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Stem and leaf anatomy of the arborescent Cucurbitaceae *Dendrosicyos socotrana* with comments on the evolution of pachycauls from lianas

M. E. Olson

Instituto de Biología, U.N.A.M., Departamento de Botánica, Ciudad Universitaria, Mexico

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Abstract. Stem and leaf anatomy of *Dendrosicyos socotrana*, the only arborescent Cucurbitaceae, are examined for correlations with life form and ecology and are used to test hypotheses regarding features adaptive in scandent plants. The stem consists mainly of ray and conjunctive parenchyma with small strands of xylem forming an anastomosing net throughout the trunk. Xylem strands bear vascular cambia that produce secondary phloem, representing the first report of successive cambia in Cucurbitales. Some features characteristic of lianas, such as very wide vessel elements with thick walls, are absent from *Dendrosicyos*. Other features, such as very wide rays and abundant axial parenchyma, are present in both *Dendrosicyos* and lianas but appear to serve differing roles in these different life forms. It is suggested that lianas have numerous features that are readily co-opted in the evolution of pachycaul trees and that the evolution of pachycauls from lianas has happened repeatedly in the core eudicots.

Key words: Adaptation, Cucurbitaceae, *Dendrosicyos socotrana*, evolution, anatomy, lianas, successive cambia, Socotra.

The arborescent *Dendrosicyos socotrana* Balf. f. is a conspicuous exception to the almost exclusive scandent habit within Cucurbitaceae. With its bloated trunk several meters tall, and few, thick main branches (Fig. 1), *D. socotrana* is an example of the pachycaul "bottle tree" habit. *Dendrosicyos* is found in shrubland communities on Socotra, the dry tropical island off the Horn of Africa, and differs from many bottle trees in having twigs and small branches that are conspicuously pendent. Although the pachycaul habit is unusual in Cucurbitaceae, it is common in many families throughout the seasonally dry tropics (particularly conspicuous examples are members of Malvaceae such as *Adansonia* in Africa, Madagascar, and Australia, and *Chorisia* in South America).

The anatomy of *Dendrosicyos* has elicited interest at least as early as 1835, with a report that "The whole diameter of their trunks consists of a soft, whitish cellular substance, so easily cut through that we could divide the largest of them with a common knife" (Wellsted 1835, quoted in Balfour 1888, p. 101). In comments accompanying the naming of the plant, Balfour (1888, p. 100) noted that "The examination of the stem would be of great morphological interest, and I hope to have some specimens from Schweinfurth's plant [in cultivation in Cairo], of which an account will be given in the Appendix''. Balfour apparently never received the specimens, but an anatomical study of *Dendrosicyos* is still of interest on various fronts. Because they are of little timber value and grow in understudied tropical dry habitats, the anatomical features of bottle trees have received little attention. One aim of this paper is therefore to examine the leaf and stem features associated with the bottle tree habit and the ecological conditions that *Dendrosicyos* experiences, especially wind and seasonal drought. A second goal is to examine the anatomy of *Dendrosicyos* in the context of anatomical features known from other Cucurbitaceae. A further goal is to examine how anatomical features of scandent ancestors are transformed in the evolution of a pachycaul tree. It is proposed that scandent plants have features conducive to the evolution of pachycaul trees and shrubs and that this phenomenon has occurred repeatedly throughout the core eudicots.

A final objective is to use *Dendrosicyos* to test hypotheses regarding anatomical features that are adaptive within the liana habit in general and Cucurbitaceae in particular. Because *Dendrosicyos* is not scandent, it is likely that features that are adaptive in a scandent morphology would be of differing selective value in a pachycaul tree. These features and hypotheses include:

1. The presence of wide, thick-walled vessels with band-like reinforcements. The very wide vessels of vines are interpreted as adaptations to meet the osmotic needs of a large leaf biomass with limited stem transectional area (Carlquist 1975, 1985, 2001a). Ewers and Fisher (1991) noted that vining species of the legume genus *Bauhinia* had wider vessel elements than species of the same genus with freestanding life forms. A similar trend has been noted between species in *Gnetum* (Fisher and Ewers 1995), and even between vining and self-supporting clones of the same genetic individual of *Toxicodendron diversilobum* (Gartner 1991). Features that have been observed to be associated with wide vessel diameter in Cucurbitaceae are increased wall thickness and bandlike wall thickenings, which may help maintain vessel integrity in the face of torsion of the stem and low xylem pressure (Carlquist 1992). Thus it might be expected that the vessels of the rigid mature stem of *Dendrosicyos* would lack band-like reinforcements and be narrower than those of scandent cucurbits.

2. Sclerified parenchyma adjacent to vessels. Carlquist (1992) postulated that vessels in Cucurbitaceae are protected by adjacent thick-walled paratracheal parenchyma cells (and libriform fibers) and cushioned by thin-walled parenchyma and ray cells in other parts of the stem. Protected within a massive trunk, the vessels of *Dendrosicyos* might be expected to be surrounded by axial parenchyma cells that are nonlignified.

3. Vasicentric tracheids. As the conductive efficiency of vessels increases with increasing diameter, the danger of air embolisms also becomes greater. Carlquist (1992) noted the presence of vasicentric tracheids in contact with the vessels of vining Cucurbitaceae as a feature of likely adaptive significance in mitigating the effect of embolized vessels. Narrow tracheids are much less likely to become embolized than vessels, so in the event of the conductive column of a vessel being broken, the vasicentric tracheids should maintain some flow to the organs supplied by their associated vessel. If, as is hypothesized above, vessels in *Dendrosicyos* are narrower than those of lianas, the selective value of vasicentric tracheids seems low.

4. Abundant axial parenchyma that buffers stem torsion. The very long stems of vines and their dependence on other plants or objects for support means that events along the length of the stem, such as a falling support branch, leave them vulnerable to pinching or breaking of the conductive column due to stem torsion. Axial parenchyma may serve a cushioning function, in which the energy of a damaging event to the stem is absorbed by thin-walled parenchyma cells, sparing the vessels from damage (Schenck 1893 *fide* Carlquist 2001a,

Putz and Holbrook 1990, Carlquist 2001a). Carlquist (1992) hypothesized that the abundant axial parenchyma in the stems of scandent Cucurbitaceae serves such a buffering function. It is of interest to examine the stem of *Dendrosicyos* for this cell type and to ask if it is serving the cushioning function that it served in the scandent ancestor, or whether it is serving a new function.

5. Very wide, tall rays. In addition to axial parenchyma, the very wide, exclusively multiseriate rays observed in scandent Cucurbitaceae have been hypothesized to provide resilience to torsion (Carlquist 1992). Fisher and Ewers (1989) noted that the very wide unlignified rays in *Coccinia* provided radially oriented zones of weakness between plates of xylem. When subjected to torsion, the stem was found to split along these rays, preserving the water column in the vessels surrounded by lignified fibers and parenchyma cells. The mature stem of *Dendrosicyos* is unlikely to experience torsion to a great degree and might therefore be expected to lack massive rays forming radial planes of weakness.

