Novelties in secretory structures and anatomy of Rhynchosia (Fabaceae)

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Manuscript received on September 13, 2013; accepted for publication on April 3, 2014

ABSTRACT
A comparative anatomical study was carried out on the secretory structures of leaflets from taxa belonging to the genus Rhynchosia - taxa difficult to delimit because of uncertain interspecific relations - in order to evaluate the potential diagnostic value of these anatomical traits for taxonomic assignment. A further objective was to establish consensual denomination for these secretory structures. The new anatomical features found in these taxa were sufficiently consistent to separate the species evaluated. The presence and localization of glandular-punctate structures bulbous-based trichomes, the number of layers in the palisade parenchyma and the arrangement of vascular units distinguish the taxa investigated and these characteristics can be extended to other species of Papilionoideae. The trichomes analyzed were described and classified into five types. Depicted in diagrams, photomicrographs, and by scanning electron microscopy, and listed for the first time at the genus and species levels. The information obtained served to effectively distinguish the taxa investigated among species of Papilionoideae.

Key words: leaflets, legume, taxonomy, trichomes.

INTRODUCTION
Investigated in several studies, secretory structures have contributed to the taxonomy of Fabaceae at various taxonomic levels (Lackey 1978, Lersten and Curtis 1994, 1995, Teixeira et al. 2000, Fortuna-Perez et al. 2012). Despite a consensus among these researchers on the importance of these secretory structures in Papilionoideae, only Solereder (1908) has pointed out that, of the 28 tribes in this subfamily, six exhibit these structures, which substantially facilitates genus separation. Nonetheless, no detailed micromorphological studies have been published addressing this feature, and appropriate terminology for these structures is lacking.

Readily seen with the naked eye on leaflets of Rhynchosia Lour. (Fabaceae – Papilionoideae), these structures are regarded as important traits in anatomical investigations (Lackey 1978, Grear 1978), complementing and supporting infrageneric taxonomic studies. Distributed in tropical and subtropical areas in both hemispheres, the genus Rhynchosia comprises 230 species (Grear 1978), grouped into two sections and six series: Copisma, comprising four series, and Arcyphyllum, with two (Grear 1978). Challenges circumscribing and identifying Rhynchosia species have been reported by Grear (1978), Fortunato (2000), and Rogalski and Miotto (2011).

According to Grear (1978) and Cristaldo et al. (2012), taxa can be mistakenly lumped together...
into a single section or assigned to different sections - e.g. Rhynchosia balansae Micheli var. balansae (Arcyphyllum series 6) and Rhynchosia balansae Micheli var. psilantha Fortunato, both assigned to Arcyphyllum series 6 based on leaflet shape and voluble habit of stem tips; Rhynchosia edulis Griseb (Copisma series 1), Rhynchosia melanocarpa Grear (Copisma series 2), and Rhynchosia minima (L.) DC. (Copisma series 3), given their similar habits, glands, and leaflet coloring; and Rhynchosia corylifolia Mart. ex Benth (Arcyphyllum series 5) and R. leucophylla Benth. (Arcyphyllum series 6), given the shape and whitish tomentose indumentum of leaflets (Grear 1978, Cristaldo et al. 2012).

Macroscopic examination of leaflets reveals glandular-punctate and bulbous-based trichomes (Polhill et al. 1981, Fortunato 2000, Cristaldo et al. 2012). Drawing on anatomical features, these structures were confirmed as secretory organs by Lackey (1978) and assigned diagnostic value for certain species. To date, however, few species in this genus have been evaluated in comparative anatomical studies.

No phylogenetic studies on the genus have been conducted and few anatomy reports on the Rhynchosia leaflets are available - e.g. Solereder (1908) and Metcalfe and Chalk (1950, 1979, 1983), along with a single investigation (Lackey 1978) of secretory structures of R. densiflora (Roth.) DC, R. phaseoloides (Sw.) DC, and R. reticulata (Sw.) DC.

The purpose of this study was to evaluate the secretory structures and anatomy of Rhynchosia, so as to establish taxon separation by addressing circumscription problems. To this end, sampling included one taxon from each series in the genus.

MATERIALS AND METHODS

Rhynchosia is a genus found in savannas, prairies, scrublands, rocky highlands, pastures, forest interiors and edges, and humid slopes (Grear 1978). Leaflets from seven taxa of Rhynchosia were obtained from herborized materials (see Appendix). Three apical leaflets were evaluated in each taxon sampling.

