*Acta Botanica Brasilica* 28(4): 583-593. 2014.
doi: 10.1590/0102-33062014abb3517

# Comparative leaf anatomy and morphology of some Brazilian species of *Crotalaria* L. (Leguminosae: Papilionoideae: Crotalarieae)

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Received: 2 February, 2014. Accepted: 9 June, 2014.

## ABSTRACT

Anatomical characters often provide strong taxonomic evidence and many times help define species whose morphological limits are in question. This study aimed to survey characters of the leaf anatomy of 16 species of *Crotalaria* L. occurring mostly in the *cerrado* (savanna) of central Brazil and in areas of *restinga* (coastal woodland, or sandy forest) along the coast of the country, in order to improve the current taxonomic circumscription of sections and species. Leaf samples were collected in the field and obtained from herbarium specimens. Standard techniques in plant anatomy were employed, including analyses under light and scanning electron microscopy. Many of the characters analyzed are relatively uniform, but some are diagnostic for species. At the section level, only trichomes with a base composed of radially distributed cells proved useful in the diagnosis of *C.* sect. *Calycinae*. The other characters analyzed showed interspecific variation, but no diagnostic value for recognition of the sections. At the species level, unlike the results found for sections, various characters have diagnostic value. Among the characters with diagnostic value at the species level, those related to texture, the venation pattern (such as the formation of areolas and insertion of the ribs secondary to midrib), and, in particular, the epidermis, showed potential for aiding the circumscription of some species.

**Key words:** Crotalarieae, plant anatomy, Papilionoideae, taxonomy

## Introduction

*Crotalaria* L. (Leguminosae: Papilionoideae) is the only genus of the tribe Crotalarieae with native representatives in South America (Wyk 1991; Wyk & Schutte 1995). The genus is one of the largest in the family Leguminosae (Lewis *et al.* 2005) and comprises some 720 species distributed mainly in tropical and subtropical areas of the southern hemisphere (Wyk 2005) with the principal center of diversity in the central-south and eastern regions of Tropical Africa (Polhill 1982). A phylogenetic study of the tribe Crotalarieae (Boatwright *et al.* 2008) confirmed the tribe as monophyletic and revealed *Bolusia* as a sister genus to *Crotalaria*. In a recent molecular systematic study based on nuclear internal transcribed spacer and the plastid markers *matK*, *psbA-trnH* and *rbcLa*, Roux *et al.* (2013) showed that *Crotalaria* is monophyletic (99% bootstrap support; posterior probability of 1.0). Systematic studies of the Crotalarieae have mainly covered external morphology and anatomy (Roux *et al.* 2011), cytogenetics (Windler 1974; Oliveira & Aguiar-Perecin 1999), or chemotaxonomy (Flores 2004).

Although infrageneric relationships are not well understood, it would appear that *Crotalaria* originated in Africa but diversified more recently into other regions of the world (Lewis *et al.* 2005). Some taxonomists (Senn 1939; Bisby 1970) have highlighted the difficulties of proposing an infrageneric classification within the group, owing to the large, highly reticulate and complex interspecific variation. In general, the most widely accepted infrageneric classification of the group is that proposed by Bisby & Polhill (1973), which is based mainly on the morphological characters of the flowers of African species. Roux *et al.* (2013) proposed a new infrageneric classification for the entire genus, based on molecular and morphological evidence, resulting in 11 recognized sections. To date, 42 species of *Crotalaria* have been recorded for Brazil (Flores 2014), and they are grouped into four sections: *Crotalaria* sect. *Calycinae* Wight & Arn.; *C.* sect. *Incanae* (Benth.) Polhill; *C.* sect. *Hedriocarpae* Wight & Arn.; and *C.* sect. *Stipulosae* (Baker f.) M.M. Roux & B.-E van Wyk. Those *Crotalaria* species include shrubs, subshrubs, and herbaceous perennials that grow in open areas, ranging from high hills to semi-deserts. The plants of *Crotalaria* can be easily recognized by the presence of simple leaves or leaves containing one to three leaflets, a rostrate keel, a style with trichomes in a single or double line toward the stigma, a monadelphous androecium (5 + 5-anther configuration), paired callosities on the standard petal and highly inflated fruit (Polhill 1982; Wyk 2005).

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Many of the species represent economically important sources of fiber, animal forage, or green manure, and some have medicinal or ornamental value (Lewis *et al*. 2005).

Anatomical studies of the subfamily Papilionoideae have provided significant support for the taxonomic classification of the group (Metcalfe & Chalk 1950; Krishnamurthy & Kannabiram 1970; Kannabiram & Krishnamurthy 1974; Kothari & Shah 1975; Lackey 1978; Crow *et al*. 1997; Teixeira & Gabrielli 2006; Fortuna-Perez *et al*. 2012). Some of these studies (e.g., Kothari & Shah 1975) have focused on the morphological diversity of specific characters, such as the types of stomata and trichomes in the tribe Hedysareae, and these have proven to quite useful in generic grouping, despite their heterogeneity. In addition, studies of leaf anatomy have made significant contributions to the systematic classification of the Leguminosae. In the case of the Phaseoleae, detailed information concerning the leaflet anatomy within the tribe not only confirmed and expanded previous knowledge, but also highlighted the usefulness of the data in taxonomic classification (Lackey 1978).

Despite the importance of anatomical detail in determining the taxonomy of some plant groups, the anatomical characteristics of the Leguminosae have not been fully explored. This is particularly so in the case of *Crotalaria*, a genus that requires better infrageneric delimitation. Among the few anatomical studies that are available for this genus, some were restricted to the leaf epidermis (Krishnamurthy & Kannabiram 1970; Kannabiram & Krishnamurthy 1974; Gill *et al*. 1982), whereas others focused on the anatomy of the cell wall of the fruits (Roux *et al*. 2011). In the present study, we examined the anatomical characters of the leaves of 16 species of *Crotalaria* occurring mostly in the *cerrado* (savanna) of central Brazil and in areas of *restinga* (coastal woodland, or sandy forest) along the coast of the country, with the objective of increasing knowledge regarding this group, specifically its current sectional classification and species circumscriptions.

