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COMPARATIVE LEAF ANATOMY AND SYSTEMATICS IN *DENDROBIUM*, SECTIONS *APORUM* AND *RHIZOBIUM* (ORCHIDACEAE)

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The specialized leaf anatomy for species of *Dendrobium* within section *Aporum* is similar to that of species in section *Rhizobium*. In both sections leaves are characterized by a unifacial or nearly unifacial surface where the exposed surface is abaxial. However, leaves in section *Rhizobium* also feature a lacuna submerged in the mesophyll and surrounded by an adaxial epidermis. In contrast, leaves in section *Aporum* merely present an internal suture that divides the leaf into bilateral halves. These two sections of *Dendrobium* are hypothesized to be sister taxa because of synapomorphies in their foliar anatomy. A cladistic analysis performed with various anatomical characters of the leaf demonstrates that both groups are monophyletic. Little resolution was found within the anatomically and morphologically distinctive *Aporum* clade.

Introduction

Dendrobium Swartz, a genus within tribe Dendrobieae, subtribe Dendrobiinae (Orchidaceae), comprises about 900 species (Bechtel et al. 1992; Mabberley 1993) that exhibit a wide range of vegetative morphology and anatomy. *Dendrobium* is distributed throughout tropical Asia, some Pacific islands, and Australasia (Dressler 1993). Plants may be epiphytic, lithophytic, or terrestrial with slender to pseudobulbous stems composed of one to several nodes. Leaves may be equitant or more or less widely separated distichously along the stem, conduplicate, laterally flattened, or cylindrical.

Schlechter ([1912] 1982) is the most widely cited authority in the classification of *Dendrobium* based on his work in the orchid flora of German New Guinea. We used Kränzlin's ([1910] 1957) classification, even though it is not favored by current workers, because it contains a more comprehensive treatment of section *Aporum* Blume. Kränzlin treats forty-nine species of which we had ten for study in addition to *Dendrobium brevimentum*, more recently described by Seidenfaden (1985).

Kränzlin divided section *Aporum* (as a subgenus) into two subgroups, *Hemiphylla* and *Holophylla*, based upon the distribution of leaves along the stem. Plants in section *Hemiphylla* possess leaves that only partially cover the stem, whereas leaves in section *Holophylla* completely cover the stem.

Species of section *Aporum* are centered around Burma, but they also occur in Malaysia, throughout the

Indonesian islands, Papua New Guinea, Andaman Islands, Thailand, Burma, Laos, Vietnam, and regions of northeastern India and southern China. Plants of *Aporum* are characterized by short rhizomes that bear fascicles of upright or pendent slender stems, sometimes obscured by overlapping leaves. Leaves are coriaceous, equitant, and laterally flattened; they vary in outline from scalpelliform (fig. 1), lanceolate, and falcate (fig. 2), to triangular. The laminae arise from a sheath that is obliquely inserted on the stem (Kränzlin [1910] 1957).

Leaf anatomy of members of section *Aporum* has not been studied extensively and was only briefly examined by Morris et al. (1996) in their treatment of subtribe Dendrobiinae and by Solereder and Meyer (1930) in their survey of the vegetative anatomy of Orchidaceae. Solereder and Meyer reported on the anatomy of Orchidaceae but made no attempt to use this information systematically. They mentioned only one species of *Aporum*, *Dendrobium aloifolium*, the leaves of which were characterized as having: (1) equitant arrangement, unifacial orientation, and an exposed abaxial epidermis; (2) glandular, sunken, multicellular hairs; (3) peripheral "bast" fiber bundles below the subepidermal cell layer ("nur periphere Bastfaserbündel, zumeist unter der subepidermalen Zellschicht"; Solereder and Meyer 1930, p. 118); (4) vascular bundles arranged in two symmetrical rows within the mesophyll; and (5) stegmata containing spherical silica bodies. In addition, they cited, as general characteristics of the *Dendrobium* leaf, calcium-oxalate raphides and druse-like structures and "anthocyanin pigments" (*anthocyanartige Farbstoffe*) in solution within the cell vacuoles. They described fiber bundles, variously thickened walls of cells comprising

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Figs. 1–5 Leaves of *Dendrobium* section *Aporum*. Bars = 10 μ m. Figs. 1 and 2 show leafy stems. Fig. 1, *D. leonis* with scalpelliform, equitant, closely overlapping leaves; \times 0.40. Fig. 2, *D. acinaciforme* with falcate, equitant, widely separated leaves; \times 0.5. Figs. 3–5 show cross sections of leaves. Fig. 3, *D. acinaciforme* with reniform suberin-like bodies (arrow) in adaxial epidermal cells; \times 1360. Fig. 4, *D. distichum* under polarized light showing thickened adaxial epidermal cell walls (arrow) impregnated with a suberin-like substance; \times 1360. Fig. 5, *D. acinaciforme* with heterogeneous fiber bundles; \times 2200.

