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WILLIAM LOUIS STERN & BARBARA S. CARLSWARD

Abstract

Laeliinae are one of the most prominent orchid subtribes, with c. 40 genera and nearly 1500 species, and contain a disparate group of taxa with widely varying morphological features. There does not appear to be a complex of characters to which one can refer in order to delineate the subtribe as a whole. Thus, it was thought that vegetative anatomy might provide clues to the monophyly of the group. The microscopic structure of the leaves, stems and roots of representatives of most of the genera was studied. It was concluded that the anatomy lacks overall uniformity and that vegetative characters alone are insufficient to assess the relationships amongst the genera. The only nearly consistent anatomical feature was the abaxial row of fibre bundles in the leaves. Thus, anatomically, as well as morphologically, Laeliinae are a mixed bag.

INTRODUCTION

What complex of features serves to characterize Laeliinae Bentham? As is usual among the orchids, floral morphology contributes the 'glue' to bring the various taxa into conformity, producing uniform clusters of plants members of which, purportedly, are related to one another. In an attempt to seek the 'glue' to understand the members of Laeliinae, the publications of Dressler (1993) and Pridgeon et al. (2005) were consulted, only to discover a patchiness of features under the heading of the subtribe. For example, these publications cite: 'Habit epiphytic or occasionally terrestrial; stems slender or pseudobulbous; leaves distichous or terminal; flowers tiny to large, resupinate or not; labellum free or attached variously to the column; column short or elongate, footless or with a column foot; anther terminal and incumbent or erect; pollinia laterally flattened or ovoid, two, four, six, eight or 12; chromosome numbers 24, 38, 40, 42 or 56, with 40 being typical.' Where is the conformity? Even when using molecular studies, Pridgeon et al. (2005) concluded that 'relationships among the main groups in Laeliinae are still unresolved'. Therefore, it was surmised that, perhaps, the 'glue' cementing members of Laeliinae could be found in the anatomy. An attempt has been made in this study to test the validity of this hypothesis.

The members of Laeliinae are tropical American plants and range throughout the warmer regions of this area, with Epidendrum magnoliae Muhl. (= E. conopseum R.Br.) extending as far north as North Carolina along the eastern coast of the USA. The larger flowered members, e.g. Cattleya Lindl., Laelia Lindl., Broughtonia R.Br. and some species of Encyclia Hook. and Epidendrum L., are highly prized and much

desired by the public and hobbyists owing to their beauty. Thus, it might be expected that they and other members of the subtribe would have been the subject of widespread anatomical research. However, except for hybridization studies, little research has been performed and practically no comprehensive anatomical studies have been completed, with the single exception of a doctoral dissertation on leaves by Baker (1972). This extensive, mature study treated the morphology and anatomy of the leaves in Laeliinae, and included an analysis of the relationships based on the accumulated data and interpretations. Baker considered 79 taxa in 36 genera, arranged alphabetically.

In Baker's observations (1972), trichomes were frequent in Laeliinae and all hairs were apparently three-celled, glandular and with sunken bases. A variably thick cuticle covered the entire leaf, and the surface texture ranged from smooth to granular to variously ornamented. Epidermal cells were irregularly polygonal in surface view, commonly isodiametric to longitudinally elongated. Cell walls in most genera were evenly thickened with occasional papillate development of the outer walls. Stomata were normally abaxial, but the amphistomatal condition occurred in several species. The stomatal apparatus was circular to elliptic, and the stomatal aperture paralleled the longitudinal axis of the leaf. Substomatal chambers were variable, seldom more than a single cell layer deep. Lengths of guard cell pairs ranged from 17 to 20 μm in Dinema polybulbon (Sw.) Lindl. [= Encyclia poybulbon (Sw.) Dressler] to 60 to 63 μm in Epidendrum parkinsonianum Hook. Baker stated that clearly differentiated subsidiary cells were absent and that the stomatal apparatus was anomocytic. A hypodermis was present in three dispositions, the most prominent of which was the cluster of inflated, elongated, anticlinal water-storage cells over the midrib, associated with unfolding tissue in conduplicate leaves. The hypodermis may have been reinforced by longitudinal files of fibre bundles.

Baker (1972) also noted that the mesophyll was composed of mixed large to small, rounded, irregularly shaped cells and often a layer of anticlinally extended cells resembling the upright cells of a palisade mesophyll. There was often a distinct gradient in cell size, number and density from ad- to abaxial, with larger, fewer and wider intercellular spaces adaxially. Fibre bundles were variously disposed in the mesophyll. Vascular bundles were organized in a single transverse row or arc. Xylem and phloem were associated with a sclerotic sheath or cap in the larger bundles, and the whole vascular bundle was encased in a circle of thin-walled, usually assimilative tissue, the bundle sheath. Raphides in idioblasts were widespread in the mesophyll. Silica bodies were conical and occurred in stegmata, specialized cells that line the fibre bundles and vascular sclerenchyma. Starch grains were sometimes present in assimilatory cells.

Reports on leaves by Chatin (1857), Krüger (1883), Metzler (1924), Duruz (1960) and Oliveira & Sajo (1999), who singled out members of Laeliinae for discussion, did not add substantially to the information provided by Baker (1972). However, although Baker cited the presence of trichomes in most Laeliinae studied, Möbius

(1887) reported a lack of hairs. Furthermore, Möbius stated that, among all the studied Laeliinae, Sophronitis perrinii (Lindl.) Van den Berg & M.W.Chase [treated as Laelia] was an exception because it had trichomes on the upper side of the leaf, whereas the leaves of other taxa were glabrous.

Williams (1979) defined subsidiary cells as extra cells found next to the guard cells, and cited Esau (1965) to indicate that these cells were morphologically distinct from other epidermal cells. All stomata were abaxial in the Laeliinae studied by Williams. All had two subsidiary cells, except for Prosthechea pygmaea (Hook.) W.E.Higgins [= Encyclia pygmaea (Hook.) Dressler], Helleriella nicaraguensis A.D.Hawkes, Homalopetalum pumilio (Rchb.f.) Schltr., Jacquiniella pedunculata Dressler and Ponera glomerata Correll, which had four subsidiary cells, two lateral and two polar, resulting from an extra set of divisions.

Weltz (1897) detailed the stem anatomy in eight genera of Laeliinae. He noted a well-developed hypodermis in the outermost and sometimes the next layer of the stem in species of six genera, and reported long hypodermal fibres in Cattleya forbesii Lindl. and C. loddigesii Lindl.; stems of C. labiata Lindl. lacked these fibres. The hypodermis of the Epidendrum spp. was homogeneous, consisting of similar appearing cells; the hypodermis of Isochilus R.Br. and Laelia consisted of long thickened cells, amongst which were intercellular spaces. An inner sclerenchymatous ring of six to eight layers, with vascular bundles inserted among the layers, occurred only in Isochilus linearis (Ruiz & Pav.) R.Br. in W.T.Aiton. Ground tissue showed little variation and usually consisted of polyhedral cells lacking intercellular spaces and inner round cells with notable interstices. Prosthechea citrina (La Llave & Lex.) W.E.Higgins [as Cattleya citrina (La Llave & Lex.) Lindl.] showed these, and differed from the typical condition solely in the presence of large air spaces in the tissue. With the exception of Isochilus, in which the vascular bundles were included in the sclerenchymatous ring noted above, innermost vascular bundles were clustered, whereas outer bundles were scattered throughout the ground tissue. Vascular bundles were collateral and species differed only in the presence or absence of a sclerenchyma sheath and bridge. All genera had silica bodies associated with vascular bundle sclerenchyma, sometimes all along the sclerenchyma surrounding the bundle, sometimes lacking along the xylem portion of the sheath and sometimes lacking entirely in the smaller bundles. Raphides of calcium oxalate occurred in most species, and chloroplasts were present in cells surrounding the vascular bundles.

Moreau's (1913) study of pseudobulbs contained little of systematic value, being a general morphological and anatomical treatment using, as examples, features drawn from plants in several subtribes, including Laeliinae. Leitgeb (1864) elaborated the structure of the aerial roots of orchids, but did not organize his study systematically. Meinecke (1894) cited an almost complete lack of tilosomes in Laeliinae, thickened endodermal cells and a parenchymatous pith.

Pridgeon, Stern & Benzing (1983) coined the term 'tilosome' for root structures formerly known by a variety of names (for example, 'Körperchen', 'kugelförmige Körper', 'Stabkörper', 'Faserkörper'), and organized their findings systematically. Among Laeliinae, tilosomes were clearly present in Prosthechea brassavolae (Rchb.f.) W.E.Higgins [as Encyclia brassavolae (Rchb.f.) Dressler], P. chimborazoensis (Schltr.) W.E.Higgins [as Encyclia chimborazoensis (Schltr.) Dressler], P. cochleata (L.) W.E.Higgins [as Encyclia cochleata (L.) Dressler), P. radiata (Lindl.) W.E.Higgins [as Encyclia radiata (Lindl.) Dressler], P. vespa (Vell.) W.E.Higgins [as Encyclia vespa (Vell.) Dressler] and Epidendrum coriifolium Lindl. Tilosomes were absent in other species studied. They speculated that the morphology of tilosomes might serve to characterize specific genera or subtribes.

Porembski & Barthlott (1988) characterized the velamen of Laeliinae as belonging to the 'Epidendrum type', featuring epivelamen cells smaller than those of the endovelamen. Endovelamen cells were extended radially (i.e. anticlinally). Large pores sometimes occupied portions of the cell wall, and tilosomes ('Stabkörper') were occasionally present. Exodermal cell walls were slightly thickened and the cortex sometimes contained 'tracheoidal idioblasts' (Stern & Judd, 2001).

Prychid, Rudall & Gregory (2004) reported silica bodies in the stegmata of leaves in several genera of Laeliinae, and noted the unusual presence of these bodies in root tissues of Cymbidium Sw., Maxillaria Ruiz & Pav. and Lycastinae Schltr.

The details in the above-mentioned studies differ, in some cases, from those developed in the current study, issues that will be addressed below. In some instances, anatomical studies have proven to be productive to support relationships (Stern et al., 1993; Stern & Judd, 2001; Stern & Carlsward, 2006) and, in others, to contradict proposed relationships (Stern et al., 1993). In other instances, anatomy has been shown to be unreliable in sorting out relationships (Stern & Morris, 1992; Stern & Whitten, 1999).

MATERIAL AND METHODS

Dressler (1993) listed 43 genera, excluding Meiracyllium Rchb.f. This genus was included in Laeliinae by Pridgeon et al. (2005) on the basis of phylogenetic studies and was one of a number of differences between their circumscription of the subtribe and that of Dressler. Research material was obtained for 32 of the genera in Dressler's circumscription and for Meiracyllium. Binomials and authorities for these species, abbreviated according to Brummitt & Powell (1992), and the parts available for study appear in Table 1. Many of the specimens were from nursery plants and other horticultural sources, and are not associated with standard herbarium vouchers. However, a complete set of preserved plant parts is stored in the spirit collection of the Selby Botanical Garden (SEL).

