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Original Article

Comparative morphoanatomical analysis of *Mikania* species

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ABSTRACT

Mikania belongs to the Asteraceae family and includes a wide range of promising pharmacological activities. Several species of *Mikania*, which is popularly known in Brazil as "*guaco*", occur in Southern Brazil and their external morphology is similar. The aim of this study was to investigate the morpho-anatomical characteristics of the leaf and stem of *Mikania campanulata*, *Mikania cordifolia*, *Mikania glomerata*, *Mikania hastato-cordata*, *Mikania microptera* and *Mikania sessilifolia* as a means of providing additional support for differentiating these taxa. The leaves and stems were investigated by employing scanning electron microscopy and light microscopy techniques. The morphological features of *Mikania* spp. leaves make it possible to differentiate between the species; nevertheless, when the plants were fragmented or pulverized the anatomical features of the leaves and stems supplied additional helpful data in this regard. The main anatomical characteristics were presence of hypodermis and lens shaped epidermal cells, set of trichomes; midrib, petiole and stem shape and vascular pattern; sclerenchymatous ring in the cortex, sclerenchymatous cells and secretory ducts in the pith.

Introduction

Mikania Willd. belongs to the Asteraceae family and the Eupatorieae tribe. It comprises 198 species in Brazil, ranging from the North to the South of the country, and is mainly found in the states of São Paulo, Rio de Janeiro, Minas Gerais, and Rio Grande do Sul (King and Robinson, 1978; Ritter and Miotto, 2005; The Plant List, 2015). *Mikania* spp. has provided a wide range of promising phamacological activities and it can be described as having expectorant, anti-inflammatory, bronchodilator, anti-hemorrhagic, antiasthmatic, analgesic, anti-mutagenic, trypanocidal, antimicrobial, antifungal, antiulcerogenic, muscle relaxant, and antirheumatic properties (Muelas-Serrano et al., 2000; Paul et al., 2000; Gasparetto et al., 2010; Pérez-Amador et al., 2010; Mourão et al., 2014; Ríos et al., 2014).

The inappropriate use of popular names can result in serious mistakes in relation to the identification of herbal drugs. The same plant often has several common names and furthermore, different species may be known by the same folk name (American Herbal Pharmacopeia, 2011). Morpho-anatomical data are used to mitigate this problem. This technique uses morphological and anatomical features to characterize and differentiate similar species (Luz et al., 2015; Wosch et al., 2015; Porto et al., 2016), especially when the botanicals are marketed in a fragmented or powdered form (American Herbal Pharmacopeia, 2011).

Mikania campanulata Gardner, *Mikania cordifolia* (L.f.) Willd., *Mikania glomerata* Spreng., *Mikania hastato-cordata* Malme, *Mikania microptera* DC., and *Mikania sessilifolia* DC., which are popularly known as "*guaco*", occur in Southern Brazil and their external morphology is similar. Hence, some confusion and/or mistakes can result in popular usage.

Previous data have revealed that *M. cordifolia* has anti-inflammatory (Peluso et al., 1995), insecticidal, trypanocidal (Arias et al., 1995; Muelas-Serrano et al., 2000), genotoxic and antiproliferative (Dias et al., 2014) activities. *M. glomerata* is the most common among the genus and it has many properties such as anti-inflammatory, antitussive and bronchodilatadory agents (Gasparetto et al., 2010). *M. sessilifolia* is used in folk medicine to treat colds and flu. There is no pharmacological or chemical

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characterization available for *M. microptera*, *M. hastato-cordata* and *M. campanulata*.

In view of the similar external morphology of *M. campanulata*, *M. cordifolia*, *M. glomerata*, *M. hastato-cordata*, *M. microptera* and *M. sessilifolia*, the intention of this paper was to study the morpho-anatomical data of the leaves and stems of these six species as a means of providing additional support for differentiating these taxa and identifying features of them which can be used as potential contaminants or substitutes for others of the genus.

Materials and methods

Plant materials

The aerial parts of at least five specimens of *Mikania campanulata* Gardner, *M. cordifolia* (L.f.) Willd., *M. microptera* DC. and *M. hastato-cordata* Malme, Asteraceae, were collected from São Maximiano Farm, which is located in the region of Serra do Sudoeste, in the city of Guaíba, state of Rio Grande do Sul, South Brazil (coordinates 30°10′ S and 51°20′ W, and 27 m altitude). *M. campanulata*, *M. cordifolia* and *M. hastato-cordata* were collected in September 2012 and *M. microptera* in December 2003. *M. glomerata* Spreng. and *M. sessilifolia* were collected in September 2014 from the Campos Gerais Region, in the city of Ponta Grossa, state of Paraná, South Brazil (25°5′ S and 50°6′ W, and 900 m altitude). The botanical materials were identified and the representative samples were registered at the Rio Grande do Sul Federal University under numbers ICN 175.095 (*M. campanulata*), ICN 116.543 (*M. cordifolia*), ICN 187.121 (*M. glomerata*), ICN 129.447 (*M. microptera*), and ICN 159.629 (*M. hastato-cordata*). *M. sessilifolia* was identified and the voucher was registered at the Herbarium of the State University of Ponta Grossa under number HUPG 10.208.

Anatomical analysis

The leaves and stems of *M. campanulata*, *M. cordifolia*, *M. glomerata*, *M. hastato-cordata*, *M. microptera* and *M. sessilifolia* were cut about 10 cm from the apex. They were then put in containers containing FAA 70 solution (Johansen, 1940), and stored in 70% ethanol (Berlyn and Miksche, 1976). The plants were segmented by hand. Transverse and longitudinal sections were stained using astra blue and/or basic fuchsine (Roeser, 1972). The leaves were clarified in frontal view of the epidermis, using Kraus and Arduin (1997) techniques. The photomicrographs were captured by an Olympus CX 31 light microscope equipped with a C 7070 control unit.