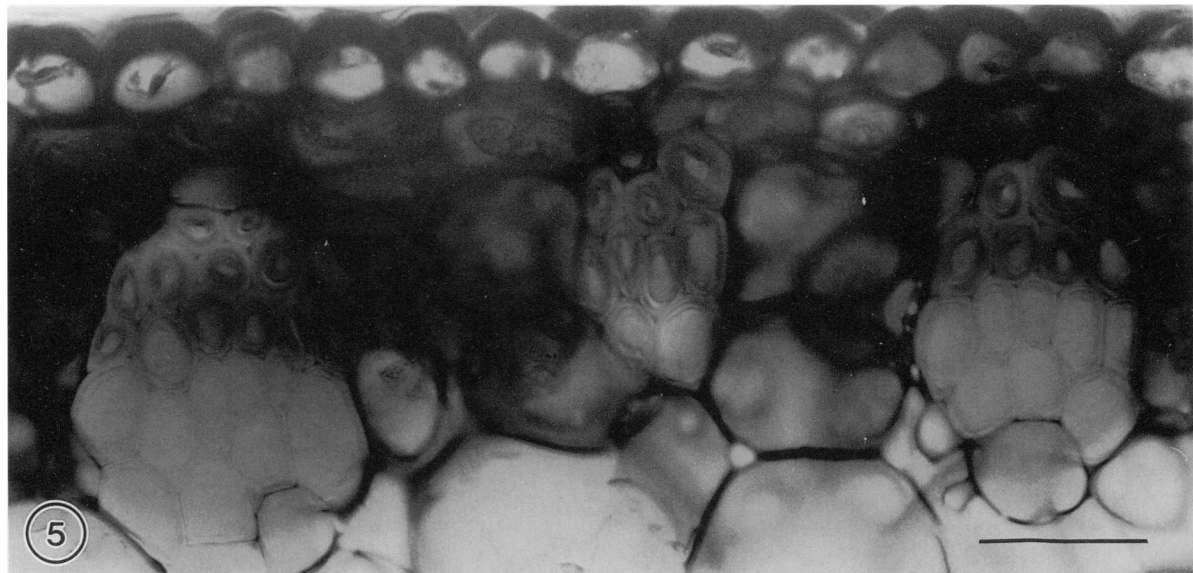
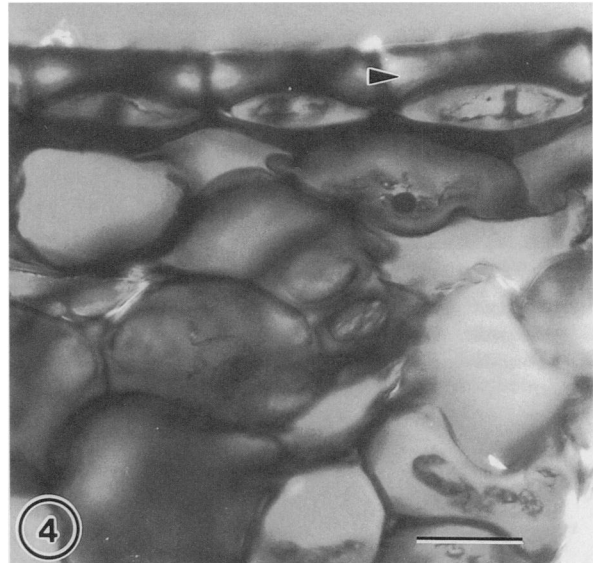
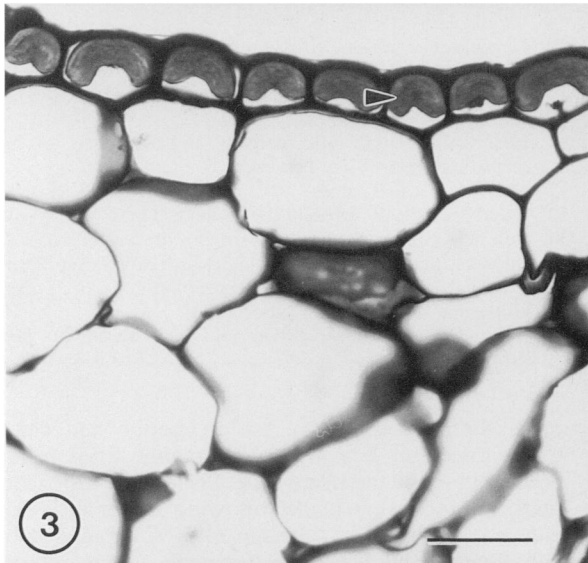
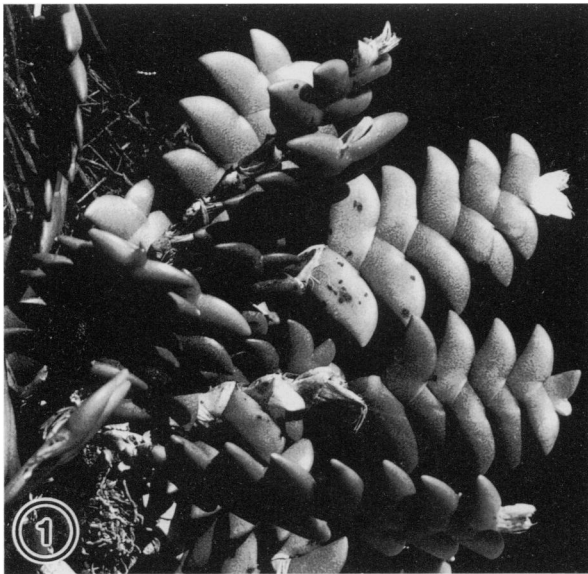


Table 1

SOURCES OF SPECIMENS

Taxon	Source ^a
<i>D. acinaciforme</i> Roxb.	WLS hort. FLAS
<i>D. aloifolium</i> (Bl.) Rchb. f.	K s.n.
<i>D. anceps</i> Sw.	WLS hort. FLAS
<i>D. brevementum</i> Seidenf.	WLS hort. FLAS
<i>D. distichum</i> (Presl) Rchb. f.	SEL 1987-0062A
<i>D. indivisum</i> (Bl.) Miq.	K 1984-3270
<i>D. leonis</i> (Lindl.) Rchb. f.	WLS hort. FLAS
<i>D. linguiforme</i> Sw.	WLS hort.
<i>D. mannii</i> Ridl.	K 1986-1031
<i>D. nathaniele</i> Rchb. f.	K 1986-5692
<i>D. rigidum</i> R. Br.	WLS hort. FLAS
<i>D. rosellum</i> Ridl.	SEL 1978-0200A
<i>D. salaccense</i> (Blume) Lindl.	WLS hort. FLAS
<i>D. toressae</i> (F. M. Bailey) Dockrill.	WLS hort.

^a WLS = William Louis Stern, private collection; K = Royal Botanic Gardens, Kew; SEL = The Marie Selby Botanical Gardens. Abbreviations of herbaria are according to Holmgren et al. (1990), and those of authors follow Brummitt and Powell (1992).

hypodermal tissue, sunken trichomes, and guard cells with small lumens and thickened walls with protruding outer ledges.

Plants in section *Rhizobium* (*Dendrobium*) possess some vegetative characteristics similar to those of *Aporum*, such as the unifacial leaf, and it has been suggested that section *Rhizobium* is the sister taxon of section *Aporum* (Stern et al. 1994). We have used foliar anatomy and morphology to derive characters that can be used in cladistic analyses of species within section *Aporum* and to provide a basis for comparison with leaves of section *Rhizobium*.

Dendrobium "has caused more problems for the taxonomist than almost any other genus of orchids." (Bechtel et al. 1992, p. 145). Traditional taxonomic studies have utilized floral characters almost exclusively to classify taxa, and *Dendrobium* is no exception. This study was undertaken with the hope that additional features derived from vegetative structure would help us to align putatively related groups. The anatomical information will be applied to assess possible evolutionary relationships between sections *Aporum* and *Rhizobium* and to assist us in the evaluation of Kränzlin's classification of section *Aporum*.

Material and methods

Plant material used in this study was obtained from the Marie Selby Botanical Gardens, Royal Botanic Gardens, Kew, and the personal collection of William Louis Stern. Table 1 contains a list of binomials with authorities for plants examined. Fresh plant material was preserved in FAA for at least 48 h and stored in 70% ethanol. Cross sections (50–60 μm) of unembedded leaves at the midpoint of the lamina and at the leaf sheath were made with a sliding microtome. Leaves were also embedded in paraffin, and cross sections (10 μm) of the lamina and leaf sheath were made with the rotary microtome. For study, thin and thick cross sections provide a more complete, three-dimensional structural framework than is possible with thin sections alone. All sections were stained with Heidenhain's hematoxylin and saf-

