Taxonomic value of foliar characters in *Dahlstedtia* Malme -
Leguminosae, Papilionoideae, Millettieae

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**ABSTRACT** – (Taxonomic value of foliar characters in *Dahlstedtia* Malme - Leguminosae, Papilionoideae, Millettieae). *Dahlstedtia* Malme (Leguminosae) is a neotropical genus, with two species recognized, *D. pinnata* (Benth.) Malme and *D. pentaphylla* (Taub.) Burk., although it has been considered a monotypic genus by some authors. Leaf anatomy was compared to verify the presence of anatomical characters to help delimit species. Foliar primordium, leaflet, petiolule, petiole, and pulvinus were collected from cultivated plants (Campinas, SP, Brazil) and from natural populations (Picinguaba, Ubatuba and Caraguatatuba, SP, Brazil *D. pinnata*). Studies on leaflet surface assessment (Scanning Electron Microscopy), as well as histology and venation analyses were carried out of dehydrated, fresh and fixed material from two species. Leaflet material was macerated for stomatal counts. Histological sections, obtained by free-hand cut or microtome, were stained with Toluidine Blue, Safranin/Alcian Blue, Ferric Chloride, Acid Phloroglucin. Secretory cavities are present in the lamina, petiolule, petiole and pulvinus. This study, associated with other available data, supports the recognition of two species in the genus *Dahlstedtia*.

**Key words**: cambial activity, *Dahlstedtia pinnata*, *Dahlstedtia pentaphylla*, parenquima paravenal

**Introduction**

The genus *Dahlstedtia* Malme (Leguminosae, Papilionoideae) belongs to tribe Millettieae (Geesink 1984), with two described species: *D. pinnata* (Benth.) Malme (type species) and *D. pentaphylla* (Taub.) Burk. Species descriptions are confused as regards floral morphology and even Burkart (1957), who recognized two species, cited specimens of one species under the other (see Malme 1905 and Burkart 1957). Some authors, such as Hutchinson (1964), Geesink (1981) and Vanni & Rodríguez (1999) considered the genus monotypic.

* Dahlstedtia* species have been recorded from Brazil, especially the Atlantic Forest. Their representatives are shrubs or trees, with conspicuous

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papilionate flowers. The corolla of \textit{D. pinnata} is pink and of \textit{D. pentaphylla} reddish, besides the presence of conspicuous lenticels on the floral branches of \textit{D. pentaphylla} and their absence in \textit{D. pinnata} (Teixeira & Gabrielli 2000).

The present study compared anatomiically the leaves of \textit{D. pinnata} and \textit{D. pentaphylla}, to seek characters that can help distinguish between the species. These characters were then related to the other data available for the genus.

### Material and methods

Foliar primordium, leaflet, petiolule, petiole and pulvinus of both species were collected from cultivated plants in Campinas, SP, Brazil. Material of \textit{Dahlstedtia pinnata} from natural populations was collected at “the State Park of Serra do Mar”, Picinguaba, Ubatuba and Caraguatatuba, State of São Paulo, Brazil and of \textit{D. pentaphylla} at “Serra da Graciosa”, Antonina, State of Paraná, Brazil. The vouchers are deposited in the Herbarium of Universidade Estadual de Campinas (UEC), São Paulo State, Brazil, under the numbers 28637, 28746, 300 and 17936.

The following herbarium material was studied:

- \textit{D. pentaphylla}: M.L. Sekiama \textit{et al}. 6610 (UEC), D.A. Great \textit{et al}. 17 (UEC), N. Figueiredo \textit{et al}. 14713 (UEC), P.H. Davis \textit{et al}. 2934 (UEC); G. Arbócz 32707 (UEC).

The material from herbarium specimens was treated according to Smith & Smith (1942). Whole leaflets were cleared and stained according to Berlyn & Miksche (1976) modified by Monteiro \textit{et al}. (1979) for venation studies.

Histological sections from herbarium specimens after treatment and from fresh material were stained with Toluidine Blue or Alcian Blue/Safranin and mounted in glycerin jelly. Fixed material was submitted to the standard techniques of infiltration with paraffin, stained with Safranin and Alcian Blue and the slides mounted in synthetic resin (Gerlach 1969).

To detect phenolic compounds and lignin, free hand sections were treated with Ferric Chloride and Acid Phloroglucin, respectively (Johansen 1940). The chemical composition of crystals was verified by 10% chloric acid, glacial acetic acid and sulfur acid (Chamberlain 1932 apud Arduin & Krauss 1997).

Leaflet material was macerated following Jeffrey’s method (Johansen 1940; Foster 1949) for stomatal counts. The Kolmogorov-Smirnov test was used (Zar 1996) to evaluate the significance of the difference found in the number of stomata/cm$^2$ of the foliolar lamina.

Photomicrographs were taken using a Zeiss II fotomicroscope and diagrams using a camera lucida.

Small pieces of leaflets were dehydrate in an ethanol series followed by critical-point drying in a Balzers CPD 030 apparatus; the specimens were mounted on aluminum stubs with colloidal carbon and coated with gold in a Balzers SCD 050 sputter coater for 280 s. The samples were observed with a Jeol JSM 5200 scanning electron microscope at 15 kv, coupled with a Sinar 67 camera.

