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Vegetative anatomy and systematics of Triphorinae (Orchidaceae)

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ABSTRACT

Triphorinae represents a group of three anatomically simple genera, the structural features of which are unspecialized. The anomocytic stomatal pattern occurs in all genera; it predominates in Triphora. A foliar hypodermis, sclerenchyma, fibre bundles and stegmata are absent. The mesophyll is homogeneous. The exodermal and endodermal cells in the roots are entirely thin-walled and tilosomes are absent. However, there are anatomical modifications that appear to be unique: root hairs in Monophyllorchis are borne on velamenal buttresses and, in Psilochilus, they arise endogenously. In the root vascular system of Psilochilus, the metaxylem occurs as a circumferential band. The surfaces of stems in Triphora are tuberculate. Mycorrhizae appear to characterize the root cortices of all genera.

Keywords: anomocytic – cladistics – orchid – root hairs – tetracytic – velamen.

INTRODUCTION

Triphorinae (Dressler) Szlach. is a subtribe of only three genera in tribe Triphoreae Dressler, which contains only one other subtribe, Diceratostelinae (Dressler) Szlach. with one genus (Pridgeon et al., 2005). Monophyllorchis Schltr., Psilochilus Barb. Rodr. and Triphora Nutt. represent subtribe Triphorinae, all of which are distributed in the New World. Monophyllorchis and Psilochilus grow in tropical America, whereas the range of Triphora extends from southern Ontario, Canada, and the far north-eastern USA south through Mesoamerica and the Caribbean into northern and central South America (Pridgeon et al., 2005). They are small, nondescript, terrestrial mycorrhizal herbs (Fig. 1), sometimes with fleshy root tubers (Rothacker, 2005), subdistichous, often reduced, nonarticulate leaves and succulent stems. There are no reports on the anatomy in the literature, except for Monophyllorchis with abaxial stomata and four or variable numbers of subsidiary cells (Williams, 1979). On the basis of molecular phylogenetic studies (Cameron et al., 1999; Chase et al., 2003), Triphoreae appear among the ‘primitive’ taxa of subfamily Epidendroideae (Dressler, 1993), but resolution among these taxa remains poor. Nevertheless, in both molecular and morphological analyses, Monophyllorchis, Psilochilus and Triphora form a monophyletic group (Cameron et al., 1999). Anatomical studies may serve to elucidate these conclusions.

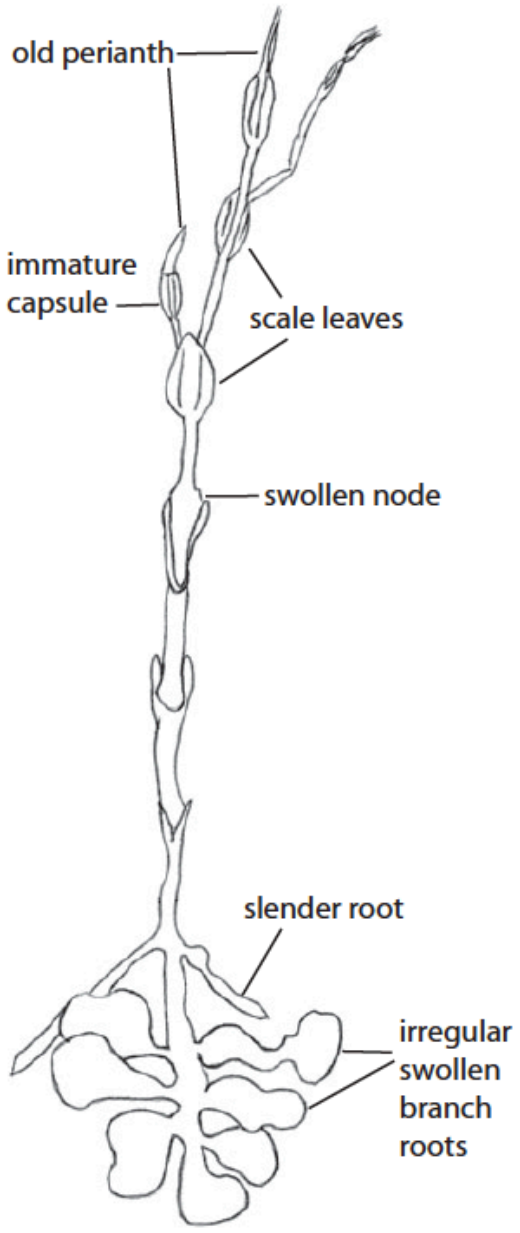


Figure 1. Triphora trianthophora. Whole plant. Swollen branch roots are highly irregular and appear to become tuberous as slender roots swell.