6. Starch storage in parenchyma of liana stems. Carlquist (1985) hypothesized that parenchyma in long vining stems could serve as a repository of starch that is in proximity to vessels along the entire length of the stem. Such starch reserves could be drawn upon to fuel massive reproductive events, leaf flushes, or raise vessel osmotic pressure to regulate conduction. Therefore the distribution and possible role of starch in the stem of *Dendrosicyos* is compared with that of vining Cucurbitaceae.

Materials and methods

In 1967, J. Lavranos collected live material of *D. socotrana* at Ras Hebak on the northern coast of Socotra. Live second-generation seedlings were kindly provided by T. Metcalf of the University of California at Davis. The plants were cultivated in a heated greenhouse in Santa Barbara, California, for an additional two years before harvesting. Phenological and growth pattern observations were made during this period. At the time of sampling, the plants had reached a height of 1.5 meters and a basal stem diameter of 12 cm. One of these plants is depicted in Fig. 1; the locations of the majority of stem samples are indicated by arrows. The sample from the area indicated by the upper arrow is referred to as the "young stem" where the stem was 2 cm in a diameter. That from the area of the lower arrow was taken from just above the widest part of the trunk and is referred to as the "mature stem". Samples taken from the distalmost branches that still had traces of epidermis are referred to as "twigs". Two sets of preparations (trans- and longitudinal sections of the three stem ages and leaves) were made, one from each of two plants, with the exception of the mature stem, which was only made from one specimen. A voucher is deposited at the Missouri Botanical Garden herbarium (MO) as Olson, s.n. Because of the highly succulent nature of the stem, it was necessary to preserve samples immediately in 70% aqueous ethanol to avoid rot and collapse of the water-filled cells. After fixation, stem chips were softened in 10% ethylene diamine prior to embedding in paraffin and sectioning on a rotary microtome at 13 μm (method of Carlquist 1982). Leaves were also fixed in 70% ethanol, dehydrated, and embedded in paraffin. Sections were stained with Northen's modification of Foster's ferric chloride-tannic acid staining series (Johansen 1940). Macerations were prepared using Jeffrey's solution (Johansen 1940) and stained in safranin. Terminology follows Carlquist (2001a). For scanning electron microscope (SEM) observation, sections were mounted on aluminum stubs, cleared in xylene, sputter coated with an Emtech K550 and observed with a Hitachi S-2460N in the SEM facility of the Instituto de Biología of the Universidad Nacional Autónoma de México. Measurements of cells are outside dimensions except for vessel diameter, for which the lumen diameter is provided. Means given are based on $n = 25$ (± standard deviation).

Results

Habit

The pendent leaders of *Dendrosicyos* are particularly easily observed in young seedlings (Fig. 1). The erect stem of the young seedling gradually bends until its apical meristem is no longer the highest point of the plant (black stem at upper left in Fig. 2). A branch that becomes the leader emerges from the highest point (light gray branch at upper left in Fig. 2). This process repeats iteratively until a massive sympodial trunk is formed, presumably the product of numerous apical meristems (as depicted in Fig. 2 at lower right, with the adjacent products of different meristems shaded differently). The leaves of the cultivated *Dendrosicyos* were never completely shed or only tardily so. This is in great contrast to other dryland pachycauls in the same greenhouse (*Moringa* spp.; *Jacaratia* spp.), which were completely leafless during the dry season.

Stem anatomy

Stem structural plan. Adult stem structural plan is characterized by vascular bands alternating with bands of conjunctive tissue, with no growth rings distinguishable (Fig. 3). Vascular bands consist of rays and plates of xylem; plates are made up of vessels, libriform fibers, vasicentric axial parenchyma, and sometimes apotracheal axial parenchyma. Phloem is produced by vascular cambia on the abaxial faces of the xylem plates. In transections, xylem plates appear to be separated from one another as islands in parenchyma. However, these strands frequently contact each other by weaving and occasionally bifurcating, forming a vascular net within the stem (Fig. 8). Most weaving of vascular strands is tangential, with the result that contact occurs mostly within vascular bands. The extension of a vascular strand radially into another band is less common.

Phellem, phelloderm, and cortex. The outermost stem of *Dendrosicyos* bears 15–25 layers of phellem and 2–5 layers of chloroplast-bearing phelloderm. The cortex of a young twig (4 mm in a diameter) was about 30 cells thick and consisted of rounded parenchyma cells generally of uniform size and shape with most of the divisions observed being transverse. The epidermis has numerous cystolith-bearing trichomes similar to those figured on the undersides of the leaves.

Cortical features persist in larger stems. In the young stem (2 cm in diameter), the phelloderm was underlaid by a cortical area with a number of cell layers similar to that in the twig but with much more divisional activity and resultant variety in cell size and shape. In the mature stem, the cortex was also about 30 cells thick, with more abundant cell division observed than in the twig or the young stem. Because of the great increase in diameter that the base of the stem undergoes, it is likely that virtually all of the cells observed in the cortex of the mature stem are the products of division of the primary cortical cells and division of the lateral meristem. Thus it is reasonable to refer to this region in the mature stem as secondary cortex. Excluding divisions of the lateral meristem, in the 2 cm stem radial divisions outnumber tangential divisions by 3:1 and oblique divisions by 2:1. In the mature stem, similar proportions were observed between radial and oblique divisions, but tangential divisions were even rarer than in the 2 cm stem.

Small fields of septate fibers were observed in the young stem every 60–300 μm around the stem, 5–10 cells beneath the phelloderm. These fibers averaged 22 (± 5.4) μm in diameter with segments 274 (± 73.8) μm long, whereas the fibers themselves exceeded the 2.5 mm height of the longisection available. Similar fibers were not observed in either the twig or the mature stem.

The lateral meristem, cambia, and their products. The outer stem is notable for its lack of a single clearly defined vascular cambium (Fig. 3). Instead, cells in a general area of the inner cortex give rise internally to ray cambia, conjunctive tissue, or vascular cambia that produce the xylem plates and associated phloem. Tangential divisions of the lateral meristem also produce small amounts of cortical parenchyma externally.

As a xylem plate increases radially, ray cambia vaguely aligned with the vascular cambia produce ray cells internally as the phloem impinges into the secondary cortex.

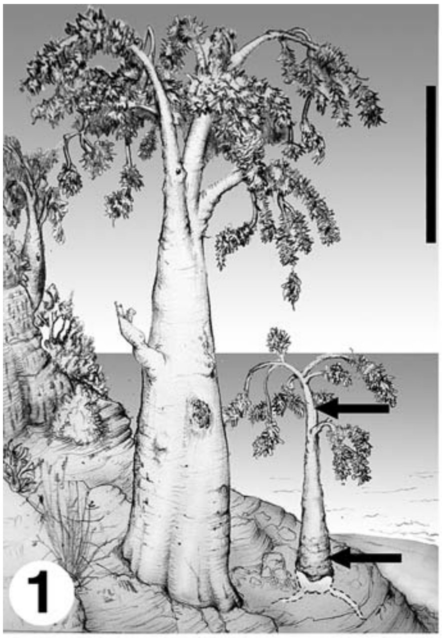
Alternately, the lateral meristem produces conjunctive parenchyma. In this way, a vascular cambium and its products become surrounded by parenchyma on all sides (Fig. 4).