Histochemical analysis

The cell content and cell wall impregnation were exposed by adopting the following standard solutions in the microchemical tests: hydrochloric phloroglucin to reveal traces of lignin (Sass, 1951); Sudan III for testing lipophilic compounds (Foster, 1949); ferric chloride to test for phenolic substances (Johansen, 1940); and iodine-iodide to test for starch (Berlyn and Miksche, 1976). The semi-permanent slides were then analyzed in the Laboratory of Pharmacognosy at the State University of Ponta Grossa to allow a detailed description of the leaf and stem tissues.

Scanning electron microscopy and energy-dispersive X-ray spectroscopy (EDS)

The scanning electron microscopy (SEM) of the leaf and stem surface was performed in high vacuum and with high accelerating voltage (15 kV). This procedure required the samples to be previously dehydrated using increasing amounts of ethanol and the critical point of CO_2. After this, they were submitted to metallization with gold (Souza, 1998).

The EDS chemical microanalysis was randomly performed in crystals and cells without crystals (control), with an X-ray detector coupled to SEM and under the same SEM operating conditions that were used to take the electron micrographs. This procedure was carried out at the multi-user laboratory of the State University of Ponta Grossa and the Electron Microscopy Center at the Federal University of Paraná.

Results and discussion

The morphology of *M. campanulata*, *M. cordifolia*, *M. glomerata*, *M. hastato-cordata*, *M. microptera* and *M. sessilifolia* was similar and the main differences can be seen in Box 1 and Fig. 1. The leaves were simple (Fig. 1) and the phyllotaxy had an opposite arrangement for all the species.

M. campanulata (Fig. 1A) had leaves that were 5–10 cm long and 4–8 cm wide. The leaves were membranous and slightly purplish on the abaxial side. They were deltate to hastate in form, with acuminate apex, hastate base, paucidentate margins, and three visible veins. They had a straight petiole that measured 5–6 cm in length.

M. cordifolia (Fig. 1B) had leaves that were 4–8 cm long and 4–8 cm wide. The studied leaves were deltoid to hastate in form, with acuminate apex, hastate to cordate base, paucidentate margin and five visible veins. The petiole was straight and 1.5–2 cm in length. The leaves of *M. glomerata* (Fig. 1C) were 10–15 cm long and 5–9 cm wide. They were lanceolate–hastate in shape and had

Box 1: Morphological characteristics of *Mikania* spp.

Leaf blade	*M. campanulata*	*M. cordifolia*	*M. glomerata*	*M. hastato-cordata*	*M. microptera*	*M. sessilifolia*
Color	Green on the adaxial side and purplish on the abaxial side	Green on both sides	Green on both sides	Green on both sides	Green on both sides	Green on both sides
Size:						
Length (cm)	5–10	4–8	10–15	4–6	6.5–13	3.5–8
Width (cm)	4–8	4–8	5–9	4–4.5	5.5–13	3–6
Shape	Deltate to hastate	Deltate to hastate	Lanceolate–hastate	Triangular to deltate	Triangular	Widely ovate
Apex	Acuminate	Acuminate	Acuminate	Acute	Acute	Acute to obtuse
Margin	Paucidentate	Paucidentate	Entire	Entire	Paucidentate	Crenate to serrate
Base	Hastate	Hastate to cordate	Cordate to hastate	Cordate to hastate	Hastate	Slightly cordate
Venation	3	5	3–5	3	5	3
Form of petiole.	Straight	Straight	Curved	Curved	Straight	Very short
Size (cm)	5–6	1.5–2	2–2.5	2–2.5	2–2.5	0.1–0.3

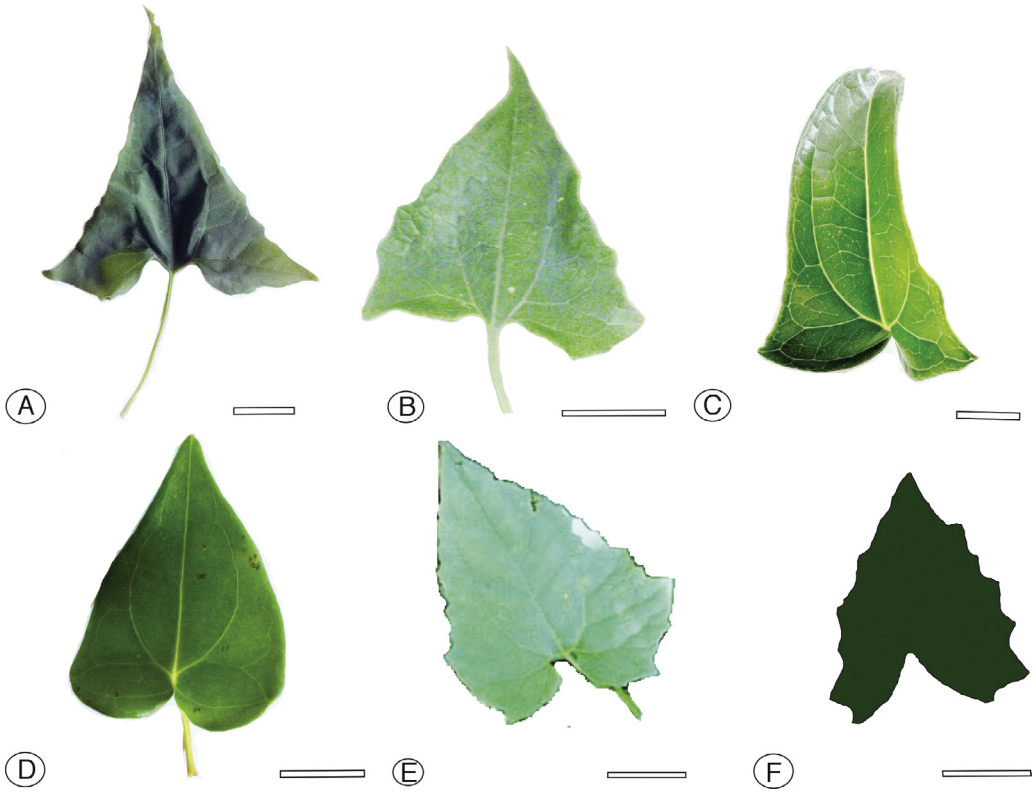

Fig. 1. *Mikania* spp. – Morphological appearance of leaves – A. *Mikania campanulata* Gardner, B. *M. cordifolia* (L.f.) Willd, C. *M. glomerata* Spreng., D. *M. hastato-cordata* Malme, E. *M. microptera* DC., and F. *M. sessilifolia* DC. Scale bar = 2 cm.

an acuminate apex, cordate to hastate base, entire margins, and 3–5 visible veins. The petiole had a curving shape and measured 2–2.5 cm in length.