MATERIAL AND METHODS

Material of all three genera was available for study. Binomials and authorities, abbreviated according to Brummitt & Powell (1992), representing species in these genera, appear in Table 1, together with the parts available for study. Methods and descriptive conventions have appeared in recently published papers (for example, Stern & Carlsward, 2006), and we have followed similar procedures here. 'Periclinal' and

'anticlinal' are used as shorthand for 'periclinally oriented' and 'anticlinally oriented.' Plant parts were preserved in FAA (70% ethanol–glacial acetic acid–commercial formalin, 9.0 : 0.5 : 0.5) and stored in 70% ethanol. Voucher specimens are housed at the Marie Selby Botanical Gardens (SEL), Sarasota, FL, USA. Transverse and longitudinal sections of leaves and transverse sections of stems and roots were cut unembedded as thinly as possible with a Reichert sliding microtome, stained in Heidenhain's iron–alum haematoxylin and counter-stained with safranin. Leaf scrapings followed Cutler's method (Cutler, 1978) and were stained with safranin. Sections and scrapings were mounted on glass slides with Canada balsam. Observations were made using a Nikon Optiphot microscope, and photographs were taken with a PixeraPro 150es digital camera attached to a Zeiss Axioskop 40 microscope. Measurements of the lengths and widths of ten guard-cell pairs were made, and these appear in Table 2.

Table 1. Species of Triphorinae studied

Taxon	Parts available
Monophyllorchis maculata Garay	L, S, R
Psilochilus cf. *macrophyllus* Ames	L, S, R
Triphora gentianoides (Sw.) Nutt. ex Ames & Schltr.	L, S, R
T. cf. *gentianoides*	L, S, R
T. trianthophora (Sw.) Rydb.	L, S, R

L, leaf; R, root; S, stem.

Table 2. Stomatal dimensions (μm)*

	Average		Range	
Taxon	Length	Width	Length	Width
Monophyllorchis maculata	40	35	35–40	32–37
Psilochilus cf. *macrophyllus*	44	42	40–47	32–50
Triphora gentianoides	40	36	37–45	30–37
Triphora trianthophora	28	23	24–30	21–27

*Stomatal measurements were not made for *T.* cf. *gentianoides*.