Taxon	Parts available
Laeliinae *sensu* Dressler (1993) and *Meiracyllium*	
Barkeria scandens (Lex.) Dressler & Halbinger	L, R
Brassavola cucullata (L.) R.Br.	L, R
Broughtonia negrilensis Fowlie	L, R, S
Cattleya forbesii Lindl.	L, R, S
Cattleya intermedia Graham ex Hook.	L, R, S
Cattleya skinneri Bateman[1]	L, R
Caularthron bicornutum (Hook.) Raf.	L, R
Constantia rupestris Barb. Rodr.	L
Dimerandra emarginata (G.Mey.) Hoehne	L, R, S
Encyclia kienastii (Rchb.f.) Dressler & G.E.Pollard	L, R
Epidendrum anceps Jacq.	L, R, S
Epidendrum nocturnum Jacq.	L, R, S
Helleriella guerrerensis Dressler & Hágsater[2]	L, R
Homalopetalum pumilio (Rchb.f.) Schltr.	L, S
Isochilus linearis (Jacq.) R.Br.[2]	L, R, S
Jacquiniella equitantifolia (Ames) Senghas	L, S

Laelia anceps Lindl.	L, R
Laelia lyonsii (Lindl.) L.O.Williams	L, R
Leptotes tenuis Rchb.f.	L, R, S
Meiracyllium trinasutum Rchb.f.	L, R
Nageliella purpurea (Lindl.) L.O.Williams[3]	L, R
Neocogniauxia monophylla (Griseb.) Schltr.[4]	L, R, S
Nidema boothii (Lindl.) Schltr.	L, R, S
Orleanesia sp.	L, R, S
Ponera juncifolia Lindl.[2]	L, R
Prosthechea boothiana (Lindl.) W.E.Higgins	L, R, S
Prosthechea radiata (Lindl.) W.E.Higgins	L, R, S
Pseudolaelia sp.	L, R, S
Psychilis macconnelliae Sauleda	L, R
Quisqueya ekmanii Dod	L, R
Rhyncholaelia digbyana (Lindl.) Schltr.	L, R
Scaphyglottis coriacea (L.O.Williams) Dressler	L, R
Scaphyglottis imbricata (Lindl.) Dressler	L
Scaphyglottis prolifera (R.Br.) Cogn.	L

Scaphyglottis reflexa Lindl.	L, R, S
Sophronitis cf. *cernua* Lindl.[5]	R
Sophronitis sincorana (Schltr.) Van den Berg & M.W.Chase[6]	L, R
Tetramicra canaliculata (Aubl.) Urb.	L, S
Arpophyllinae[7]	
Arpophyllum giganteum Hartw. ex Lindl.	L, R, S

1 = *Guarianthe skinneri* (Bateman) Dressler & W.E.Higgins
2 Placed in subtribe Ponerinae by Pridgeon *et al.* (2005)
3 = *Domingoa purpurea* (Lindl.) Van den Berg & Soto Arenas
4 Included in subtribe Pleurothallidinae by Pridgeon *et al.* (2005)
5 = *Cattleya* cf. *cernua* (Lindl.) Van den Berg
6 = *Cattleya sincorana* (Schltr.) Van den Berg
7 Included in subtribe Laeliinae by Pridgeon *et al.* (2005)

Table 1. Species of Laeliinae and Arpophyllinae (functional outgroup) studied (L, leaf; R, root; S, stem)

Methods and descriptive conventions follow those elaborated in recently published papers (Stern & Judd, 2001; Yukawa & Stern, 2002; Stern, Judd & Carlsward, 2004), and similar procedures were used here. The terms 'periclinally oriented' and 'anticlinally oriented' are reduced to 'periclinal' and 'anticlinal,' respectively. The terms small, medium and large, with respect to the sizes of substomatal chambers, refer to their dimensions compared with adjacent mesophyll cells (small, volume of one or two mesophyll cells; large, volume of four or five mesophyll cells). With reference to radiating rows of protoxylem and protophloem in the vascular cylinder of roots, these are referred to as 'poles.' In most cases, only a single species of a genus formed the basis of our observations. Thus, only the generic name is used in the text. Where more than one species of a genus was studied, the complete binomial is stated, except for Meiracyllium, in which both species are virtually anatomically identical.

Plant parts were preserved in FAA (70% ethanol–glacial acetic acid–commercial formalin, 9:0.5:0.5) and stored in 70% ethanol. Transverse and longitudinal sections of leaves and transverse sections of roots and stems were cut unembedded as thinly as possible with a Reichert sliding microtome, stained in Heidenhain's iron–alum–haematoxylin and counter-stained with safranin. Tilosomes (Pridgeon et al., 1983) occurred in the roots of some species but, without the scanning electron

microscope, their minute structure could not be characterized. Cutler's (1978) method was followed to prepare leaf scrapings, and the resulting pieces were stained with safranin. Sections of tissue and scrapings were mounted on glass slides with Canada balsam. Observations were made using a Nikon Optiphot microscope, and photographs were prepared with an Olympus BH-2 epifluorescent microscope and a QImaging Retiga 2000R digital camera. Measurements of the lengths and widths of ten guard cell pairs appear in Table 2. The C-shaped guard cells of Sophronitis sincorana (Schltr.) Van den Berg & M.W.Chase did not lend themselves to measurements comparable with those of other taxa. Although the idioblasts containing raphide packets remained intact throughout processing, when ferric ammonium sulphate was used as a mordant, raphides were dissolved. However, raphides could be seen in stained leaf scrapings when no mordant was used.

Taxon	Average		Range	
	Length	Width	Length	Width
Laeliinae				
Barkeria (ad)	52	36	50–55	30–42
Barkeria (ab)	48	31	42–52	50–55
Broughtonia (ad)	43	45	40–41	32–55
Broughtonia (ab)	42	43	37–50	37–57
Cattleya forbesii	28	30	30–35	25–40
Cattleya intermedia	30	32	27–35	27–40
Cattleya skinneri	40	35	35–42	30–42
Caularthron	48	40	40–50	30–42
Dimerandra	26	25	22–27	25–27

Epidendrum anceps	29	23	22–32	15–42
Epidendrum nocturnum	35	38	30–37	35–42
Helleriella	54	53	50–60	50–55
Laelia anceps	54	52	47–62	40–62
Laelia lyonsii	51	51	50–62	42–57
Meiracyllium	34	30	27–37	22–40
Neocogniauxia	45	38	40–50	32–42
Nidema	30	31	25–40	25–37
Orleanesia	50	49	40–57	40–55
Ponera	43	37	40–47	30–45
Prosthechea boothiana	32	29	30–35	25–35
Prosthechea radiata	40	27	37–42	27–35
Pseudolaelia (ad)	47	41	42–50	32–47
Pseudolaelia (ab)	44	44	40–47	42–50
Psychilis	45	38	37–50	35–42
Quisqueya	48	41	45–52	37–47
Rhyncholaelia	61	64	52–67	60–70
Scaphyglottis coriacea	34	29	30–37	27–32

Scaphyglottis imbricata	33	33	30–37	30–37
Scaphyglottis prolifera	39	34	35–40	32–37
Arpophyllinae				
Arpophyllum giganteum	47	48	40–52	45–60

Table 2. Stomatal dimensions (μm)

Eleven features of vegetative anatomy were used to construct a character matrix for the cladistic analysis of 37 species in Laeliinae (Tables 3, 4). All characters used were readily divisible into discrete states, and multistate characters were considered to be unordered. Following the molecular analyses of van den Berg et al. (2005), Arpophyllinae Dressler (Arpophyllum giganteum Hartw. ex Lindl.) was used for outgroup comparison (although this subtribe was included in Laeliinae by Pridgeon et al., 2005). Maximum parsimony analyses were performed using the computer program PAUP* 4.0b10 (Swofford, 1999). Heuristic searches were performed with 1000 random addition sequence replicates, saving ten trees per replicate. For branch swapping, the tree bisection and reconnection (TBR) algorithm was used, saving multiple trees (MULTREES on). Bootstrap analyses utilized 1000 replicates, with ten random addition replicates (TBR swapping) per bootstrap replicate.

Number	Anatomical character
1	Foliar stomatal distribution: abaxial (0), ad- and abaxial (1)
2	Guard cell shape: reniform (0), C-shaped (1)
3	Foliar fibre bundle distribution: ad- and abaxial (0), adaxial + abaxial + mesophyll (1), abaxial + mesophyll (2), abaxial (3), absent (4)
4	Foliar hypodermis distribution: ad- and abaxial (0), adaxial (1), abaxial (2), absent (3)
5	Mesophyll organization: heterogeneous (0), homogeneous (1)
6	Foliar stegmata distribution: fibre bundles (0), vascular bundles (1), fibre bundles and vascular bundles (2), absent (3)
7	Cauline stegmata distribution: absent (0), present (1)
8	Pseudobulbs: absent (0), present (1)
9	Tilosomes: present (0), absent (1)
10	Cortical cell wall banding: present (0), absent (1)
11	Exodermal cell wall thickenings: ⌒-thickened (0), ○-thickened (1), thin (2)

Table 3. Anatomical characters used in the cladistic analysis of Laeliinae. Plesiomorphic states are listed first (0), followed by unordered apomorphic states (1, 2, 3)

Taxon	Character										
	1	2	3	4	5	6	7	8	9	10	11
Barkeria scandens	0	0	3	3	1	2	1	0	1	1	2
Brassavola cucullata	1	0	3	0	0	2	?	0	1	1	1
Broughtonia negrilensis	0	0	1	2	1	0	0	1	1	1	2
Cattleya forbesii	1	0	3	0	0	2	1	1	1	1	0
Cattleya intermedia	1	0	3	0	0	2	1	1	1	1	0
Cattleya skinneri	1	0	2	0	0	2	?	1	1	0	2
Caularthron bicornutum	1	0	0	3	1	2	?	1	1	1	2
Constantia rupestris	1	0	3	3	1	2	?	1	?	?	?
Dimerandra emarginata	1	0	0	3	0	0	1	0	1	0	1
Encyclia kienastii	1	0	4	3	1	1	?	1	1	1	1
Epidendrum anceps	1	0	3	3	1	2	1	0	1	1	0
Epidendrum nocturnum	1	0	0	3	1	2	1	0	1	1	0
Helleriella guerrerensis	1	0	4	1	1	1	?	0	1	1	0
Homalopetalum pumilio	1	0	4	3	1	3	0	0	?	?	?
Isochilus linearis	1	0	4	?	1	1	?	0	1	1	2

Jacquiniella equitantifolia	1	0	3	3	1	2	0	0	?	?	?
Laelia anceps	1	0	1	0	0	2	?	1	0	0	2
Laelia lyonsii	?	?	?	?	?	?	?	1	0	0	1
Leptotes tenuis	1	0	3	0	1	2	1	0	1	1	0
Meiracyllium trinasutum	0	0	4	0	1	1	?	0	1	0	0
Nageliella purpurea	0	0	4	3	1	1	?	1	1	1	2
Neocogniauxia monophylla	1	0	4	0	0	3	0	0	1	1	1
Nidema boothii	1	0	0	3	1	2	1	1	1	1	1
Orleanesia sp.	1	0	0	3	1	2	1	0	1	1	0
Ponera juncifolia	1	0	4	1	0	1	?	0	1	1	2
Prosthechea boothiana	1	0	0	3	1	2	1	1	1	1	1
Prosthechea radiata	1	0	3	3	1	2	1	1	1	1	2
Pseudolaelia sp.	0	0	3	3	1	2	0	1	1	1	2
Psychilis macconnelliae	1	0	1	3	1	0	?	1	1	0	1
Quisqueya ekmanii	1	0	1	3	1	0	?	0	0	1	1
Rhyncholaelia digbyana	1	0	3	0	0	2	?	1	1	0	1
Scaphyglottis coriacea	1	0	2	1	0	0	?	0	1	1	1
Scaphyglottis imbricata	1	0	3	0	0	1	?	0	?	?	?

