VEGETATIVE ANATOMY OF CALYPSOEAE (ORCHIDACEAE)

WILLIAM LOUIS STERN¹ & BARBARA S. CARLSWARD²,³

¹Department of Biological Sciences, Biscayne Bay Campus, MSB 357, Florida International University, North Miami, Florida 33181, USA
²Department of Biological Sciences, Eastern Illinois University, Charleston, Illinois 61920-3099, USA
³Corresponding author: bscarlsward@eiu.edu

ABSTRACT. Calypsoeae represent a small tribe of anatomically little-known orchids with a wide distribution in the Western Hemisphere. Leaves are present in all genera, except Corallorhiza and Wullschlaegelia both of which are subterranean taxa. Stomata are abaxial (ad- and abaxial in Aplectrum) and tetracytic (anomocytic in Calypso). Fiber bundles are absent in leaves of all taxa examined except Govenia tingens. Stegmata are present in leaves of only Cremastra and Govenia. Roots are velamentous, except in filiform roots of Wullschlaegelia. Vegetative anatomy supports a relationship between Wullschlaegelia and Corallorhiza but does not support the grouping of winter-leaved Aplectrum and Tipularia nor proposed groupings of genera based on pollinarium features.

ADDITIONAL KEYWORDS: Leaf, stem, root, fiber bundle, velamen, stegmata

Introduction

Calypsoeae (Camus) Dressler [Corallorhizinae in Dressler (1981) except Calypso] is a small tribe consisting of approximately 12 genera and 62 species (Freudenstein 2005). Plants are terrestrial, mostly cormous and/or rhizomatous, leafless and rootless in some genera, and usually mycorrhizal. Several of these genera are monotypic or oligotypic, Govenia being the richest with about 30 species. Plants are distributed widely from Europe, northern Asia, and North America to tropical Central America and the Caribbean, Brazil, and Argentina. Calypsoeae are absent from Africa, Australia, and islands of the East Indies and Pacific Ocean. Yoania australis, a New Zealand endemic, was renamed Danhatchia australis by Garay and Christenson, and placed in Cranichideae (Freudenstein 2005).

Little is known of the anatomy of the groups and except for Corallorhiza (Carlsward & Stern 2008), Wullschlaegelia (Stern 1999), and to a certain extent Yoania (Campbell 1970), only brief mention is made of anatomy for Aplectrum by Holm (1904), Soleder & Meyer (1930), Porembski & Barthlott (1988); for Oreorchis by Porembski & Barthlott (1988); for Tipularia by Holm (1904), Soleder & Meyer (1930), Porembski & Barthlott (1988); and for Wullschlaegelia by Johow (1885), Soleder & Meyer (1930), Møller & Rasmussen (1984); Govenia by Prideon, Stern & Benzing (1983), Porembski & Barthlott (1988); for Oreorchis by Porembski & Barthlott (1988); for Tipularia by Holm (1904), Soleder & Meyer (1930), Porembski & Barthlott (1988); and for Wullschlaegelia by Johow (1885), Soleder & Meyer (1930).

The rhizome of Yoania australis is covered with scale leaves, but the plant lacks expanded, chlorophyllous leaves (Campbell 1970) as do Corallorhiza and Wullschlaegelia. The rhizome has short conical projections bearing long hairs, resembling Corallorhiza and Cremastra. There is a parenchymatous cortex bounded internally by an endodermis with Casparian strips. Vascular tissue consists of two to six collateral bundles embedded in parenchyma. Among these genera, the only anatomical features of any substantive value are the foliar stegmata in Cremastra and Govenia reported in this study and the spiranthosomes in cortical parenchyma cells from fusiform roots of Wullschlaegelia, noted by Stern (1999). Other characters occur widely in Orchidaceae.

Two groups of Calypsoeae have been identified based upon the origin of the stalk supporting the pollinia and molecular data (Freudenstein 2005).
Pollinia in *Aplectrum*, *Cremastra*, *Corallorhiza*, *Govenia*, and *Oreorchis* are attached to a stalk (stipe) derived from the apex of the rostellum, i.e., a hamulus. Pollinia in *Calypso*, *Yoania*, and possibly *Changnienia*, are regular, i.e., the stalk is derived from tissues of the anther bed on the column. There is no stalk supporting the pollinia of *Wullschlaegelia*. Along with *Tipularia*, the last three genera also have spurred lips. Winter leaves appear in *Aplectrum* and *Tipularia*, genera that occur in deciduous woodlands, and depend upon sunlight reaching leaves during winter. This feature may also be indicative of a close relationship between these two genera (Freudenstein 2005).