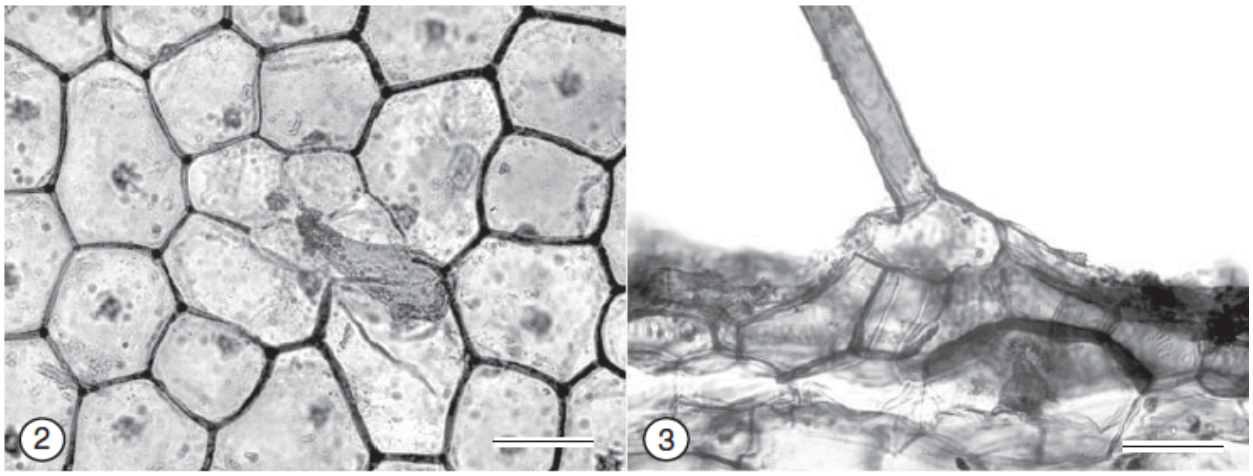
ANATOMY
MONOPHYLLORCHIS

Leaf

Surface: HAIRS present, sparse, both surfaces, bicellular, basal cell elongate, apical cell glandular-clavate (Fig. 2). EPIDERMIS: adaxial cells polygonal, walls straight-sided; abaxial cells polygonal, walls straightsided and curvilinear. STOMATA abaxial, largely tetracytic, few anomocytic and anisocytic.

Section: CUTICLE less than 2.5 mm thick, more or less smooth. EPIDERMIS: adaxial cells variable, oval, angular; abaxial cells smaller mostly oval. STOMATA superficial, substomatal chamber moderate, about the same size as adjacent mesophyll cells or

smaller; outer ledges small, inner ledges minute. HYPODERMIS absent. FIBRE BUNDLES absent. MESOPHYLL homogeneous, four to seven cells wide; cells thin-walled, circular towards middle of leaf, oval towards margins. Intercellular spaces mostly small, triangular. Raphide idioblasts, circular in transverse section, three to five times the length of chlorophyllous cells. VASCULAR BUNDLES: collateral in a single row, small, few, with little conductive tissue; mid-vein bundle with a few separated tracheary elements; compact xylem strand absent. STEGMATA and SCLERENCHYMA absent. Bundle sheath cells thin-walled with chloroplasts.



Figures 2, 3. *Monophyllorchis maculata.* Fig. 2. Abaxial leaf scraping with clavate glandular hair. Scale bar, 50 µm. Fig. 3. Transverse section of root with hair borne on velamenal buttress. Scale bar, 100 µm.