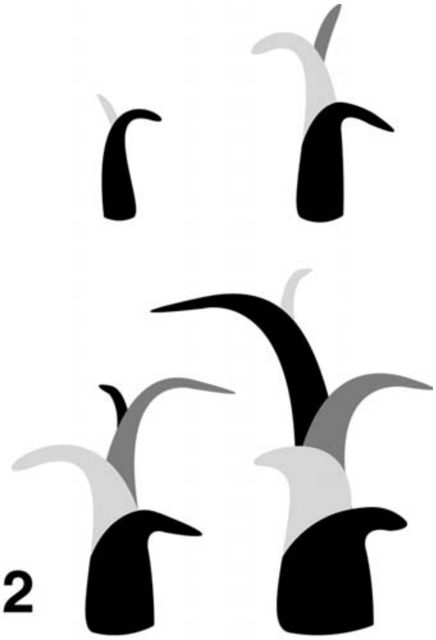
In the young stem, the plates of xylem formed in the first season of growth are counter to the orientation of mature xylem plates, being associated with cambia that produce intraxylary phloem toward the pith. These plates reach their largest tangential dimensions close to the pith and are narrowest at their outermost extent (Fig. 6). Just external to these xylem plates are small patches of xylem each with 1–3 vessels. Each of these patches has a small cambium that is usually on the abaxial face of the patch, producing phloem toward the outside of the stem. Occasionally, these small cambia are located on the adaxial face of the patch and produce phloem toward the pith, as do the innermost xylem plates.

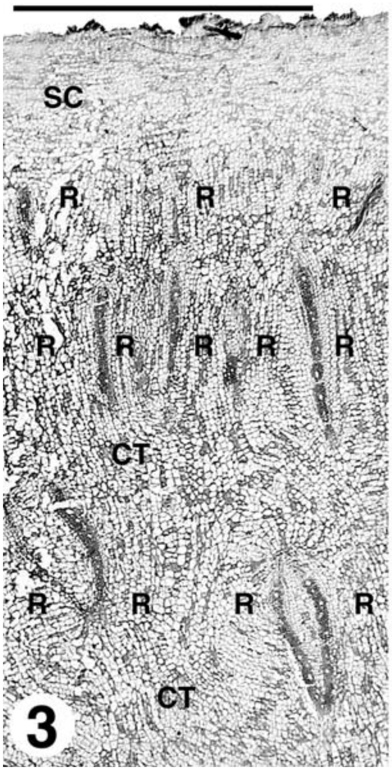
Occasional strands of interxylary phloem ranging in size from 3–20 cells wide were observed in the ray cells between xylem plates (Fig. 7). Phloem strands produced by vascular cambia include sieve tube elements and companion cells, which are often exceeded in number and size by phloem parenchyma. Small strands of phloem similar to those figured as occurring in rays occur sporadically in the outer cortical parenchyma. These strands may represent incipient formation of vascular cambia.

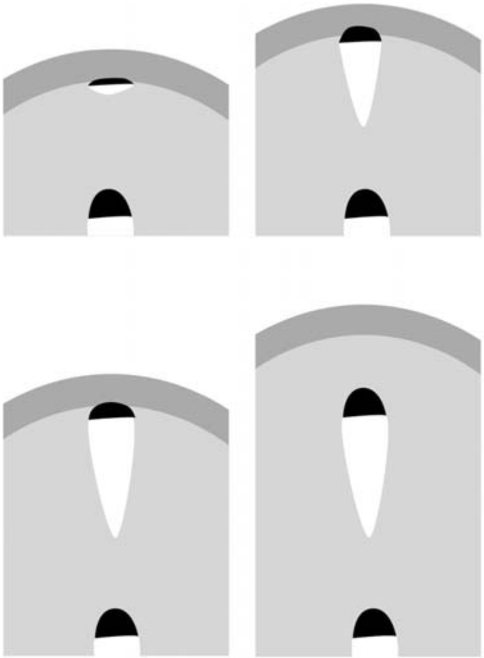
Vessels. In the mature stem, mean vessel element dimensions are 180 (±79.6) µm long and 48 (±12) µm in diameter. Vessels were solitary in 84% of the cases examined. The remainder were in mostly radial or tangential pairs or, much more rarely, radial multiples or globular clusters of up to five vessels. Vessel density is 2 (±2) vessels per mm^2, occupying 0.7% per unit area of transection. Perforation plates are simple with a distinct border and usually have an angle of 90°–45° with respect to the longitudinal axis of the vessel element. Lateral wall pitting is alternate. Vessel-vessel pit cavities are narrowly oval, sometimes approaching rhomboidal, with narrowly oval apertures 4 (±0.8) µm wide and 1 (±0.4) µm tall. Pits at vessel-axial parenchyma interfaces are 10 (±4.1) µm wide and 4 (±0.8) µm tall and have usually very broadly oblong cavities with wide, oval, or oblong apertures (Figs. 10, 11), forming distinct borders 1–2 µm wide. Sometimes 2–4 pit apertures are interconnected within the same groovelike pit cavity. Vessel-axial parenchyma pits are sufficiently broad as to approach scalariform arrangement in some areas (Fig. 10). Although vessels were sometimes observed to contact libriform fibers, no vessel-fiber pitting was observed. Vessels are usually slightly radially oblong and angular in cross section, with each side of the polygon corresponding to the face of a contacting cell. Mean vessel wall thickness is 2 (±0.8) µm. Vessels are sometimes obstructed by numerous small (ca. 50 µm in diameter) tyloses. Mean vessel dimensions in the young stem were 75 (±21.4) µm diameter with a wall thickness of 3 (±0.8) µm.

Libriform fibers. *Dendrosicyos* exhibits a kind of fiber dimorphism. Large-pitted parenchyma-like fibers that are usually non-septate form the bulk of this cell type (Fig. 12). Occasionally intermingled with parenchyma-like fibers are very long fibers that are always septate and usually bear only small pits (Fig. 13; to distinguish them from the parenchyma-like fibers, which are only occasionally septate, these cells are referred to here as "septate fibers"). Mean dimensions of parenchyma-like fibers are 482 (±139.3) µm long and 25 (±6.2) µm in diameter. None of the septate fibers persisted through the maceration process intact; mean dimensions of the fragments were 1329 (±378.3) µm long and 22 (±5.4) µm in diameter, figures sufficing to demonstrate that the septate fibers are much longer and somewhat narrower than the parenchyma-like ones. The mean number of segments observed in these fragments was 6, and the mean length of each segment was 274 (±73.8) µm. Parenchyma-like fibers have more abundant and larger pits: parenchyma-like fiber pits are 4 (±1.8) µm wide in their largest dimension, whereas those of septate fibers are 3 (±0.8) µm wide (Fig. 13). Pits in both fiber types are exclusively non-bordered (Fig. 13 shows pits of parenchyma-like fibers). Mean libriform fiber wall thickness is 2 (±0.6) µm (septate vs. parenchyma-like fibers were not distinguished in transection, but the rarity of septate fibers means that this figure likely represents the wall thickness of the non-septate fibers). Parenchyma-like fibers are broadly fusiform or with cylindrical main









bodies with long intrusive tails that together equal or exceed half of the total cell length. Tails often bifurcate or have other irregular ramifications at their tips (Fig. 12).

