* or *Gnetum* was a vascentric distribution of tracheids seen.

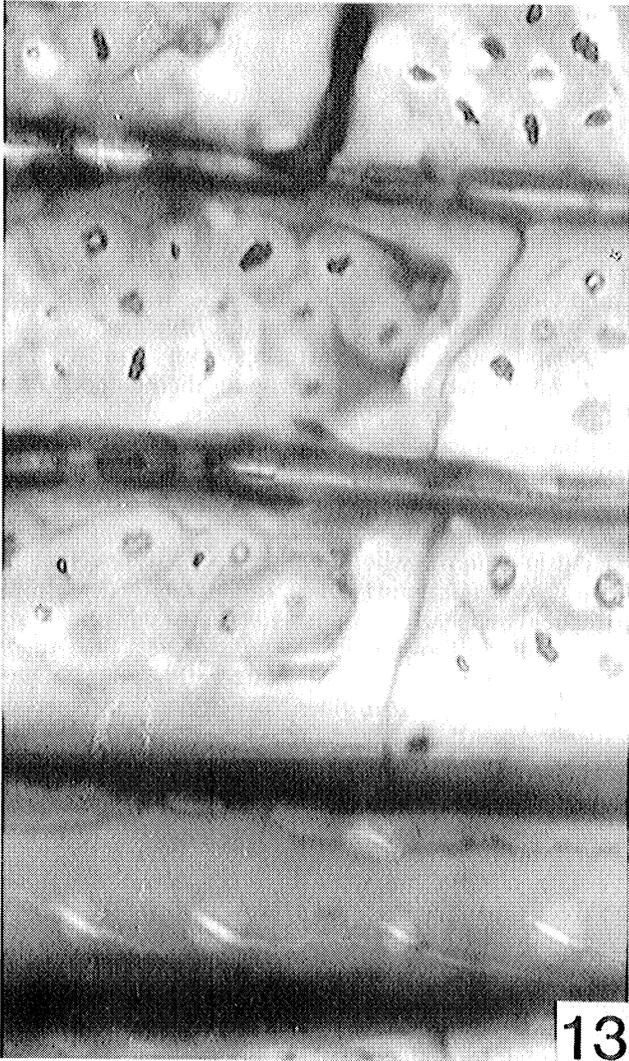
The observation that both *Ephedra* and *Gnetum* have fiber-tracheids together with tracheids, a condition otherwise unreported in gymnosperms, suggests this feature may be plesiomorphic in Gnetales, in which case lack of fiber-tracheids in *Welwitschia* would have to be interpreted as an autapomorphy.

Gnetales are distinctive in having both multiseriate and uniseriate rays, although in *Ephedra*, multiseriate rays predominate and uniseriate rays are relatively scarce. In *Welwitschia*, rays are more difficult to ob-

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Figs. 13–16 Light photomicrographs of *Gnetum* to show differentiation between cell types. Fig. 13, *Gnetum gnemon*, Carlquist 8088; radial section of root showing, bottom to top, a tracheid, two fiber-tracheids containing septa and starch, and a pair of cells from an axial parenchyma strand, separated by a lignified wall, which is vertical in this photograph. Fig. 14, *Gnetum cuspidatum* Blume, transection of stem, showing three cell types in axial secondary xylem: tracheids have thick walls; fiber-tracheids have walls of intermediate thickness; and axial parenchyma cells (some of which contain crystals) have very thin walls, some of which are not clearly delineated. Fig. 15, *Gnetum schwackeanum*, Fariño 425; transection of stem, showing a portion of secondary xylem in which axial parenchyma cells (thin gray walls) are relatively abundant. Fig. 16, *Gnetum leyboldii*, McPherson 10003; transection of juncture between secondary phloem (above) and secondary xylem (below), to show that the files of phloem parenchyma (narrower cells with thin walls) are intercontinuous with files of narrow cells in the secondary xylem. Wide cells in the secondary phloem = sieve cells; wide cells in the secondary xylem = tracheids. Further explanation in text. Figs. 13, 14, 16, Magnification scale above figure 7; fig. 15, scale above figure 15 (divisions = 10 μ m).