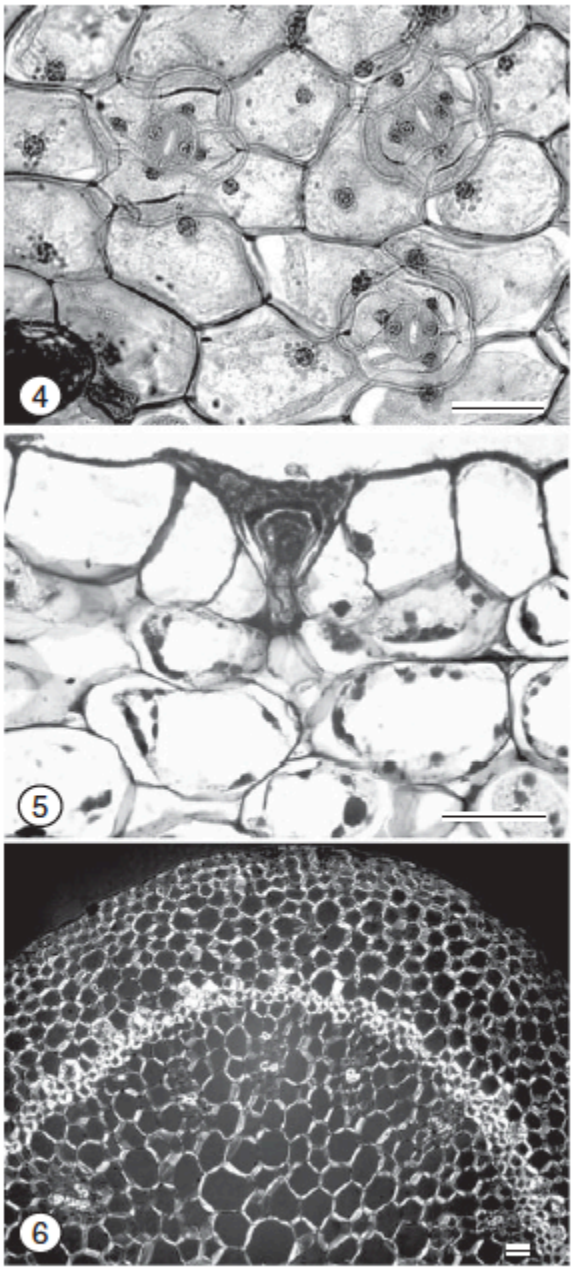
Stem

HAIRS and STOMATA absent. CUTICLE less than 2.5 mm thick, smooth. EPIDERMAL CELLS thin-walled, oval. HYPODERMIS absent. CORTEX: cells thin-walled, mostly circular to oval with small, triangular, inter cellular spaces, outer cells with chloroplasts; cells continuous with cells of ground tissue between vascular bundles. ENDODERMIS and PERICYLE absent; ill-defined layer of tangentially flattened cells lacking Casparian strips just external to the ring of vascular bundles. GROUND TISSUE cells thin-walled, polygonal with few intercellular spaces; cells possibly contain minute droplets of lipid, circular in face view, hemispherical in side view. VASCULAR BUNDLES about 12, collateral, in a single ring containing little conductive tissue, tracheary elements reduced to a few cells. SCLERENCHYMA and STEGMATA absent. Raphides occur in unmodified cortical cells.

Root

VELAMEN two to three cells wide, cells variably shaped, walls marked with radially elongated, teardrop-shaped and slit-like openings. Root hairs marked similarly, bases inflated, borne on severalcelled velamenal buttresses (Fig. 3). TILOSOMES absent. EXODERMAL CELLS entirely thin-walled; passage cells unusually large, conspicuous. CORTICAL CELLS about 20, seriate, thin-walled, circular to oval, polygonal where crowded, intercellular spaces triangular, small; hyphae and dead fungal masses in isolated sections. ENDODERMIS uniseriate, cells polygonal, angular and oval to rounded; Casparian strips present; thin-walled throughout. PERICYCLE uniseriate, cells small,

variably shaped; thick-walled, thin-walled opposite xylem. VASCULAR CYLINDER eight-arch, xylem groups alternating with phloem arcs around the periphery. Vascular tissue embedded in parenchyma. PITH cells parenchymatous, thinwalled, rounded to polygonal without intercellular spaces.



Figures 4–6. *Psilochilus* cf. *macrophyllus*. Fig. 4. Abaxial surface leaf scraping exhibiting both anisocytic and tetracytic stomatal patterns. Scale bar, 50 µm. Fig. 5. Transverse section of leaf showing sunken, glandular, three-celled hair. Scale bar, 50 µm. Fig. 6. Transverse section of stem with sclerenchyma band between cortex and ground tissue, shown under polarized light. Scale bar, 100 µm.

PSILOCHILUS

Leaf

Surface: HAIRS three-celled, both surfaces, clavate, apex swollen, glandular, sunken. EPIDERMIS: adaxial cells polygonal, walls straight-sided, curvilinear around hair bases; abaxial cells polygonal, many with rounded corners, walls straight-sided and curvilinear. STOMATA abaxial, equally divided between tetracytic and anisocytic organization (Fig. 4), few anomocytic.