M. hastato-cordata (Fig. 1D) had leaves that were 4–6 cm long and 4–4.5 cm wide. The leaves were triangular to deltate in form with acute apex, cordate to hastate base, entire margins, and three visible veins. They had a straight petiole that measured 2–2.5 cm in length. The leaves of *M. microptera* (Fig. 1E) were 6.5–13 cm long and 5.5–13 cm wide. They were triangular in shape with acute apex, hastate base, paucidentate margins and five visible veins. The petiole was 2–2.5 cm in length.

M. sessilifolia (Fig. 1F) had leaves that were 3.5–8 cm long and 3–6 cm wide. The leaves were widely ovate in form, with acute to obtuse apex, slightly cordate at the base, crenate to serrate margins and three visible veins. They had a very short petiole that measured 0.1–0.3 cm in length.

In botanical products, substitution, impurities and contaminants are types of adulteration. Adulteration can consist of the partial or complete replacement of one species for a different species, excessive quantity of impurities, deteriorated or poor quality material, as well as contaminants or any condition that would otherwise reduce the quality of the product (American Herbal Pharmacopeia, 2011).

There have been many well-publicized accounts of the unintentional and intentional contamination of botanicals by incorrect vegetable species. The problems of accidental contamination and economically-motivated tampering are questions that must be addressed when dealing with herbal drugs that have been gotten from traders or when the origin of the botanicals is inexact (Reynertson and Mahmood, 2015). Morpho-anatomical features have been used to vouch for the quality and authenticity of herbal drugs (Wosch et al., 2015; Araújo et al., 2015; Guerreiro et al., 2015).

The botanical analysis of medicinal species is the first test conducted by the herbal industry and it is designed to identify the species of plant. Its morphological features are important in terms of authenticity. However, anatomical profiles have been used to classify and differentiate species, especially when they are in fragmented or powdery form. The identification of *Mikania* genus is complex because of its morphological similarities and this has resulted in the indiscriminate use of different plants for the same therapeutic purposes (Amorin et al., 2014; Araújo et al., 2015).

Several *Mikania* have a similar morphology, and these include *M. glomerata* Spreng. and *M. laevigata* Sch. Bip. ex Baker (Budel et al., 2009), *M. lanuginosa* DC. and *M. hirsutissima* DC. (Amorin et al., 2014), *M. confertissima* Sch. Bip. ex Baker, *M. hatschbachii* G.M. Barroso and *M. glomerata*, and *Mikania hirsutissima* and *M. microlepsis* Baker (Oliveira et al., 1994).

The anatomical characteristics of the leaves shown in Box 2 and the stem anatomical features shown in Box 3 can be used to differentiate the *Mikania* species.

Anatomically, from a frontal view of the foliar blade, the anticlinal epidermal cell walls were thin and sinuous (Fig. 2B, G, H, I) and slightly wavy (Fig. 2A, C) on the abaxial side and slightly wavy on the adaxial side (Fig. 2D–F, J–L) for all the species. In addition, the epidermis was covered by a smooth cuticle, but was slightly striated near the stomata (Fig. 3K, N, O).

Depending on the occurrence of the stomata *M. campanulata*, *M. cordifolia*, *M. glomerata* and *M. hastato-cordata* had hypostomatic leaves and *M. microptera* and *M. sessilifolia* had amphistomatic leaves and anomocytic and anisocytic types of stomata were encountered (Fig. 2A–C, G–I, K, L).

Hypostomatic leaves are frequent in *Mikania* (Neves and Sá, 1991; Oliveira et al., 1994; Budel et al., 2009). However, amphistomatic leaves have also been found (Oliveira et al., 1994; Amorin et al., 2014; Araújo et al., 2015). Anomocytic stomata are common in *Mikania* (Rodrigues et al., 1996; Oliveira et al., 1999; Budel et al., 2009; Gasparetto et al., 2010; Amorin et al., 2014; Araújo et al.,

Box 2: Anatomical features showing differentiation between the leaves of *Mikania* spp.

	M. campanulata	*M. cordifolia*	*M. glomerata*	*M. hastato-cordata*	*M. microptera*	*M. sessilifolia*
Hypoder-mis	Absent	Absent	Present	Absent	Absent	Absent
Lens-shaped epidermal cells (adaxial)	Present	Absent	Absent	Absent	Absent	Absent
Presence of stomata	Hypostomatic	Hypostomatic	Hypostomatic	Hypostoma-tic	Amphistoma-tic	Amphistoma-tic
Capitate glandular trichome	Present	Present (rare)	Present	Present	Present	Present
Uniseriate glandular trichome	Absent	Present	Present	Present	Present	Present
Uniseriate glandular trichome with broad -based cell	Present	Absent	Absent	Absent	Absent	Absent
Conical glandular trichome	Present	Present	Absent	Present	Present	Present
Midrib shape in cross-section	Biconvex shape with obtuse projection on the adaxial side (prominent and rounded convexity on the abaxial side)	Biconvex shape, slightly convex on the adaxial side (rounded on the abaxial side)	Biconvex shape, slightly convex on the adaxial side (rounded on the abaxial side)	Slightly flat, convex shape	Biconvex shape with obtuse projection on the adaxial side (prominent and angular convexity on the abaxial side)	Slightly flat, convex shape
Midrib vascular patterns	3–5 free collateral vascular bundles in an open arc	3–5 free collateral vascular bundles in an open arc	3–5 free collateral vascular bundles in an open arc	3–5 free collateral vascular bundles in an open arc	3–5 free collateral vascular bundles in an open arc	One vascular bundle in the center
Petiole shape in cross-section	Flat-convex shape with two ribs on the adaxial side	Concave-convex shape in tranverse section (rounded on the abaxial side)	Concave-convex shape in transection (rounded on the abaxial side)	Concave-convex shape in transection (rounded on the abaxial side)	Concave-convex shape in transection (rounded on the abaxial side)	Flat-convex shape with two ribs on the adaxial side and three ribs on the abaxial side
Petiole vascular pattern	5–11 free collateral vascular bundles/U-shaped	About 10 free collateral vascular bundles in an open arc	5–11 free collateral vascular bundles/U-shaped	5–11 free collateral vascular bundles/U-shaped	5–11 free collateral vascular bundles/U-shaped	About 10 free collateral vascular bundles in an open arc

Box 3: Anatomical features showing differentiation between the stems of *Mikania* spp.