Table 2

CHARACTERS USED IN CLADISTIC ANALYSIS OF SECTIONS
APORUM AND *RHIZOBIUM*

1. Cells surrounding hair base: thin- and thick-walled (0), thin-walled only (1).
2. Stomatal apparatus: paracytic (0), tetracytic and cyclocytic (1), mainly paracytic with some anomocytic (2) [unordered].
3. Cells surrounding substomatal chamber: thin-walled (0), thin- and thick-walled (1).
4. Abaxial cuticle: thin, less than 3 μm (0); thick, more than 3.5 μm (1).
5. Epidermal cell shape (x-s): rounded, width:length ratio less than 46 (0); elongate, width:length ratio greater than 54 (1).
6. Fiber bundles: absent (0), present with heterogeneous cell composition (1).
7. Water-storage cells: absent (0), present with banded thickenings (1), present with pleats (2) [unordered].
8. Distribution of stigmata: present along vascular bundles only (0), absent (1), present along vascular bundles and fiber bundles (2), present along fiber bundles only (3) [unordered].
9. Leaf orientation: bifacial (0), primarily abaxial epidermis exposed with lacuna or incipient lacuna (adaxial groove) present (1), only abaxial epidermis exposed and no lacuna (2) [unordered].
10. Hypodermis: absent (0), composed of thick- and thin-walled banded cells (1), composed of thin-walled banded and unbanded cells (2), composed of thin-walled unbanded cells (3) [unordered].
11. Leaf width:length ratio: narrow, 0–4 (0); wide, 43–70 (1); intermediate, 10–38 (2) [unordered].
12. Mesophyll canal: absent (0), present (1).
13. Basal leaf sheath: present (0), absent (1).
14. Suture within mesophyll: absent (0), present (1).
15. Suberin-like substance associated with adaxial epidermal cells: absent (0), present inside the epidermal cells (1), present in the epidermal cell walls (2) [unordered].
16. Major vascular bundle at leaf pole (x-s): absent (0), present (1).

ranin (Stern et al. 1994). Differentiation and dehydration were carried out in a graduated ethanol series followed by clearing in limonene (Hemo-De, Fisher Scientific Company). Leaf scrapings were made following Cutler (1978), stained with a 1% solution of safranin in 50% ethanol, dehydrated with ethanol, and cleared in limonene. Surface observations of leaves were made from these scrapings. All sections and scrapings were mounted on microscope slides with Canada balsam and photographed with a Nikon HFX-DX photomicrographic system.

Lengths and widths of 10 guard-cell pairs were measured from one leaf of each species. Because of the limited plant material, other observations were based on several sections from one or two leaves of the same plant. We used "hypodermis" as a nonspecific term to identify the distinctive cell layers beneath the epidermis in the sense of Esau (1960, 1977). Centric leaf, as described by Metcalfe (1979), refers to one completely covered by an abaxial surface while the adaxial surface has been completely eliminated. In the centric leaf, bilaterally arranged vascular bundles are in opposite pairs with xylem regions facing the median longitudinal axis of the leaf section. Wall outlines of water-storage cells appear pleated in sectional view and are referred to as pleated water-storage cells. Adaxial features were recorded from tissues exposed within the leaf sheath.

Features of foliar anatomy and morphology (table 2) were used to construct the character matrix (table 3) employed in the cladistic analysis of sections *Aporum* and *Rhizobium*. As discussed by Seidenfaden and Wood (1992), leaf morphol-

Table 3

CHARACTER MATRIX USED IN CLADISTIC ANALYSIS OF SECTIONS *APORUM* AND *RHIZOBIUM*

Taxon (section)	12345	67890	12345	6
<i>Dendrobium salaccense</i> (<i>Grastidium</i>).....	00000	00000	00000	0
<i>D. linguiforme</i> (<i>Rhizobium</i>).....	01010	02011	11100	0
<i>D. rigidum</i> (<i>Rhizobium</i>).....	01010	02111	10100	0
<i>D. toressae</i> (<i>Rhizobium</i>).....	01010	01012	10100	0
<i>D. acinaciforme</i> (<i>Aporum</i>).....	12000	22323	10011	1
<i>D. aloifolium</i> (<i>Aporum</i>).....	10001	12223	20000	1
<i>D. anceps</i> (<i>Aporum</i>).....	12001	12223	20000	1
<i>D. brevementum</i> (<i>Aporum</i>).....	10001	12223	20010	1
<i>D. distichum</i> (<i>Aporum</i>).....	02010	12223	20002	1
<i>D. indivisum</i> (<i>Aporum</i>).....	10011	12223	20010	1
<i>D. leonis</i> (<i>Aporum</i>).....	02110	12223	20010	1
<i>D. mannii</i> (<i>Aporum</i>).....	12001	12223	20011	1
<i>D. nathaniele</i> (<i>Aporum</i>).....	12010	12223	20010	1
<i>D. rosellum</i> (<i>Aporum</i>).....	12001	12223	20002	1

ogy is variable within each species of section *Aporum*; therefore we used it sparingly in this analysis to prevent misinterpretation of data. We did not use guard-cell pair dimensions in the cladistic analysis because they were continuous, and discrete states could not be delimited (table 4). Variability in stomatal and trichome densities, presumably a response to environmental conditions, precluded their use in our analysis. We used *Dendrobium salaccense*, a species in section *Grastidium*, as the outgroup (Maddison et al. 1984) following the analysis of Morris et al. (1996). In that work *D. salaccense* was chosen as the functional outgroup because it was hypothesized to be a paraphyletic relative to the remaining taxa in *Dendrobiinae* and because it exhibited a relatively simple vegetative structure presumed to be plesiomorphic.

We used the computer program Hennig86 (Farris 1988) to perform a branch-and-bound analysis (implicit enumeration, ie), with extended branch swapping (bb*). All characters were unordered. ACCTRAN optimization was used to situate characters that were equivocally placed from the standpoint of parsimony (Wiley et al. 1991).

Results

LEAF SURFACE

EPIDERMIS. Parenchyma cells thin-walled with anticlinal walls varying from straight-sided to curvilinear for all species. Abaxial—cells more or less isodiamet-

ric, average width: length ratio = 33 μm : 50 μm . Adaxial—cells tangentially elongate, average width: length ratio = 35 μm : 143 μm .

STOMATA. Abaxial only, paracytic in *Denrobium aloifolium*, *D. indivisum*, and *D. brevementum*; mostly paracytic with few anomocytic in all other species examined. Average stomatal lengths range from 25.9 μm to 37.8 μm ; average stomatal widths range from 20.4 μm to 31.7 μm (table 4).

LEAF SECTION

CUTICLE. Abaxial—smooth, thick in *D. leonis*, *D. nathaniele*, and *D. distichum* (average = 3.9 μm); minutely and irregularly verrucose, thick (2.7–6.7 μm) in *D. indivisum*; smooth, thin for all other species (average = 2.8 μm). Adaxial—smooth, thin (average = 0.9 μm).