**Material and methods**

We had available for study representatives of seven of the 12 genera included in Calypsoeae by Freudenstein (2005). *Corallorhiza* and *Wullschlaegelia* are treated in two other publications (Stern 1999, Carlsward & Stern 2008). Binomials and authorities, abbreviated according to Brummitt and Powell (1992), representing these genera appear in Table 1, along with organs available for study. Methods and descriptive conventions have appeared in recently published papers (e.g., Stern & Carlsward 2006), and we have followed similar procedures here. “Periclinal” and “anticlinal” have been used as shorthand for “periclinally orientated” and “anticlinally orientated.” Plant parts were preserved in FAA (70% ethanol-glacial acetic acid-commercial formalin, 9.0:0.5:0.5) and stored in 70% ethanol. 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 hematoxylin, 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 are given in Table 2.

**Anatomy results**

LEAF — Surface: HAIRS absent in *Aplectrum*, *Cremastra*, and *Govenia tingens*. In *Calypso* two-celled thin-walled hairs ad- and abaxial, the base embedded among cells in a nest of small epidermal cells; adaxial: basal cell clear, apical cell bulbous, darkly staining (Fig. 1A); abaxial: basal cell much shorter than blunt-tipped darkly staining, elongated apical cell (Fig. 1B). Hairs ad- and abaxial, two-celled in *Govenia superba*, thin-walled arising from a cluster of small epidermal cells; apical cell blunt-tipped. *Tipularia* has two-celled (?) hairs on both leaf surfaces arising from a group of small epidermal cells. Hair bases embedded among epidermal cells. STOMATA abaxial, except ad- and abaxial in *Aplectrum*. Occasionally a few stomata may appear adaxially on leaves in taxa that normally bear abaxial stomata. Stomatal apparatus is

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Collector/Voucher</th>
<th>Parts available</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aplectrum hyemale</em> (Muhl. ex Willd.) Nutt.</td>
<td>B. Carlsward s.n./SEL</td>
<td>L, S, R, Rh, C</td>
</tr>
<tr>
<td><em>Calypso bulboasa</em> (L.) Oakes</td>
<td>R. Halse 7141/OSC</td>
<td>L, S, R, C</td>
</tr>
<tr>
<td><em>Corallorhiza maculata</em> (Raf.) Raf.</td>
<td>K. Chambers 5597/OSC</td>
<td>Rh</td>
</tr>
<tr>
<td><em>C. odontorhiza</em> (Willd.) Nutt.</td>
<td>M. W. Morris &amp; R. Carter 4098/FLAS</td>
<td>Rh</td>
</tr>
<tr>
<td><em>C. wisteriana</em> Conrad</td>
<td>M. W. &amp; M. S. Morris 4102/FLAS</td>
<td>Rh</td>
</tr>
<tr>
<td><em>Cremastra appendiculata</em> (D. Don) Makino</td>
<td>R. Determann s.n./SEL</td>
<td>L, S, R, Rh, C</td>
</tr>
<tr>
<td><em>Govenia superba</em> (La Llave &amp; Lex.) Lindl.</td>
<td>Hort./SEL</td>
<td>L, S, R, C</td>
</tr>
<tr>
<td><em>G. tingens</em> Poepp. &amp; Endl.</td>
<td>W. M. Whitten 91284/FLAS</td>
<td>L, S, R, C</td>
</tr>
<tr>
<td><em>Tipularia discolor</em> (Pursh) Nutt.</td>
<td>W. L. Stern s.n./FLAS</td>
<td>L, S, R, C</td>
</tr>
<tr>
<td><em>Wullschlaegelia aphylla</em> (Sw.) Rchb.f.</td>
<td>R. L. Dressler 4940/FLAS</td>
<td>S, R</td>
</tr>
<tr>
<td><em>W. calcarata</em> Benth.</td>
<td>R. L. Dressler 4646/FLAS</td>
<td>S, R</td>
</tr>
<tr>
<td><em>W. calcarata</em> Benth.</td>
<td>M. G. Born 41/U</td>
<td>S, R</td>
</tr>
</tbody>
</table>