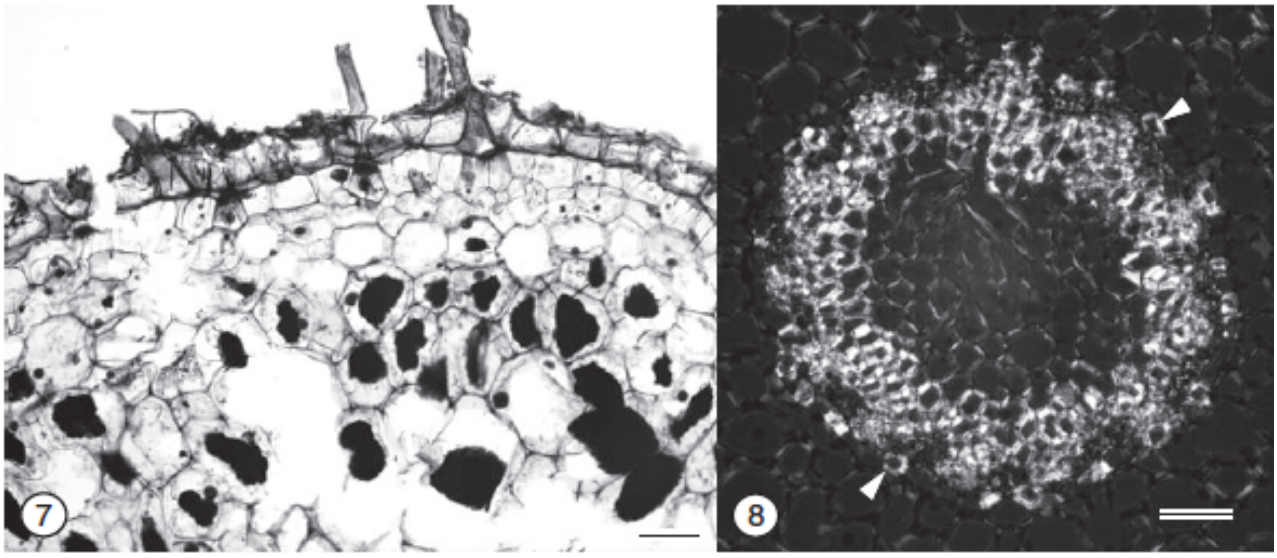
Section: CUTICLE less than 2.5 mm thick, smooth. EPIDERMIS: adaxial cells squarish, abaxial rectangularpericlinal. HAIRS three-celled, sunken, glandular (Fig. 5). STOMATA superficial, substomatal chamber small, outer ledges small, inner ledges absent. HYPODERMIS absent. FIBRE BUNDLES absent. MESOPHYLL homogeneous, four to six cells wide, cells thin-walled, circular, oval, irregular, intercellular spaces mostly triangular. Raphide idioblasts circular in transverse section, elongated, saccate in longitudinal section, about seven times as long as associated chlorenchyma cells. VASCULAR BUNDLES few, collateral in one row, conductive tissue sparse, xylem unitary. SCLERENCHYMA and STEGMATA absent. Bundle sheath cells thin-walled, poorly defined.

Stem

HAIRS clavate, sunken, as in leaf. STOMATA absent. CUTICLE less than 2.5 mm thick, smooth. EPIDERMAL CELLS square and rectangular. CORTEX eight to ten cells wide, cells polygonal to rounded, thin-walled with chloroplasts throughout, intercellular spaces triangular; small, polygonal SCLERENCHYMA CELLS in two or three layers between cortex and ground tissue (Fig. 6). ENDODERMIS and PERICYCLE absent. VASCULAR BUNDLES collateral, 15 in one ring. STEGMATA absent.

Root

VELAMEN uniseriate. Cells anticlinal, rectangular; walls pitted with circular, oval and elongated openings. Velamen cell walls thicker than exodermal cell walls. Root hairs arising adjacent to exodermal passage cells and passing through velamen into substrate (Fig. 7). TILOSOMES absent. EXODERMAL CELLS thin-walled, outer tangential and radial walls thicker than inner tangential walls. Cells nucleate, but passage cell nuclei much larger than nuclei in other cells. CORTEX: three-layered (Fig. 7); outer layer one to three cells wide, cells thin-walled, oval and circular, fungus-free; middle layer two to four cells wide, cells thin-walled, oval and circular, fungus-filled with masses of dead hyphae and pelotons; inner layer five to six cells wide, cells thin-walled, mostly circular, fungus-free; all cell layers with triangular intercellular spaces. ENDODERMIS uniseriate, cells square, mostly thin-walled, an occasional cell with an O-thickened wall (Fig. 8). Remnants of Casparian strips present. PERICYCLE subtending ENDODERMIS; cells smaller than endodermal cells and thin-walled. Conductive elements of VASCULAR CYLINDER organized in a scalloped ring; points of the scallop represent protoxylem; the continuous ring metaxylem (Fig. 8). Embayments between protoxylem points phloic. Metaxylem ring enclosing PITH of circular and polygonal thin-walled cells with triangular intercellular spaces. Raphides in unmodified cortical cells.