The samples were rehydrated in glycerin, washed several times in distilled water (Smith and Smith 1942), serially dehydrated in ethanol, and stored in 0.7 ethanol (Kraus and Arduin 1997).

Sections were cut across the leaf blade of the terminal leaflet. Cross-sections of the leaflets in the apical, mid- and basal portions of the midrib were obtained by freehand sectioning using a steel blade and were stained with Astra blue and safranin, based on Kraus and Arduin (1997).

The documentation was prepared with the aid of a photonic microscope with a camera lucida attachment, using projected micrometric scales. Five replicates of measurements were made for each trichome type in each taxon using an appropriate program and a Leica photomicrography optical microscope.

For scanning electron microscopy, 1 cm² samples in 0.7 ethanol were critical-point dried using carbon dioxide, fixed on the sample holder, and coated with a fine layer of gold (Denton Vacuum Desk IV, LLC). The samples were analyzed and photomicrographs were obtained using a device (JEOL JSM-6380LV) from the Multiuser Scanning Electron Microscopy Laboratory of the Universidade Federal de Mato Grosso do Sul at Campo Grande.

RESULTS

The results obtained indicate that several anatomical traits are variable to all taxa (Table I) and thus of diagnostic value down to the generic or specific level. Observed in cross-section, the epidermis is uniseriate and variable in all taxa evaluated (Table I). The outline of epidermal cells in all species is sinusous on the abaxial surface and irregular in the adaxial portion of the leaflet blade (Figs. 1 - 2).

Leaflet midrib varies across taxa (Figs. 3 - 9), e.g. the shape and number of the vascular bundle and the collenchymas and sclerenchymas distribution.
**TABLE I**
Distribution of mesophyll tissues and secretory trichomes in leaflets of *Rhynchosia* species.

<table>
<thead>
<tr>
<th>Secretory structures</th>
<th>Shape epidermal cells</th>
<th>Shape vascular bundle</th>
<th>Collenchyma midrib</th>
<th>Sclerenchyma midrib</th>
<th>Number vascular units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ad S E B BB</td>
<td>Ab S E B BB</td>
<td>Ab</td>
<td>Ad</td>
<td>Ab</td>
</tr>
<tr>
<td><em>R. balansae</em> var. balansae</td>
<td>+ + - - + + + -</td>
<td>Rectangular</td>
<td>Rectangular</td>
<td>Oval</td>
<td>+</td>
</tr>
<tr>
<td><em>R. balansae</em> var. psilantha</td>
<td>+ - - + + + -</td>
<td>Ellongated</td>
<td>Ellongated</td>
<td>Narrow oval</td>
<td>-</td>
</tr>
<tr>
<td><em>R. minima</em></td>
<td>+ + + + + + + +</td>
<td>Cubic</td>
<td>Ellongated</td>
<td>Oval</td>
<td>+</td>
</tr>
<tr>
<td><em>R. edulis</em></td>
<td>+ + - + + + + +</td>
<td>Cubic</td>
<td>Ellongated</td>
<td>Elliptical</td>
<td>+</td>
</tr>
<tr>
<td><em>R. melanocarpa</em></td>
<td>+ + - + + + + +</td>
<td>Rounded</td>
<td>Rounded</td>
<td>Elliptical</td>
<td>+</td>
</tr>
<tr>
<td><em>R. corylifolia</em></td>
<td>+ + - + + + + -</td>
<td>Ellongated</td>
<td>Cubic</td>
<td>Irregularly elliptical</td>
<td>-</td>
</tr>
<tr>
<td><em>R. leucophylla</em></td>
<td>+ + - - + + + -</td>
<td>Ellongated</td>
<td>Cubic</td>
<td>Orbicular</td>
<td>+</td>
</tr>
</tbody>
</table>

Ab: abaxial surface; Ad: Adaxial surface; S, E, B, BB: trichome types. S: Spherical capitate trichomes, with circular apical cells, have unicellular stalks; E: Ellipsoid capitate trichomes have oblong shaped heads, and unicellular stalks; B: Bulky capitate trichomes, large terminal secretory cells, arranged in epidermal depressions; BB: Bulbous-based trichomes with cells apically elongated and terminal cells slightly rounded, basal cells large; C: Continuous sclerenchyma midrib; D: discontinuous sclerenchyma midrib. +: Presence; -: absence.