Table 1. Species of Calypsoeae studied. L, leaf; S, stem; R, root; Rh, rhizome; C, corm.
basically tetracytic in *Aplectrum*, *Cremastra*, *Govenia*, and *Tipularia* with a few anomocytic configurations. Lateral cells of the stomatal apparatus in *G. tingens* often elongated serpent-like to intruded between adjoining epidermal cells (Fig. 1C); in *Tipularia* lateral cells and sometimes apical cells protrude among other epidermal cells. In *Calypso*, stomatal apparatuses entirely anomocytic (Fig. 1D). Guard cells typically reniform and stomata parallel the long axis of the guard-cell pair. Average stomatal lengths range from 42-47 μm to 50-62 μm for different taxa. 

**Table 2. Stomatal dimensions (μm)**

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Average Length</th>
<th>Average Width</th>
<th>Range Length</th>
<th>Range Width</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aplectrum hyemale</em> ad.</td>
<td>45</td>
<td>40</td>
<td>42-47</td>
<td>35-45</td>
</tr>
<tr>
<td><em>Aplectrum hyemale</em> ab.</td>
<td>46</td>
<td>39</td>
<td>31-52</td>
<td>32-42</td>
</tr>
<tr>
<td><em>Calypso bulbosa</em></td>
<td>50</td>
<td>45</td>
<td>42-55</td>
<td>40-50</td>
</tr>
<tr>
<td><em>Cremastra appendiculata</em></td>
<td>42</td>
<td>35</td>
<td>37-47</td>
<td>27-37</td>
</tr>
<tr>
<td><em>Govenia superba</em></td>
<td>59</td>
<td>48</td>
<td>50-80</td>
<td>42-50</td>
</tr>
<tr>
<td><em>Govenia tingens</em></td>
<td>62</td>
<td>52</td>
<td>57-67</td>
<td>47-62</td>
</tr>
<tr>
<td><em>Tipularia discolor</em></td>
<td>58</td>
<td>45</td>
<td>50-62</td>
<td>37-50</td>
</tr>
</tbody>
</table>

42 μm in Cremastra to 62 μm in Govenia tingens and widths from 39 μm in Aplectrum to 52 μm in Govenia tingens. Epidermal cells polygonal on both surfaces; abaxial cells in Calypso may be elongated; walls straight-sided or curvilinear.

Section: CUTICLE smooth, somewhat granulose in Calypso; 2.5 μm to less than 2.5 μm thick. Epidermal cells mostly isodiametric to periclinal in Govenia and to a certain extent in Tipularia. STOMATA superficial; substomatal chambers large in Tipularia, Govenia, and Calypso; moderate in Aplectrum and Cremastra. Stomatal ledges usually poorly defined in section; outer ledges apparent in Aplectrum, Calypso, Cremastra, Govenia, and Tipularia but most pronounced in Aplectrum. Inner ledges obscure but apiculate in G. tingens and Tipularia. FIBER BUNDLES absent throughout, except in G. tingens. HYPODERMIS uniseriate adaxially, cells globose or inflated, sparsely provided with chloroplasts in Aplectrum; biseriate adaxially in Tipularia, outer layer of more or less inflated upright cells (Fig. 2A), inner layer cells isodiametric; cells of both layers rich in chloroplasts. Hypodermis absent in Calypso, Cremastra, and Govenia. MESOPHYLL homogeneous, 4-7 cells wide, cells thin-walled, mostly oval and circular with small triangular and polyhedral intercellular spaces; in Tipularia, cells and associated intercellular spaces are organized as in a eudicotyledon (Fig. 2A). Raphide-bearing idioblasts circular in TS (Fig. 2B), saccate, blunt-ended in LS. VASCULAR BUNDLES collateral in a single series. In larger vascular bundles of Aplectrum, Cremastra, and Govenia both xylem and phloem subtended by patches of thin-walled sclerenchyma. On the xylem side in Aplectrum and Govenia, these produce bulges (TS) resulting in ridges on the adaxial leaf surface (Fig. 2B) but not on the phloem side. Midvein in Aplectrum, Cremastra, and Govenia is subtended opposite phloem by a massive cluster of sclerenchyma cells creating a pronounced keel (Fig. 2B). Vascular bundles in Calypso not associated with sclerenchyma; in Tipularia sclerenchyma is associated only with the xylem. STEGMATA absent from Aplectrum, Calypso, and Tipularia but present in Cremastra and Govenia. Conical, rough-surfaced silica bodies in steigmata occur along sclerenchyma opposite xylem and phloem in Cremastra, along phloem sclerenchyma only in G. tingens. Bundle sheath cells circular, thin-walled, and chloroplast-bearing in all taxa; chloroplasts do not occur in some bundle sheath cells of Calypso.