Figures 7, 8. *Psilochilus* cf. *macrophyllus* transverse section of root. Scale bars, 100 µm. Fig. 7. Hairs arising endogenously below the velamen, usually opposite an exodermal passage cell, and passing through it into the substratum (note the three-layered cortex). Fig. 8. Encircling scalloped metaxylem vascular cylinder and endodermal cells with O-thickened walls (arrowheads), shown under polarized light.

TRIPHORA

Leaf

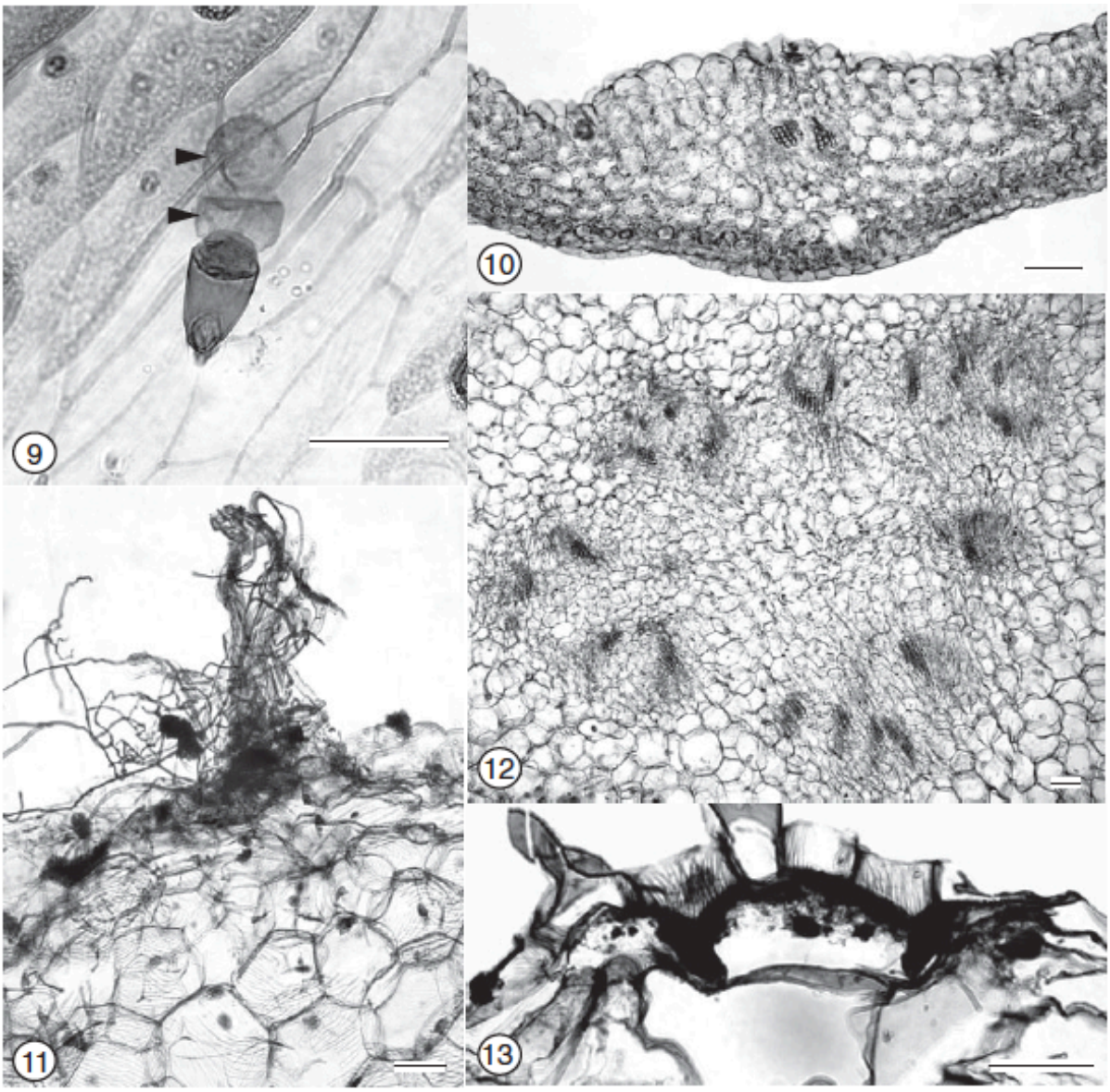
Surface: HAIRS present, both surfaces, uniseriate, three-celled; two apical cells thin-walled, squarish, basal cell thicker walled, clavate, secretory (Fig. 9). EPIDERMIS: adaxial cells polygonal, walls straightsided; abaxial cells polygonal with rounded corners, many elongated parallel with veins, cell walls mostly curvilinear. STOMATA abaxial, mostly anomocytic.