	M. campanulata	*M. cordifolia*	*M. glomerata*	*M. hastato-cordata*	*M. microptera*	*M. sessilifolia*
Cross-sectional stem shape	Circular	Hexagonal	Circular	Circular with 2 valecules at opposite sides	Hexagonal with 6 acute wings	Hexagonal with 6 rounded wings
Sclerenchymatous ring	Absent	Present	Absent	Absent	Absent	Absent
Secretory ducts in the pith	Absent	Present	Present	Absent	Absent	Absent
Sclerenchymatous cells in the pith	Absent	Absent	Absent	Present	Absent	Absent
Prismatic crystals	Absent	Present	Absent	Absent	Absent	Absent

2015). However, anisocytic (Budel et al., 2009) and actinocytic types (Neves and Sá, 1991) have been described for *Mikania* species.

In the present study, two types of glandular trichomes and one type of non-glandular trichome were found in the leaves of all the studied species. With regard to the glandular trichomes, the first type was capitate uniseriate or biseriate and secretion occurred within the subcuticular space (Fig. 3A, G–I, K, M, O and Fig. 4A, C, F, G). They were frequently positioned in a depression caused by invaginations of the adjacent epidermal cells (Figs. 3K, M, O and 4A, C, F, G). This trichome was encountered in all the species; however, it is more abundant in *M. microptera* and *M. sessilifolia*, and is rare in *M. cordifolia*.

The other glandular trichome encountered in the studied species was multicellular, uniseriate and filamentous, and formed about six cells. They were either straight or curved and had a terminal cell that varied from a spherical (Fig. 3D, H–J, M) to a spatulate shape (Fig. 3C). They often appeared in the epidermis depression (Figs. 3J, M and 4E). In *M. campanulata*, this kind of trichome had broad-based cell (Fig. 3H).

The non-glandular trichome was conical and was long or short and straight or curved (Figs. 3B, F, I, L and 4B, F). It had a striated cuticle that could be clearly observed in *M. sessilifolia* (Fig. 3L). In the present study, only *M. glomerata* did not have this type of trichome.

Glandular and non-glandular trichomes are often found in *Mikania* (Milan et al., 2006; Gasparetto et al., 2010; Appezzato-da-Glória et al., 2012; Amorin et al., 2014; Araújo et al., 2015). In the present study was observed that the set of trichomes can help to differentiate de species.

In the present study the leaf blade in cross-section had a uniseriate epidermis in *M. campanulata* (Fig. 5A), *M. cordifolia* (Fig. 5B), *M. hastato-cordata* (Fig. 5D), *M. microptera* (Fig. 5E), and *M. sessilifolia* (Fig. 5F). The hypodermis was only observed in *M. glomerata* (Fig. 5C, C′). Additionally, it was only in *M. campanulata* that the upper epidermis cells had a very conspicuous lenticular shape (Figs. 3A, E, H, 4A and 5A). In all the studied species, the epidermis was covered by a thin cuticle and the stomata were located at the same level as the other epidermal cells.

In *Mikania*, the epidermal system is often formed by a single epidermal layer, although the hypodermis appears in a few species such as *M. confertissima*, *M. glomerata*, *M. hatschbachii*, *M. hookeriana* (Oliveira et al., 1994), and *M. laevigata* (Budel et al., 2009). Oliveira et al. (1994) reported that the presence of this stratum can be used to classify the *Mikania* species into two groups.

The leaves of the analyzed species were of a dorsiventral mesophyll type. The palisade parenchyma consisted of 1–2 layers and the spongy parenchyma displayed several strata and some small intercellular spaces. Minor collateral vascular bundles were detected