## Materials and methods

### Materials

We sampled leaves from a total of 48 specimens, belonging to 16 species of *Crotalaria*, occurring in open habitats: most are from the *cerrado* and *campo rupestre* (dry rocky grassland) formations, both in the Cerrado Biome in central Brazil, although two specimens were collected from the sandy *restingas* of the southeastern Atlantic coast of Brazil. The choice of species of each section was made according to the representativeness, selecting those with occurrence in Brazil. Samples of three mature leaves of each species were collected in the field or were obtained from herbarium material. A list of voucher specimens is given in Tab. 1. We compared species according to their morphological and anatomical characteristics, using a presence/absence table (Tab. 2).

### Light microscopy

Samples were collected from the leaves of the second to fifth nodes of the stem, and the leaflets obtained from herbarium specimens were rehydrated by heating in aqueous glycerin (50%) for 3 min. All material was fixed in 50% formalin/acetic acid-alcohol, dehydrated in an ethanol series, and stored in 70% ethanol (Johansen 1940). The middle region of the leaf blade was studied from freehand sections and subsequently stained with 1% Astra Blue in 50% ethanol and 1% Safranin in 50% ethanol, as modified from Kraus *et al*. (1998).

To analyze the epidermis and stomata type, 100 mm$^2$ samples were subjected to diaphanization and staining with 1% Safranin in 50% ethanol (Franklin 1945). Leaf blades were clarified with a technique modified from Shobe & Lersten (1967), and the venation pattern of the leaf blade was classified according to the guidelines established by the Leaf Architecture Working Group (1999), Ellis *et al*. (2009) and Hickey & Wolfe (1975). Trichomes were classified according to their overall morphology (Theobald *et al*. 1979).

### Scanning electron microscopy

A scanning electron microscopy study was conducted to visualize the cuticle and trichomes. Small pieces of leaves were fixed on aluminum stubs using double-sided adhesive, and were coated with a thin layer (ca. 25 nm) of gold-palladium, as described by Silveira (1989). The scanning electron microscopy micrographs were taken through a Zeiss DSM 940 (Carl Zeiss, Oberkochen, Germany) at an accelerating voltage of 10 kV.

## Results

### Venation

The primary venation pattern in all of the studied species of *Crotalaria* is pinnate, whereas the secondary venation pattern is brochidodromous and characterized by secondary veins that do not terminate at the margin but join to form arches (Fig. 1A-P).

The insertion of secondary veins along the principal vein varies considerably (Tab. 2). In *Crotalaria lanceolata*, *C. martiana*, *C. micans*, *C. pallida*, *C. rufipila* and *C. vitellina*, all secondary veins are decurrent to the primary vein (Fig. 2A), whereas in *C. breviflora*, *C. flavicoma*, *C. juncea*, *C. stipularia*, *C. velutina*, *C. maypurensis* and *C. miottoae* only the proximal secondary veins are so. Less frequently, uniformly spaced excurrent veins appear distributed across the leaf blade (Fig. 2B) in *C. incana*, *C. paulina* and *C. spectabilis*. Intersecondary veins (Fig. 2C) are present in all species, and their proximal courses are always parallel to the main secondary veins. The tertiary veins (Fig. 2C) of the intercostal region are percurrent in all species (Fig. 2C),

**Table 1.** List of the analyzed species, with voucher, collection site and herbarium (codes according to Thiers 2009). *BRA Brazil,* (including state abbreviation).

| Taxon | Collector | Collection site | Herbarium |
|---|---|---|---|
| *C. breviflora* DC. | *Devecchi 74* | BRA. MG. Santana do Riacho | SPF |
| | *Devecchi 32* | BRA. MG. Santana do Riacho | SPF |
| | *Zappi 1980* | BRA. MG. Santana do Pirapama | SPF |
| *C. flavicoma* Benth. | *Arbo 4664* | BRA. MG. Santana do Riacho | SPF |
| | *Pirani 4204* | BRA. MG. Santana do Riacho | SPF |
| | *Siniscalchi 31* | BRA. MG. Santana do Riacho | SPF |
| *C. incana* L. | *Devecchi 52* | BRA. MG. Santana do Riacho | SPF |
| | *Devecchi 64* | BRA. MG. Santana do Riacho | SPF |
| | *Devecchi 51* | BRA. MG. Santana do Riacho | SPF |
| *C. juncea* L. | *Pirani 3812* | BRA. MG. Santo Hipólito | SPF |
| | *Casagrande 1* | BRA. SP. Piracicaba | UEC |
| | *Douglas 2* | BRA. SP. Piracicaba | SPF |
| *C. lanceolata* E. Mey. | *Devecchi 25* | BRA. MG. Santana do Pirapama | SPF |
| | *Devecchi 71* | BRA. MG. Jaboticatubas | SPF |
| | *Devecchi 48* | BRA. MG. Santana do Riacho | SPF |
| *C. martiana* Benth. | *Devecchi 76* | BRA. MG. Conceição do Mato Dentro | SPF |
| | *CFCR13838* | BRA. MG. Santa Bárbara | SPF |
| | *CFCR13233* | BRA. MG. Joaquim Felício | SPF |
| *C. maypurensis* Kunth | *Devecchi 26* | BRA. MG. Santana do Pirapama | SPF |
| | *Zappi 2758* | BRA. MG. Santana do Pirapama | SPF |
| | *Souza 28198* | BRA. MG. Itacambira | SPF |
| *C. micans* Link | *Devecchi 49* | BRA. MG. Santana do Riacho | SPF |
| | *Devecchi 66* | BRA. MG. Jaboticatubas | SPF |
| | *Groppo 799* | BRA. MG. Joaquim Felício | SPF |
| *C. miottoae* A.S. Flores & A.M.G. Azevedo | *Flores 1036* | BRA. MG. Camanducaia | SPF |
| | *Flores 420* | BRA. MG. Santana do Riacho | SPF |
| | *Bianchini 330* | BRA. MG. Camanducaia | SPF |
| *C. pallida* Aiton | *Devecchi 33* | BRA. MG. Santana do Riacho | SPF |
| | *Devecchi 47* | BRA. MG. Santana do Riacho | SPF |
| | *Cordeiro s.n.* | BRA. SP. Itanhaém | SPF |
| *C. paulina* Schrank | *Panizza s.n.* | BRA. SP. São Paulo | SPF |
| | *Vitta 696* | BRA. MG. Santana do Riacho | UEC |
| | *Grotta s.n.* | BRA. SP. Sorocaba | SPF |
| *C. rufipila* Benth. | *CFSC6048* | BRA. MG. Santana do Riacho | SPF |
| | *Zappi 1882* | BRA. MG. Santana do Pirapama | SPF |
| | *Devecchi 28* | BRA. MG. Santana do Riacho | SPF |
| *C. spectabilis* Roth | *Devecchi 31* | BRA. MG. Santana do Riacho | SPF |
| | *Devecchi 17* | BRA. MG. Santana do Riacho | SPF |
| | *Devecchi 10* | BRA. MG. Santana do Riacho | SPF |
| *C. stipularia* Desv. | *Devecchi 63* | BRA. MG. Santana do Riacho | SPF |
| | *Baldiglian s.n.* | BRA. SP. Bertioga | SPF |
| | *Devecchi 79* | BRA. MG. Gouveia | SPF |
| *C. velutina* Benth. | *CFSC11656* | BRA. MG. Santana do Riacho | SPF |
| | *Devecchi 29* | BRA. MG. Santana do Riacho | SPF |
| | *Devecchi 154* | BRA. MG. Santana do Riacho | SPF |
| *C. vitellina* Ker Gawl. | *Zappi 735* | BRA. MG. Santana do Pirapama | SPF |
| | *Pirani 4018* | BRA. MG. Diamantina | SPF |
| | *CFCR6150* | BRA. MG. Diamantina | SPF |