Axial parenchyma. Three types of axial parenchyma cells are found in *Dendrosicyos*. Vasicentric scanty parenchyma contacts the radial walls of the vessels, with libriform fibers barely contacting the tangential walls. In other cases, especially when vessels are abundant, vasicentric parenchyma cells form a complete sheath around vessels (Fig. 6) or even extends between vessels. These cells are of highly irregular shape (Fig. 11 shows such a cell from a maceration) with constrictions between the large vessel-axial parenchyma pits and walls 1 (±0.6) μm mean thickness. Vasicentric parenchyma-libriform fiber pits are small and oval (Figs. 9, 11). Clear cases of vasicentric parenchyma cells divided into strands were not observed. The second type of axial parenchyma in *Dendrosicyos* is apotracheal, in bands that interrupt the plates of xylem. Bands of apotracheal axial parenchyma were by far most common in the young stem (two bands are indicated by arrows in Fig. 6) and were rare in the mature stem (note the lack of such bands in the xylem plate in Fig. 5). The third type of axial parenchyma is conjunctive tissue (labeled "CT" in Fig. 3; also at bottom of Fig. 5) that fills the area between the vascular bands. These cells were rarely observed to be in strands of two, but are usually not so divided. In the material available, bands of conjunctive tissue equaled the vascular bands in radial extent or were narrower.

Rays. Very wide multiseriate rays are initiated between xylem plates. Even the primary rays are multiseriate, with no uniseriate precursors (Fig. 6 shows part of one such ray and its point of contact with the pith). These rays may be 20–50 cells wide or more and are often 5 mm or more in height. Cells are for the most part procumbent, though square and upright cells may be found, especially on the ray periphery. No uniseriate wings were observed on multiseriate rays, and uniseriate rays are lacking. Ray cell pit density is not evenly distributed on all faces and appears to be least on the tangential faces of the cells. Ray and conjunctive parenchyma cells, which form the bulk of the stem of *Dendrosicyos*, are not distinguishable based on shape and are not expected to be functionally different.

Crystals, starch, and storying. Crystals were not observed in the stem, but starch granules are common in cortex, ray, conjunctive parenchyma, and pith cells (Figs. 5–8), less so in libriform fibers (Fig. 9 at top). Clear cases of storying were not detected in any of the preparations made.

Leaf anatomy

Leaves average 10 cm in length and diameter, and bear prickly margins. The mean thickness of the leaf from the upper cuticle to the lower cuticle and away from veins or surface

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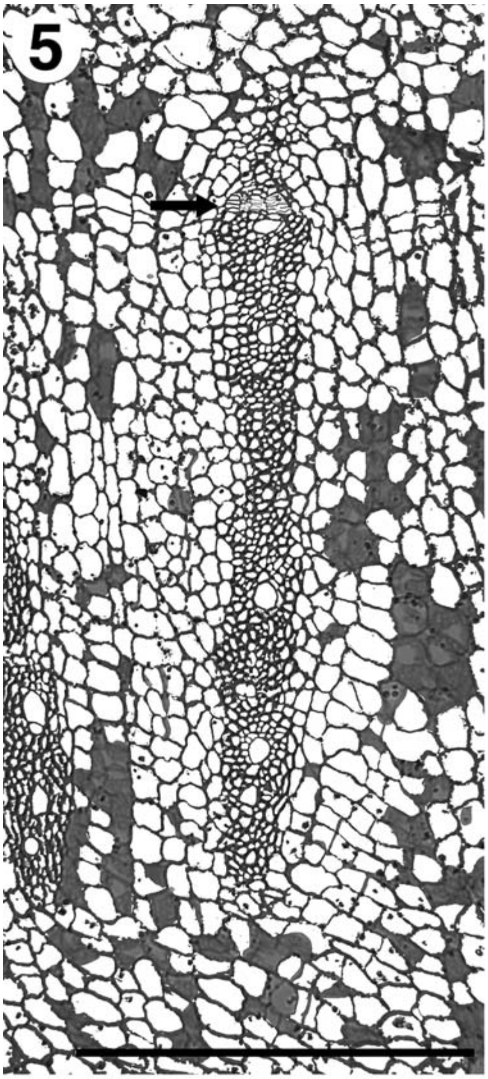
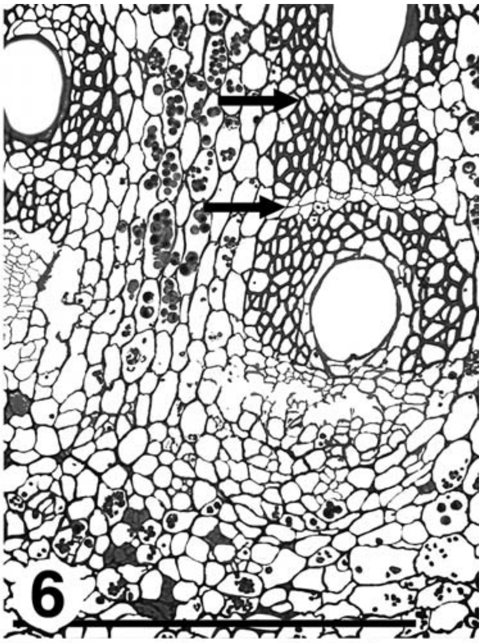
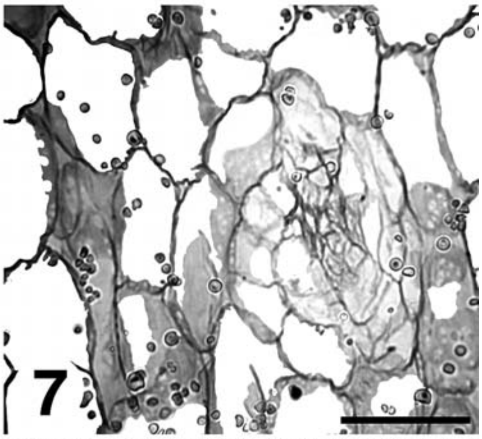
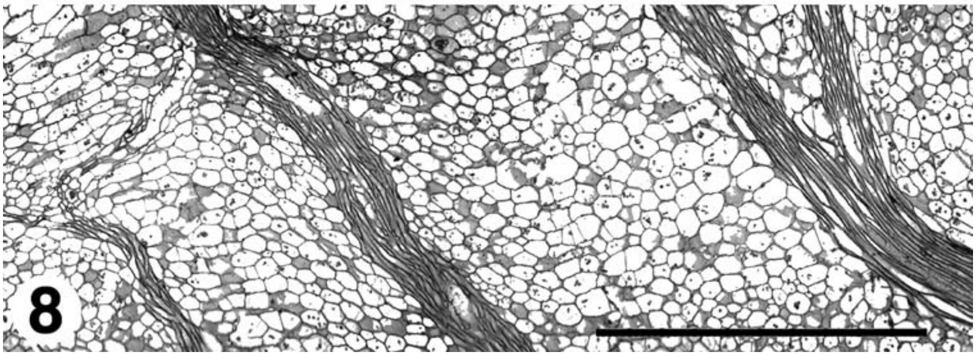
Figs. 1–4. *Dendrosicyos socotrana*, habit and growth patterns. **1** Habit. Mature plant in middle, one of the seedlings from which samples were drawn is shown at right. Low arrow indicates location of "mature" sample. Upper arrow indicates location of "young stem" sample. Note rigid lower trunk and flexible, pendent upper stems. Scale bar = 1m. **2** Contributions of successive leaders in producing the trunk of *Dendrosicyos*. Tissue derived from different apical meristems is given different shading. **3** Stem transection to show structural plan of bands of conjunctive tissue (*CT*) alternating with vascular bands that consist of rays (*R*) and xylem plates (dark strips of cells between rays). Outer surface of stem is shown at top; most of the light cells in this area are part of the secondary cortex (*SC*). Scale bar = 3 mm. **4** Diagram of xylem plate ontogeny. Dark gray = 2° cortex; light gray = ray and conjunctive parenchyma; black = phloem; white = xylem; black-white interface = vascular cambium. Following a band of conjunctive tissue, a new vascular cambium is produced by the lateral meristem (top left). This cambium produces xylem to the inside as the stem expands (upper right and lower left). The xylem plate becomes distanced from the cortex when the lateral meristem produces another band of conjunctive tissue (lower right) projections is c. 300 μm. The epidermis of the upper leaf surface is composed of a single layer of cells of ca. 30 μm mean cell height (Figs. 14, 15). These cells have slightly lignified cell walls, with the faces corresponding to the upper leaf surface covered with a thick cuticle (Fig. 16). Cuboidal or slightly rhomboidal crystals are occasionally found singly in the epidermal cells (two are shown beneath pustule in Fig. 14). Stomata occur sparsely on the upper leaf