Scaphyglottis prolifera	1	0	1	0	1	2	?	1	?	?	?
Scaphyglottis reflexa	1	0	0	0	0	2	1	0	1	0	1
Sophronitis cf. cernua	?	?	?	?	?	?	?	1	1	1	1
Sophronitis sincorana	1	1	1	0	1	2	?	1	0	1	1
Tetramicra canaliculata	1	0	3	3	0	2	0	0	?	?	?
Arpophyllum giganteum	0	0	0	0	0	0	0	0	0	0	0

Table 4. Character states for taxa used in the cladistic analysis of Laeliinae. Arpophyllinae (*Arpophyllum giganteum*) was used as an outgroup for polarization

ANATOMY

Laeliinae

Leaf

Surface: Hairs absent throughout, except glandular, sunken, extremely rare on both surfaces in Meiracyllium. Stomata abaxial, except adaxial and abaxial in Barkeria Knowles & Westc., Broughtonia, Meiracyllium, Nageliella L.O.Williams and Pseudolaelia Porto & Brade. Isobilateral leaves, where, in transverse section, the adaxial surface is reduced to a more or less wide sulcus and the abaxial surface is rounded, occur in Brassavola R.Br., Encyclia, Jacquiniella Schltr., Leptotes Lindl., Scaphyglottis reflexa Lindl. and Tetramicra Lindl. The exposed portion of the leaf is abaxial and stomata surround the surface. The stomatal apparatus is fundamentally tetracytic (Fig. 1), but additional cell divisions often occur in the lateral and sometimes polar subsidiary cells in several species, occasionally giving rise to a circle of subsidiary cells as in Broughtonia and Neocogniauxia Schltr. Varied stomatal configurations appear frequently in Constantia Barb. Rodr., owing to multiple divisions of subsidiary cells. In Laelia anceps Lindl. and Meiracyllium, the stomatal apparatus is mostly anomocytic; in Sophronitis sincorana, Nidema Britton & Millsp. and Orleanesia Barb. Rodr., both tetracytic and anomocytic conditions occur. Many divisions of subsidiary cells give rise to bizarre arrangements in Laelia lyonsii (Lindl.) L.O.Williams and Quisqueya Dod. Guard cells are typically reniform and stomata parallel the long axis of the guard cell pair, but, in Sophronitis sincorana, guard cells are C-shaped and guard cell arms extend to form the coves of the two facing 'C's. Average lengths of guard cell pairs (Table 4) range from 26 μm in Dimerandra Schltr. to 61 μm in Rhyncholaelia Schltr., and widths from 23 μm in

Epidendrum anceps to 64 μm in Rhyncholaelia. Epidermal cells are polygonal on both surfaces and walls are straight-sided or curvilinear; corners are rounded or sharply angled.
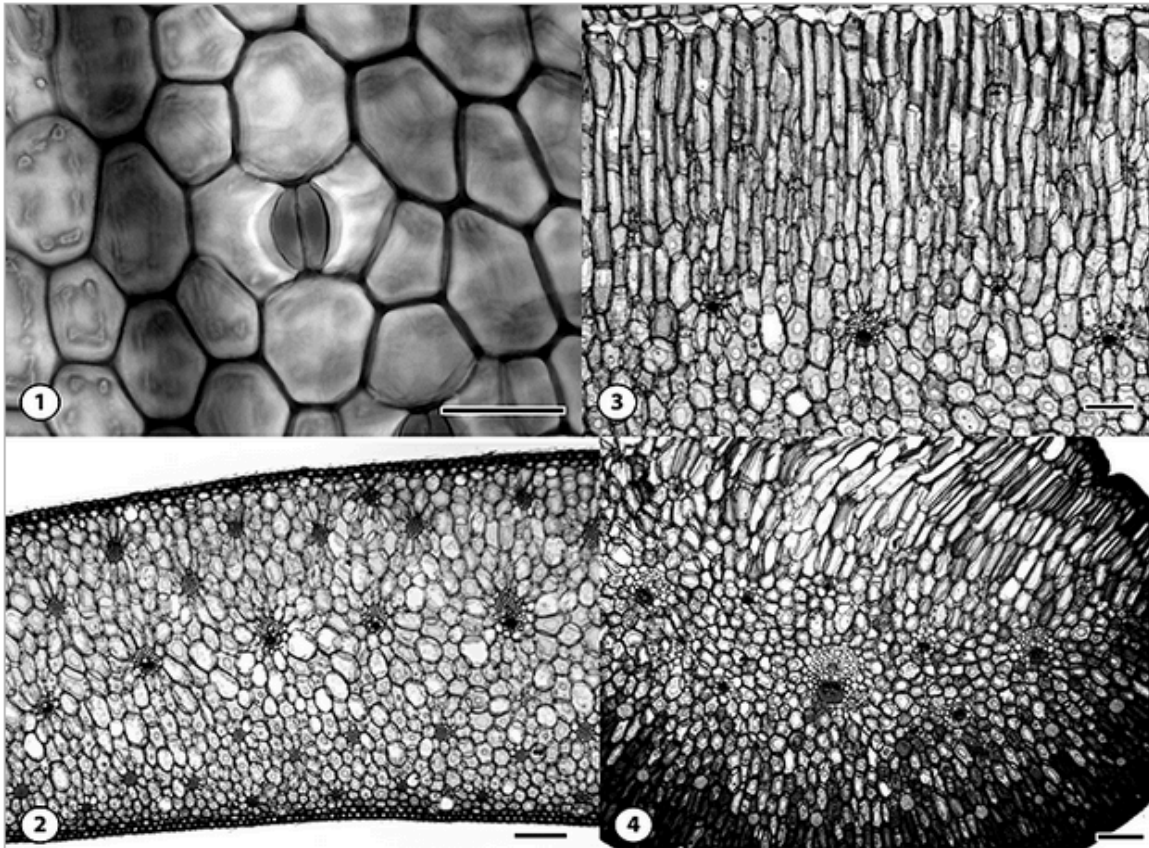

Figure 1–4. Leaf transverse section. Fig. 1. Caularthron bicornutum leaf scraping, abaxial surface, showing tetracytic stomatal apparatus. Scale bar, 50 μm. Fig. 2. Broughtonia negrilensis with several adaxial rows of fibre bundles and a single abaxial row. Scale bar, 200 μm. Fig. 3. Cattleya skinneri with heterogeneous mesophyll and vascular bundles showing robust phloem sclerenchyma and weaker xylem sclerenchyma. Scale bar, 200 μm. Fig. 4. Brassavola cucullata isobilateral leaf with encircling upright cells. Scale bar, 200 μm.

Section: Cuticle mostly between 2.5 and 5.0 μm thick, but up to 50 or 60 μm thick in Laelia lyonsii, Nageliella and Rhyncholaelia; smooth in most taxa, but ridged perpendicular to the axis of the cell in Barkeria. Ridges in Barkeria contain minute, empty vesicles. Cuticle is rough in Broughtonia, Cattleya forbesii, C. intermedia Graham, C. skinneri Bateman, Leptotes, Neocogniauxia, Psychilis Raf., Rhyncholaelia, Scaphyglottis coriacea (L.O.Williams) Dressler and S. reflexa; rough-papillose in Isochilus; and mammillate in Nageliella. Epidermal cells variable, periclinal, square, isodiametric, usually regular and consistently shaped within taxa, but irregularly formed in Encyclia, Homalopetalum, Ponera Lindl. and Scaphyglottis reflexa. Cell surfaces are finely grooved perpendicular to the long axis in Epidendrum anceps

and E. nocturnum Jacq.; notches occur between the upper surfaces of adaxial cells in E. anceps; adaxial cells are regularly larger than abaxial cells; in Pseudolaelia, epidermal cells on both surfaces appear to be similarly sized. Stomata usually superficial; slightly sunken in Brassavola, Caularthron, Constantia, E. nocturnum, Helleriella A.D.Hawkes, Jacquiniella, Rhyncholaelia, Scaphyglottis coriacea, S. imbricata (Lindl.) Dressler, S. prolifera and Sophronitis sincorana; sunken in Laelia anceps, Leptotes and Scaphyglottis reflexa. Substomatal chambers usually tiny; small to moderate in Cattleya skinneri, Constantia, Homalopetalum, Ponera, Prosthechea boothiana (Lindl) W.E.Higgins, Quisqueya, Scaphyglottis prolifera and Sophronitis sincorana; large in Helleriella, Nageliella, Neocogniauxia, Pseudolaelia and Rhyncholaelia. Stomatal ledges are usually undeveloped, obscure or absent; inner and outer ledges are more or less pronounced in Helleriella and Pseudolaelia, outer ledges prominent in Meiracyllium. Cuticular 'horns' regularly overarch the stomatal pore giving the appearance of outer ledges. Fibre bundles present in most taxa (Table 5), variably in three positions, except lacking in Encyclia, Helleriella, Homalopetalum, Isochilus, Meiracyllium, Nageliella, Neocogniauxia and Ponera. There is usually a single row of bundles along either or both surfaces, except one to three adaxial rows in Broughtonia (Fig. 2), two adaxial rows in Brassavola, two adaxial and two abaxial rows in Scaphyglottis prolifera, and one or two in Epidendrum nocturnum and Leptotes. In addition to the abaxial row of small fibre bundles in Constantia, there are two large bundles, one each at the leaf margin. Scaphyglottis imbricata shows an abaxial row of fibre bundles alternating with rows of stomata. In S. prolifera, a row of small bundles appears adjacent to each epidermis followed internally by a row of somewhat larger bundles and a few isolated fibres and tiny bundles in the mesophyll. Fibre bundles in isobilateral leaves, as defined above, encircle the rounded abaxial surface and are absent from the more or less restricted adaxial surface in Brassavola, Jacquiniella, Leptotes, Scaphyglottis reflexa and Tetramicra. Hypodermis: typical hypodermis usually absent; when present, it may be uniseriate throughout, uniseriate and biseriate in part, or biseriate overall (Table 6). In Cattleya forbesii, the hypodermis is biseriate ad- and abaxially, cells with birefringent outer walls alternate with cells having non-birefringent outer walls. In C. intermedia, the adaxial series of cells is continuous, the abaxial discontinuous with fine birefringent banding parallel to the width of the cells. Adaxial hypodermal cells in C. skinneri have birefringent cell walls, but no banding. The adaxial hypodermis in Scaphyglottis imbricata consists of short, linear rows of thick-walled fibres alternating with one or more thin-walled cells. Water-storage cells with birefringent walls in Sophronitis sincorana form the second adaxial hypodermal layer. In the isobilateral leaves of Leptotes, the uniseriate hypodermis encircles the leaf. Adaxial cells in Neocogniauxia resemble the upright cells of a heterogeneous mesophyll and exhibit birefringent, ladder-like bars and wavy walls. The birefringent abaxial cells alternate with non-birefringent abaxial cells. Hypodermal cells encircle isobilateral leaves of Scaphyglottis reflexa. Scalariform cell wall thickenings occur in second layer cells of Rhyncholaelia. It is questionable whether rows of fibre bundles beneath the epidermis constitute all or part of the hypodermis. Fibre bundles occur subdermally in many taxa (Table 5), and their position will be discussed below with reference to their possible