and the external tertiary veins (i.e. those outside the arches formed by the secondary veins) join to form loops (Fig. 2C).

The areoles can be classified into two groups according to the stage of development, i.e. well developed as in *C. miottoae* (Fig. 2H) and moderately developed as in the other species studied (Fig. 2I). Within the areoles, when present, it is possible to identify three types of freely ending veinlets (FEVs): those without branches (Fig. 2D); those with a single branch (Fig. 2E); and those with dendritic ramifications (Fig. 2F). In some species, FEVs are absent (Fig. 2G).

The terminal portion of the free vascular endings can be simple (Fig. 2D), as in *Crotalaria breviflora*, *C. martiana*, *C. pallida*, *C. paulina*, *C. stipularia*, *C. velutina* and *C. vitellina*, or can bear a tracheoid idioblast (Fig. 2I), as in the remaining species studied. However, simple terminations are also observed in species presenting tracheoid idioblasts.

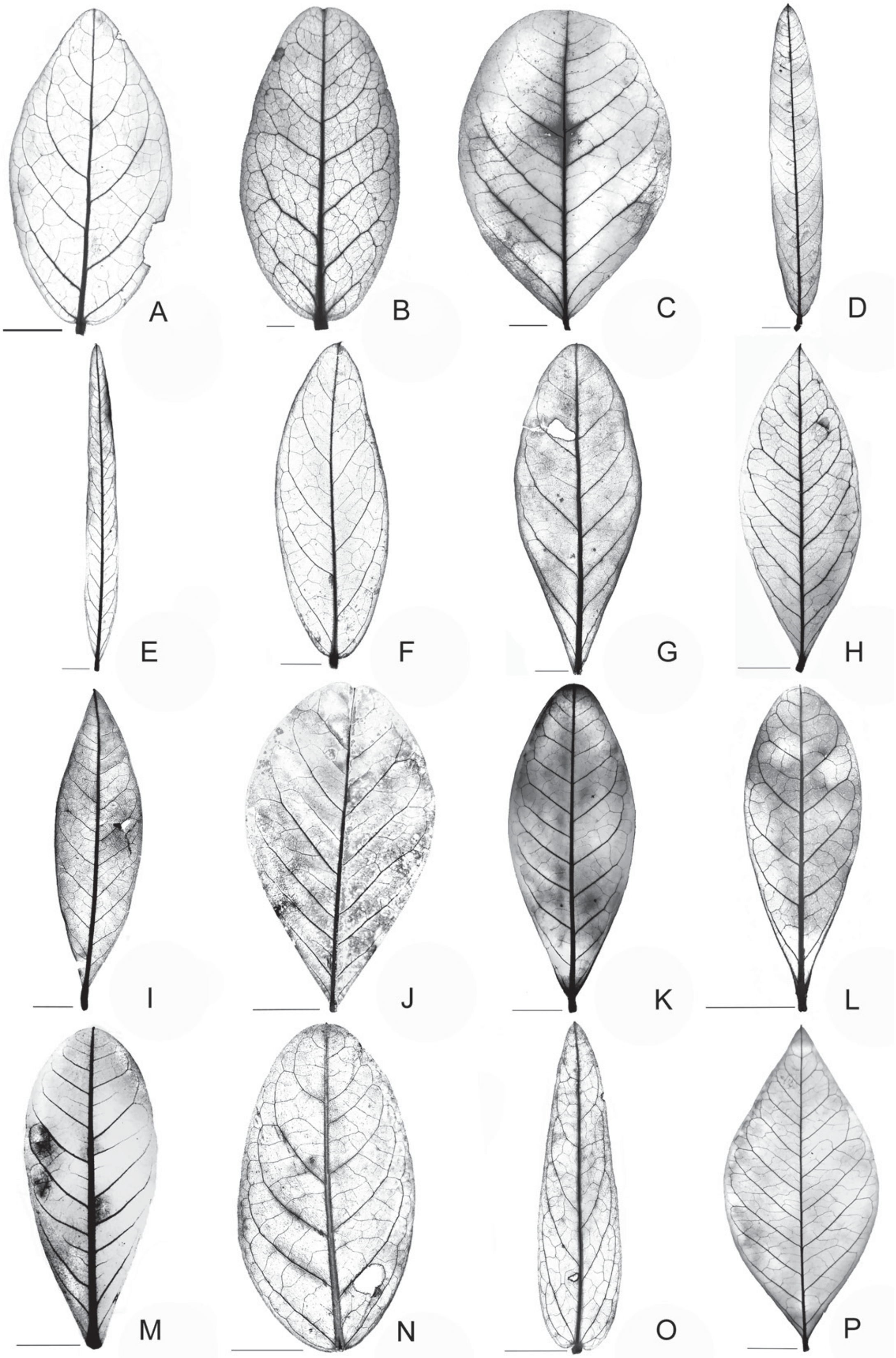

**Figure 1.** Diaphanized leaves and the pinnate-brochidodromous pattern present in 16 species of *Crotalaria* L.: **A.** *C. breviflora* DC.; **B.** *C. flavicoma* Benth.; **C.** *C. incana* L.; **D.** *C. juncea* L.; **E.** *C. lanceolata* E. Mey.; **F.** *C. martiana* Benth.; **G.** *C. maypurensis* Kunth; **H.** *C. micans* Link; **I.** *C. miottoae* A.S. Flores & A.M.G. Azevedo; **J.** *C. pallida* Aiton; **K.** *C. paulina* Schrank; **L.** *C. rufipila* Benth.; **M.** *C. spectabilis* Roth; **N.** *C. stipularia* Desv.; **O.** *C. velutina* Benth.; and **P.** *C. vitellina* Ker Gawl. Scale: 1 cm.