surface (one of these is shown in Fig. 15). The upper surface of the leaf does not bear trichomes, but multicellular pustules are common (Fig. 14). These pustules are 2–5 cells tall and overlie the main epidermal cell layer, which may become discontinuous beneath the pustules as in Fig. 14. The cells of the pustules have walls that are comparable in thickness to the other epidermal cells, but each is usually filled with a mass of striated material, presumably calcareous cystoliths (cf. Metcalfe and Chalk 1950). Beneath the epidermis are two palisade layers totaling ca. 120 μm thick, characterized by numerous open spaces (Fig. 15). Small cuboidal crystals are common in these cells (upper arrow in Fig. 14). The spongy mesophyll is ca. 330 μm think and is composed of 5–6 layers of highly irregular cells with large open spaces between them. Chloroplasts are common in the cells of both mesophyll layers but are slightly more so in the palisade layers. The epidermis of the lower surface is similar to that on the upper surface, with the cells in the single layer being slightly smaller, averaging 23 (± 5) μm in height (Figs. 14, 17). Stomata, which are anomocytic, are much more common on the lower surface than on the upper. Figure 17 shows three stomata in transection, and Fig. 18 shows three in a paradermal section. Stomata on the lower surface average 23 (± 6) μm in their largest dimension and 17 (± 4) μm in their smallest, with a mean stomatal aperture of 11 (± 4) μm in its largest dimension. Stomata are flush or nearly so with the leaf surface. Pustules are not found on the lower leaf surface, but two- to seven-celled trichomes are conspicuous in this area. Most such trichomes have a basal cell that is constricted with respect to the diameter of the rest of the trichome (Fig. 17). The tips of these trichomes are often bulbous or pointed (Fig. 17), with the apical 1–2 cells being occupied by cystoliths.

Discussion

Stem anatomical correlations with habit and ecology. The most conspicuous feature of the stem of *Dendrosicyos* in connection with the bottle tree habit is the large quantity of ray and conjunctive parenchyma clearly associated with water and photosynthate storage as a means of surviving prolonged drought. One consideration of such storage is expansion and contraction of the stem associated with uptake and use of stem water reserves. Although the stem of *Dendrosicyos* is not ribbed like that of a columnar cactus, the undulating walls of ray, axial, and cortical parenchyma suggest a mechanism to cope with drastic seasonal changes in stem diameter. Presumably the undulations in the cell walls are reduced as the cell water content and stem diameter increases in the rainy season, with an opposite trend in the dry season.

In plants with a single cambium, rays are typically involved in radial conduction from the single phloem area to or from more central parts of the stem. But the necessity for extensive radial translocation in plants with successive cambia seems small due to the comprehensive enervation of the stem by phloem strands and the lack of a single subcortical phloem system (Carlquist 2001a).