hypodermal origin. Mesophyll mostly homogeneous, some heterogeneous (Table 7). Cells thin-walled. Cells in the homogeneous condition circular, oval or irregular. Upright cells of the palisade layer in the heterogeneous condition resemble those in dicotyledonous leaves (Fig. 3). The shorter, rounded cells of the abaxial layer are not like those of the spongy layer of heterogeneous dicotyledonous leaves. The latter consist of cells separated by more or less large and irregular intercellular spaces that do not occur in Laeliinae. In the homogeneous mesophyll, cells of the adaxial layer only sparsely chlorophyllous, large, separated by wider intercellular spaces; cells of the abaxial layer richly chlorophyllous, small, rounded, crowded with narrower intercellular spaces. Intercellular spaces triangular (best seen in longitudinal section). Crystalliferous idioblasts ubiquitous, circular in transverse section, saccate in longitudinal section. In the isobilateral leaf of Brassavola, upright cells encircle the leaf (Fig. 4), those that are adaxial are considerably longer than those abaxial; central cells homogeneous, most circular, tightly packed. Achlorophyllous upright cells constitute two-thirds of the area of the transverse section of the leaf in Scaphyglottis reflexa, forming a fan-like layer centred on the adaxial invagination. The abaxial region consists of greatly crowded, oval, thin-walled chlorophyllous cells. In conduplicate leaves, cells adaxial to the midvein vascular bundle are strongly inflated and anticlinal, forming a closing layer. Water-storage cells occur in the mesophyll of Encyclia, Jacquiniella, Leptotes and Neocogniauxia. Cell walls with birefringent bands occur in Brassavola, Homalopetalum, Leptotes, Meiracyllium and Rhyncholaelia. Bands are non-birefringent in Scaphyglottis reflexa. Isolated fibres occur in Helleriella and Isochilus. Mesophyll of Helleriella is stratified: (1) the adaxial portion consists of four or five layers of crowded, richly chlorophyllous, circular cells; (2) the middle part has five to eight layers of crowded, sparsely chlorophyllous, loosely organized oval cells oriented parallel with the epidermis; (3) the abaxial layer resembles the adaxial layer. Vascular bundles collateral, generally in a single linear series; double series with larger bundles abaxial in Caularthron, Epidendrum anceps, Prosthecea radiata, occasionally E. nocturnum, Psychilis and Rhyncholaelia; two or three series in Brassavola. Vascular bundles paired, one row along each side of the V-shaped leaf of Jacquiniella and a large bundle, one on either side of the leaf invagination; bundles in an arcuate series in Encyclia, Leptotes, Ponera, Scaphyglottis reflexa and Tetramicra. Sclerenchyma caps both xylem and phloem in all taxa, but predominates adjacent to the phloem pole (Figs 3, 4). Sclerenchyma surrounds vascular bundles in Cattleya forbesii, Leptotes and Scaphyglottis imbricata; sometimes absent in the smallest vascular bundles and/or present only at the phloem pole. Stegmata with conical, rough-surfaced silica bodies typically occur along vascular sclerenchyma and fibre bundles (Table 8). In Jacquiniella, stegmata occur only along thick-walled sclerenchyma of the bundle pair adjacent to the adaxial invagination. In Cattleya intermedia, stegmata occur only along the sclerenchyma of the two marginal vascular bundles. There are no stegmata associated with sclerenchyma caps in the vascular bundles between the leaf margins. Circular or oval bundle sheath cells are thin-walled and often have chloroplasts. In Constantia, Psychilis, Nageliella and Quisqueya, bundle sheath cells are elongated or oblong, radiating from the vascular bundle. Chloroplast-containing, thin-walled bundle sheath cells and non-

chloroplast-containing, thick-walled bundle sheath cells occur in Laelia anceps. Bundle sheath cells are all thick-walled in Scaphyglottis imbricata. There are no distinct bundle sheath cells in Ponera, cells surrounding the vascular bundles merge into normal mesophyll cells. Bundle sheaths are frequently discontinuous.

Taxon	Adaxial	Abaxial	Mesophyll centre
Laeliinae			
Barkeria	–	+ (1)	–
Brassavola	–	+ (2)	–
Broughtonia	+ (1–3)	+ (1–3)	+
Cattleya forbesii	–	+ (1)	–
Cattleya intermedia	–	+ (1)	–
Cattleya skinneri	–	+ (1)	+
Caularthron	+ (1)	+ (1)	–
Constantia	–	+ (1)	–
Dimerandra	+ (1)	+ (1)	–
Encyclia	–	–	–
Epidendrum anceps	–	+	–
Epidendrum nocturnum	+ (1 or 2)	+ (1 or 2)	–
Helleriella	–	–	–
Homalopetalum	–	–	–
Isochilus	–	–	–

Jacquiniella	–	+ (1)	–
Laelia anceps	+ (1)	+ (1)	+
Laelia lyonsii	+(1)	+f(1)	–
Leptotes	–	+ (1 or 2)	–
Meiracyllium	–	–	–
Nageliella	–	–	–
Neocogniauxia	–	–	–
Nidema	+ (1)	+ (1)	–
Orleanesia	+ (1)	+ (1)	–
Ponera	–	–	–
Prosthechea boothiana	+ (1)	+ (1)	–
Prosthechea radiata	–	+	–
Pseudolaelia	–	+	–
Psychilis	+ (1)	+ (1)	+
Quisqueya	+ (1)	+ (1)	+
Rhyncholaelia	–	+ (1)	–
Scaphyglottis coriacea	–	+ (1)	+
Scaphyglottis imbricata	–	+ (1)	–

Scaphyglottis prolifera	+ (2)	+ (2)	+
Scaphyglottis reflexa	+	+ (1)	–
Sophronitis sincorana	+ (1)	+ (1)	+
Tetramicra	–	+ (1)	–
Arpophyllinae			
Arpophyllum giganteum	–	+ (1)	+ (2 or 3)

Table 5. Foliar fibre bundle distribution (number of rows)

Taxon	Adaxial	Abaxial
Laeliinae		
Barkeria	–	–
Brassavola	1	1
Broughtonia	–	1
Cattleya forbesii	2	2
Cattleya intermedia	2	2
Cattleya skinneri	1 or 2	1
Caularthron	–	–
Constantia	–	–
Dimerandra	–	–

Encyclia	–	–
Epidendrum anceps	–	–
Epidendrum nocturnum	–	–
Helleriella	1	–
Homalopetalum	–	–
Isochilus	–	–
Jacquiniella	–	–
Laelia anceps	1 or 2	1 or 2
Laelia lyonsii	2	1
Leptotes	1	1, isobilateral
Meiracyllium	1 or 2	1 or 2
Nageliella	–	–
Neocogniauxia	1	1
Nidema	–	–
Orleanesia	–	–
Ponera	1	–
Prosthechea boothiana	–	–
Prosthechea radiata	–	–

Pseudolaelia	–	–
Psychilis	–	–
Quisqueya	–	–
Rhyncholaelia	1 or 2	1 or 2
Scaphyglottis coriacea	1 or 2	–
Scaphyglottis imbricata	1	1
Scaphyglottis prolifera	2	2
Scaphyglottis reflexa	1	1
Sophronitis sincorana	2	1
Tetramicra	–	–
Arpophyllinae		
Arpophyllum giganteum	1	1

Table 6. Foliar hypodermal distribution (number of layers)

Taxon	Homogeneous	Heterogeneous
Laeliinae		
Barkeria	+	
Brassavola		+

Broughtonia	+	
Cattleya forbesii		+
Cattleya intermedia		+
Cattleya skinneri		+
Caularthron	+	
Constantia	+	
Dimerandra		+
Encyclia	+	
Epidendrum anceps	+	
Epidendrum nocturnum	+	
Helleriella	+	
Homalopetalum	+	
Isochilus	+	
Jacquiniella	+	
Laelia anceps		+
Laelia lyonsii		+
Leptotes	+	
Meiracyllium	+	

Nageliella	+	
Neocogniauxia		+
Nidema	+	
Orleanesia	+	
Ponera		+
Prosthechea boothiana	+	
Prosthechea radiata	+	
Pseudolaelia	+	
Psychilis	+	
Quisqueya	+	
Rhyncholaelia		+
Scaphyglottis coriacea		+
Scaphyglottis imbricata		+
Scaphyglottis prolifera	+	
Scaphyglottis reflexa		+
Sophronitis sincorana	+	
Tetramicra		+
Arpophyllinae		
Arpophyllum giganteum		+

Table 7. Mesophyll organization in leaf

Taxon	Vascular bundles		Fibre bundles
	Xylem	Phloem	
Laeliinae			
Barkeria	+	+	+
Brassavola	+	+	+
Broughtonia	–	–	+
Cattleya forbesii	+	+	+
Cattleya intermedia	Marginal*	Marginal*	+
Cattleya skinneri	+	+	+
Caularthron	+	+	+
Constantia	+	+	+
Dimerandra	–	–	+
Encyclia	–	+	N/A
Epidendrum anceps	+	+	+
Epidendrum nocturnum	+	+	+
Helleriella	+	+	N/A
Homalopetalum	–	–	–
Isochilus	+	+	N/A

Jacquiniella	Marginal[*]	Marginal[*]	+
Laelia anceps	+	+	+
Laelia lyonsii	–	–	+
Leptotes	+	+	+
Meiracyllium	+	+	N/A
Nageliella	+	+	N/A
Neocogniauxia	–	–	–
Nidema	+	+	+
Orleanesia	+?	+?	+
Ponera	+	+	N/A
Prosthechea boothiana	+	+	+
Prosthechea radiata	+	+	+
Pseudolaelia	+	+	+
Psychilis	–	–	+
Quisqueya	–	–	+
Rhyncholaelia	+	+	+
Scaphyglottis coriacea	–	–	+
Scaphyglottis imbricata	+	+	–

Scaphyglottis prolifera	+	+	+
Scaphyglottis reflexa	–	+	+
Sophronitis sincorana	+	+	+
Tetramicra	+	+	+
Arpophyllinae			
Arpophyllum giganteum	–	–	+

N/A, No fibre bundles along which stegmata may occur.

* Stegmata occur only in vascular bundles near leaf margin; they do not occur in vascular bundles between the leaf margins.