**Table 2.** Morphoanatomical characters of the 16 analyzed species of *Crotalaria* L.

| Section/ specie<br>Characters | *C.* sect. *Calycinae* | | | | | | | | *C.* sect. *Incanae* | *C.* sect. *Stipulosae* | | *C.* sect. *Hedriocarpae* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *C. breviflora* | *C. flavicoma* | *C. juncea* | *C. martiana* | *C. paulina* | *C. spectabilis* | *C. stipularia* | *C. velutina* | *C. incana* | *C. maypurensis* | *C. micans* | *C. miottoae* | *C. rufipila* | *C. vitellina* | *C. lanceolata* | *C. pallida* |
| Leaf organization: simple (0); compound (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Leaf attachment: sessile (0); petiolate (1) | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Leaf texture: chartaceous (0); membranous (1); coriaceus (2) | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Primary venation pattern pinnate: absent (0); present (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Secondary venation pattern simple brochidodromous: absent (0); present (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Insertion of secondary veins to midvein: decurrent (0); excurrent (1); decurrent proximal secondaries (2) | 2 | 2 | 2 | 0 | 1 | 1 | 2 | 2 | 1 | 2 | 0 | 2 | 0 | 0 | 0 | 0 |
| Intersecondary veins: absent (0); present (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Tertiary vein of intercostal region percurrent: absent (0); present (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Stage of development of the areoles: good (0); moderate (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| Freely ending veinlets: simple (0); tracheoid idioblast (1) | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Marginal ultimate venation (tertiary veins): discontinuous (0); looped (1) | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 |
| Occurrence of stomata: amphystomatic (0); hypostomatic (1) | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Stomata located at the same level of the epidermal cells: absent (0); present (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Occurrence of trichomes: on both sides (0); only one side (1) | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| Arrangement of mesophyll: dorsiventral (0); homogenous (1) | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lobed cells of spongy parenchyma: absent (0); present (1) | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Thickness of periclinal wall of the epidermal cells: not thickened (0); on one side (1); on both sides (2) | 2 | 2 | 2 | 2 | 1 | 2 | 0 | 2 | 0 | 2 | 1 | 1 | 0 | 0 | 1 | 1 |
| Midrib region concave-convex in transverse section: absent (0); present (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| The central and lateral veins limited externally by endodermis: absent (0); present (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Lignified cells associated with vascular tissues: absent (0); present (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| Lateral veins without lignified cells: absent (0); present (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Secondary veins with fibers adjacent to the phloem: absent (0); presente (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| Region of the central vein with collenchyma: only one side (0); on both sides of midrib (1) | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Arrangement of the palisade parenchyma: in one layer (0); in two layer (1) | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 |
| Epidermis uniseriate on both side: absent (0); present (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Epidermis in transverse section: adaxial cells ≥ 2x abaxial cells (0); adaxial cells = abaxial cells (1) | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| Anticlinal walls cells in frontal views on adaxial surface: sinuous (0); straight (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| Anticlinal walls cells in frontal views on abaxial surface: sinuous (0); straight (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 |
| Projections (small papillae) in epidermal cells at region of the midrib: absent (0); present (1) | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Trichome base comprised of cells radially distributed: absente (0); present (1) | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Epicuticular waxes on adaxial surface: thin film (0); superimposed (1) | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 |
| Epicuticular waxes on abaxial surface: thin film (0); superimposed (1) | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |

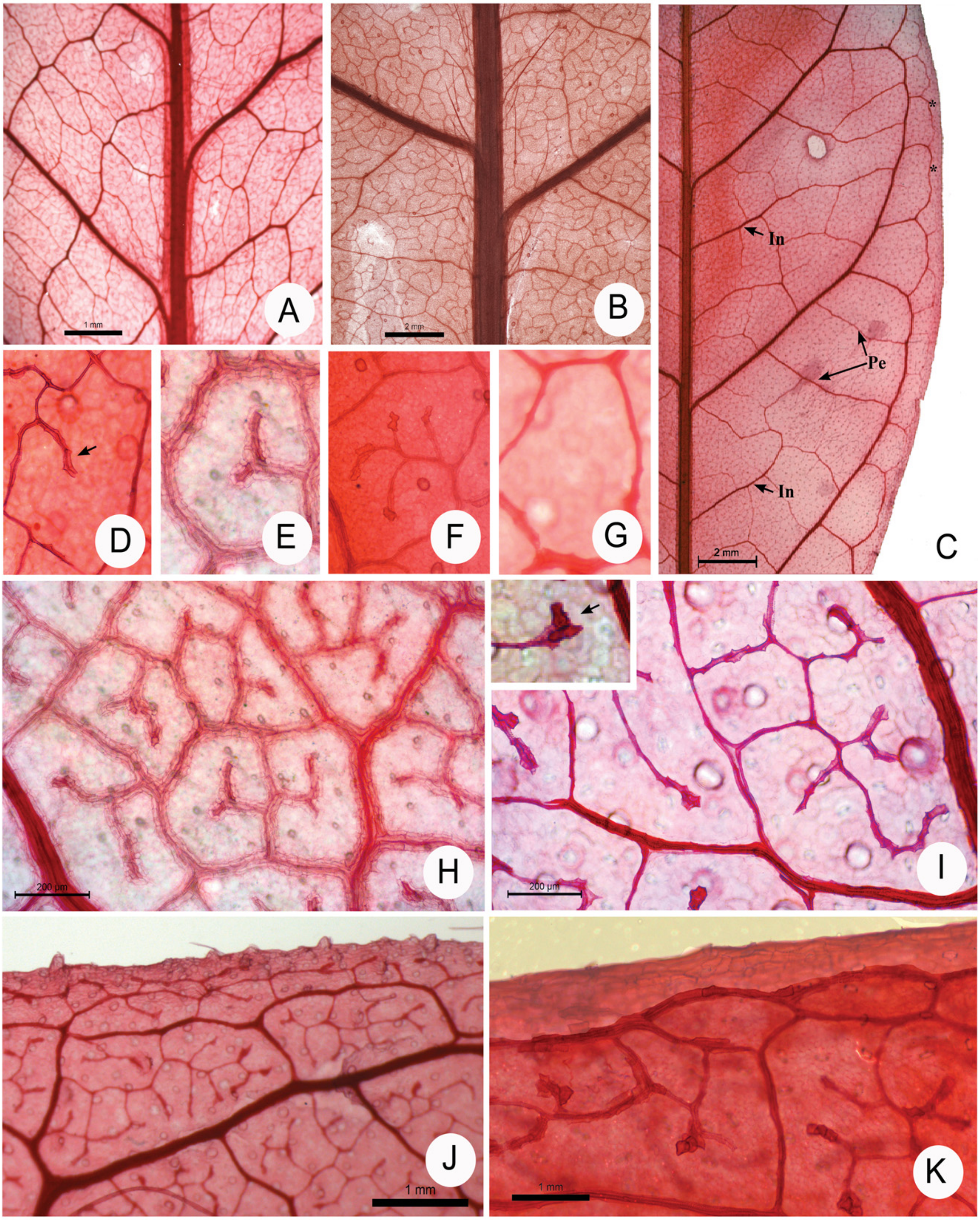

**Figure 2.** Details of leaf architecture: **A.** decurrent insertion of secondary veins in *Crotalaria flavicoma* Benth.; **B.** excurrent insertion of secondary veins in *C. incana* L.; **C.** intersecondary veins (In) with their proximal courses parallel to the main secondary veins, tertiary veins percurrent (Pe), external tertiary veins joined to form loops (*) in *C. rufipila* Benth.; **D-G.** Freely ending veinlets (FEVs)—**D.** FEVs without branches (arrow) in *C. velutina* Benth., **E.** FEVs with a single branch in *C. miottoae* A.S. Flores & A.M.G. Azevedo, **F.** FEVs with dendritic ramifications in *C. incana* L. and **G.** FEVs absent in *C. flavicoma*; **H.** areoles well developed in *C. miottoae*; **I.** areoles moderately developed, with FEV bearing a tracheoid idioblast in the terminal portion (arrow) in *C. flavicoma*; **J.** marginal terminal veins discontinuous in *C. flavicoma*; and **K.** marginal terminal veins loop-forming in *C. maypurensis* Kunth.

In the majority of species, the marginal terminal veins are discontinuous (Fig. 2J), although some species exhibit loop-forming veins (Fig. 2K) along almost the entire length of the margin.

### *Trichomes*

Trichomes are present on both surfaces of the leaves of all of the species studied, except for *Crotalaria lanceolata*, *C. maypurensis*, *C. micans* and *C. paulina*, in which they appear only on the abaxial surface. Although *C. miottoae* and *C. pallida* present trichomes on both surfaces, the structures are rare on the adaxial surface and are distributed predominantly near the midrib (Fig. 3A). The trichomes are non-glandular, uniseriate and filiform in all of the studied species, although the structures can be short or long, and the cuticular layer of the body can be smooth (Fig. 3F) or granular (Fig. 3G). The structures comprise a cellular base (Fig. 3B), with a body consisting of a short subterminal cell with a thickened wall and a further elongated terminal cell (Fig. 3C). In some cases, the trichome base is comprised of radially distributed cells (Fig. 3D) or not (Fig. 3E).

### *Leaf blade*

Transversal sections revealed that, in all of the studied species, the leaf epidermis is uniseriate on both surfaces. In most of the species, cells of the adaxial surface presented walls with contours that were ≥ 2× higher than were those of cells on the abaxial surface (Fig. 4A), although in *Crotalaria maypurensis*, *C. micans*, *C. spectabilis*, *C. stipularia* and *C. velutina*, the cell walls of the abaxial and adaxial surfaces showed similar contours. It is possible to observe thickening of the external periclinal walls of epidermal cells on the abaxial and adaxial surfaces (Fig. 4A), except in *C. lanceolata*, *C. micans*, *C. miottoae*, *C. pallida* and *C. paulina*, in which such thickening is observed only on the adaxial surfaces, as well as in *C. incana*, in which no such thickening can be detected. Epidermal cells on the adaxial surfaces of leaves of *C. breviflora* exhibit projections (small papilla) that are restricted to the region of the midrib (Fig. 4B).

Frontal views of the dissociated epidermis reveal the presence of two types of cell contour. Therefore, whereas most species present anticlinal cell walls with straight contours (Fig. 4C) on both surfaces of the leaves, *Crotalaria incana*, *C. miottoae*, *C. pallida* and *C. vitellina* exhibit sinuous cell walls (Fig. 4D) on both surfaces, and *C. rufipila* show sinuous cell walls only on the adaxial surface.

All of the studied species present amphistomatic leaves with the exception of *Crotalaria breviflora*, the leaves of which were hypostomatic. However, the distribution of stomata is sparse on the adaxial surfaces of *C. miottoae*, *C. spectabilis* and *C. vitellina*, and was generally limited to areas near to the main veins (Fig. 3A). In all species, the stomata are located at the same level as the epidermal cells.

The leaves of 14 of the 16 species studied presented a dorsiventral mesophyll (Fig. 5A), a homogeneous mesophyll being observed only in *Crotalaria spectabilis* and *C. velutina* (Fig. 5B). In the species with a dorsiventral mesophyll, the palisade parenchyma frequently consists of two layers (Fig. 5A), although it appears sometimes as a single layer (Fig. 5C), as in *C. flavicoma*, *C. incana*, *C. miottoae* and *C. vitellina*.