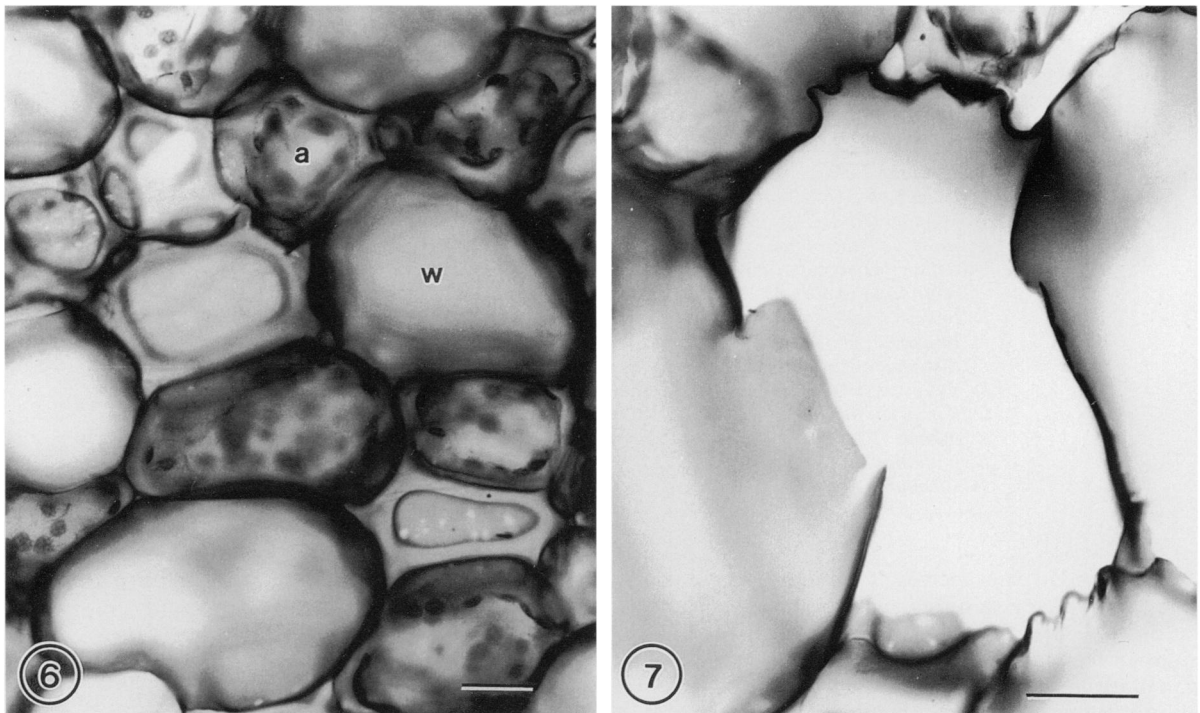
HAIRS. Uniseriate, bicellular, glandular, sunken, abaxial, and adaxial; cells surrounding base typically thin-walled, except in *D. leonis* and *D. distichum*, where both thick- and thin-walled cells occur. Hairs sometimes appear raised owing to exudate but are typically even with other epidermal cell surfaces.

EPIDERMIS. Abaxial—cells thin-walled, outer walls variably thickened, polygonal, and tangentially elongate in *D. aloifolium*, *D. mannii*, *D. indivisum*, *D. brevementum*, *D. rosellum*, and *D. anceps*; polygonal and rounded in *D. acinaciforme*, *D. nathaniele*, *D. distichum*, and *D. leonis*. Adaxial—cells thin-walled, polygonal, and tangentially elongate, except in *D. acinaciforme* and *D. indivisum*, where the cells are more or less isodiametric; those of *D. mannii* and *D. acinaciforme* enclose reniform, suberin-like bodies (fig. 3). Cell walls of *D. distichum* and *D. rosellum* are impregnated with a suberin-like substance where the outer wall is thicker than the inner (fig. 4). Suberin-like substances are absent in other species studied.

Table 4

DIMENSIONS OF GUARD-CELL PAIRS FOR SPECIES OF SECTION *APORUM*

Taxa	Range (μm)		Mean (μm)	
	Length	Width	Length	Width
<i>D. acinaciforme</i>	27–31	24–31	28	26
<i>D. aloifolium</i>	23–28	19–23	26	20
<i>D. anceps</i>	29–33	24–31	31	28
<i>D. brevementum</i>	27–29	21–29	28	25
<i>D. distichum</i>	33–43	29–34	38	32
<i>D. indivisum</i>	32–37	25–31	35	29
<i>D. leonis</i>	25–28	23–28	27	25
<i>D. mannii</i>	27–35	27–31	31	29
<i>D. nathaniele</i>	31–37	29–32	33	31
<i>D. rosellum</i>	29–35	28–36	33	32



Figs. 6, 7 Cross sections, leaves of *Dendrobium* section *Aporum*. Bar = 10 μm . Fig. 6, *D. rosellum* showing undifferentiated mesophyll composed of water-storage cells and assimilatory cells; $\times 940$. Fig. 7, *D. rosellum* mesophyll with pleated water-storage cell; $\times 1500$. a = assimilatory cell, w = water-storage cell.

STOMATA. Abaxial only: superficial, cells surrounding substomatal chamber thin-walled and chlorenchymatous, except in *D. leonis*, where both thin- and thick-walled chlorenchymatous cells occur. Outer ledges prominent, inner ledges inconspicuous.