**Figures 1 - 2** - Photomicrographs with details of the leaflet blade of *Rhynchosia*, superficial view, evidencing abaxial and adaxial surfaces, respectively, of the leaflets. Fig. 1: Epidermal cells with shape slightly sinuous in *R. minima*. Fig. 2: Epidermal cells with shape irregular in *R. edulis*. Non-glandular trichomes (White arrow), secretory trichomes (asterisk) and base of non-glandular trichomes (black arrow).

(Table I). Papillose epidermal cells are seen at midrib level, except in *R. corylifolia*.

Secretory and non-glandular trichomes were distributed on the blade of all taxa evaluated (Figs. 10-18). Non-glandular trichomes are single, unicellular, and non-branched, consisting of one to three cells, with cubic base cells and elongated apical cells (Figs. 17 and 18).

Secretory trichomes (Table I; Figs. 10-16) can be differentiated according to shape and size, and
can be classified into four types. Capitate types (Figs. 10-14) have multicellular heads and uni- or multicellular stalks, bulbous base type (Figs. 15 and 16) has a rounded base consisting of apically elongated cells.

The capitate trichomes in this study are very variable in head shape and can be subdivided into three types facilitating their classification: spherical, ellipsoid and bulky capitate trichomes.

Spherical capitate trichomes, with circular apical cells, have unicellular stalks (Figs. 10, 11 and 19). Ellipsoid capitate trichomes have oblong shaped heads and unicellular stalks, differing from typical capitate trichomes (Figs. 12 and 21). These trichomes occurred on the leaflets of all taxa investigated. Bulky capitate trichomes (Figs. 13, 14, 23-28) are characterized by a group of large secretory terminal cells partly arranged below the level of other epidermal cells, sometimes connected to vascular units by a parenchymal sheath, on the epidermis of the abaxial surface of all species, and also on the adaxial surface of *R. minima*.

Figures 3 - 9 - Diagrams of the midrib cross-section, evidencing the distribution of tissues of *Rhynchosia* species.
Bulbous-based trichomes, are another type of secretory trichome (Figs. 15, 16, 29-32), these have apically elongated cells and slightly rounded terminal cells (Fig. 32) and are characterized by a group of large basal cells forming an ample, rounded secretory structure known as the bulbous base. These trichomes range from 96 to 202 µm (131 ± 54.88) in height and from 16 to 33 µm (25 ± 6.803) in width and are distributed on the epidermis of both blade surfaces, except in R. balansae var. balansae and R. balansae var. psilantha. These trichomes were unevenly distributed in the species evaluated and were absent from the apical and basal portions of R. corylifolia and R. leucophylla leaflets (Table I).

Figure 10 - 18 - Diagrams of the types of trichomes occurring in leaflets of Rhynchosia species. Fig. (10, 11) Spherical capitate trichomes: apical cells semicircular and stem unicellular; (12) Ellipsoid capitate trichomes: oblong, head spherical and stem; (13 and 14) Bulky capitate trichomes: terminal secretory cells large, arranged in epidermal depressions; (15 and 16) Bulbous-based trichomes: cells apically elongated and terminal cells slightly rounded, basal cells large; (17 and 18) Non-glandular trichomes. Bar 30µm.
Figure 19 - 28 - Details of spherical and ellipsoid capitates trichomes (black arrow) and bulky capitates trichomes (asterisk) inserted in epidermal depressions (black arrow), evidenced in Rhynchosia species. 19. R. balansae var. psilantha; 20. R. minima; 21. R. corylifolia; 22. R. edulis; 23. R. leucophylla; 24 and 25. R. minima; 26 and 27; R. melanocarpa; 28. R. edulis.
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Figures 29 - 32 - Bulbous-based trichomes evidencing the rounded base (black arrow), cells apically elongated (key) and terminal secretory cell (white arrow) in species 29-32. *R. edulis*.