The midrib region shows a concave-convex contour in all of the studied species (Fig. 5E). The vascular system exhibits a half-moon shape with the xylem and phloem polarized in the adaxial and abaxial surfaces, respectively. Lignified cells associated with vascular tissues (perivascular cells) are observed in all species (Fig. 5F) except *Crotalaria maypurensis*. Lignification of the perivascular cells occurs initially in the abaxial flanks lateral to the phloem, and in some cases they form a continuous cylinder (Fig. 5E). In the region of the central vein, it is possible to observe collenchyma on both surfaces or just on the abaxial surface, as in *C. spectabilis*. The vascular tissues in the lateral veins are also polarized, although no lignified perivascular cells similar to those of the central vein were observed here. The central and lateral veins are externally limited by a bundle sheath (endodermis; Fig. 5D), which is more visible in the smaller veins.

## Discussion

Among the characters studied, the most valuable in helping delimitate the sections of *Crotalaria* was the presence of trichomes with bases comprising radially distributed cells. Apart from two exceptions, this feature is observed only in species classified in *C.* sect. *Calycinae*. Although interspecific variation is observed in all of the other characters studied, those have no diagnostic value with respect to the recognition of sections.

In contrast to the results obtained for sections, a number of characters show diagnostic value for the circumscription of *Crotalaria* at the specific level. Among these characters are those related to texture, venation patterns (areoles and type of insertion of secondary veins adjoining the main vein) and, most particularly, the characteristics of the epidermal cells (Tab. 2).

Kannabiram & Krishnamurthy (1974) investigated leaves of 10 species of *Crotalaria* and described the consistent presence of epidermal cells with straight walls (in front view) on the adaxial surfaces and sinuous walls on the abaxial surfaces. However, the present study demonstrates that this pattern is variable, because, in some species, cells with similar wall contours (predominantly straight) can be observed on both surfaces of the leaves. Therefore, *C. juncea* and *C. micans* present epidermal cells with straight walls on the abaxial and adaxial surfaces, whereas *C. rufipila* exhibits epidermal cell walls with sinuous and straight contours on the adaxial and abaxial surfaces, respectively. Two trichomes

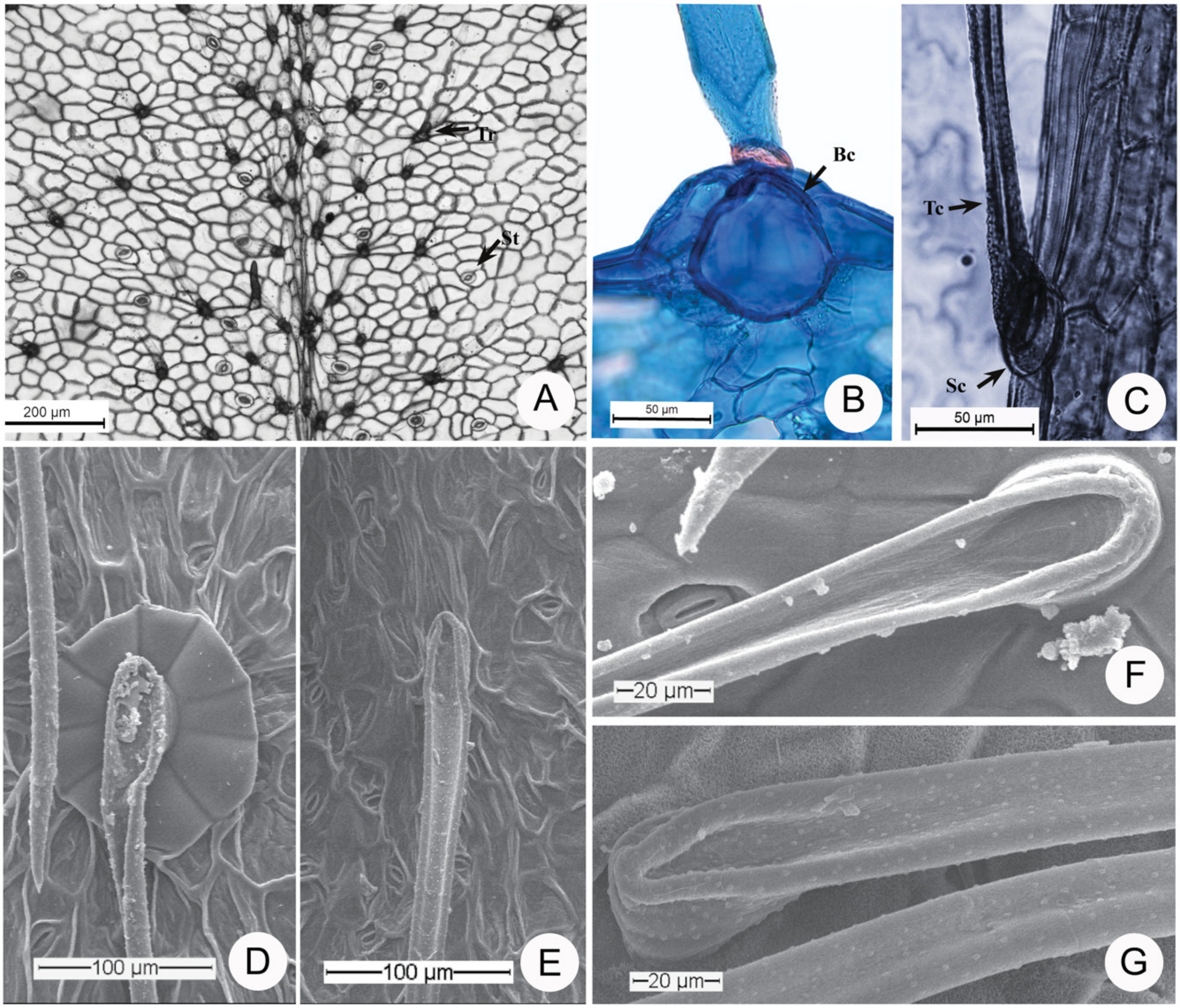

**Figure 3.** Types of trichomes in *Crotalaria* spp.: **A.** frontal view of the adaxial surface with trichomes (Tr) and stomata (St) distributed predominantly near the midrib in *C. miottoae* A.S. Flores & A.M.G. Azevedo; **B.** trichome comprising a basal cell (Bc), body consisting of a short subterminal cell with a thickened wall and an elongate terminal cell in *C. martiana* Benth.; **C.** trichome with a short subterminal cell (Sc) and an elongate terminal cell (Tc) in *C. vitellina* Ker Gawl.; **D.** scanning electron microscopy (SEM) micrograph of adaxial surface of *C. stipularia* Desv., showing trichome with cells distributed radially in the base; **E.** SEM micrograph of abaxial surface showing trichomes without cells distributed radially in the base; **F.** SEM micrograph showing details of the trichome with a smooth surface in *C. juncea* L.; and **G.** SEM micrograph showing details of the trichome with a granular surface in *C. flavicoma* Benth.