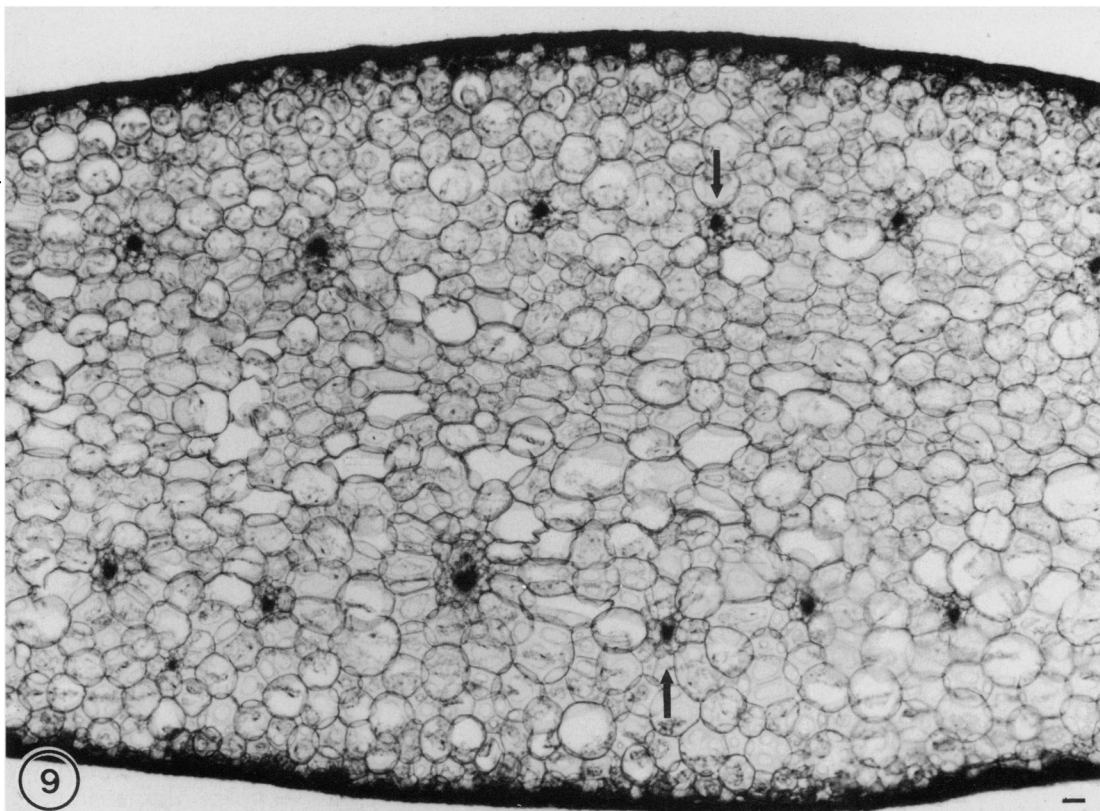
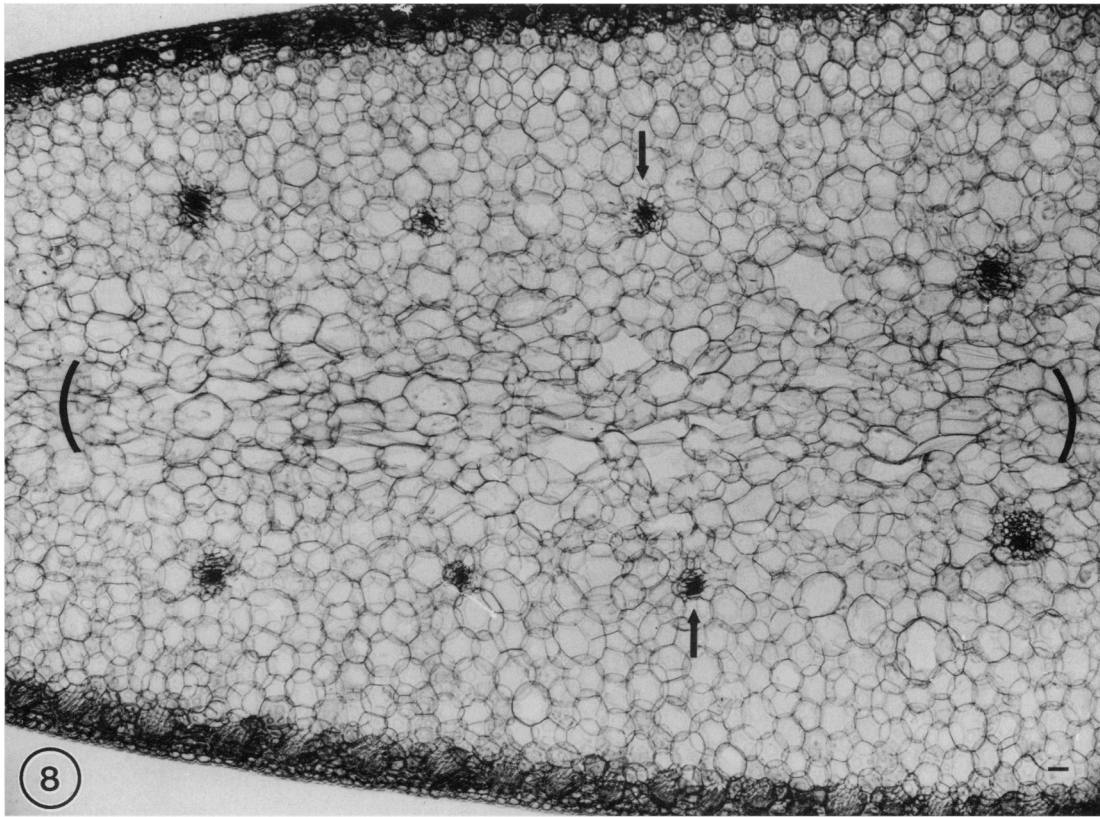
HYPODERMIS. Abaxial only, two- or three-layered, cells thin-walled, chlorenchymatous, rounded, smaller, and more densely staining than those of the mesophyll; intercellular spaces small and numerous.

FIBER BUNDLES. Abaxial only: heterogeneous bundles of both small, thick-walled fibers and large, thin-walled fibers alternate with small homogeneous bundles composed of either thick- or thin-walled fibers in all species (fig. 5). Thick- and thin-walled cells vary from rounded to polygonal in both heterogeneous and homogeneous bundles. Stegmata containing rough-surfaced, spherical silica bodies are associated with inner and outer surfaces of heterogeneous and homogeneous fiber bundles in all species.

MESOPHYLL. Undifferentiated, composed of smaller assimilatory cells and scattered, larger water-storage cells (fig. 6); assimilatory cells living, thin-walled, rounded with conspicuous primary pit fields, chlorenchymatous; water-storage cells nonliving, thin-walled, rounded with conspicuous primary pit fields. Water-storage cells with smooth or pleated (fig. 7) walls grade in size from small to large from the periphery toward the center of the leaf. In *D. acinaciforme*, *D.*

nathaniele, *D. mannii*, *D. indivisum*, *D. brevimentum*, and *D. leonis*, water-storage cells adjacent to the mid-axis or suture are noticeably elongated and cell walls are pleated (fig. 8); there is no discernible suture in *D. anceps*, *D. aloifolium*, *D. indivisum*, *D. brevimentum*, *D. distichum*, and *D. rosellum*, and all water-storage cells are rounded (fig. 9). Starch grains absent in all species studied. Intercellular spaces small and numerous. Raphides present in saccate idioblasts scattered infrequently throughout the mesophyll.

VASCULAR BUNDLES. Collateral, primary phloem abaxial, primary xylem oriented toward leaf axis. A suture separates symmetrically arranged, regularly spaced small and large vascular bundles in *D. acinaciforme*, *D. leonis*, *D. nathaniele*, *D. mannii*, *D. indivisum*, and *D. brevimentum* (fig. 8). The leaf is divided bilaterally, regardless of the presence of a suture, by one row of vascular bundles on either side of the midline in *D. brevimentum*, *D. aloifolium*, *D. anceps*, *D. distichum*, *D. indivisum*, *D. leonis*, *D. mannii*, and *D. nathaniele* (fig. 9); *D. acinaciforme* and *D. rosellum* possess two or three rows of vascular bundles. All species have a single, major vascular bundle at only one pole of the leaf section (fig. 10). Sclerenchyma predominates at phloem regions of vascular bundles; it is poorly developed at xylem regions of the larger vascular bundles and absent next to the xylem of the smaller vascular bundles. Stegmata containing rough-surfaced, spherical silica bodies are associated with phloic sclerenchyma of the larger bundles of *D. leonis*,



Figs. 8, 9 Cross sections, leaves of *Dendrobium* section *Aporum*. Bar = 10 μm . Fig. 8, *D. leonis* showing suture (parentheses) in mesophyll surrounded by symmetrically arranged vascular bundles (arrows); $\times 216$. Fig. 9, *D. anceps* showing mesophyll lacking suture and symmetrically arranged vascular bundles (arrows); $\times 216$.