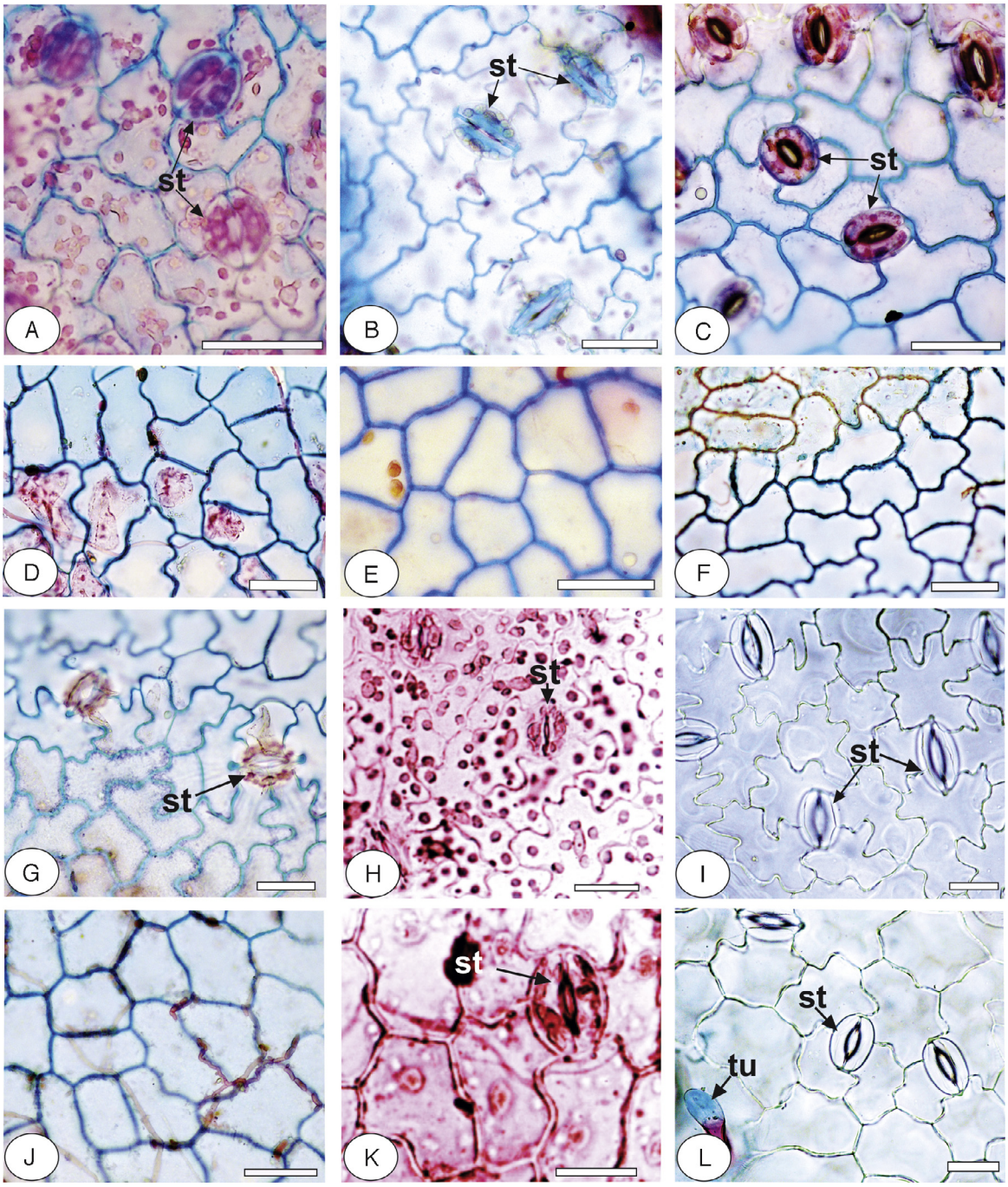

Fig. 2. *Mikania* spp. View of the leaf surface, showing stomata (st) and uniseriate glandular trichome (ut) – Abaxial side – A. *M. campanulata*, B. *M. cordifolia*, C. *M. glomerata*, G. *M. hastato-cordata*, H. *M. microptera*, I. *M. sessilifolia*. Adaxial side – D. *M. campanulata*, E. *M. cordifolia*, F. *M. glomerata*, J. *M. hastato-cordata*, K. *M. microptera*, L. *M. sessilifolia*. Scale bar = 20 μm.

immersed in the mesophyll and surrounded by the parenchymatic sheath (Fig. 5B–F). Secretory ducts were observed near the vascular bundles.

This arrangement of the chlorenchyma was also confirmed in several *Mikania* (Rodrigues et al., 1996; Budel et al., 2009; Amorin et al., 2014; Araújo et al., 2015). However, Milan et al. (2006) found that the arrangement of the mesophyll of *M. glomerata* varied in accordance with the different regions of the leaf blade.

Some anatomical differences were observed in the shape of the midrib. *M. campanulata* had a biconvex shape with a obtuse projection on the adaxial side and prominent and rounded convexity on the abaxial side (Fig. 6A). *M. cordifolia* (Fig. 6B) and *M. glomerata* (Fig. 6C) had a biconvex shape and were slightly convex on the adaxial side and rounded on the abaxial side. *M. hastato-cordata* (Fig. 6D) and *M. sessilifolia* (Fig. 6F) had a slightly flat convex shape and *M. microptera* had a biconvex shape with an obtuse projection on the adaxial side and prominent and angular convexity on the abaxial side (Fig. 6E).

The uniseriate epidermis of all the taxa was covered by a smooth cuticle and the stoma were positioned at the same level as the other epidermal cells, except in *M. cordifolia*, where the stomata were located above the other epidermal cells. Oliveira et al. (2000) described this stomata complex as looking like a tower. A variable