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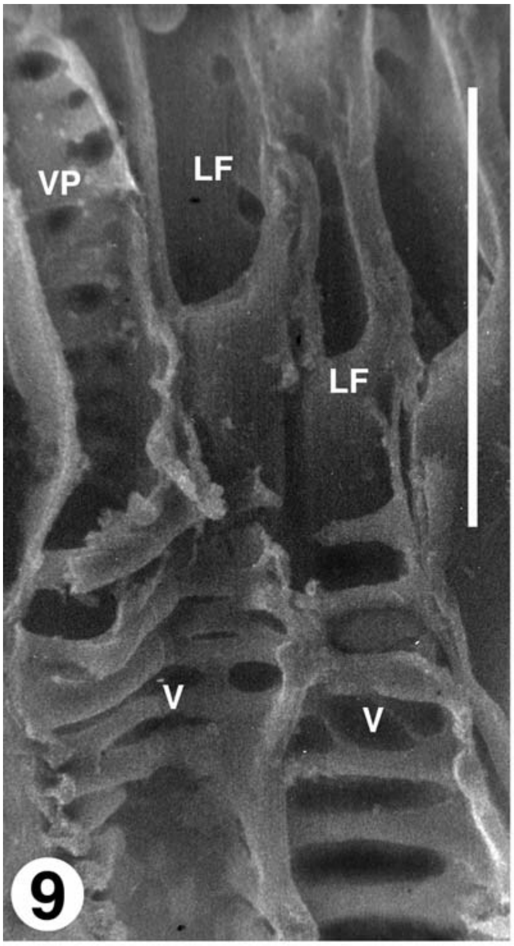
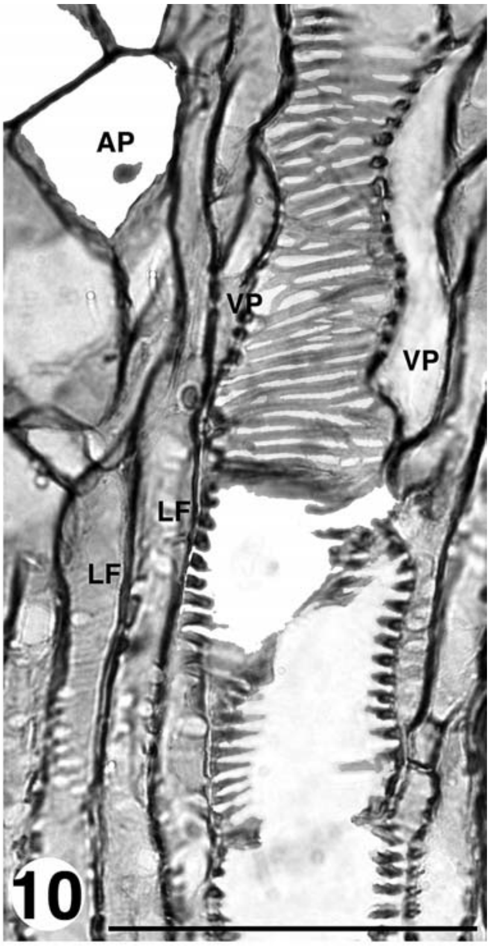
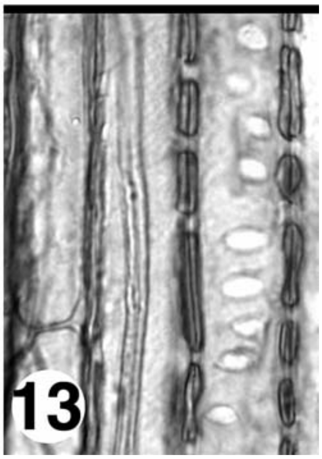
Figs. 5–8. *Dendrosicyos socotrana*, stem anatomy. **5** Stem transection, image oriented with outermost extent at top, showing one entire xylem plate with part of another at bottom left. Abaxial to xylem is a vascular cambium (arrow) producing secondary phloem toward the outside of the stem. This cambium coincides roughly in alignment with the ray cambia on either side. Scale bar = 1 mm. **6** Transection showing pith at bottom and intraxylary phloem being produced from vascular cambia adaxial to xylem plates (torn cells). Bands of apotracheal axial parenchyma interrupt xylem plates (arrows). Starch granules are common in pith and rays. Scale bar = 500 μm. **7** Strand of interxylary phloem with no associated xylem from a transection of mature stem. Scale bar = 100 μm. **8** Tangential section of mature stem to show very wide, tall rays, bifurcation of xylem plates (as at right) and reconnection (as at left). Scale bar = 1 mm

In *Dendrosicyos*, the somewhat lighter pitting on tangential than on other faces of ray cells suggests that, rather than radial translocation, these cells may be chiefly involved in storage of photosynthates supplied by local phloem strands and the possibly rapid translocation of storage products to nearby vessels associated with reproductive events or leaf flushes.

The presence of the gelatinous fibers that characterize reaction wood might be expected







in a heavy, water-laden stem with little lignified tissue, especially one with the pendent branches of *Dendrosicyos* (cf. *Euphorbia*, Carlquist 1970; *Fouquieria*, Carlquist 2001b). The absence of this wall type may be a phylogenetic contingency. Reaction wood has not been reported in Cucurbitaceae, and appears to be nonexistent or rare in the flexible stems of lianas (cf. Metcalfe and Chalk 1950, Höster and Liese 1966).

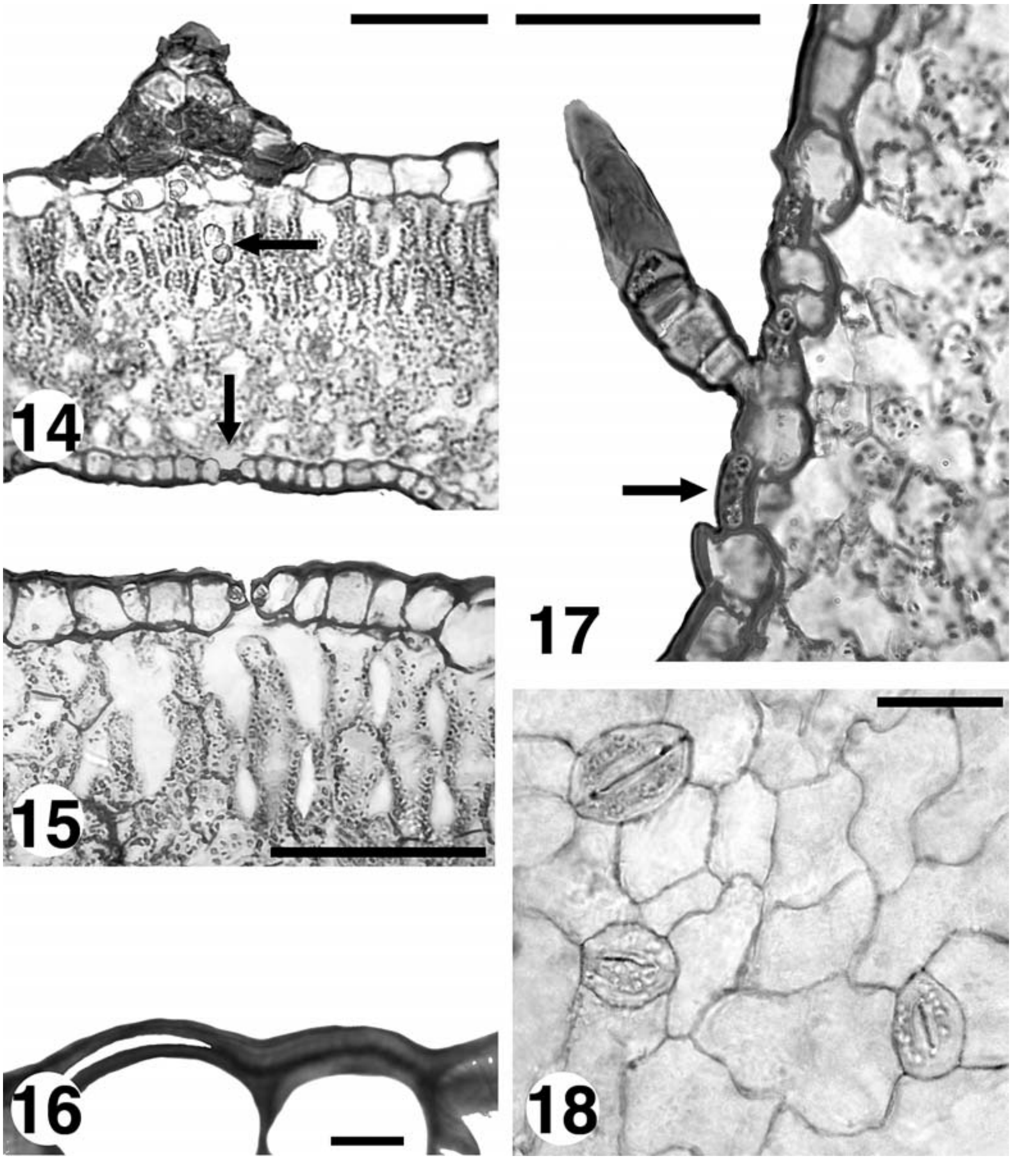
The abundance of radial divisions in the cortical cells is reflective of the large increase in circumference that the trunk of *Dendrosicyos* undergoes. Likewise, radial divisions in the cells of rays and conjunctive tissue are also involved in the increase in circumference of the massive stem. The comparatively low number of tangential divisions observed within the cortical parenchyma, rays, and conjunctive tissue is compensated for by divisions in the lateral meristem, which contributes to the increase in diameter of the stem. Oblique divisions are likewise more common than tangential divisions in the cortex and appear to fill the increase in volume created by stem expansion.