Table 8. Foliar stegmata distribution

Stem

Hairs and Stomata absent. Cuticle smooth, thickness variable, 5.0 μm in Jacquiniella, 30.0 μm in Orleanesia, up to 50.0 μm in Prosthechea radiata; rough in Leptotes, Pseudolaelia and Scaphyglottis reflexa. Epidermal cells mostly thin-walled; thick-walled in Epidendrum nocturnum, Orleanesia and Pseudolaelia; largely periclinal, oval; angular in E. anceps, Leptotes, Neocogniauxia, Nidema, Orleanesia, Prosthechea radiata, Pseudolaelia and Tetramicra; conical in E. nocturnum; outer edges of adjacent cells flanged in Leptotes. Hypodermis one or two cells wide; thick-walled in Barkeria, Cattleya forbesii, C. intermedia, Dimerandra, E. anceps, E. nocturnum, Neocogniauxia, Pseudolaelia and Scaphyglottis reflexa. Cortex lacking in most taxa; six to eight cells wide, cells thin-walled, variably shaped, containing occasional cruciate starch grains in Broughtonia; two to four cells wide in C. forbesii and C. intermedia; three to five cells wide in Jacquiniella; 11 or 12 cells wide in Tetramicra; 17–19 cells wide in Pseudolaelia. Fibre bundles encircle the ground tissue internal to the cortex in Broughtonia. Ground tissue: large, roundish, fewer, thin-walled empty water-storage cells surrounded by many small, oval to circular assimilatory cells with tiny, triangular intercellular spaces; water-storage cells have banded cell wall thickenings in Homalopetalum. Ground tissue cells are more or less evenly sized in Orleanesia and Tetramicra. Chloroplasts are frequent in outer cells and cruciate starch grains are commonly deposited in assimilatory cells. Vascular bundles many, collateral, mostly scattered throughout the ground tissue. Bundles encircle the stem and are scattered randomly in the ground tissue of Jacquiniella, Nidema, Pseudolaelia, Scaphyglottis reflexa and Tetramicra. In Leptotes, bundles are

mostly centrally clustered with others forming an ill-defined peripheral series; nine bundles in an outer ring surround a central cluster of nine bundles in Neocogniauxia. Generally, a crescentiform mass of sclerenchyma caps both xylem and phloem of vascular bundles; phloem sclerenchyma is more robust than xylem sclerenchyma. Phloem sclerenchyma is almost always present; xylem sclerenchyma is absent in some taxa. In taxa in which both xylem and phloem sclerenchyma is present in larger bundles, xylem sclerenchyma is frequently absent in smaller bundles; in some cases, sclerenchyma is entirely lacking in smaller bundles. Sclerenchyma completely encircles the vascular bundles of Jacquiniella and Scaphyglottis reflexa. It is prominent along the phloem side of central vascular bundles and appears to encircle bundles of the ground tissue in Neocogniauxia. There are relatively few sclerenchyma cells at either pole in Homalopetalum, and only a few opposite xylem in Orleanesia. Stegmata (Table 9) bearing conical, rough-surfaced silica bodies occur in most taxa associated only with phloem sclerenchyma.

Taxon	Phloem	Xylem
Laeliinae		
Barkeria	+	-
Broughtonia	-	-
Cattleya forbesii	+	-
Cattleya intermedia	+	-
Dimerandra	+	-
Epidendrum anceps	+	-
Epidendrum nocturnum	+	-
Homalopetalum	-	-
Jacquiniella	-	-
Leptotes	+	-

Neocogniauxia	–	–
Nidema	+	–
Orleanesia	+	–
Prosthechea boothiana	+	–
Prosthechea radiata	+	+
Pseudolaelia	–	–
Scaphyglottis reflexa	+	–
Tetramicra	–	–
Arpophyllinae		
Arpophyllum giganteum	–	–

Table 9. Cauline stegmata distribution

Root

Velamen mostly three to six cells wide, ranging from three cells in Cattleya forbesii, Leptotes, Ponera and Pseudolaelia to eight to ten cells in Helleriella, Laelia anceps and P. boothiana. Cell walls thin, thickish to thick in Barkeria, Brassavola, Caularthron, Laelia lyonsii, Nidema, Pseudolaelia and Psychilis. Epi- and endovelamen regions may each contain cells of different orientations (Fig. 5), passing from one orientation to another (Table 10). Overall, epivelamen cells are usually isodiametric and endovelamen cells anticlinal. Velamenal thickenings are tenuous strips of cell wall material, often branched, anastomosing, looped, spirally oriented or forming slits parallel with the shorter or longer dimension of the cell. Cell wall thickenings in Nidema are broad; in Orleanesia, they are ladder-like; epivelamen cells in Isochilus are short, pentagonal with wide, thickening bands perpendicular to the longer axis of the cell; endovelamen cells in Leptotes are accompanied by large intercellular spaces and strands of cell wall material forming a reticulum. Tilosomes of undetermined detail present in Laelia, Quisqueya and Sophronitis sincorana. Exodermal cells anticlinal or isodiametric; cell wall thickenings various (Table 11). Some thick-walled cells have a broad lumen, others a very narrow lumen (Fig. 6). Passage cells intermittent, thin-walled with large

nuclei. Cortex stratified, four to six cells wide in Caularthron, 16–19 cells in E. nocturnum, up to 26 cells in Helleriella. Cells thin-walled; outer and inner layer cells smaller, isodiametric, polygonal or periclinal-oval; outer layer cells lacking intercellular spaces; middle layer cells larger, oval and circular with numerous triangular intercellular spaces; masses of dead hyphae common; living pelotons in Leptotes and Nageliella. Middle layer cells with broad, birefringent bands of cell wall material in C. skinneri, Laelia, Meiracyllium, Rhyncholaelia and Scaphyglottis reflexa; branched, narrow and wide birefringent bands in Caularthron; ladder-like branched bands in Psychilis. Empty water-storage cells with birefringent cell walls in middle cortical layer of E. nocturnum, Isochilus, Nidema, Orleanesia, Ponera, Psychilis, Quisqueya, Rhyncholaelia, Scaphyglottis coriacea and Sophronitis. Variably shaped, mostly polygonal, thick-walled cells with multilaminate walls occur scattered throughout the middle cortex of Helleriella (Fig. 7). Some outer layer cells of Laelia lyonsii isodiametric, inline image-thickened with laminated walls. Outer cells of middle layer often with chloroplasts in several taxa; starch grains in Meiracyllium. Endodermal and pericyclic cells variously oriented and their cell walls differently thickened (Table 12). Cell walls of both endodermis and pericycle are thick opposite the phloem and thin opposite the xylem of the vascular cylinder. Pericyclic cells are smaller than endodermal cells. Endodermal cells are usually isodiametric; anticlinal in Barkeria, Neocogniauxia and Nidema. They are ○-thickened opposite the phloem; thin-walled in Barkeria, C. skinneri, Laelia anceps, Pseudolaelia and Sophronitis sincorana. Pericycle cells usually resemble endodermal cells. They are thin-walled in C. skinneri, Caularthron, Laelia anceps, Nageliella, Pseudolaelia and Sophronitis sincorana. Vascular cylinder: xylem poles range from seven in Encyclia to 24 in Laelia lyonsii; in most roots, there are ten or more xylem poles. Vascular tissue is embedded in thin- or thick-walled sclerenchyma in all taxa, except surrounded by parenchyma in Barkeria (Fig. 8), Epidendrum anceps and Pseudolaelia. In Meiracyllium and Sophronitis cf. cernua, phloem is invested in sclerenchyma and xylem in parenchyma. Pith cells parenchymatous, thin-walled, circular to oval with triangular intercellular spaces in most taxa; cell walls are somewhat thickened in Isochilus and Quisqueya; polygonal with thick walls in Scaphyglottis reflexa and Rhyncholaelia; sclerotic, thick-walled, angular, lacking intercellular spaces in Encyclia; starch grains in Epidendrum nocturnum and Helleriella. Cells with one to three large cruciate starch grains occupying the entire lumen in Leptotes (Fig. 9).
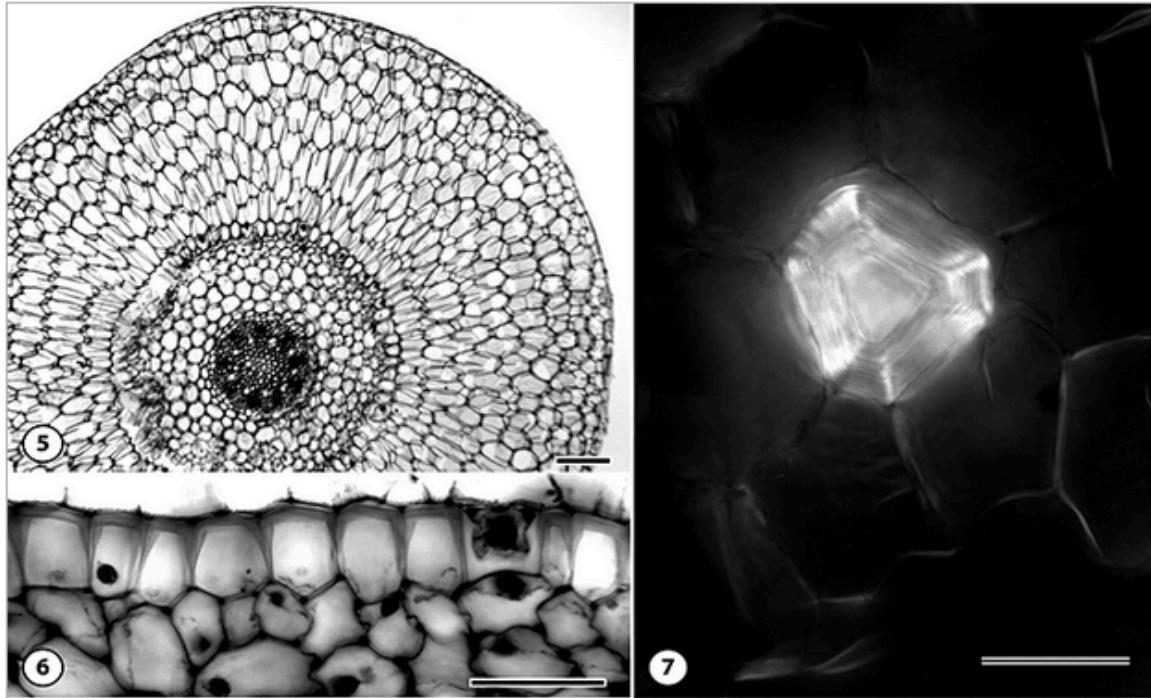
Figure 5–7. Root transverse section. Fig. 5. Prosthechea boothiana with velamen cells of different configurations. Scale bar, 200 μm. Fig. 6. Leptotes tenuis with heavily inline image-thickened exodermal cell walls. Scale bar, 50 μm. Fig. 7. Helleriella guerrerensis cortex under polarized light with multilaminate, thick-walled idioblast. Scale bar, 50 μm.

Taxon	Epivelamen	Endovelamen
Laeliinae		
Barkeria	iso	anti → iso
Brassavola	iso	anti
Cattleya forbesii	anti	anti
Cattleya skinneri	iso → anti	anti
Dimerandra	Cells lacking	anti
Epidendrum anceps	iso	anti

Epidendrum nocturnum	iso → anti	anti
Helleriella	peri	iso → anti
Isochilus	iso	iso → anti
Laelia anceps	iso	anti
Laelia lyonsii	iso	anti
Leptotes	iso	Reticulate
Meiracyllium	?	?
Nageliella	iso	anti
Nidema	iso	iso → anti
Orleanesia	iso → anti	anti
Ponera	peri	iso
Prosthechea boothiana	iso/peri	iso → anti
Prosthechea radiata	iso	anti
Pseudolaelia	anti	anti
Psychilis	iso	anti
Quisqueya	anti	anti
Reichenbachanthus	iso → peri	anti
Rhyncholaelia	iso	anti

Scaphyglottis coriacea	iso → anti	anti
Sophronitis cf. cernua	anti	iso → anti
Sophronitis sincorana	anti	anti
Arpophyllinae		
Arpophyllum giganteum	iso	anti

Table 10. Orientation of velamen cells in root (anti, anticlinal; iso, isodiametric; peri, periclinal)

Taxon	Thin	○-Thickened	⌒-Thickened
Laeliinae			
Barkeria	+		
Brassavola		+	
Broughtonia	+		
Cattleya forbesii			+
Cattleya intermedia			+
Cattleya skinneri	+		
Caularthron	+		
Dimerandra		+	
Encyclia		+	

Epidendrum anceps			+
Epidendrum nocturnum			+
Helleriella			+
Isochilus	+		
Laelia anceps	+		
Laelia lyonsii		+	
Leptotes			+
Meiracyllium			+
Nageliella	+		
Neocogniauxia		+	
Nidema		+	
Orleanesia			+
Ponera	+		
Prosthechea boothiana		+	
Prosthechea radiata	+		
Pseudolaelia	+		
Psychilis		+	
Quisqueya		+	