STEM — Subterranean storage, perennating, and connective organs (rhizomes) several in Calypsoeae, as noted by Freudenstein (2005), and represented in our research materials. These organs correspond to cauline structure in that vascular bundles, containing adnate strands of xylem and phloem, are associated in a usually parenchymatous matrix. They are arranged in different configurations in contrast with root morphology where conductive tissues are typically concentrated in a central vascular cylinder. There are a rhizome and corm in Aplectrum and Cremastra and a corm in Calypso, Govenia, and Tipularia. The organizational pattern of each organ is described separately for each taxon.

Aplectrum rhizome — HAIRS none. STOMATA present, superficial, substomatal chamber large.
CUTICLE smooth to rugose, 2.5 μm thick. EPIDERMAL CELLS mostly isodiametric, subtended by a uniseriate HYPODERMIS in some areas; cells larger than epidermal cells but smaller than ground tissue cells. CORTEX none. ENDODERMIS discontinuous around the vascular cylinder; cells isodiametric, entirely thin-walled, lacking intercellular spaces; there are possibly Casparian strips. Presence of a PERICYCLE is questionable. GROUND TISSUE cells thin-walled, circular, oval, and variably shaped; cruciate starch grains in assimilatory cells; thin-walled, circular; enucleate water-storage cells scattered about, intercellular spaces triangular (Fig. 3A). Twenty-five to 30 widely separated VASCULAR BUNDLES distributed within the endodermis surrounded by a parenchymatous matrix of thin-walled, nucleated, small angular cells lacking intercellular spaces. SCLERENCHYMA and STEGMATA none.

Calypso corm — HAIRS two-celled, thick-walled, apical cell clavate, darkly-staining (Fig. 3B). STOMATA absent. CUTICLE smooth, 5.0 μm thick. EPIDERMAL CELLS squarish, isodiametric. HYPODERMIS uniseriate, cells tending toward periclinal. CORTEX, ENDODERMIS, PERICYCLE absent. GROUND TISSUE cells with thick and thin walls, oval, angular and circular; larger, enucleate water-storage cells and smaller, nucleated assimilatory cells lacking starch grains; intercellular spaces few. VASCULAR BUNDLES many, collateral, widely scattered. Xylem arcuate in some bundles, phloem centered in the arms of the arc. SCLERENCHYMA and STEGMATA none.

Cremastra rhizome — HAIRS emerge as tufts of unicellular strands from raised, truncate, pyramidal, multicellular cushions along the periphery of the stem (Fig. 3C). STOMATA and CUTICLE indistinguishable. EPIDERMAL CELLS squarish, isodiametric.
CORTEX many cells wide; cells crowded, various, angular, oval, circular, irregular; walls thickish; water-storage cells circular, empty. Cruciate starch grains in assimilatory cells. ENDODERMAL CELLS surrounding vascular bundles, entirely thin-walled, rectangular, square, some roundish, isodiametric, with Casparian strips (Fig. 3D). PERICYCLE discontinuous, cells like endodermal cells without Casparian strips. GROUND TISSUE of oval and angular thin- and thick-walled parenchyma cells. VASCULAR TISSUE organized as an irregular series of discontinuous arcs in which collateral vascular bundles are interspersed. SCLERENCHYMA and STEGMATA absent.

Cremastra corm — HAIRS and STOMATA absent. CUTICLE none. EPIDERMAL CELLS periclinal. ENDODERMAL CELLS angular, thin-walled, surrounding each vascular bundle. GROUND TISSUE with larger, almost circular, empty, water-storage cells and smaller, thin-walled, oval and variously shaped, assimilatory cells containing cruciate starch grains; intercellular spaces tiny, triangular, and various. VASCULAR BUNDLES many, collateral, scattered. SCLERENCHYMA and STEGMATA absent.