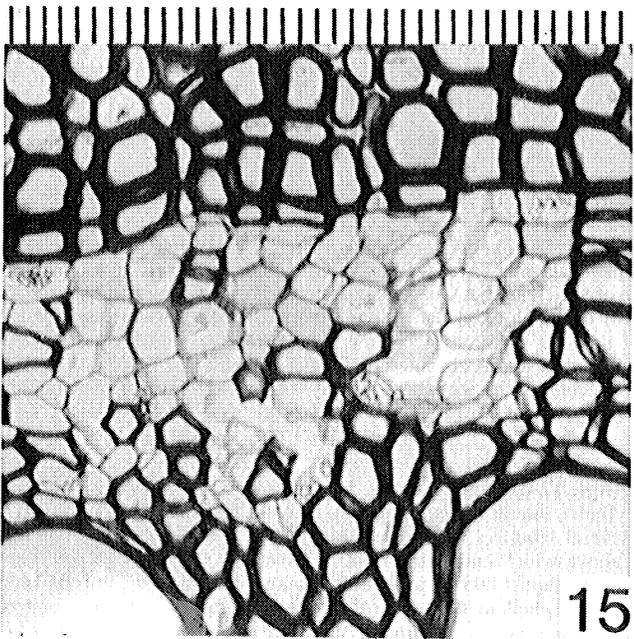
Figs. 17–20 Light photomicrographs of stem transections of *Gnetum* (figs. 17–19) and *Ephedra*. Fig. 17, *Gnetum schwackeanum*, Fariño 425; portion of outer stem to show (top to bottom), sclerenchymatous cylinder, lateral meristem region, recently formed vascular strand (five vessels visible in secondary xylem), and conjunctive tissue in which several laticifers are present. Fig. 18, *Gnetum cuspidatum*, Carlquist 8091; portion of vascular strand (phloem fibers are dark, near center), above which and to the right of which are radially oriented files of cells related to lateral meristem activity. Fig. 19, *Gnetum latifolium* Blume, Carlquist 8087; transection of outer portion of underground stem; a thin gray band about one-fourth from the top of the photograph delimits phellem (above) from cortex; in cortex, there are scattered gelatinous fibers and (lower right) a few sclereids. Fig. 20, *Ephedra californica*, cortex of primary stem; arrows point to gelatinous fibers, which are pale gray. Figs. 17, 19, Scale above figure 17 (divisions = 10 μ m); figs. 18, 20, scale above figure 15.