Capitate trichomes of spherical and ellipsoid types (Figs. 10-12, 19, and 21) ranged from 15 to 42 µm (25.31 ± 7.92) in height and from 14 to 18 µm (15.84 ± 1.45) in width, and were smaller than bulky capitate trichomes (vesicular glands) (Figs. 13, 14, 20, and 22-28). They varied from 20 to 67 µm (44.94 ± 12.13) in height and from 18 and 57 µm (37.31 ± 9.36) in width, with wide variations in shape.

Non-glandular trichomes with one to three cells (Figs. 17, 18, and 33-36) were homogenously distributed on the blade in all species, and measured from 64 to 296 µm (139.7 ± 54.7). Their apical cell is consistently larger than basal cells. When inserted at the epidermis level, the apical cell is larger than and distinct from adjacent cells.

DISCUSSION

Papillose epidermal cells are evident in the midrib region of the leaf epidermis, except in *R. corylifolia*, and are present on the abaxial leaflet surface in all taxa, representing an important taxonomic trait for the group of species investigated. Papillose cells are also an additional useful trait for separating species of *Dalbergia* L. (Farooqui et al. 1989) and from those of *Myrocarpus* Allemão, *Myroxylon* L.f. or *Myropermum* Jacq. (Sartori and Tozzi 2002).

Marked vascular system variation, particularly in the sclerenchymatous sheath, differentiates *Rhynchosia* species. Variable arrangement of the sclerenchymatous sheath in species of *Tephrosia*, *Alysicarpus*, and *Rhynchosia* has been reported only by Metcalfe and Chalk (1950), particularly for tribe Phaseoleae.

Trichomes of spherical and ellipsoid types were termed capitate by Abu-Asab and Cantino (1987), because these trichome heads are twice higher than stalk length. The dimensions of capitate trichomes (15 to 42 µm in length and 14 to 18 µm in width)
Figures 33 - 36 - Details of non-glandular trichomes with apical cell (black arrow) and terminal cell (white arrow) in species 33 and 34. *R. edulis*. 35 and 36. *R. balansae var. balansae*. 
and bulley capitate (20 to 67 µm in length and 18 to 57 µm in width) allow these two structures to be set apart. Both the in-depth evaluation of these trichomes and the denomination suggested herein are novel for the taxa investigated.

Secretory trichomes, usually termed glandular-punctate trichomes or vesicular glands (Lackey 1978), feature a head size twice as large that of stalk, are located in depressions in the epidermis, and have been reported as having diagnostic value for the tribe. In the present study, these glands were named bulky capitate trichomes, as their former denomination applied to a vast range of secretory structures. In a detailed anatomical study of species of *Indigofera* L. (Marquiafavel et al. 2009), seven types of secretory trichomes, generically referred to as punctiform glands at the time, were identified and named.

Capitate trichomes, bulbous-based trichomes, and non-glandular trichomes, found in all seven taxa investigated, are being reported for genus *Rhynchosia* for the first time. Lackey (1978) reported bulky capitates trichomes and bulbous-based trichomes only for *R. densiflora*, *R. phaseoloides*, and *R. reticulata*. Glandular trichomes have taxonomic value for *Cajaniae* (Polhill et al. 1981) and represent a unifying characteristic for members of this subtribe (Lackey 1978, Solereder 1908).

According to Lackey (1978), bulbous-based trichomes are not a trait common to every genus of Phaseoleae, being absent, for instance, from genera *Baukea*, *Flemingia*, *Carissoa*, *Eriosema* and *Paracalyx*, as well as from *Rhynchosia densiflora*. The present study revealed an absence of this type of trichome from *R. balansae* var. *balansae*, *R. balansae* var. *psilantha*, and from the abaxial surface of *R. leucophylla*, making bulbous-based trichome a useful taxonomic characteristic that sets *R. leucophylla* apart from *R. corylifolia*. Metcalfe and Chalk (1950) reported that glandular trichomes with large, spherical heads, whether uni- or biseriate and featuring a bulbous base, are common only in genera such as *Desmodium*, *Eleiotis*, *Hallia*, *Ononis*, *Rhynchosia*, and *Tephrosia*, of tribe Phaseoleae.

In the taxonomic revision of the genus *Rhynchosia*, Grear (1978) reported pubescence as a variable trait across species, confirmed in the present study by the observation of non-glandular trichomes of differing lengths. According to Metcalfe and Chalk (1950) and Solereder (1908), non-glandular trichomes, including uniseriate structures with a basal cell accompanied by a long terminal cell, can exhibit wide variations - traits corroborated by the present investigation.