types—unicellular (Procópio *et al*. 2003) and multicellular uniseriate (Metcalfe & Chalk 1950)—have been described in some *Crotalaria* species. Among the *Crotalaria* species studied here, including *C. incana*, in which Procópio *et al.* (2003) described unicellular trichomes, we observed only multicellular uniseriate trichomes. Procópio *et al.* (2003) also observed that leaves of *Crotalaria incana* showed two-layer palisade parenchyma, although that feature not was confirmed in the present study, in which all analyzed samples showed a single-layered palisade parenchyma. Amphistomatic leaves predominate in all of the species investigated, with the exception of *C. breviflora*, which has hypostomatic leaves. Variation regarding the occurrence of stomata on just one or both surfaces of the leaves of *Crotalaria* species has been described previously by Metcalfe & Chalk (1950).

A dorsiventral mesophyll is observed in all of the species studied, except for *Crotalaria spectabilis* and *C. velutina*, in which the mesophyll is homogeneous, a pattern that has been described previously in the Leguminosae (Cowan 1981; Crow *et al.* 1997; Metcalfe & Chalk 1950). The presence of lignified cells in association with the vascular tissues of the central vein, which is observed in most of the studied species, has been reported by Alvarez *et al.* (2001) for *Swartzia brachyrhachis* Harms and *S. laurifolia* Benth. The distribution of these lignified cells, differentiating initially in the lateral flanks and sometimes forming a continuous cylinder, is the most common pattern observed in the studied species. This

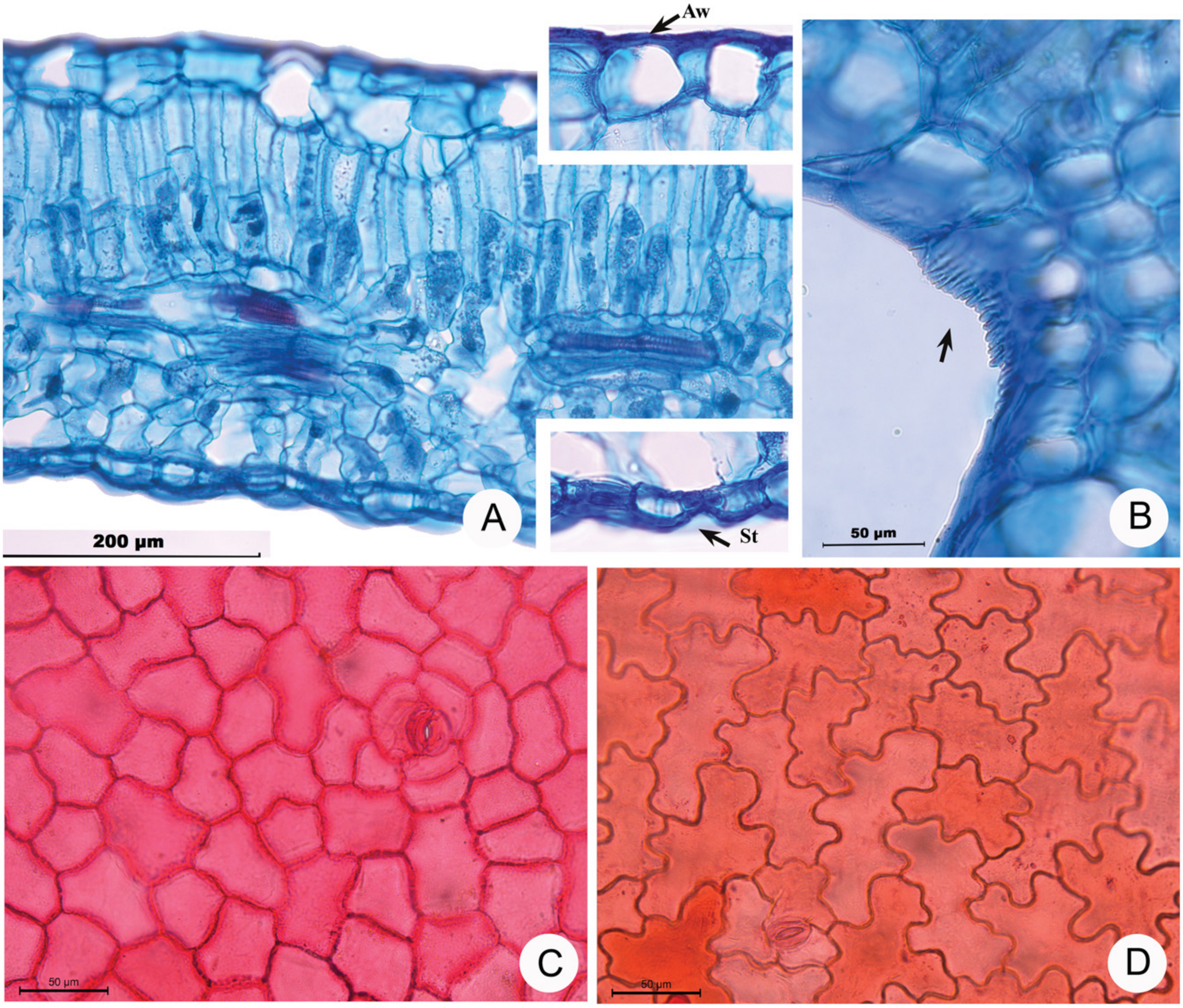

**Figure 4.** Leaf epidermis in *Crotalaria* spp.: **A.** uniseriate epidermis with cell contours that are ≥ 2× higher on the adaxial surface than on the abaxial surface in *C. breviflora* DC., details showing stomata on abaxial surface; **B.** epidermal cells showing projections (papillae), restricted to the region of midrib on the adaxial surface (arrow) in *C. breviflora*; **C.** epidermal cells in frontal view with straight anticlinal walls in *C. breviflora*; and **D.** epidermal cells in frontal view with sinuous anticlinal walls in *C. vitellina* Ker Gawl.
St – stomata; Aw – anticlinal wall.

feature has been described previously by Metcalfe & Chalk (1950), although it is somewhat variable in the Papilionoideae.