Section: CUTICLE less than 2.5 mm thick, smooth. EPIDERMIS: adaxial cells squarish; abaxial cells squarish and rectangular. STOMATA superficial, substomatal chambers small, outer ledges small, inner ledges absent. HYPODERMIS absent. FIBRE BUNDLES absent. MESOPHYLL homogeneous, four to six cells wide, cells thin-walled, circular and oval, intercellular spaces triangular and irregular. Raphide idioblasts circular in transverse section, elongated in longitudinal section, four to six times the length of chlorophyllous cells. VASCULAR BUNDLES collateral in one row. Xylem in two to four groups in midvein (Fig. 10) and other larger veins, unitary in smaller veins. STEGMATA and SCLERENCHYMA absent. Bundle sheath cells illdefined, with chloroplasts.

Aerial stem

Surface tuberculate. HAIRS and STOMATA absent.
CUTICLE less than 2.5 mm thick, smooth. EPIDERMAL CELLS thin-walled, round to angular. HYPODERMIS: uniseriate, cells thin-walled round to oval, smaller than cortical cells. CORTEX: five to six cells wide, cells thin-walled, round to angular, intercellular spaces triangular. Cortex and pith merge between vascular bundles. ENDODERMIS and PERICYCLE absent. GROUND TISSUE cells thin-walled, circular to angular,

intercellular spaces absent. VASCULAR BUNDLES collateral, eight in a circle. SCLERENCHYMA and STEGMATA absent.



Figures 9–13. *Triphora gentianoides*. Figs 10–12. Scale bars, 100 µm. Fig. 9. Leaf scraping with three-celled hairs, the two apical cells are thin-walled and clear (indicated by arrowheads), the basal cell is thicker walled, clavate and secretory. Scale bar, 50 µm. Fig. 10. Transverse section of leaf showing two tracheary groups in midvein. Fig. 11. Transverse section of tuberculate subterranean stem having tufts of unicellular hairs and showing tangled fungal hyphae. Fig. 12. Transverse section of vascular cylinder in subterranean stem with U-shaped and paired vascular bundles. Fig. 13. Transverse section of root with pectinated velamenal cell walls. Scale bar, 50 µm.

Subterranean stem

Surface tuberculate, excrescences with tufts of unicellular HAIRS and hyphae (Fig. 11). STOMATA and CUTICLE absent. EPIDERMAL CELLS thin-walled, square. HYPODERMIS absent. CORTEX about 15 cells wide, cells thin-walled, rounded to polygonal, intercellular spaces small, triangular, raphide bundles in unmodified cells,

cells with dead fungal masses and pelotons, cortical cells contiguous with central ground tissue between vascular bundles. ENDODERMIS and PERICYCLE absent. GROUND TISSUE cells thin-walled, rounded, angular, polygonal, smaller than cortical cells, lacking intercellular spaces. VASCULAR BUNDLES collateral several in a ring surrounding the ground tissue. Bundles U-shaped, lobed and paired (Fig. 12). SCLERENCHYMA and STEGMATA absent.