Integration of leaf and stem features. The leaves of *Dendrosicyos* have lignified epidermal cell walls and a thick cuticle. These significant investments in leaf tissue suggest that *Dendrosicyos* does not share the strategy of many deciduous dryland plants, which have highly mesomorphic leaves that are produced immediately following rains and are shed just as suddenly (cf. *Fouquieria* Carlquist 2001b). Likewise, very low conductive area and narrow vessels do not suggest that *Dendrosicyos* transpires at rates comparable to a mesophyte during the rainy season. The mean vessel diameter of a broad sampling of woody mesophytes (108 μm; Carlquist 1975: 206) greatly exceeds that of *Dendrosicyos*. However, neither are the leaves of *Dendrosicyos* highly xeromorphic, lacking such features as stomatal crypts or dense indumentum. Instead, dead leaves clothe the branch tips, likely providing shade and still air around living leaves, buffering them from water loss. Thus a combination of anatomical and phenological traits combine to form leaves that probably persist longer than those of many other dryland pachycauls in similar environments. Leaf longevity has been linked to reduced photosynthetic rate (Reich et al. 1992). The narrow vessels of *Dendrosicyos* relative to more rapid-growing tropical dryland pachycauls (e.g. *Moringa*, Olson and Carlquist 2001) may be related to a relatively lower rate of photosynthesis.

Features of scandent Cucurbitaceae that are absent in *Dendrosicyos*. Of the features hypothesized to be adaptive in a scandent morphology, the first three categories discussed in the introduction are absent from the arboreal stem of *Dendrosicyos*:

1. Wide, thick-walled vessels with band-like reinforcements. The vining Cucurbitaceae examined by Carlquist (1992) had a mean vessel

Figs. 9–13. *Dendrosicyos socotrana*, stem cell shape and pitting. **9** SEM image from a tangential section showing vessel-vasicentric parenchyma pitting as seen from the inside of the two vessels labeled "*V*". On the far wall of the vasicentric parenchyma cell labeled "*VP*" can be seen vasicentric parenchyma-libriform fiber pitting. The three thick-walled cells at upper middle and right are libriform fibers (labeled "*LF*"), and show a few fiber–fiber pits. Part of a starch grain can be seen in left fiber at top. Scale bar = 50 μm. **10** Radial section showing vessel-vasicentric parenchyma pits on the vessel elements at center. Vasicentric parenchyma cells on either side of the vessel at top are labeled "*VP*". Fiber–fiber pits can be seen at lower left. Scale bar = 100 μm. **11** Vasicentric parenchyma cell from maceration to show irregular shape and constriction of cell between vessel-parenchyma pits (broad light patches). Smaller, lighter patches on far wall of cell are parenchyma-fiber pits. Scale bar = 50 μm. **12** Portion of parenchyma-like fiber from maceration to show broadly fusiform shape, abundant pits, and bifurcate tip. Scale bar = 100 μm. **13** Tangential section showing parts of three libriform fibers. The two cells at right are parenchyma-like fibers with large, simple pits. On the left is a septate fiber with a septum at lower left and no pits visible in this image. Scale bar = 50 μm



Figs. 14–18. *Dendrosicyos socotrana*, leaf anatomy. **14** Leaf transection, upper surface at top. Dark cells forming pustule at top bear cystoliths. The upper arrow indicates two crystals. Lower arrow highlights stoma in lower surface. Scale bar = 100 µm. **15** Leaf transection showing stomata in upper leaf surface and palisade cells with abundant air spaces. Scale bar = 100 µm. **16** Leaf transection showing outer surface of epidermal cells and thick cuticle (slightly detached at left). Scale bar = 10 µm. **17** Leaf transection showing three stomata (the lowermost one indicated by an arrow), spongy mesophyll at right, and trichome with cystolith-bearing cells at its apex. Scale bar = 50 µm. **18** Leaf paradermal section showing three anomocytic stomata. Scale bar = 25 µm

diameter of 164 µm among the four species. In great contrast, the nonscandent *Dendrosicyos* has a much smaller mean vessel diameter of 48 µm in the mature stem. It may be assumed that in the seasonally dry habitat of *Dendrosicyos*, large vessels and their attendant vulnerability to embolism are a liability while the arborescent habit of *Dendrosicyos* obviates the requirement for large vessels by permitting a higher number of vessels. The combined mean vessel wall thickness of the four vining species that Carlquist (1992) examined was

4.9 µm, greatly exceeding the wall thickness found in the mature stem of *Dendrosicyos* of 2.36 µm. Consistent with the hypothesis of Carlquist (1992) regarding the adaptive role of bandlike thickenings on vessel walls of vines is the absence of similar features in the mature stem of *Dendrosicyos*. The young stem of *Dendrosicyos* was found to have vessels that are 64% wider than those of the mature stem, with slightly thicker walls, suggesting that the long, slender branches are subject to similar considerations of limited stem area per unit leaf mass as are scandent plants. The very low vessel density, likely one of the lowest ever recorded for a tree, suggests that vessel elements are not of significance in support of the stem. Therefore, selection for increased mechanical support is probably not the factor responsible for narrowing of vessels with respect to scandent ancestors, but rather it is selection for conductive safety.

2. Sclerified parenchyma adjacent to vessels. Significantly, no dimorphism in parenchyma wall thickness was observed in *Dendrosicyos*, with the vasicentric parenchyma being similar in wall thickness to the conjunctive and ray parenchyma cells. With vessels being less vulnerable to crushing or twisting inside of the thick trunk of *Dendrosicyos*, thick-walled parenchyma cells around vessels appear to be of little adaptive value.

3. Vasicentric tracheids. Consistent with this hypothesis is the observation that vasicentric tracheids are absent in *Dendrosicyos*. Relative to a vine, the large number of narrow vessels in *Dendrosicyos* seem much less likely to embolize. Also, the large quantity of water-filled parenchyma and parenchyma-like fibers in *Dendrosicyos* could supply vessels with water, preventing excessively low xylem pressures.