The terminology of Hickey (1973) was followed to describe the leaf architecture.

### Results

The leaves are imparipinnate, alternate, pulvinate, with reduced or caducous stipules, opposite leaflets, 5-7-9 in \textit{D. pinnata} and 5-7 in \textit{D. pentaphylla}. The leaflets are symmetrical, narrowly elliptic, with attenuate apices, cuneate bases and entire margins (Fig. 1-2). Leaflet sizes vary from 9-13 cm long to 4-6 cm wide; the petiolule is 3-5 cm long; the petiole is 15-25 cm long; the rachis is 6,5-10,9 cm long and the pulvinus is 0,5-1,2 cm long. The larger sizes of leaf, petiolule, petiole, rachis and pulvinus were found in \textit{D. pinnata}.

Leaflet surface is smooth in both adaxial (Fig. 3, 5) and abaxial sides (Fig. 4, 6), with wax deposits of

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several forms (Fig. 3-6). No trichomes are observed. Venation is pinnate accompanied by brochidodromous secondary venation, with midvein of moderate size, following a straight, unbranched course (Fig. 7). The position of the first point of primary vein radiation is basal with moderate acute and nearly uniform angle of divergence (Fig. 1, 2, 7). Loop-forming branches of secondary veins join superjacent secondaries at moderate acute angles forming orthogonal reticulate pattern (Fig. 1, 2, 7). Secondary

Figures 3-6. Leaflet surface of *Dahlstedtia* Malme species (MEV micrographs). Adaxial (3) and abaxial (4) sides of *D. pinnata* (Benth.) Malme. Adaxial (5) and abaxial (6) sides of *D. pentaphylla* (Taub.) Burk. Bars = 3, 4 = 10 µm; 5, 6 = 50 µm.
veins follow a curved abruptly course (Fig. 7). Tertiary veins originating from secondary veins at right angles also form orthogonal reticulate pattern (Fig. 8, 9). The higher vein orders are distinct and also show orthogonal reticulate pattern (Fig. 8, 9). Ultimate veins branch once and the marginal ultimate venation is fimbriate (Fig. 8). Areoles are well-developed, pentagonal and large (1-2 mm) (Figs. 8, 9). Glands are present in D. pentaphylla (Fig. 9) and absent in D. pinnata.

Leaflet stomata are paracytic (Fig. 10) and occur only abaxially (Fig. 4). The number of stomata/cm² is 134 ± 30 (mean ± standard deviation) for D. pentaphylla and 125 ± 29 (mean ± standard deviation) for D. pinnata. The Kolmogorov-Smirnov test is 0.258 **p << 0.01, indicating that the difference is significant.

Mesophyll is dorsiventral with approximately four layers (Fig. 11). The median layer has conspicuous spongy parenchyma cells (Fig. 11-12), with fewer chloroplasts than the other layers. These cells are horizontally enlarged; in paradermal sections, they are lobed and have conspicuous intercellular spaces (Fig. 12).

Secretary cavities (Fig. 9, 13-14) are only recorded for Dahlstedtia pentaphylla, and are characterized by a lumen surrounded by a one-layered epithelium. They are present in the palisade and bundle parenchyma in the whole leaflet lamina, in the cortical parenchyma of the petiolo and petiole, and in the peripheral parenchyma of the pulvinus (Fig. 14).

Secretary and tector trichomes occur throughout the epidermis at several developmental stages in the foliar primordium. The tector trichomes are uniseriate and falcate. The secretary trichomes (Fig. 13) vary in size, because the head can have four to six cells and the peduncle, two to three cells. The head cells have more evident nuclei, granular content and are more stained than the other trichome cells. No trichomes are present in the adult leaf.

The central bundle shape (Fig. 15-19) and the sclerenchyma structure (Fig. 15-19) vary along the foliar organs (Table 1). A vascular cambium (Fig. 20) is present in the petiolo, petiole and pulvinus (Table 1), originating a secondary structure in the vascular system. The xylem fibres increase in number and the rays are already formed (Fig. 21-22).

Crystalliferous and phenolic idioblasts occur along the leaf (Table 1). The crystals are prismatic (Fig. 23) and of calcium oxalate, and occupy the cell lumen almost completely. Both types of cells are very long, and form chains, when observed in longitudinal sections. Only in Dahlstedtia pinnata crystalliferous idioblasts are located in the medulla of the petiole.

**Discussion**

In comparison with the vegetative axis (Teixeira & Gabrielli 2000), more useful characters are provided from leaves to distinguish between the two species of Dahlstedtia. The presence of secretary cavities in D. pentaphylla and their absence in D. pinnata was an important character for diagnosis of the species (Table 2). Secretary cavities were similarly located in all the examined representatives of D. pentaphylla, and they are an easily observed character for species identification with the use of free hand sections. Clarified leaflets should be used carefully, because the presence of conspicuous spaces in the mesophyll of D. pinnata may be confused with glandular punctuations, as in Brazilian Lonchocarpus Kunth. species (Teixeira et al. 2000).