The taxonomic importance of trichomes in Leguminosae, and particularly of secretory structures, has been pointed out in a number of studies, as particular types of trichomes are often useful for delimiting species (Fortuna-Perez et al. 2012, Teixeira and Gabrielli 2000, Teixeira et al. 2000, Teixeira and Rocha 2009), genera (Lersten and Curtis 1993, 1994, 1995), and tribes (Lackey 1978).

None of the secretory structures found in the present study had previously been detailed micromorphologically in the genus investigated. The research literature (Solereder 1908, Metcalfe and Chalk 1950, Lackey 1978, Polhill et al. 1981, Fanh 1979, Pyykko 1966) highlights the usefulness of these structures in taxonomic studies, and the present study provides morphological, anatomical, and ultrastructural details for group separation, which can further support phylogenetic studies.

**Taxonomic Implications of Anatomical Traits of Leaflets of Rhynchosia Species**

The set of anatomical data addressed in the present study effectively separates the *Rhynchosia* taxa investigated. The shape of the epidermal cells and vascular bundles, sclerenchyma discontinuity, absence of collenchymas and ellipsoid capitated trichomes in *R. balansae* var. *psilantha* differentiates this variety from *R. balansae* var. *balansae*.

*Rhynchosia edulis*, *R. melanocarpa*, and *R. minima* are considered morphologically similar, but
cross-sectional epidermal cells, shape vascular budles, organization of collenchymas and sclerenchyma separate these species. Presence of bulky capitate trichomes on the adaxial surface is relevant to distinguish *R. minima* from the other species investigated. *Rhynchosia minima* was the only taxon to exhibit, on both leaflet surfaces, all the epidermal indumenta investigated in the present study, a feature that distinguishes it from the other species.

Orbicular shape of the main vascular bundle, presence of epidermal papillae, absence of bulbous-based trichomes on the abaxial surface and continuity sclerenchyma in *R. leucophylla* consistently differentiates this species from *R. corylifolia*, which has bulbous-based trichomes on the abaxial surface, discontinuous sclerenchyma and an irregularly elliptical main vascular bundle, but lacks epidermal papillae.

The results of the present study can be generalized to other species in tribes of the same family, as no changes in the types proposed are found in other genera of this tribe. Moreover, the presence or absence of these structures is a strong differential feature among species of Papilionoideae. Recognition of secretory structures associated with midrib shape has diagnostic value for the species investigated, contributing towards elucidating taxonomic problems generally encountered by experts. These results indicate that studies of this nature should be encouraged, yielding responses to issues on species complexes or even allowing these species to be grouped into higher taxonomic categories or specific clades.

ACKNOWLEDGMENTS

The authors thank physicist Luis Felipe Plaça of the Scanning Electron Microscopy Laboratory of the Universidade Federal de Mato Grosso do Sul. Thanks are extended to the Brazilian Institutional Program for Student Scientific Research Grants (PIBIC-CNPq/UFMS) for the grant awarded to author W.V.

RESUMO

Estudo anatômico comparativo foi realizado nas estruturas secretoras de folíolos de táxons pertencentes ao gênero *Rhynchosia* – taxa de difícil delimitação por possuir relações interespecíficas incertas – a fim de avaliar o valor diagnóstico potencial dessas características anatômicas para atribuição taxonômica. Outro objetivo foi estabelecer denominação consensual para estas estruturas secretoras. As novas características anatômicas encontradas nestes taxa foram suficientemente consistentes para separar as espécies avaliadas. A presença e localização de glândulas puntiformes, tricomas base bulbosa, o número de camadas do parênquima paliçádico e o arranjo das unidades vasculares diferenciam os taxas analisados e estas características podem ser estendidas para as demais espécies de Papilionoideae. Os tricomas analisados foram descritos e classificados em cinco tipos.

Os dados obtidos estão documentados em diagramas, fotomicrografias e microscopia eletrônica de varredura, estes foram listados pela primeira vez para o gênero. As informações obtidas serviram para distinguir eficazmente os taxa investigados e entre as espécies de Papilionoideae.

Palavras-chave: folíolos, legumes, taxonomia, tricomas.

REFERENCES


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APPENDIX


An Acad Bras Cienc (2015) 87 (1)