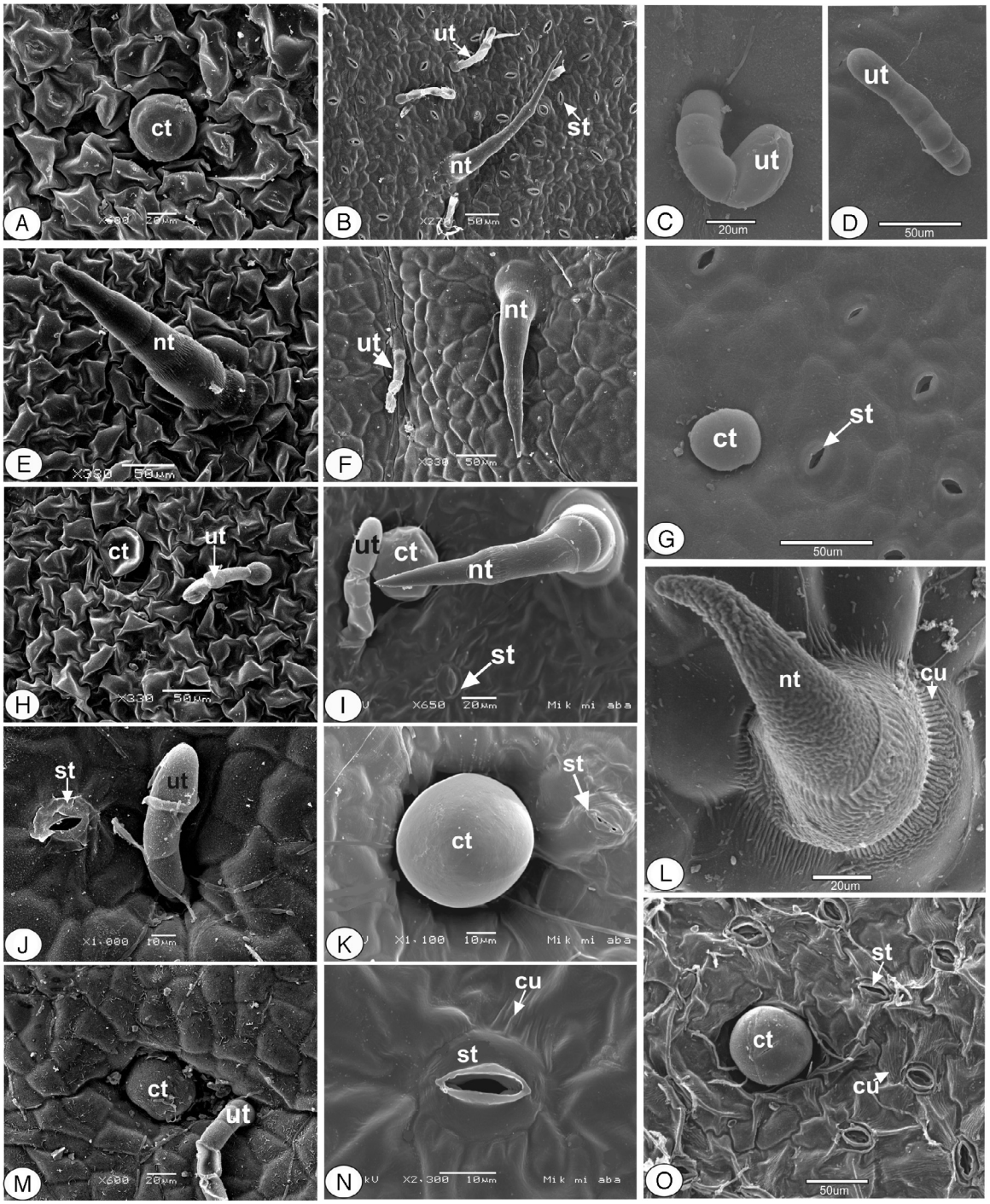

Fig. 3. *Mikania* spp. View of the leaf surface – scanning electron microscopy (SEM) showing striated cuticle (cu), capitate glandular trichome (ct), stomatum (st), non-glandular trichome (nt), uniseriate glandular trichome (ut). A, E, H. Adaxial side of *M. campanulata*; B. Abaxial and F. Adaxial side of *M. cordifolia;* C and D. Adaxial side and G. Abaxial side of *M. glomerata*; J. Abaxial and M. Adaxial side of *M. hastato-cordata*; I, K, N. Abaxial side of *M. microptera*, L. Adaxial side and O. Abaxial side of *M. sessilifolia.*

number of angular collenchyma on both sides was observed in all the species (Fig. 6A–F).

With regard to the vascular pattern, only *M. sessilifolia* had a collateral vascular bundle at the center of the midrib (Fig. 6F). The other studied species showed 3–5 free vascular bundles in an open arc (Fig. 6A–E). These vascular bundles were surrounded by an endodermis which had starch grains. Secretory ducts were located near or between the vascular bundles and were formed of 4–12 cells with a uniseriate epithelium in all the species.

In the present study, the petiole of *M. cordifolia*, *M. glomerata, M. hastato-cordata* and *M. sessilifolia* had a concave-convex shape in transverse section and were rounded on the abaxial side (Fig. 7B–D, F). *M. campanulata* had a flat-convex shape with two ribs on the adaxial side (Fig. 7A). *M. microptera* had a flat-convex shape with two ribs on the adaxial side and three ribs on the abaxial side (Fig. 7E).

M. campanulata (Fig. 7A), *M. glomerata* (Fig. 7C), *M. hastato-cordata* (Fig. 7D) and *M. microptera* (Fig. 7E) had 5–11 free collateral vascular bundles that were U-shaped, and *M. cordifolia* (Fig. 7B) and *M. sessilifolia* (Fig. 7F) had about ten free collateral vascular bundles in open arc. The epidermis had the same previously-described characteristics for the leaf blade.