Reichenbachanthus		+	
Rhyncholaelia		+	
Scaphyglottis coriacea		+	
Sophronitis cf. cernua		+	
Sophronitis sincorana		+	
Arpophyllinae			
Arpophyllum giganteum			+

Table 11. Exodermal cell wall thickening in root

	Endodermis	Pericycle
Taxon	wall thickening/shape	wall thickening/shape
Laeliinae		
Barkeria	Thin/iso–anti	Thin/iso
Brassavola	o/iso	o/iso
Broughtonia	o/iso	o/peri
Cattleya forbesii	o/iso	o/iso
Cattleya intermedia	o/iso	o/iso

Cattleya skinneri	Thin/peri	Thin/peri
Caularthron	○/iso	Thin/peri
Dimerandra	○/iso	○/iso
Encyclia	○/iso–peri	○/peri–iso
Epidendrum anceps	○/iso	○/iso–poly
Epidendrum nocturnum	○/iso	○/poly
Helleriella	○/iso	○/peri
Isochilus	○/iso	○/poly
Laelia anceps	Thin/iso–poly	Thin/poly
Laelia lyonsii	○/iso	○/peri
Leptotes	○/iso	○/poly–peri
Meiracyllium	○/iso	○/iso
Nageliella	○/iso	Thin/peri–iso–poly
Neocogniauxia	○/anti	○/peri
Nidema	○/anti	○/poly–iso
Orleanesia	○/iso	○/peri–iso–poly
Ponera	○/iso	○/poly
Prosthechea boothiana	○/iso	○/iso

Prosthechea radiata	o/iso	o/iso–poly
Pseudolaelia	Thin/iso	Thin/iso
Psychilis	o/iso	o/poly–iso
Quisqueya	o/iso	o/iso
Reichenbachanthus	o/iso	o/peri
Rhyncholaelia	o/iso	o/iso–peri
Scaphyglottis coriacea	o/iso	o/peri–iso–poly
Sophronitis cf. *cernua*	o/iso	o/peri
Sophronitis sincorana	Thin/poly	Thin/poly
Arpophyllinae		
Arpophyllum giganteum	o/poly	o/poly

Table 12. Endodermis and pericycle cell features in root (anti, anticlinal; iso, isodiametric; peri, periclinal; poly, polygonal)
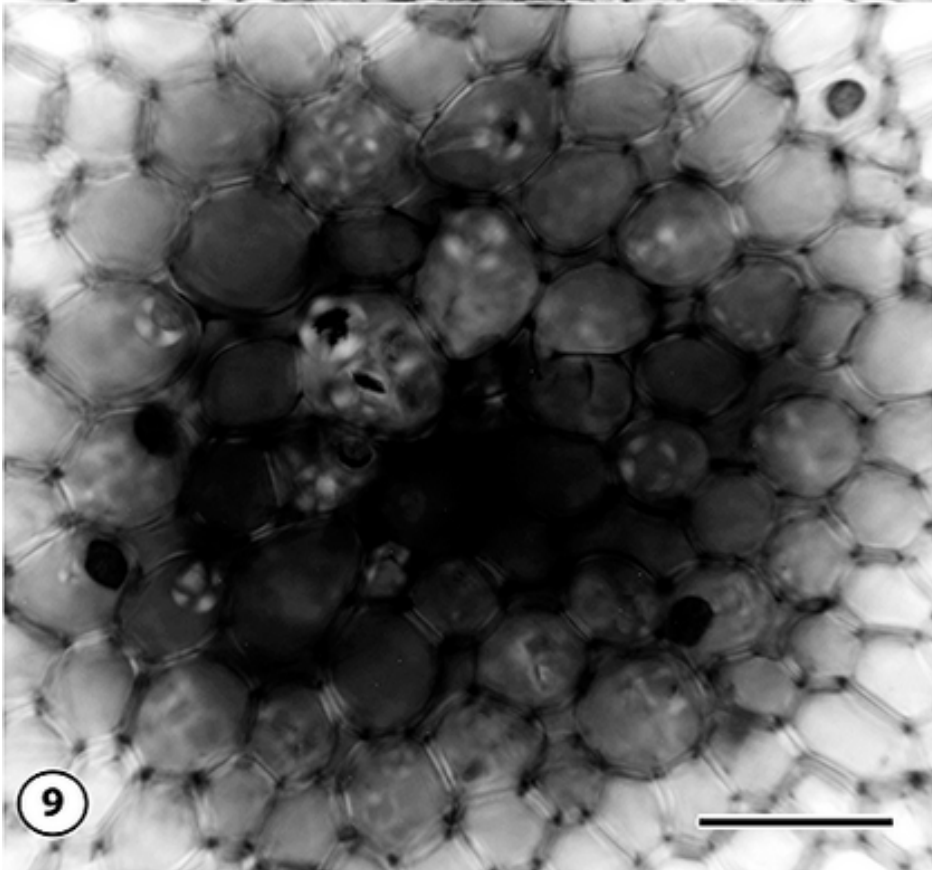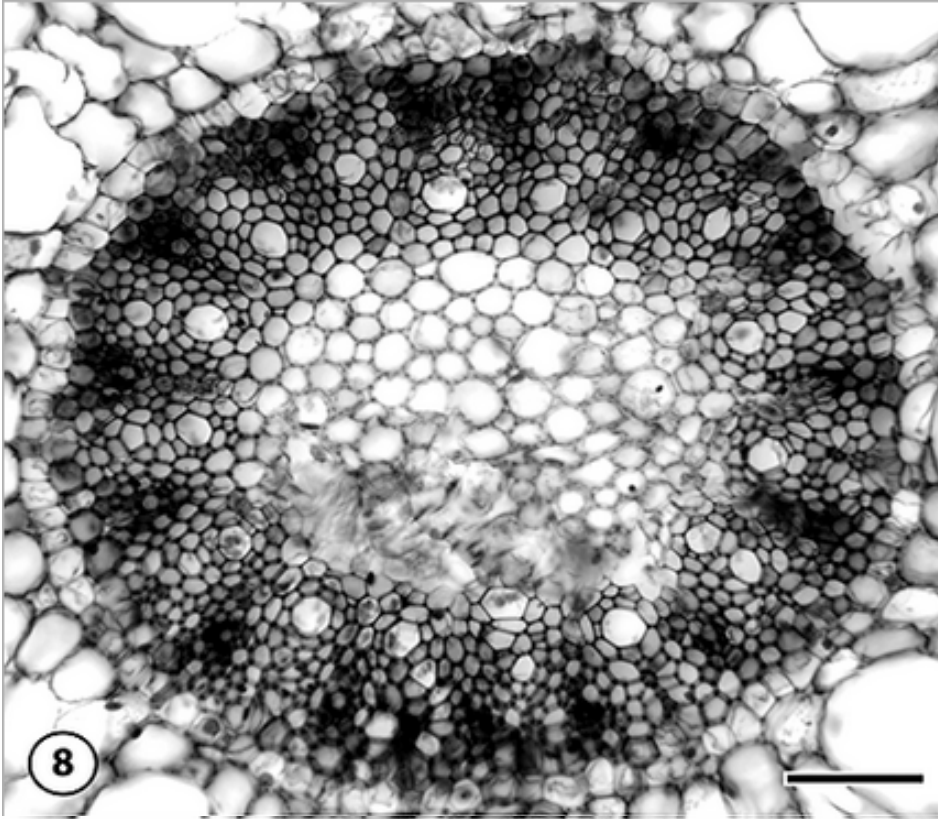
Figure 8, 9. Root transverse section. Fig. 8. Barkeria scandens vascular cylinder showing conductive cells embedded in parenchyma. Scale bar, 100 μm. Fig. 9. Leptotes tenuis pith showing one to three large, cruciate starch grains in each cell. Scale bar, 30 μm.