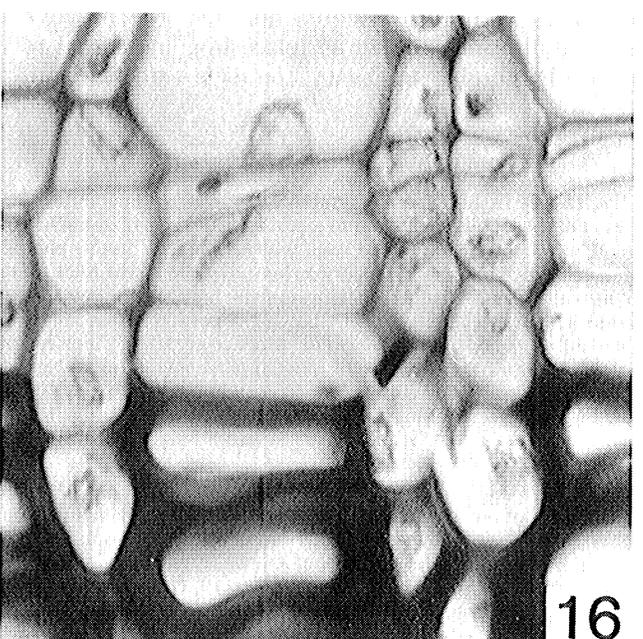
13



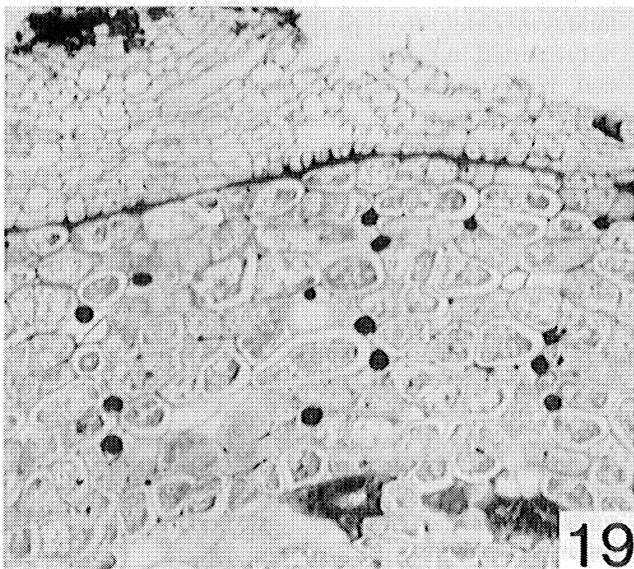
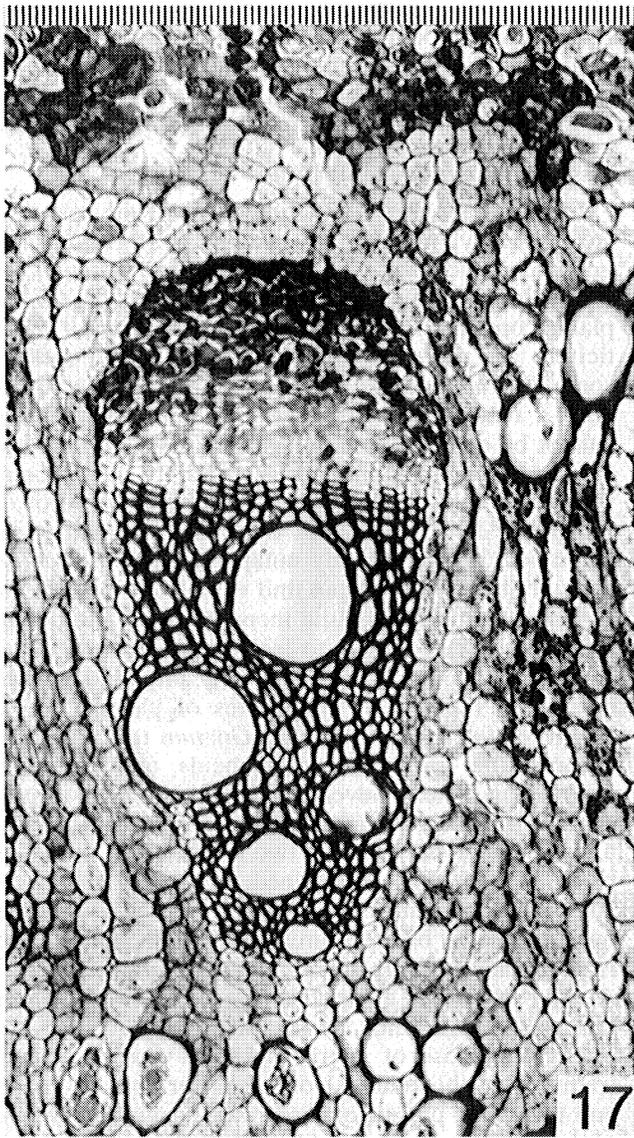
14



15



16



serve, and certainly multiseriate rays are present, but at least a few of these may begin as uniseriate rays. The presence of both multiseriate and uniseriate rays in *Gnetum* has been cited as a point of resemblance to angiosperms, but it may be a plesiomorphic feature in Gnetales. In addition, it may occur more widely within gymnosperms than has generally been appreciated. Presence of both types of rays is common in cycads (Greguss 1968). More pertinent is whether both multiseriate and uniseriate rays are present in the gymnosperms that are placed close to Gnetales in recent cladograms, especially those orders of gymnosperms likely to be sister groups of Gnetales. Both uniseriate and biseriate rays are present in the wood of Bennettitales (Wieland 1906). *Pentoxylon* has some biseriate and multiseriate rays as well as uniseriate ones (Vishnu-Mittre 1957), *Bucklandia* has rays uniseriate to triseriate (Bose 1953), and Doyle and Donoghue (1986) score Caytoniales as having at least some multiseriate rays. Thus, presence of multiseriate rays intermixed with uniseriate rays appears widespread among gymnosperms commonly considered close to Gnetales, and this feature is therefore best not interpreted as a synapomorphy that would link Gnetales only with angiosperms.

The other wood or stem feature of major potential phylogenetic significance is the presence of successive cambia (figs. 17, 18) that originate in parenchyma derived from a kind of lateral meristem activity. I do not place the cambial modifications described by Lev-Yadun and Aloni (1993) for *Ephedra* in this category; those conditions appear to be distortion of cambial orientation owing to increasingly contorted conformation of the aging stem. The origin of lateral meristem activity in all *Gnetum* lianoid species is in inner cortex, as claimed by La Rivière (1916) and as confirmed in more recent studies (Carlquist and Robinson 1995; Carlquist 1996a, 1996b). The origin of new cambia in the bark of *G. gnemon* is similar in that cortical tissue is involved, although the time of origin of these new cambia is evidently much delayed compared with origin of those in the lianoid species. The lateral meristem files in *Welwitschia*, however, are derived from phelloderm rather than cortex. Therefore, the variations in cambial activity within Gnetales are likely not synapomorphies or symplesiomorphies but a series of autapomorphies.