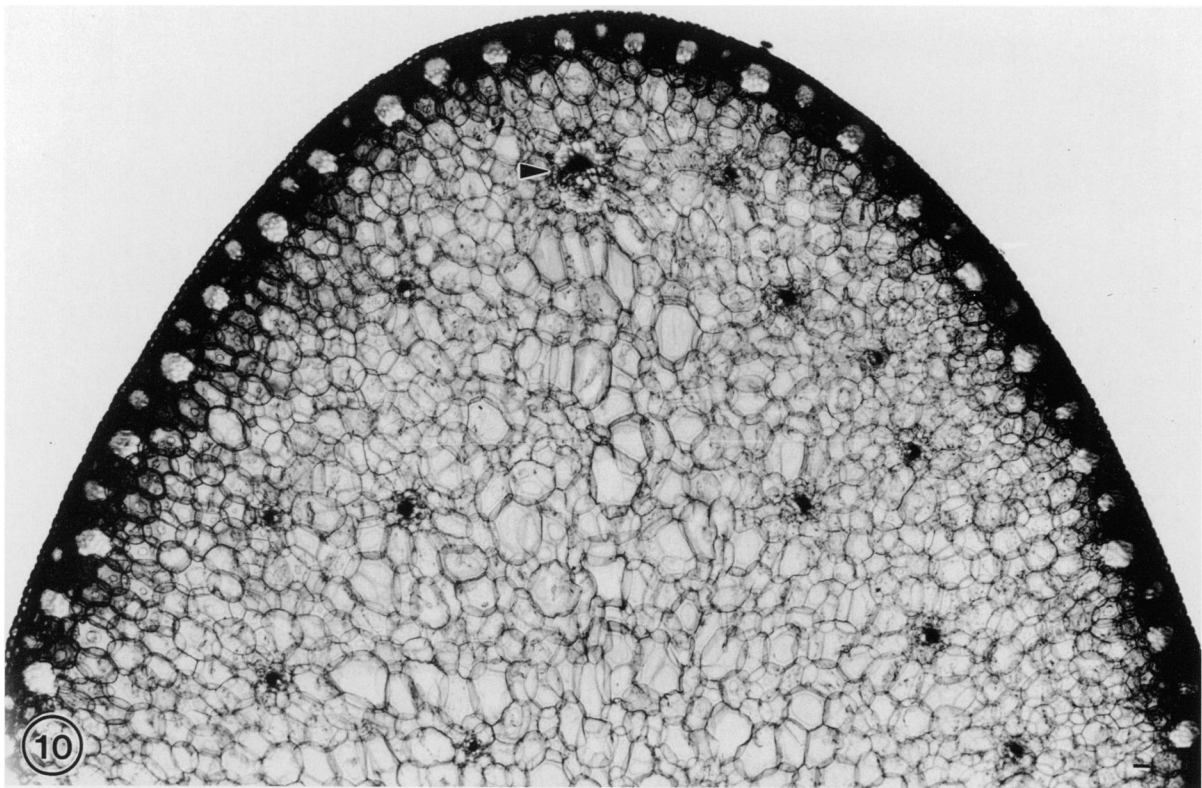


Fig. 10 Cross section, leaf of *Dendrobium* section *Aporum*. Bar = 10 μ m. *Dendrobium acinaciforme* showing major vascular bundle at one pole (arrowhead); \times 192.

D. anceps, *D. brevementum*, *D. aloifolium*, *D. distichum*, *D. indivisum*, *D. mannii*, *D. rosellum*, and *D. nathaniele*; they are absent along vascular-bundle sclerenchyma in *D. acinaciforme*. Bundle sheath uniseriate, composed of thin-walled chlorenchyma cells in all species.

Discussion

CLADISTICS

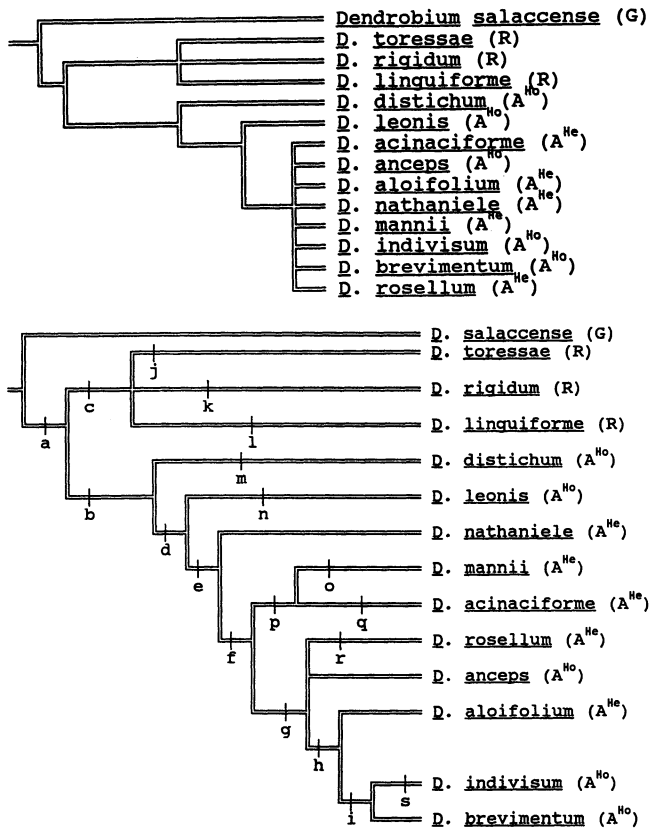
Features of leaf anatomy were used to construct a character matrix (table 3) as the basis for a cladistic analysis of species within Kränzlin's subgroups *Hemiphylla* and *Holophylla* (section *Aporum*) and between sections *Rhizobium* and *Aporum* (table 2). The branch-and-bound analysis resulted in the discovery of 68 equally parsimonious trees (length = 33, consistency index = 0.75, and retention index = 0.81). A strict consensus tree is shown in figure 11.

The strict consensus of the 68 trees showed little resolution among the species of section *Aporum*, although *Dendrobium distichum* and *D. leonis* were placed basally within section *Aporum* in all generated cladograms (fig. 11). *Dendrobium distichum* is separated from the rest of *Aporum* because of the suberin-like substance that impregnates the cell wall in this species. The remaining species formed a clade based on the shared presence of a suture embedded within the mesophyll, a character that reversed in some species. *Dendrobium leonis* was distinguished by the pres-

ence of thick-walled, chlorenchymatous cells that surround the substomatal chamber. The remaining species that were studied formed a clade on the basis of thin-walled chlorenchymatous cells that surround the hair bases. Configuration of the stomatal apparatus, abaxial cuticle thickness, abaxial epidermal cell shape, presence of a suture within the mesophyll, and form/presence of a suberin-like substance were homoplasious characters within this clade, here referred to as the *D. nathaniele*-*D. brevementum* clade. Apomorphies for the clade comprising sections *Rhizobium* and *Aporum* were the presence of pleated water-storage cells and a thick cuticle. Ordering the character states of leaf orientation would result in the unifacial leaf being an additional synapomorphy for the two clades. Characters supporting the monophyly of section *Aporum* and section *Rhizobium* are provided in figure 12 (see also "Comparative anatomy" below). One of the 68 equally parsimonious trees is presented in figure 12. Using this tree, more resolution is evident within the *D. nathaniele*-*D. brevementum* clade, but these subclades are supported by very homoplasious and/or single characters and are not supported in other equally parsimonious trees. Note that the monophyly of neither of Kränzlin's subgroups of *Aporum* is supported.