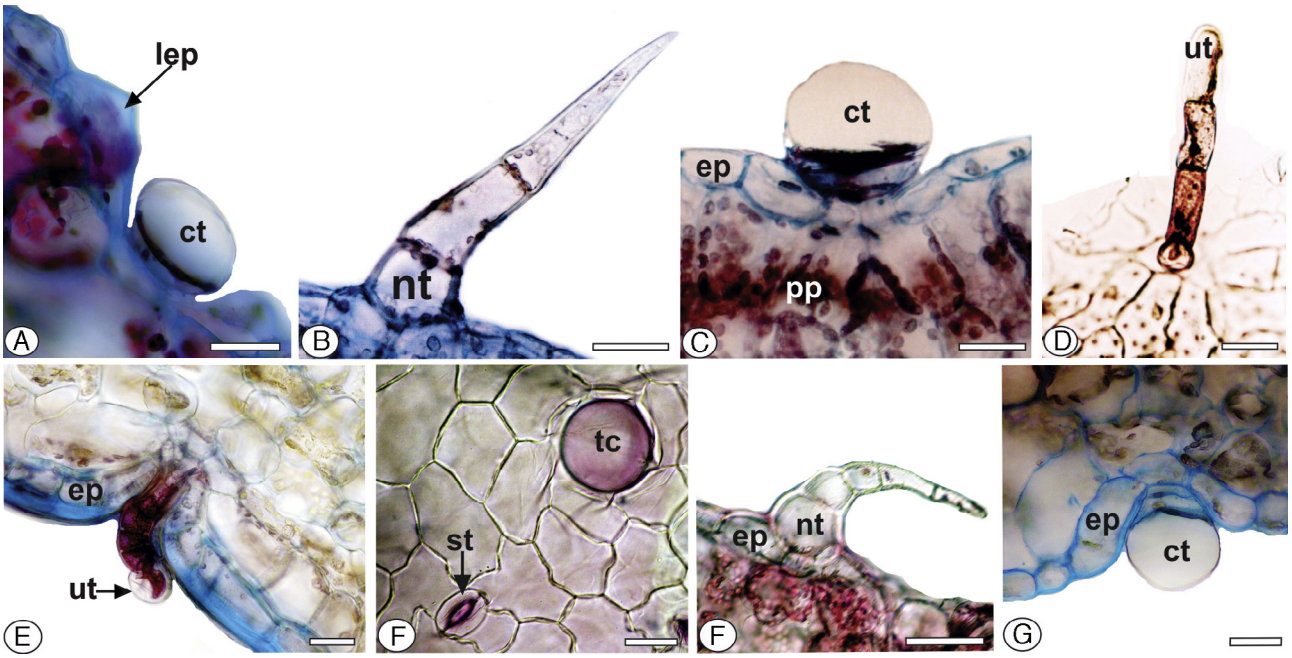

Fig. 4. *Mikania* spp. A–C, E, E, G. Trichomes in cross-section and D, F. Trichomes in frontal view. A. Capitate glandular trichome (ct) in *M. campanulata*, B. Non-glandular trichome (nt) in *M. cordifolia*, C. Capitate glandular trichome (ct) in *M. glomerata*, D. Uniseriate glandular trichome (ut) in *M. microptera*, E. Uniseriate glandular trichome (ut) in *M. hastato-cordata*, F. Capitate glandular trichome (ct) and stomatum (st) in *M. sessilifolia*, F. Non-glandular trichome (nt) in *M. sessilifolia*, G. Capitate glandular trichome (ct) in *M. sessilifolia*. Scale bar = 20 μm.

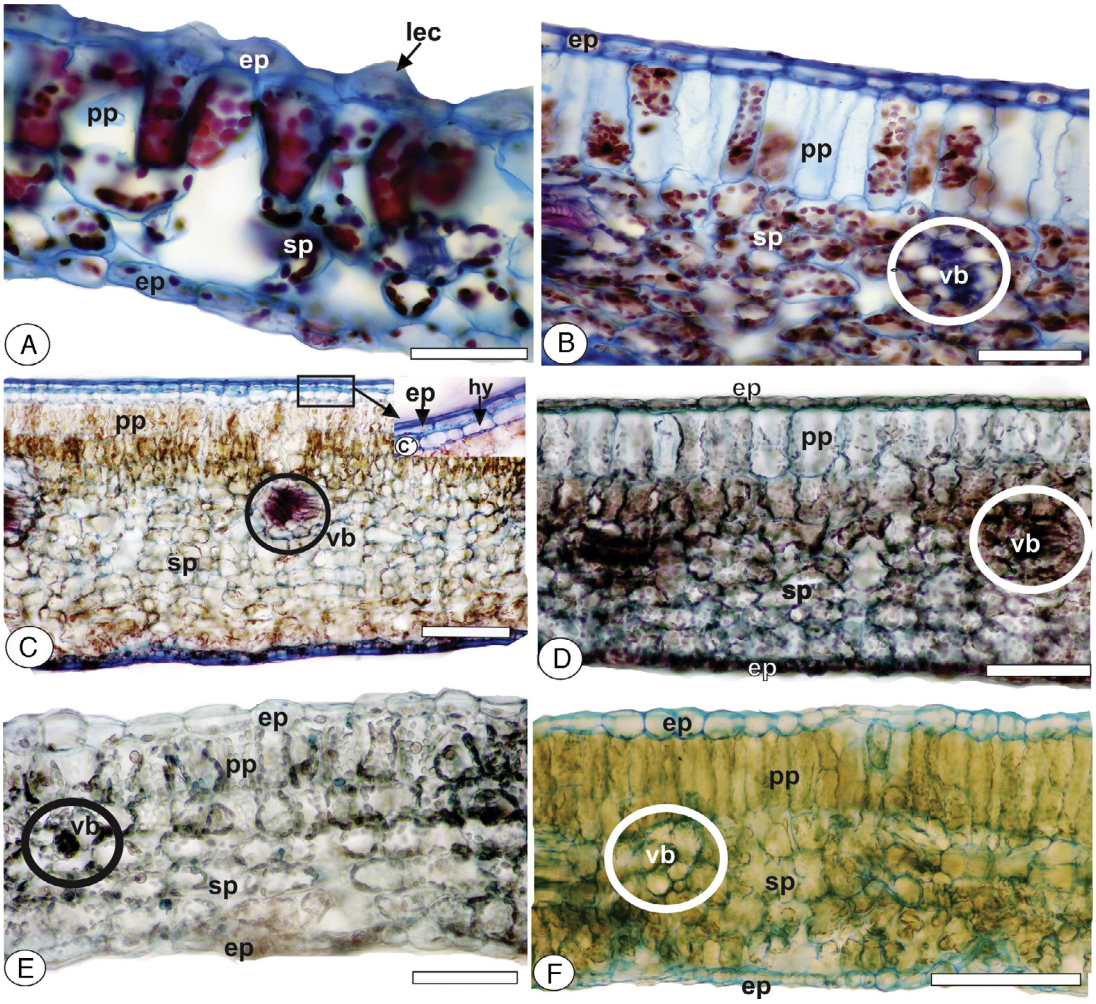

Fig. 5. *Mikania* spp. Leaf blade in cross-section, showing epidermis (ep), hypodermis (hy), palisade parenchyma (pp), spongy parenchyma (sp), vascular bundle (vb). A. *M. campanulata*, B. *M. cordifolia*, C. *M. glomerata*, D. *M. hastato-cordata*, E. *M. microptera*, F. *M. sessilifolia*. Scale bar = 200 μm.