Govenia superba corm — HAIRS and CUTICLE absent. STOMATA present in G. tingens, subtended by small substomatal chambers. EPIDERMAL CELLS isodiametric. CORTEX, ENDODERMIS, PERICYCLE none. GROUND TISSUE: Large numbers of wide, circular, thin-walled water-storage cells surrounded by much smaller, thin-walled, oval, rectangular, square, and variously shaped assimilatory cells lacking starch grains. VASCULAR BUNDLES collateral, many, scattered. SCLERENCHYMA and STEGMATA absent.

Tipularia corm — HAIRS, STOMATA, and CUTICLE absent. STOMATA present in G. tingens, subtended by small substomatal chambers. EPIDERMAL CELLS isodiametric. CORTEX, ENDODERMIS, and PERICYCLE none. GROUND TISSUE of larger, circular, thick-walled water-storage cells and much smaller, irregularly shaped assimilatory cells with cruciate starch grains. Intercellular spaces absent. VASCULAR BUNDLES collateral, many, scattered. SCLERENCHYMA and STEGMATA absent.

Discussion

Aplectrum stands alone among Calypsoeae, owing to its combination of amphistomatous leaves, lack of foliar hairs, and absence of stegmata. Calypso, too, is unique with its exclusively anomocytic stomatal apparatuses, absence of vascular bundle sclerenchyma and lack of stegmata. Corallorhiza and Wullschlaegelia are characterized by their leaflessness and subterranean habit. Govenia tingens lacks foliar hairs and is the only taxon studied with foliar fiber bundles lined with stegmata. Wullschlaegelia has roots of two distinct kinds, fusiform and filiform. The latter lack a velamen. Cremastra lacks hairs on leaves but
has tufts of unicellular hairs that arise from cushions along the rhizome.

*Crémastra*, *Govenia*, and *Tipularia* have abaxial tetracytic stomata; *Calypso* has mostly abaxial, and a few adaxial anomocytic stomatal apparatuses. Foliar hairs are present on both surfaces in *Calypso*, *Govenia superba*, and *Tipularia*. A foliar hypodermis occurs in *Aplectrum* and *Tipularia*. Sclerenchyma appears along both xylem and phloem sides of vascular bundles in *Aplectrum*, *Crémastra*, and *Govenia*, but only on the xylem side in *Tipularia*. *Govenia* and *Tipularia* have the largest stomatal dimensions; measurements for the other taxa are substantially smaller (Table 2).

Freudenstein (2005) suggested that the appearance of winter leaves in *Aplectrum* and *Tipularia* may be evidence of a close relationship between these two genera. Indeed, among the taxa studied, only these two are represented by a foliar hypodermis consisting of enlarged, thin-walled, globose or expanded cells with chloroplasts. Evert (2006) observed that cells of the inner layer (s) of a multiple epidermis (i.e., a hypodermis) commonly contain few or no chloroplasts. Indeed, the cells of hypodermises sometimes appear to lack contents entirely, especially when they serve as water-storage cells. In the cases of winter leaves of *Aplectrum* and *Tipularia*, however, the hypodermal cells are chlorophyllous, especially in *Tipularia*. The hypotheses that establish two groups of genera in Calypsoeae, based upon origins of the pollen stipes and presence of spurred lips, cannot be substantiated by vegetative anatomy. Dressler’s Wullschlaegelieae (1980) and Lindley’s Corallorhizidae (1853) are groupings more congruent with our findings.
ACKNOWLEDGEMENTS. The authors thank Kenton L. Chambers, Oregon State University, for the superb specimen of Calypso bulbosa that he provided for our research. For various facilities and use of research equipment, we acknowledge with appreciation George Bowes and the Department of Botany, University of Florida. David Lee, formerly of Florida International University, provided laboratory space for the early phases of this research. The Kampong of the National Tropical Botanical Garden in Coconut Grove, Florida, provided laboratory room. Finally, we'd like to thank Alec Pridgeon, an anonymous reviewer, and J. Richard Abbott for their help with the manuscript.

LITERATURE CITED