COMPARATIVE ANATOMY

This analysis, combined with the investigation of Morris et al. (1996), provides convincing evidence that



Figs. 11, 12 Cladograms of *Dendrobium* sections *Aporum* and *Rhizobium*. Fig. 11, Strict consensus of 68 trees. Fig. 12, One of 68 equally parsimonious cladograms. Synapomorphies supporting each clade include: a = 4(1), 7(2); b = 2(2), 6(2), 8(2), 9(2), 10(3), 11(2), 16(1); d = 14(1); e = 1(1); f = 4(0), 5(1); g = 14(0); h = 2(0); i = 14(1); j = 10(2), 7(1); k = 8(1); l = 12(1); m = 15(2); n = 3(1); o = 5(0); p = 15(1); q = 8(3), 11(1); r = 15(2); s = 4(1). (G) = section *Grastidium*, (R) = section *Rhizobium*, (A^{Ho}) = section *Aporum*, subgroup *Holophylla*, (A^{He}) = section *Aporum*, subgroup *Hemiphylla*. The superscripts pertain to these subgroups.

sections *Aporum* and *Rhizobium* are monophyletic sister taxa. The anatomical study of Stern et al. (1994), based on foliar anatomy of section *Rhizobium*, supports the monophyly of that group.

Synapomorphies found in this study to support the monophyly of the *Aporum* clade include the presence of: (1) fiber bundles with heterogeneous cell composition; (2) mainly paracytic stomatal apparatus; (3) hypodermis of small, thin-walled, chlorenchyma cells; (4) intermediate leaf width:length ratio = 10–38; (5) distribution of stigmata along vascular sclerenchyma and fiber bundles; (6) major vascular bundle at leaf pole; and (7) unifacial leaf lacking a lacuna or evidence of a lacuna.

We chose anatomical and morphological features of the leaf for a phylogenetic comparison between sections *Aporum* and *Rhizobium*. Some of the similarities of these hypothesized sister taxa (Morris et al. 1996) are: (1) leaves unifacial, or nearly so; (2) presence of an abaxial hypodermis; (3) water-storage cells in the mesophyll; (4) rough-surfaced, spherical silica bodies

in stigmata; and (5) vascular bundles symmetrically arranged within the mesophyll. Many members of section *Aporum* exhibit a bilaterally symmetrical leaf divided by a suture distinguished by a series of elongate water-storage cells parallel with the median longitudinal axis of the leaf section. In contrast, advanced members of section *Rhizobium* have a lacuna embedded within the mesophyll dividing the leaf bilaterally, much as the suture does in members of section *Aporum*. The lacuna represents a canal that runs through the leaf from its point of attachment to the stem. Basal members of *Rhizobium* exhibit what Stern et al. (1994) interpreted as an incipient lacuna, a groove on the upper surface of the leaf characterized by an adaxial epidermis.

Formation of the unifacial leaf and internal suture characteristic of species in section *Aporum* might have resulted evolutionarily following the steps suggested below. Ordering the character states that correspond to leaf orientation would result in a morphocline, in which the hypothetical bifacial leaf would fold inward along the midvein, bringing the adaxial surfaces together to form a nearly unifacial leaf characteristic of the hypothetical ancestor for *Rhizobium* and *Aporum*. The adaxial epidermis might have been reduced further while still maintaining an incipient lacuna as a groove on the upper surface. The incipient lacuna, characteristic of basal *Rhizobium* members, by continued infolding of the leaf margins, would result in the unifacial leaf characteristic of advanced members of *Rhizobium*, in which there is a true lacuna embedded in the mesophyll surrounded by an adaxial epidermis.

The unifacial leaf of *Aporum* may have evolved from the same generalized unifacial leaf of the hypothetical ancestor for *Rhizobium* (fig. 13), or the two different unifacial leaves characteristic of sections *Aporum* and *Rhizobium* may have evolved in parallel. It is unlikely that unifacial leaves of *Aporum* evolved via the degeneration of the lacuna seen in leaves of section *Rhizobium* because such a hypothesis would require *Rhizobium* to be paraphyletic. Paraphyly of section *Rhizobium* is not supported by this cladistic analysis or the analyses performed by Stern et al. (1994) and Morris et al. (1996). The suture present in some studied species of section *Aporum* might represent relictual evidence of the adaxial infolding in the same bifacial ancestral group hypothesized for *Rhizobium*. A major vascular bundle at one pole of the leaf section could symbolize the ancestral midvein.

Metcalf (1971) discussed a similar speculative developmental concept in leaves of Cyperaceae using certain taxa as hypothetical morphological stages. He noted that the condition in which the area of adaxial surface is reduced compared with the abaxial surface results in an “isobilateral” leaf. The “isobilateral” leaf has vascular bundles in opposite pairs. There are two “pseudo-margins,” one represented by the putative midrib at one pole, the other by two fused “true margins” at the opposite pole. The “centric” condition is an extreme development from the isobilateral config-

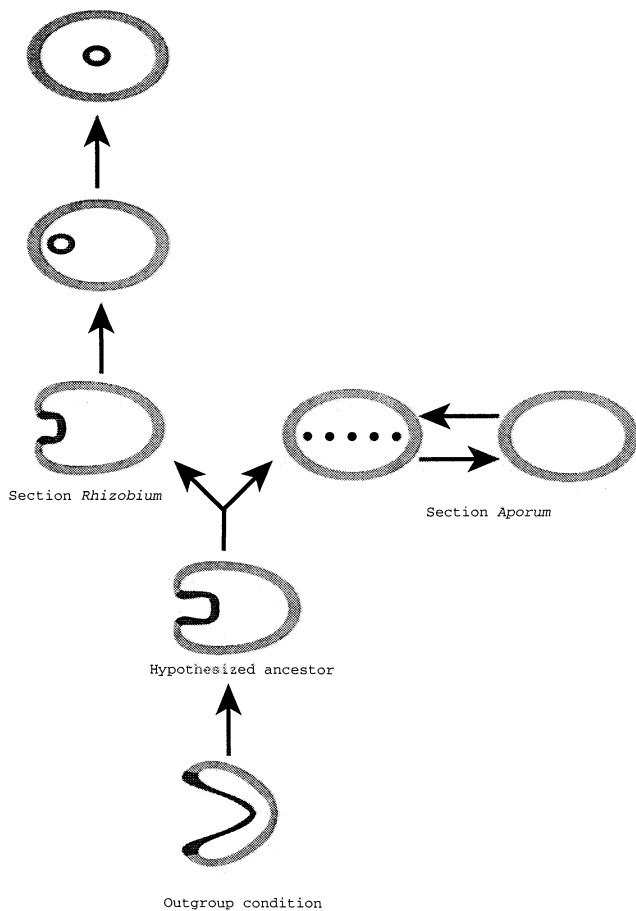


Fig. 13 Hypothesis of leaf infolding for *Dendrobium* sections *Aporum* and *Rhizobium*. Adaxial surface black, abaxial surface grey, and dotted line represents suture.

uration in which the adaxial surface is eliminated altogether (Metcalf 1979). Metcalf also speculated about the occurrence of this phenomenon within Orchidaceae. His interpretations appear analogous to our proposal for the formation of unifacial leaves in section *Aporum*.