Secretary cavities have already been observed in the leaves of genera related to Dahlstedtia, such as Lonchocarpus and Derris Lour. (Solereder 1908; Metcalfe & Chalk 1950; Teixeira et al. 2000), and they have been studied in detail in tribes Amorpheae and Psoraleae (Turner 1986), and cited in Poiréa Vent. (Müller unpublished), Zornia J.F. Gmel. (Kothari & Shah 1975), Myrocarpus frondosus Allemão, Myroxylon peruiferum L. f. (Freire 1984) and Poincianella Britton & Rose (Lersten & Curtis 1994). Secretary structures have been used in taxonomic studies due to their variety of morphological types and their constant position in the plant body and taxon analyzed (Solereder 1908; Metcalfe & Chalk 1950; Fahn 1979). In legumes, secretary cavities were used, at subgeneric level, to define a group of species in Poincianella (Lersten & Curtis 1994) and at suprageneric level, to maintain the “status” of tribes Amorpheae and Psoraleae (Turner 1986).

Other leaf characters deserve comments: the number of stomata and the distribution of crystalliferous idioblasts. The differences found in the stomata number/cm² between the two species of Dahlstedtia were significant (Kolmogorov-Smirnov test). Despite the similarity of the morphology and type of crystal between species, there was difference in the distribution of crystalliferous idioblasts in the petiole (Table 2). Therefore, these characters may also be informative for the delimitation of these species.

The presence of glandular trichomes and idioblasts containing phenolic compounds is a similarity in both

Bars = 15 = 125 µm; 16 = 500 µm; 17-19 = 200 µm.
Table 1. A comparison of anatomic characteristics in the leaf lamina, petiolule, petiole and pulvinus of *Dahlstedtia* Malme species.

<table>
<thead>
<tr>
<th>Leaf parts</th>
<th>Central bundle shape</th>
<th>Sclerenchyma</th>
<th>Cambial activity</th>
<th>Distribution of crystalliferous idioblasts</th>
<th>Distribution of phenolic idioblasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf lamina</td>
<td>closed (Fig. 15)</td>
<td>ring of lignified fibres (Fig. 15)</td>
<td>-</td>
<td>parenchyma tissue of the larger bundles</td>
<td>among the phloem parenchyma cells and the central bundle</td>
</tr>
<tr>
<td>Petiolule</td>
<td>open with curved edges (Fig. 16)</td>
<td>ring of gelatinous fibres (Fig. 16)</td>
<td>+</td>
<td>subepidermal cells in the perivascular tissue</td>
<td>among the phloem and central parenchyma cells</td>
</tr>
<tr>
<td>Petiole</td>
<td>closed (Fig. 17)</td>
<td>grooves of lignified fibres (Fig. 17)</td>
<td>+</td>
<td>among the collenchyma and the parenchyma</td>
<td>among the phloem and central parenchyma cells</td>
</tr>
<tr>
<td>Pulvinus upper half</td>
<td>closed and very grooved (Fig. 18)</td>
<td>arc of lignified fibres (Fig. 18-19)</td>
<td>+</td>
<td>subepidermal and perivascular tissues</td>
<td>phloem parenchyma cells</td>
</tr>
<tr>
<td>lower half</td>
<td>open and enlarged (Fig. 19)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

species as are the characteristics of dorsiventral mesophyll, hypostomatic leaflet and paracytic stomata.

The vascular system of the pulvinus, the petiole, the petiolo and the leaf lamina (see Fig. 15-19) are similar in *D. pinnata* and *D. pentaphylla*, although the vascular system patterns of the petiole in Leguminosae are variable at tribal, generic or specific level (Watari 1934; Dormer 1945). The petiole of *Dahlstedtia* is hold by the medium and lateral traces, while the stipules are hold by the lateral ones.

The median layer of the mesophyll observed in *Dahlstedtia* is similar to a very developed spongy parenchyma. Morphologically, this tissue can be compared to the paraveinal mesophyll (Fisher 1967) or extended bundle sheath system, the most recent designation (Kevekordes et al. 1988). The extended bundle sheath system has already been described in 52 legume species (Kevekordes et al. 1988) and characterized as one-layered, horizontally displayed and with lobed cells that occur between the spongy and palisade parenchyma (Solereder 1908; Metcalfe & Chalk 1950; Fisher 1967), of procambial origin (Weston & Cass 1973). Despite the morphological similarity, this tissue deserves developmental studies to clarify its origin in *Dahlstedtia*.

Cambial activity in the petiolo, petiole and pulvinus of *Dahlstedtia* species was confirmed by the presence of a radial structure observed in radial sections. Most reports of cambial activity in leaves refer to Gymnosperms (Mauseth 1988). The few dicotyledon species presenting cambial activity in the leaves, *Laurocerasus officinalis* Roem., *Ligustrum vulgare* L., *Ulmus montana* With. and *Viburnum rhytidophyllum* Hemsl., were studied by Shtromberg (1959).

This leaf anatomical study, associated to other available data (see table 2), agrees with Burkart’s taxonomic position (1957), according to which *Dahlstedtia* comprises at least two species: *D. pinnata* and *D. pentaphylla*.

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