Arpophyllinae

Leaf

Surface: Hairs absent. Epidermal cells polygonal, square, rectangular, thick-walled; some very thick-walled almost occluding the lumen (Fig. 10). Stomata abaxial. Stomatal apparatus tetracytic, polar cells conspicuous, large. Lateral cells (Fig. 11) one to several, occasionally none. Guard cell pairs average 47 μm long and 48 μm wide (Table 4).
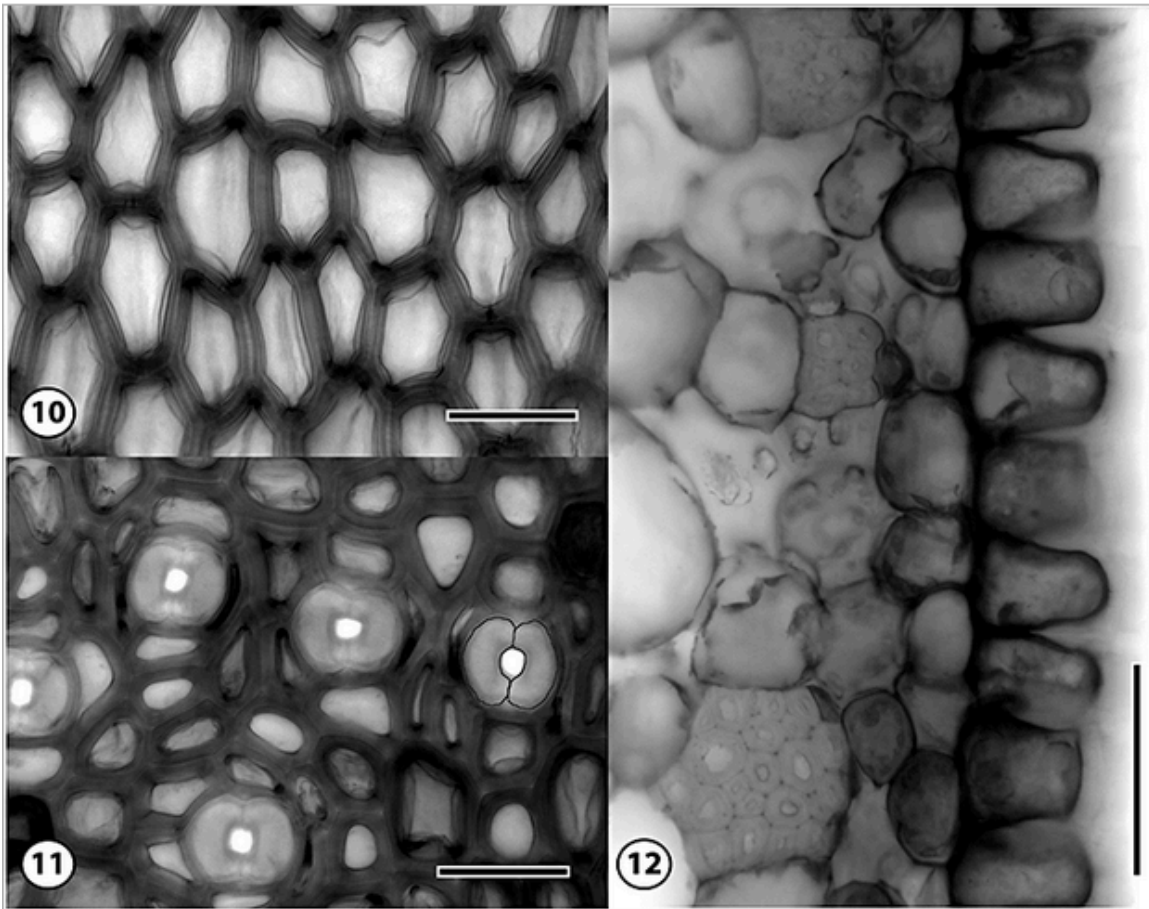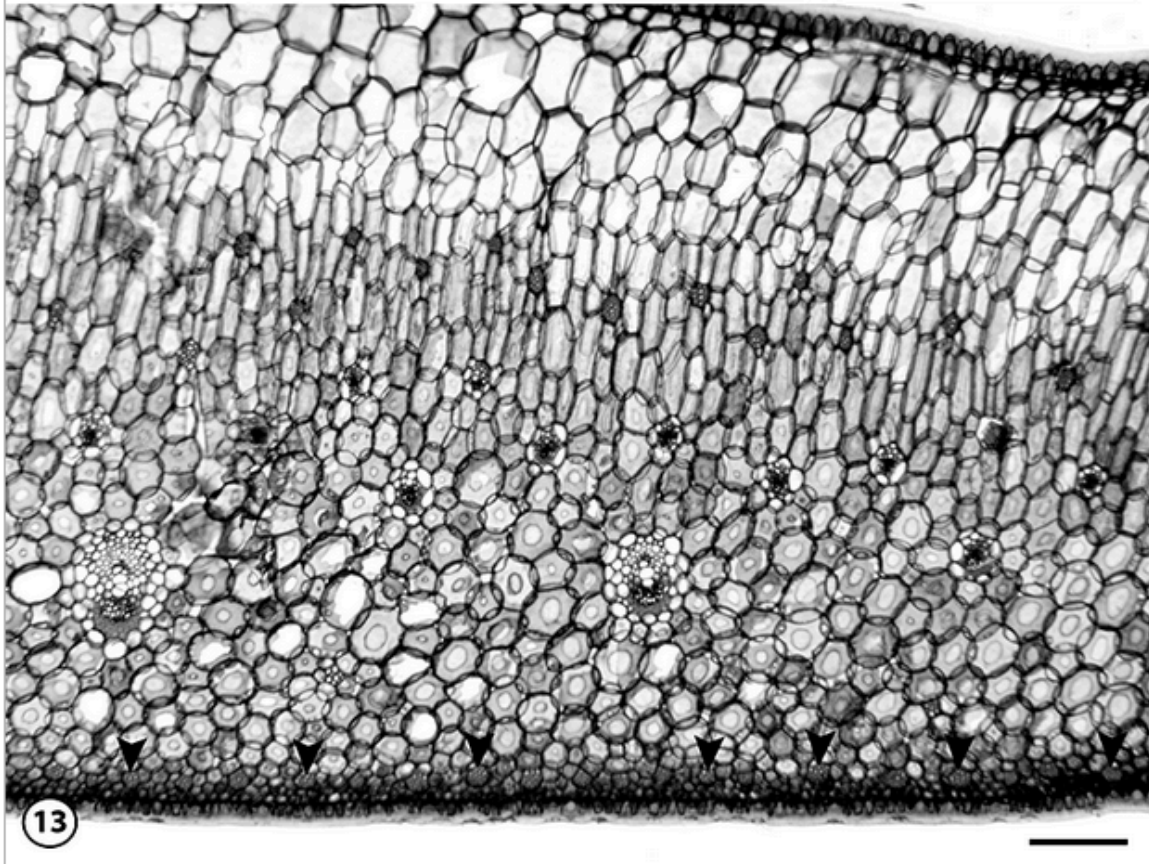
Figure 10–13. Leaves of Arpophyllum giganteum. Fig. 10. Adaxial surface with thick-walled epidermal cells. Scale bar, 50 μm. Fig. 11. Abaxial surface showing stomatal apparatus with several thick-walled lateral subsidiary cells subtending guard cells (outlined in one pair on right side). Scale bar, 50 μm. Fig. 12. Transverse section showing dentiform abaxial epidermal cells. Scale bar, 50 μm. Fig. 13. Transverse section showing localized tripartite region of mesophyll with two or three rows of mid-mesophyll fibre bundles and a single row of abaxial bundles (arrowheads). Scale bar, 200 μm.

Section: Cuticle smooth, both surfaces, except abaxial surface, intermittently concave opposite stomata; adaxial surface c. 7.5 μm thick, abaxial surface c. 5.0 μm thick. Epidermal cells: adaxial cells somewhat anticlinal, apices obtusely rounded; abaxial cells dentiform (Fig. 12) with obvious spaces impregnated with cuticular material. Stomata sunken; stomatal chambers small to non-existent; stomatal ledges obscure. Fibre bundles in two or three irregular rows mid-mesophyll and a single regular abaxial row (Fig. 13) one or two cells removed from the abaxial hypodermis. Hypodermis uniseriate both surfaces, discontinuous; adaxial cells significantly larger than abaxial cells. Mesophyll heterogeneous; palisade layer seven to nine cells wide, cells elongated, upright; abaxial cells circular to oval, intercellular spaces triangular and variously shaped, cells with conspicuous primary pit fields. In some areas of the mesophyll, adaxial to the upright cells, there are several layers of irregularly formed, rounded cells giving rise to a tripartite mesophyll (Fig. 13) in

these places. Vascular bundles collateral in three rows: uniseriate row of widely spaced larger bundles located entirely within the adaxial mesophyll; biseriate row of smaller bundles located mostly in the abaxial mesophyll and in the transition region between the abaxial mesophyll and the upright cells. Sclerenchyma occurs only along the abaxial surface of the phloem (Fig. 14). In the larger bundles, sclerenchyma of thick-walled fibres forms an abaxial crescent up to six cells wide; in the smaller bundles, sclerenchyma crescents are one to three cells wide. Stegmata containing conical, rough-surfaced silica bodies occur only along fibre bundles. Continuous bundle sheaths are conspicuous around both larger and smaller vascular bundles. Bundle sheath cells are thin-walled and lack chloroplasts.
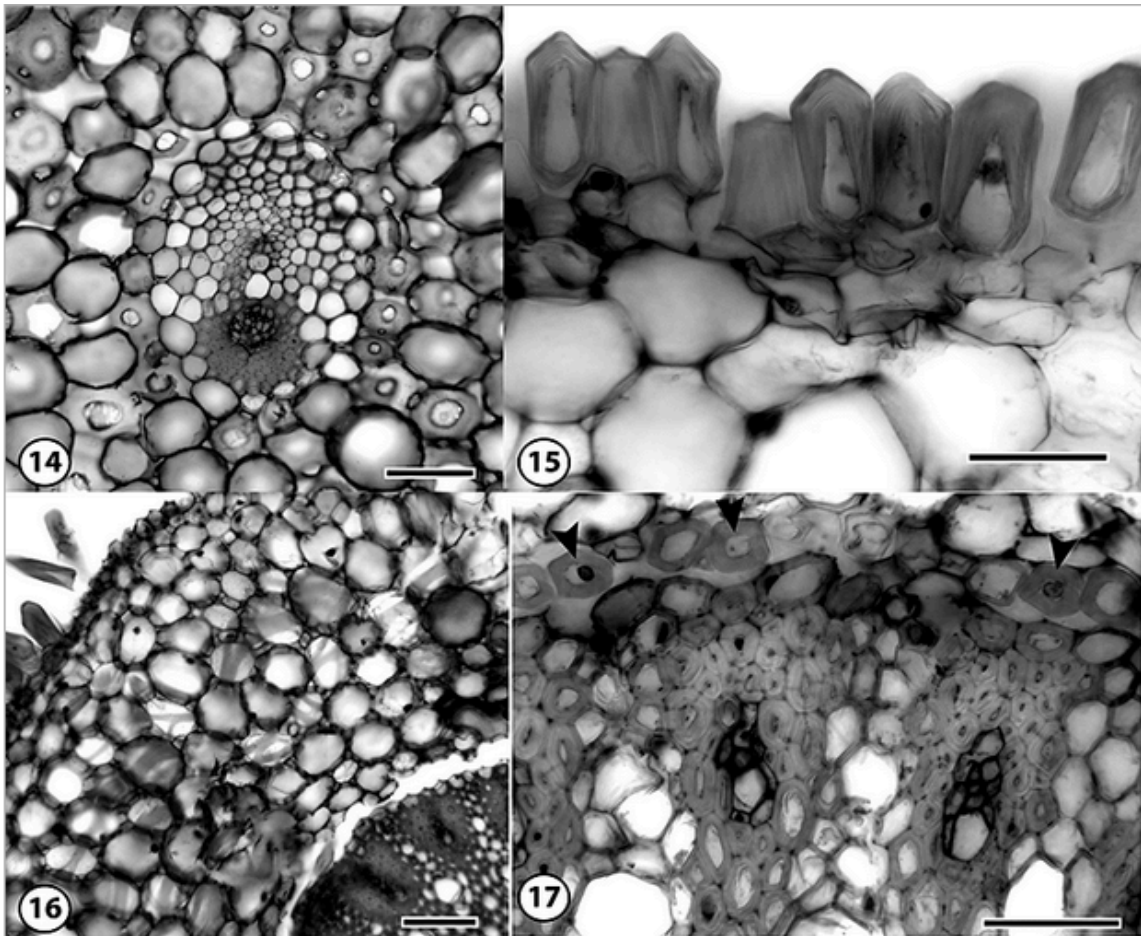
Figure 14–17. Arpophyllum giganteum. Fig. 14. Transverse section of leaf vascular bundle showing robust phloem sclerenchyma. Scale bar, 100 μm. Figs 15–17. Root transverse section. Fig. 15. inline image-Thickened exodermal cells with very thick walls. Scale bar, 50 μm. Fig. 16. Cortical cells showing broad, branched, cell wall bands. Scale bar, 100 μm. Fig. 17. Vascular cylinder with ○-thickened endodermal cells displaying heavily thickened walls (arrowheads) and thick-walled sclerenchyma surrounding clusters of phloem cells. Scale bar, 50 μm.

Root

Velamen five to nine cells wide. Epivelamen cells more or less isodiametric; endovelamen cells more or less anticlinal, barrel-shaped to polygonal. All cells are marked with closely placed, linearly branched, slender, birefringent cell wall thickenings. Cells are infested with hyphae. Tilosomes spongy (Pridgeon et al., 1983). Exodermal cells anticlinal, inline image-thickened with very thick walls (Fig. 15). Cortex 11, 12 cells wide, cells circular, thin-walled, heavily marked with broad, branched birefringent bands (Fig. 16); innermost cells smaller than middle layer cells, oval with triangular intercellular spaces, outermost cells smaller than middle layer cells, polygonal, lacking intercellular spaces. Endodermal cells (Fig. 17) square to polygonal, thick-walled opposite phloem clusters, thin-walled opposite xylem rays. Pericycle cells like endodermal cells, but thinner walled. Vascular cylinder 24-arch, radial rows of xylem cells alternate with elongated, oval phloem clusters. Vascular tissue embedded in thick-walled sclerenchyma, except metaxylem cells surrounded by thin-walled sclerenchyma. Pith cells circular, parenchymatous, thin-walled with triangular intercellular spaces.