Stern et al. (1994) discussed the formation of unifacial leaves in section *Rhizobium* and suggested that those members with an internal lacuna evolved in a manner similar to the hypothesized infolding of leaves in section *Aporum* supported here. Such a developmental series could result in a unifacial leaf and would therefore constitute a synapomorphy for sections *Rhizobium* and *Aporum*. Unifacial leaves in sections *Rhizobium* and *Aporum* are differently inserted along the stem and vary in overall form. Therefore, we did not assume, a priori, that the unifacial condition in these two groups was homologous. Nevertheless, the results of cladistic analyses do suggest that the common ancestor of plants in sections *Rhizobium* and *Aporum* had unifacial leaves.

Members of section *Rhizobium* contain symmetrically arranged foliar vascular bundles, a condition also found in section *Aporum*. The hypodermis in *Aporum*

and *Rhizobium* differs in cellular composition. In the *Aporum* group it comprises thin-walled, chlorenchymatous cells, while in section *Rhizobium* the hypodermis consists of either thick-and/or thin-walled unbanded cells or thin-walled banded and unbanded cells. Thin-walled, banded cells of the hypodermis in *Rhizobium* may serve as additional water-storage tissue, while thick-walled, unbanded cells may function in support.

Unbanded water-storage cells of the mesophyll are pleated in section *Aporum*, and in *Rhizobium*, water-storage cells typically are pleated and lack banded thickenings or, infrequently, they may have these (Stern et al. 1994). With some exceptions, stegmata are found throughout the genus *Dendrobium* (Solereder and Meyer 1930; Morris et al. 1996) in various distributional patterns. These silica-containing cells are associated with the fiber bundles and vascular-bundle sclerenchyma of leaves in section *Aporum*, but they only occur along the vascular-bundle sclerenchyma of leaves in section *Rhizobium* because fiber bundles are absent in this group. Silica-body-containing stegmata, as well as the fiber bundles and vascular-bundle sclerenchyma, may serve as additional structural reinforcement to hold the leaves erect on stems during periods of water stress in plants of sections *Rhizobium* and *Aporum*. The elongated raphide-containing cells of the mesophyll noted in section *Aporum* are of wide distribution in Orchidaceae and, as such, are of little or no systematic value.

The most obvious unifying characteristic of sections *Rhizobium* and *Aporum* is the unifacial orientation of the leaf surface, where only the abaxial epidermal surface is exposed. Unifacial leaves are not unique to these sections of *Dendrobium* but occur in other genera of orchids, such as *Epidendrum* and *Maxillaria* (Solereder and Meyer 1930). The unifacial condition might be considered ecologically analogous to amphistomaty, in which all leaf surfaces are covered by stomata. Mott et al. (1982) have suggested that amphistomaty might be correlated with thick, sun leaves as an adaptation to high leaf conductance and therefore high photosynthetic potential. This hypothesis might also be applicable to the thick, unifacial leaves of sections *Aporum* and *Rhizobium*. Amphistomaty and unifacial leaves are found in other groups of plants, such as succulent CAM (crassulacean acid metabolism) plants (Kluge and Ting 1978) and are correlated with high light levels (Mott et al. 1982).

Salisbury (1927) suggested that amphistomaty might be an adaptation to dry habitats, as the coriaceous condition of the leaves he observed seemed to indicate. Coriaceousness, the presence of fiber bundles, and wall thickenings of cells surrounding the substomatal chamber in *D. leonis* and hair bases in *D. leonis* and *D. distichum*, as well as the suberin-like substance that occurs in adaxial epidermal cells of some members of *Aporum*, could also be adaptations to xeric conditions. The walls of adaxial epidermal cells in *D. distichum* and *D. rosellum* are impregnated with this

suberin-like substance. Esau (1965) and Fahn (1990) noted that suberin may occur in the cell wall matrix, and Metcalfe (1960) described suberin deposits in cell walls of leaves in Gramineae. Exudate produced by the glandular hairs in section *Aporum* could be an adaptation to a xeric habitat, as hypothesized by Ehleringer (1984), but it also might serve as a deterrent to herbivory, as pointed out by Kelsey et al. (1984). Exudations may be composed of “oils” (Solereder and Meyer 1930) or substances that might prevent herbivory, such as terpenes and phenolics (Kelsey et al. 1984). Sunken, glandular hairs occur throughout the genus *Dendrobium* as well as in other genera of Orchidaceae, such as *Bulbophyllum*, *Epidendrum*, and *Maxillaria* (Solereder and Meyer 1930).

Although all members of section *Aporum* are epiphytic and therefore subjected to water stress (Carlquist [1975] disputes this), the habitats of plants mentioned by Seidenfaden and Wood (1992) indicate wet, shaded conditions. In this humid environment, the abaxial stomata of unifacial leaves could enhance gas exchange by providing a greater surface for diffusion of carbon dioxide into the leaf and transpiration of excess water vapor from the leaf in a moisture-laden atmosphere than would occur in bifacial leaves. Still, the ecological significance of the hypothesized xeromorphic adaptations in leaves of section *Aporum* lacks an unequivocally supportable explanation.

Stern et al. (1994) also questioned the purported significance of the xeromorphic features they observed in leaves of section *Rhizobium*, vis-à-vis the environmental conditions under which many of these species live. A plausible hypothesis accounting for these apparent discrepancies would be that the common ancestor of the *Rhizobium* and *Aporum* groups developed a unifacial leaf through stages similar to the ones hypothesized by Metcalfe (1971) in response to an ancestral, xerophytic environment. Because the xeromorphic foliar conditions were not disadvantageous to present-day plants in sections *Rhizobium* and *Aporum*, they have been retained. This scenario might also explain why the basal members of *Aporum*, i.e., *D. leonis* and *D. distichum*, exhibit several unique xeromorphic adaptations.

Conclusions

Although our results from foliar anatomy illustrate that sections *Rhizobium* and *Aporum* are monophyletic sister taxa, supporting the analysis of Morris et al. (1996), there was very little resolution within section *Aporum* (fig. 11). The only consistent resolution among the species studied in the *Aporum* clade is the basal placement of *Dendrobium leonis* and *D. distichum*. The absence of resolution probably results from the relatively small number of characters used, as well as the homoplasious nature of some of these characters. Our results indicate that neither of Kränzlin's groups, i.e., *Holophylla* and *Hemiphylla*, can be considered monophyletic. Some of the members within *Holophylla* are nested within the unresolved clade consisting primarily of *Heterophylla* species (figs. 11, 12). Further studies involving floral morphology, as well as additional research on vegetative anatomy and morphology incorporating additional taxa, and molecular investigations could possibly provide data for further resolution of the *Aporum* clade.

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