Features shared by scandent Cucurbitaceae and *Dendrosicyos*. The remainder of the features discussed in the introduction appear to have an adaptive role both in scandent plants and in the pachycaul *Dendrosicyos*. The examination of *Dendrosicyos* therefore does not provide information that is consistent with or that will falsify these hypotheses (but that these features are adaptive in pachycaul trees is nevertheless testable using comparative methods):

4. Abundant parenchyma that buffers stem torsion. *Dendrosicyos* stems do support very large quantities of conjunctive parenchyma, but the lower stems are certainly not subject to torsion to the degree that would be experienced by a vine. Parenchyma in *Dendrosicyos* seems to serve mainly in the adaptive role of storage of water and starch and mechanical support via turgor pressure.

Therefore, examination of this trait in the mature stem of *Dendrosicyos* reveals little about the role of parenchyma in scandent cucurbits. However, the terminal branches of *Dendrosicyos*, while not scandent, are flexible and pendent and might be expected to be subject to some of the selective pressure affecting vines, especially considering the extremely high winds reported during the southwest monsoon (Miller and Guarino 1994, Gwynne 1968, reports that there are an average of 23 gale days in July around Socotra). Indeed, it is much more common to find the plates of xylem in the young stem to be interrupted by bands of apotracheal axial parenchyma. Such a configuration in the easily-bent terminal branches seems likely to serve the same buffering function that such cells are hypothesized to serve in scandent species. The distribution of axial parenchyma in young stems thus appears consistent with the hypothesis of Carlquist (1992).

5. Wide, tall rays. Extremely wide, tall rays are found in the stem of *Dendrosicyos*, but it seems unlikely that they are serving as zones of weakness along which the stem fractures under torsion for at least two reasons. First, the highly interconnected vascular system forms a dense net through the parenchymatous stem, probably strengthening the stem rather than creating planes of weakness. Second, it seems unlikely that the lower stem of *Dendrosicyos* would experience sufficient torsion as to make fracture planes of adaptive value. The very wide and tall rays of *Dendrosicyos* must

therefore be serving a different purpose, most likely filling an adaptive role identical to that of conjunctive parenchyma.

6. Starch storage in stem parenchyma. Two lines of reasoning suggest that storage of photosynthates in the stem of *Dendrosicyos* is adaptive in ways that may be similar to that in lianas. First, as in many lianas, leaf and floral production is highly seasonal and a massing of nutrients would no doubt help fuel such episodic events. Also, as in many lianas, the root system of *Dendrosicyos* appears to provide limited volume for storage relative to stem volume.

Co-opting of features of scandent plants to pachycauls. The last three numbered categories of features described above have apparently adaptive roles in both scandent plants and in the pachycaul *Dendrosicyos*. Whereas abundant parenchyma and rays serve both a protective and a storage function in scandent plants, it seems most likely that these cell types are involved in storage only in *Dendrosicyos*. This storage probably fuels episodic flowering or vegetative events in the members of both life form classes. These cell types were present in scandent ancestors for reasons adaptive in that biomechanical context, but are seen in new roles in *Dendrosicyos*. Such features can be said to be evolutionarily co-opted or exapted.

The abundance of ray and axial parenchyma in the stems of many vines seem to be features readily co-opted for life as a dryland pachycaul. If this is the case, then repeated evolution of species with pachycaul habits from scandent ancestors would be expected. This is probably the case in such genera as *Cyphostemma* (Vitaceae), *Adenia* (Passifloraceae), *Pyrenacantha* (Icacinaceae), and other taxa mostly from Africa and Madagascar.

The evolution of increased stature in island plants has been associated with the invasion of moist habitats from drier ones (Givnish 1995). Carlquist (1974) noted that the main exception to this trend are arborescent stem succulents. Indeed, the selective forces driving the evolution of arborescence in *Dendrosicyos* do not seem to be entirely addressed by any of the prominent models proposed to explain evolutionary increase in stature. For example, the open, rocky habitat of *Dendrosicyos* does not seem a probable context for evolutionary height increase driven by competition with neighbors for light, as is likely in denser, moister habitats. Alternatively, massive storage may be allocated to stems when rocky substrates prevent extensive formation of underground tubers. *Dendrosicyos* and numerous other pachycauls occur largely on rock (e.g. *Moringa*, some *Adansonia*, etc.).

Systematics. *Dendrosicyos* is the first taxon reported with successive cambia in Cucurbitaceae and apparently in the Cucurbitales (cf. Carlquist and Miller 2001). Given the great range of morphological diversity in Cucurbitaceae, that successive cambia have not been previously reported may indicate the need for more extensive sampling rather than the rarity of the phenomenon.

The assumption that *Dendrosicyos* has evolved from scandent ancestors has never been challenged, but the relationships of this species to the rest of the family are not clear. *Dendrosicyos* is currently included within the tribe Melothrieae (Jeffrey 1962), but there is evidence that this group is para- (or poly-) phyletic (S. Renner, pers. comm). It seems possible that any one of the several cucurbits possessing conical aboveground tubers and annual vines in the drylands of the western Indian Ocean (e.g. *Corallocarpus*, *Xerosicyos*, *Zygosicyos*, etc.) could be the sister taxon to *Dendrosicyos*.

Until such phylogenetic issues are resolved, the evolutionary polarities of the anatomical features in *Dendrosicyos* will remain hypothetical. For example, the ray and conjunctive parenchyma of *Dendrosicyos* have converged to a striking degree on similar cell form and probably function presumably because of selective pressure related to storage. The rays of *Dendrosicyos* seem clearly homologous to the very broad rays of cucurbits described by previous authors (see Carlquist 1992). In all these species, rays are exclusively multiseriate and occur between plates of xylem. Whether conjunctive tissue in *Dendrosicyos* is homologous to banded axial parenchyma or other tissue will be more readily addressed once the sister taxon of *Dendrosicyos* is identified and the diversity of anatomical modes in Cucurbitaceae more extensively sampled.

Summary. The absence in *Dendrosicyos* of very broad, thick-walled vessels with band-like reinforcements, lignified parenchyma in proximity to vessels and vasicentric tracheids offers a comparative confirmation of the hypothesis that these features are adaptive in lianas. Abundant parenchyma, very large rays, and starch storage in the stem appear to be features that were adaptive in the scandent ancestors of *Dendrosicyos* and have been evolutionarily coopted to serve adaptive roles in a new biomechanical context. It is hypothesized that these features facilitate the evolution of the pachycaul habit from vines and that this is the case not only in *Dendrosicyos* but also in a diversity of families. These evolutionary hypotheses depend on their phylogenetic assumptions; the assumptions made here should be tested with broad-scale phylogenetic reconstruction within Cucurbitaceae.

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Address of the author: Mark E. Olson (e-mail: molson@ibiologia.unam.mx), Instituto de Biología, U.N.A.M., Departamento de Botánica, Circuito exterior s/n, Ciudad Universitaria, Copilco, Coyoacán, A.P. 70-367 México, Distrito Federal, C.P. 04510 Mexico.