In some cases, anatomical characteristics taken in combination can be quite valuable in the recognition of different taxa. An example in which the consideration of morphological and anatomical characters has aided the circumscription of morphologically similar taxa is provided by *Crotalaria miottoae*. Flores & Tozzi (2005) reported that, despite the significant morphological similarity to *C. vitellina* and *C. micans*, the delimitation of *C. miottoae* could be successfully achieved through distinctive anatomical leaf characters (Tab. 2). Another example involves *C. maypurensis* and *C. micans*, species that are morphologically quite similar and difficult to distinguish (Lewis 1987; Gómez-Sosa 2000) because of significant intraspecific variation. Although these species do share some similarities in leaf anatomy, a number of characters present distinctive states (Tab. 2).

The characters of the leaves analyzed in this study are of potential value for the circumscription of a number of *Crotalaria* species. This is particularly valuable in the cases of species that are share similar external morphological features. Consequently, characters such as the degree of development of areoles and the presence of a coating of epicuticular wax on the abaxial surface corroborate the circumscription of *C. miottoae*, whereas the presence of perivascular fibers in the central and secondary veins, with fibers adjacent to the phloem, allow a distinction to be drawn between *C. micans* and *C. maypurensis*. Further anatomical studies regarding other species of this large genus are needed in order to improve the taxonomy of *Crotalaria*.

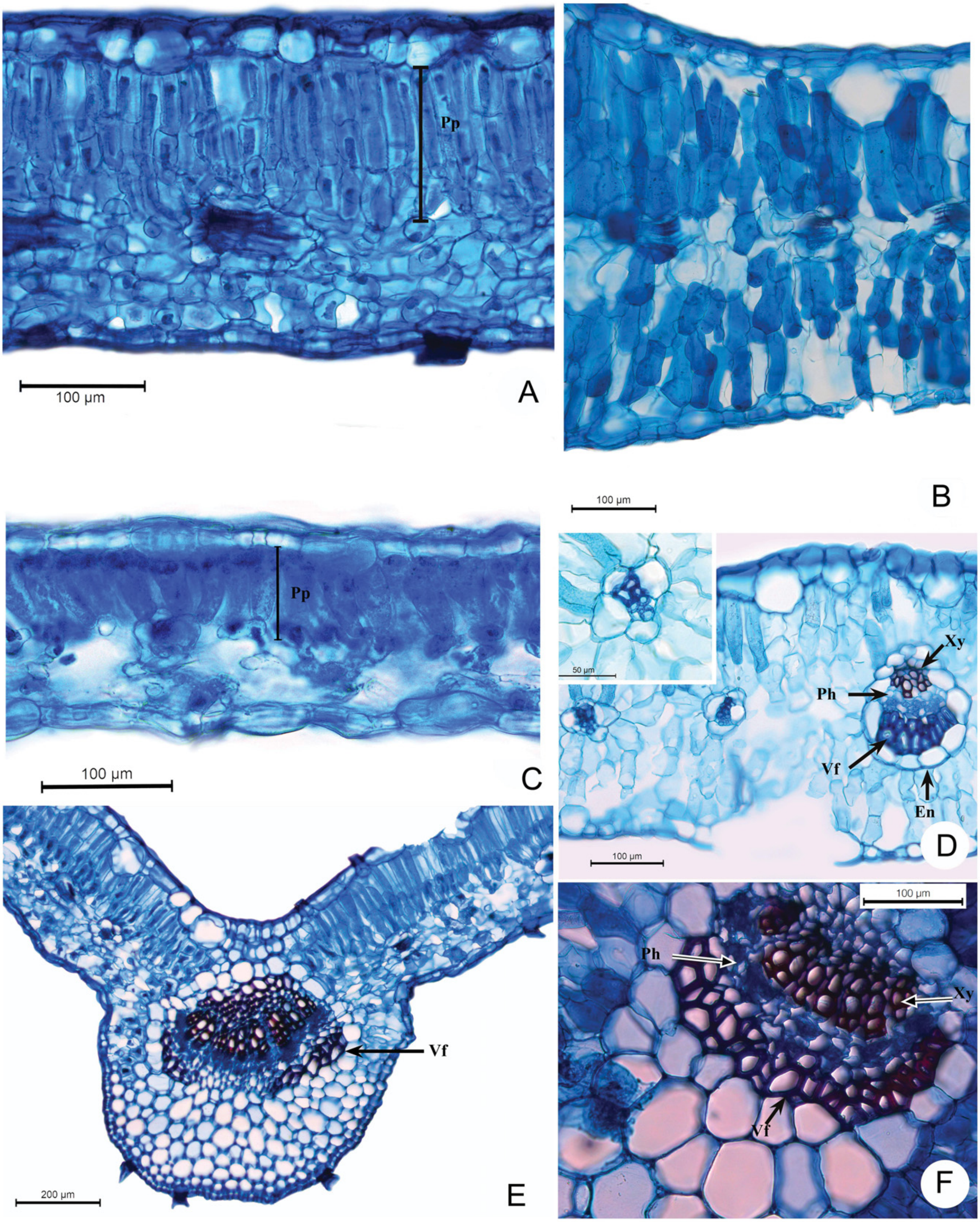

**Figure 5.** Leaf blade characters: **A.** dorsiventral mesophyll with two layers of palisade parenchyma on the adaxial surface in *C. paulina* Aiton; **B.** homogeneous mesophyll in *C. velutina* Benth.; **C.** palisade parenchyma with a single layer in *C. vitellina* Ker Gawl.; **D.** vascular bundles with perivascular fibers and endodermis in *C. spectabilis* Roth, details showing smaller vascular bundles limited by endodermis and no vascular bundles; **E.** midrib region showing concave-convex petiole outline, in transverse section, with lignified cells associated (perivascular cells) in *C. breviflora* DC.; and **F.** details of the midrib region of *C. velutina*, showing the perivascular cells forming a continuous cylinder.
Pp – palisade parenchyma; Xy – xylem; Ph – phloem; Vf – vascular fibers; En – endodermis.

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