RESULTS AND DISCUSSION

Anatomy

Overall, our observations of leaf anatomy agreed with those of Baker (1972), with two exceptions: the presence of hairs and the configuration of the stomatal apparatus. According to Baker, trichomes are frequent on leaves in Laeliinae, but we failed to find evidence of these in any of the taxa examined (except Meiracyllium), in scrapings or sections. Möbius (1887) was unable to demonstrate hairs, except for leaves of Sophronitis perrinii, and Solereder & Meyer (1930) found them in an Epidendrum species. The discrepancy between our findings and those of Baker can probably be explained by the observation that the terminal cell in the hairs of Laeliinae is thin-walled and easily detached, and that hairs are ephemeral in mature leaves (Baker, 1972). It is entirely possible that hairs were removed during the leaf scraping process and that they were so sparse as to have been overlooked in section.

Baker stated that he could find no clearly differentiated subsidiary cells and that the stomatal apparatus was anomocytic. However, we found that subsidiary cells stood out in bold relief in many taxa (Fig. 1). The anomocytic stomatal organization appears in some taxa, sometimes together with the tetracytic condition. In our experience, however, most stomatal configurations in Laeliinae are tetracytic (Fig. 1).

The question of whether fibre bundles can form part or all of the hypodermis needs to be examined. Yukawa & Stern (2002) provided a classification of subepidermal foliar sclerenchyma for Cymbidium and described four hypodermal arrangements, in each of which there were fibres or fibre bundles immediately below the epidermis. They considered these to be hypodermal inclusions. Baker (1972) also included fibre bundles in the hypodermis, and, in the current study, we have shown that fibre bundles occur subdermally in many taxa (Table 5, Fig. 2). According to

Esau (1965) and Evert (2006), hypodermis is a general term for a layer or layers of cells beneath the epidermis if morphologically distinct from underlying layers. This definition permits the inclusion as hypodermis of subdermal fibres, fibre bundles and assimilatory and water-storage cells.

Fibre bundles (Table 5) occur in leaves of almost all taxa, consistently along the abaxial surface, sporadically along the adaxial surface and in the mesophyll centre. Encyclia, Helleriella, Homalopetalum, Isochilus, Meiracyllium, Nageliella, Neocogniauxia and Ponera totally lack fibre bundles. Hypodermis (Table 6) occurs in leaves of several taxa: Brassavola, Broughtonia, Cattleya, Helleriella, Isochilus, Jacquiniella, Laelia anceps, Meiracyllium, Neocogniauxia, Ponera, Rhyncholaelia, Scaphyglottis and Sophronitis sincorana. It is absent in Prosthechea and Epidendrum species, Nidema, Orleanesia, Pseudolaelia, Psychilis, Quisqueya and Tetramicra. Mesophyll (Table 7) is mostly homogeneous, with the notable exception of heterogeneity in Brassavola, Cattleya, Dimerandra, Laelia anceps, Neocogniauxia, Ponera, Rhyncholaelia, Scaphyglottis coriacea, S. imbricata, S. reflexa and Tetramicra. Stegmata, associated with vascular sclerenchyma in leaves (Table 8), occur in all taxa, except Broughtonia, Dimerandra, Homalopetalum, Neocogniauxia, Psychilis, Quisqueya and Scaphyglottis coriacea. Stegmata are associated with fibre bundles (Table 8), except in Homalopetalum, Neocogniauxia and Scaphyglottis imbricata. Stegmata occur with cauline vascular bundle sclerenchyma, except in Broughtonia, Homalopetalum, Jacquiniella, Neocogniauxia and Tetramicra. Exodermal cells (Table 11) are thin-walled in Barkeria, Broughtonia, C. skinneri, P. radiata, Isochilus, Laelia anceps, Nageliella, Ponera and Pseudolaelia. In all other taxa, exodermal cells are variously thick-walled. Stomata (Table 4) occur only on the abaxial sides of leaves, except for both sides in Barkeria, Broughtonia, Meiracyllium, Nageliella and Pseudolaelia.

Distinctive anatomical features occur in several taxa: thin-walled endodermal cells throughout the roots of Barkeria; in C. intermedia, stegmata associated with vascular sclerenchyma only in bundles near leaf margins, not in bundles intermediate between these; two large fibre bundles adjacent to leaf margins in Constantia; amphistomatal leaves in Barkeria, Broughtonia, Meiracyllium, Nageliella and Pseudolaelia; epidermal cells conical in stems of E. nocturnum; mesophyll trizonate in Helleriella; circular to polygonal to polymorphic cells with thick anisotropic, stratified walls scattered among the normal thin-walled cortical cells in roots of Helleriella; widely separated, circular, thin-walled empty cells distributed subepidermally along leaf edges in Homalopetalum; epivelamen cells with few, short, thick bands of cell wall material perpendicular to the long axis of cells in velamina of Isochilus; stegmata in leaves of Jacquiniella associated with sclerenchyma of paired vascular bundles alongside the foliar groove, other vascular bundles lack stegmata; guard cells in the stomatal apparatus of Sophronitis sincorana C-shaped; in Leptotes, epidermal cells of stems are flanged at their edges (transverse section), cell walls of velamen in a reticulum, pith cells with one to three large cruciate starch grains occupying the entire lumen; foliar cuticle in Nageliella papillate and 60 μm thick; stomatal apparatus in Neocogniauxia cyclocytic;

subsidiary cells in leaves of Ponera greatly flattened parallel to the guard cells; tilosomes in roots of Laelia, Quisqueya and Sophronitis sincorana; in roots of Sophronitis cf. cernua and Meiracyllium, phloem is surrounded by sclerenchyma, xylem by parenchyma.

The anatomy of Laeliinae, as constituted above, lacks overall uniformity and, in this respect, mirrors the view posited with respect to the taxonomic delimitation of the group. The only nearly consistent anatomical feature is the abaxial row of fibre bundles in the leaves. This character probably has little phylogenetic meaning, because fibre bundles serve a mechanical function in support of the leaves and systematically occur throughout many epiphytic Orchidaceae. They are absent, however, in several genera noted above. Overall, Laeliinae is anatomically a mixed bag and, contrary to our hypothesis, anatomy does not serve to tie together the taxa of Laeliinae.

Cladistics

Heuristic analyses of 34 genera using 11 vegetative anatomical and morphological characters (Tables 3, 4) produced 460 equally parsimonious trees with length (L) = 56, consistency index (CI) = 0.34 and retention index (RI) = 0.69. The strict consensus (Fig. 18) of these genera showed little resolution, and no clade was supported with greater than 50% bootstrap values. The C. forbesii + C. intermedia and C. skinneri + Laelia anceps + Rhyncholaelia clades were defined by the presence of pseudobulbs. Within the C. skinneri + Laelia anceps + Rhyncholaelia clade, C. skinneri was sister to Laelia anceps based on thin-walled exodermal cells. The close association of Cattleya, Laelia and Rhyncholaelia seen in our analyses has also been supported by molecular data (Pridgeon et al., 2005).

By contrast with previous molecular studies (Pridgeon et al., 2005), in which Laelia lyonsii, Psychilis, Quisqueya, Scaphyglottis prolifera and Sophronitis sincorana were scattered among several alliances within Laeliinae, our analyses supported the union of these genera based on the presence of tilosomes, adaxial and abaxial foliar fibre bundle distribution, and $\bigcirc$-thickened exodermal cell walls. Psychilis and Laelia lyonsii formed a clade united by cortical cell wall thickenings within the roots, and this clade was sister to Quisqueya based on the presence of foliar stegmata distribution only along the fibre bundles. Scaphyglottis prolifera and Sophronitis sincorana were supported by the adaxial and abaxial distribution of the foliar hypodermis.

The low CI values of the shortest trees, low bootstrap percentages and high homoplasy seen in the vegetative characters used in these analyses suggest that vegetative characters alone are insufficient to assess the relationships amongst the genera of Laeliinae. However, these vegetative characters may still be useful in examining the patterns of structural evolution within this diverse subtribe of orchids.
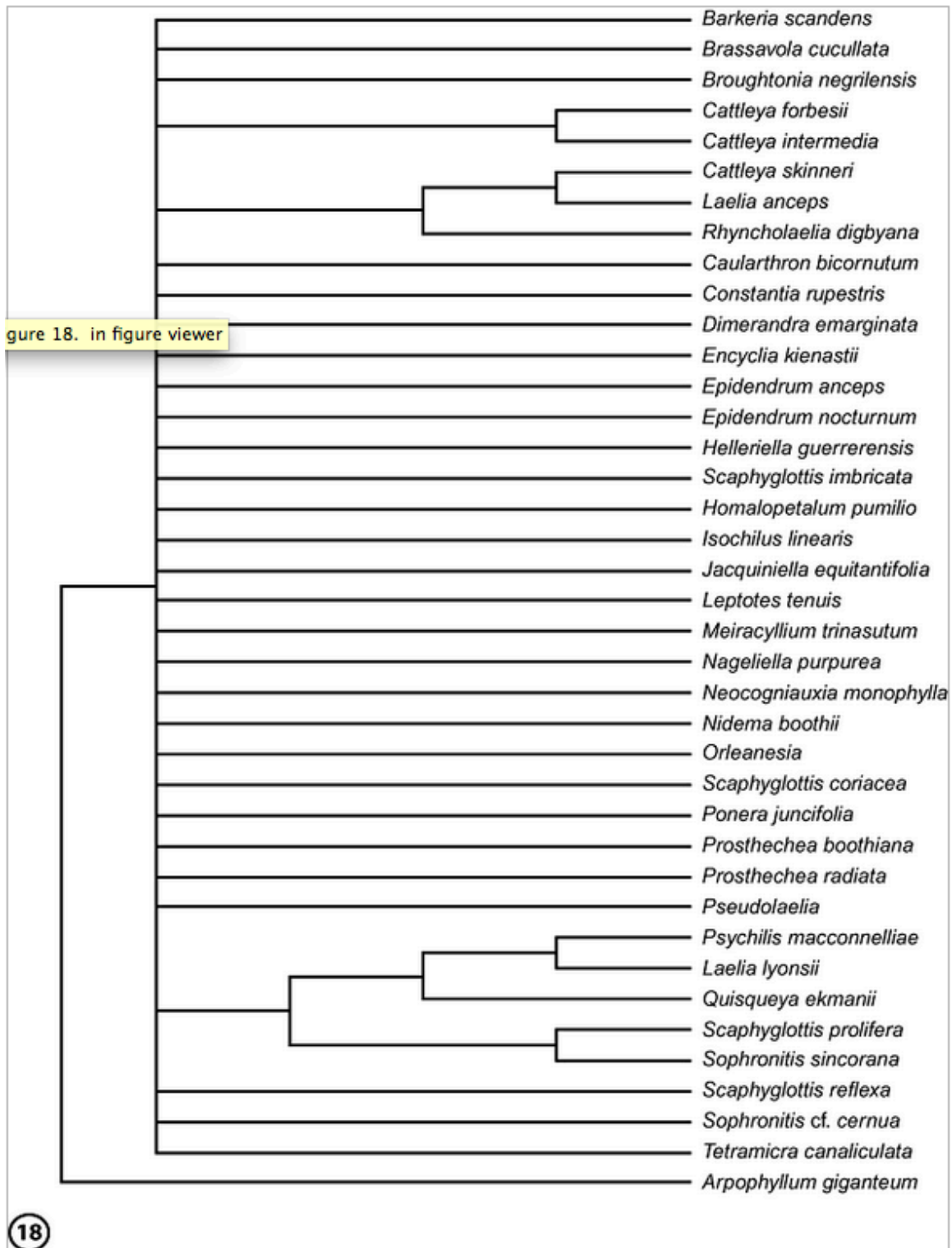
Figure 18. Strict consensus of 460 trees from the cladistic analysis of anatomical and morphological characters.

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