Wood and stem features of Gnetales do not, therefore, indicate any synapomorphies with angiosperms, in my opinion. The opinions of Thompson (1918) and of Bailey (1944, 1953) that vessels arose independently in angiosperms and in Gnetales are supported here, on the basis of new concepts as well as previously cited evidence. Gnetales appear to have originated from a vesselless gymnosperm group with both multiseriate and uniseriate rays. This interpretation is reinforced by the demonstration of tori in pits of at least five species of *Gnetum* and the demonstration that *Gnetum* and *Ephedra* have both tracheids and fiber-tracheids, yet without vasicentric distribution of tra-

cheids as in all angiosperms that have tracheid dimorphism. The cladograms by Albert et al. (1994), Doyle et al. (1994), and Nixon et al. (1994) that indicate that the likely sister group of Gnetales is a vesselless group of gymnosperms are thereby supported by wood data.

Relationships within Gnetales

Gnetales are rich in autapomorphies with respect to stem and xylem anatomy. Laticifers (fig. 17) are a good example of a feature restricted to *Gnetum*. Although developmentally younger stages of *Gnetum* plants contain laticifers commonly (Martens 1971), laticifers are not evident in older stems and roots of some *Gnetum* species. Laticifers thus must be considered a feature related to development, and absence cannot be claimed for any species of *Gnetum* without study of developmental stages; laticifers should be assumed to be likely to be present at some stage of development in all species of *Gnetum*. Vesturing in pits of vessels is also a likely autapomorphy for *Gnetum*. As with laticifers, one can find vessels in some species in which vesturing is quite inconspicuous, but no species studied was judged to lack vesturing altogether.

Several wood features, especially some of those that are newly reported in this series of papers, suggest affinity between *Ephedra* and *Gnetum* (e.g., presence of both fiber-tracheids and tracheids, and foraminate perforation plates). Several bark features, such as presence of diffusely distributed gelatinous fibers, suggest a resemblance between the two genera. Alternative explanations may be entertained, however: richness in autapomorphies in *Welwitschia* (which has phellem but lacks other bark features) suggests it has departed from an ancestral gnetalean stock in numerous ways. Among the likely autapomorphies in *Welwitschia* are lack of fiber-tracheids, vessels with simple perforation plates like those of lateral walls of vessels, grooves (rather than thickenings) on inner surfaces of vessels and tracheids, presence of secretory cavities, presence of large crystal-bearing fibrosclereids, and origin of lateral meristem from phelloderm. One peculiar feature not reported in earlier decades appears to link *Ephedra* and *Welwitschia*: occurrence of minute intercellular calcium oxalate crystals lining intercellular features. This feature is otherwise unknown in vascular plants, at least in the form reported in these two genera. Perhaps occurrence of the minute intercellular crystals in *Ephedra* and *Welwitschia* is a plesiomorphy, a feature lost in ancestors of *Gnetum* as that phylad shifted into mesic localities. *Gnetum* has crystals within cells of wood, phloem, and bark.

Although much information valuable in major phylogenetic and ecological contexts emerged from the survey of bark and wood at a species level, very few features likely to be of taxonomic significance at infrageneric levels were uncovered. In part, sampling of *Ephedra* and *Gnetum* is too poor to delimit these features at present. One indication that some features of interest may await discovery is the presence of peculiar ray astrosclereids in *Gnetum klossii* Merrill. *Gne-*

tum gnemon appears rather different from the lianoid species of *Gnetum* in bark and wood characters, but study of the sister species of *G. gnemon*, *Gnetum costatum* K. Schum. is needed, and much infraspecific diversity within *G. gnemon* remains to be explored. Attention should be focused on whether Markgraf's (1930) sections and subsections—which correspond with habit and geography—are confirmed by newer data. The placement of the New World lianas in the same section as *G. gnemon* rather than in the section that includes the Indomalesian and Asiatic lianoid *Gnetum* species is one feature of Markgraf's (1930) system that invites reinvestigation. Although wood and bark data seem to offer a number of features of major phylogenetic and ecological significance, molecular data will likely be of prime importance for infrageneric systematics of *Ephedra* and *Gnetum*. At present, we have few reliable hints about evolution and speciation

patterns within these two remarkably widespread and divergent genera, and even our concepts about what constitutes a species within these genera are tentative.

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