Root tuber
Enlarged, fleshy; surface tuberculate. VELAMEN (deteriorated) at least two-layered, cell walls pectinated (Fig. 13). TILOSOMES absent. EXODERMAL CELLS entirely thin-walled. CORTEX: 20–25 cells wide, cells thin-walled, polygonal to rounded, raphide bundles in unmodified cells, intercellular spaces few, masses of dead hyphae and pelotons in outermost 15 cell layers; innermost layers free of fungi. ENDODERMAL CELLS with Casparian strips, thin-walled. PERICYCLE two or three layers, cells thin-walled. VASCULAR CYLINDER 33-arch, xylem groups with narrow tracheary elements alternating with phloem clusters adjacent to extensive arcs of intervening parenchyma. PITH cells thin-walled, closely resembling cortical cells, intercel lular spaces absent; few isolated tracheary elements scattered within pith. Starch grains absent.

RESULTS AND DISCUSSION

The genera of Triphorinae are anatomically simple: there is no foliar hypodermis; there are no fibre bundles, sclerenchyma or stegmata in the leaves; the mesophyll is homogeneous throughout; and the conductive tissue is scanty. The anomocytic stomatal pattern is the least specialized in that epidermal cells surrounding the guard-cell pair are assorted randomly (Williams, 1979). The leaves in each of the three genera show at least some anomocytic configurations, but in the leaves of Triphora the anomocytic pattern predominates. In Monophyllorchis, the tetracytic pattern predominates and, in Psilochilus, the stomatal plan is equally divided between the tetracytic and anisocytic conditions, showing some degree of organization. The stomatal size (Table 2) in Triphora trianthophora is significantly smaller than in the other taxa reported.

The endodermis and pericycle are absent in all stems. A sclerenchyma band is present in the stem of Psilochilus; there is no sclerenchyma in the stems of Monophyllorchis or Triphora. Exodermal cells in roots are thin-walled (outer tangential and radial walls barely thickened in Psilochilus). Endodermal cells are thin-walled. In Monophyllorchis, root hairs are borne on a multicellular buttress of velamen cells. Root hairs in Psilochilus arise internally opposite exodermal passage cells and proceed through the velamen, in contrast with the typical situation in which root hairs are superficial, originating as cells of the outer layer of the velamen. The vascular tissues in the roots of Monophyllorchis and Triphora are organized in typical fashion as alternating groups of xylem and phloem cells arranged around the periphery of the axis. However, in the roots of Psilochilus, the xylem forms a continuous ring, rather than isolated in discrete patches alternating with clusters of phloem cells. In summary, although the anatomy of Triphorinae is generally unremarkable, several features stand out: buttressed root hairs of Monophyllorchis, unusual internal origin of root hairs in Psilochilus atypical circumferential band of

metaxylem in roots of this same genus, and tubercular stem and root surfaces of Triphora.

Recent cladistic analyses of rbcL sequences indicate a sister-group relationship between Diceratostele and the three genera in Triphorinae (Dressler, 1979; Cameron et al., 1999; Chase et al., 2003). On the basis of these molecular data and following Szlachetko (1995), Pridgeon et al. (2005) assigned Diceratostele subtribal rank within Triphoreae. Although there are no anatomical synapomorphies shared betwee Diceratostele and the three genera of Dressler's (1990) Triphoreae, our observations support the placement of Diceratostele in its own subtribe, Diceratostelinae. Diceratostele (Stern et al., 1993), with its adaxial and abaxial sclerenchyma bundle caps in leaf veins, stegmata associated with these fibres, stegmata related to cortical fibre bundles in stems and thick-walled exodermal cells in roots, make this genus anatomically anomalous in Triphoreae. Dressler (1993) considered Triphoreae (Monophyllorchis, Psilochilus and Triphora) among his 'primitive' epidendroids containing few derived features. The apparent anatomical simplicity noted in these three genera supports his hypothesis that they are a 'distinctively primitive group,' although difficult to place. The only derived anatomical features presen in this relatively unremarkable group are the internally derived root hairs, circumferential metaxylem in roots and tetracytic/anisocytic stomatal patterns in Psilochilus; buttressed root hairs in Monophyllorchis; and tubercular stem and root surfaces in Triphora.

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