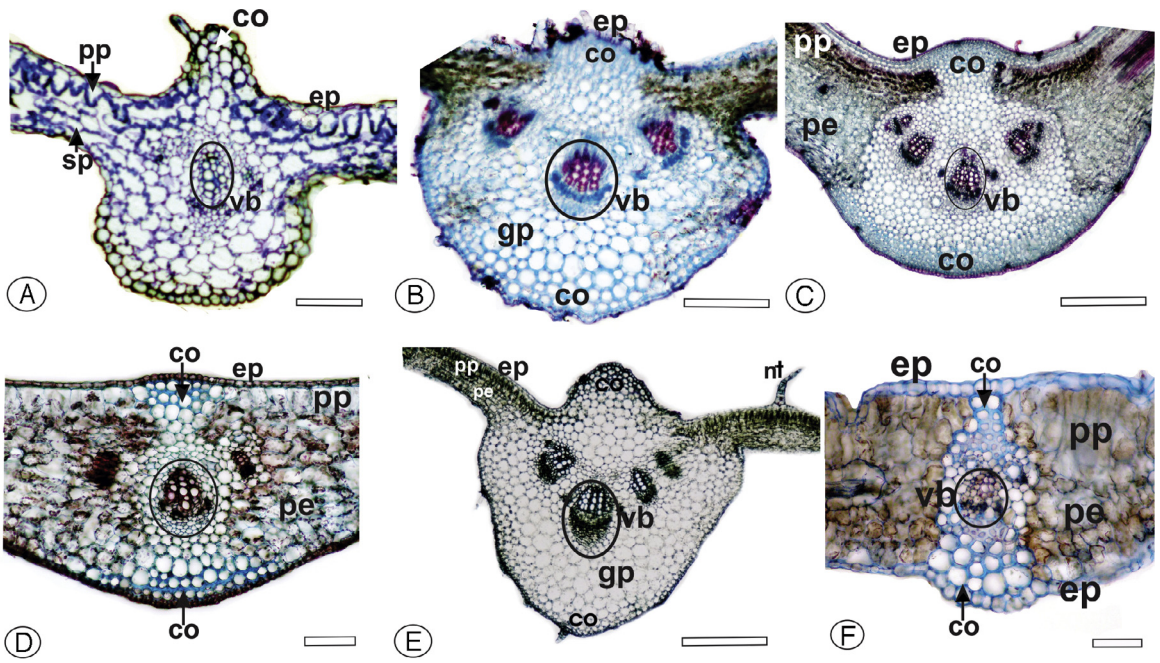

Fig. 6. *Mikania spp*. Midrib in cross-section, showing collenchyma (co) epidermis (ep), ground parenchyma (gp), palisade parenchyma (pp), spongy parenchyma (sp), vascular bundle (vb). A. *M. campanulata*, B. *M. cordifolia*, C. *M. glomerata*, D. *M. hastato-cordata*, E. *M. microptera*, F. *M. sessilifolia*. Scale bar = 200 μm.

Variable strata of angular collenchyma appeared under the epidermis.

The shape and the vascular pattern of the midrib and petiole help to differentiate taxa into genus as reported by Wosch et al. (2015) and Porto et al. (2016). In the present study, these characteristics were one of the most relevant in the anatomical study.

In the present study, the cross-sectional stem shape was hexagonal in *M. cordifolia* (Fig. 8B), *M. microptera* (Fig. 8E) and *M. sessilifolia* (Fig. 8F), but had six acute wings in *M. microptera* (Figs. 8E, K) and six rounded wings in *M. sessilifolia* (Figs. 8F and 9I). On the other hand, a circular stem shape was observed in *M. campanulata* (Fig. 8A), *M. glomerata* (Fig. 8C) and *M. hastato-cordata* (Fig. 8D). In addition, *M. campanulata* had two valecules on opposite sides (Fig. 8A).

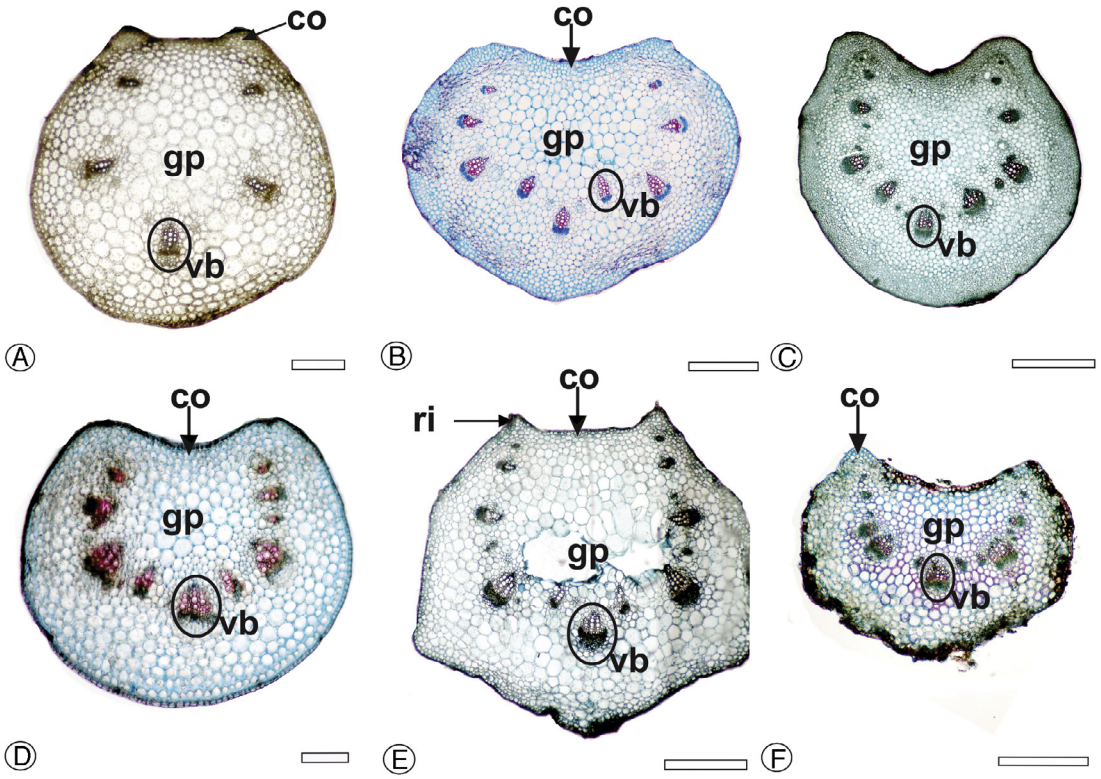

Fig. 7. *Mikania* spp. Petiole in cross-section, showing collenchyma (co), ground parenchyma (gp) and vascular bundle (vb). A. *M. campanulata*, B. *M. cordifolia*, C. *M. glomerata*, D. *M. hastato-cordata*, E. *M. microptera*, F. *M. sessilifolia*. Scale bar = 200 μm (A–E), 50 μm (F).

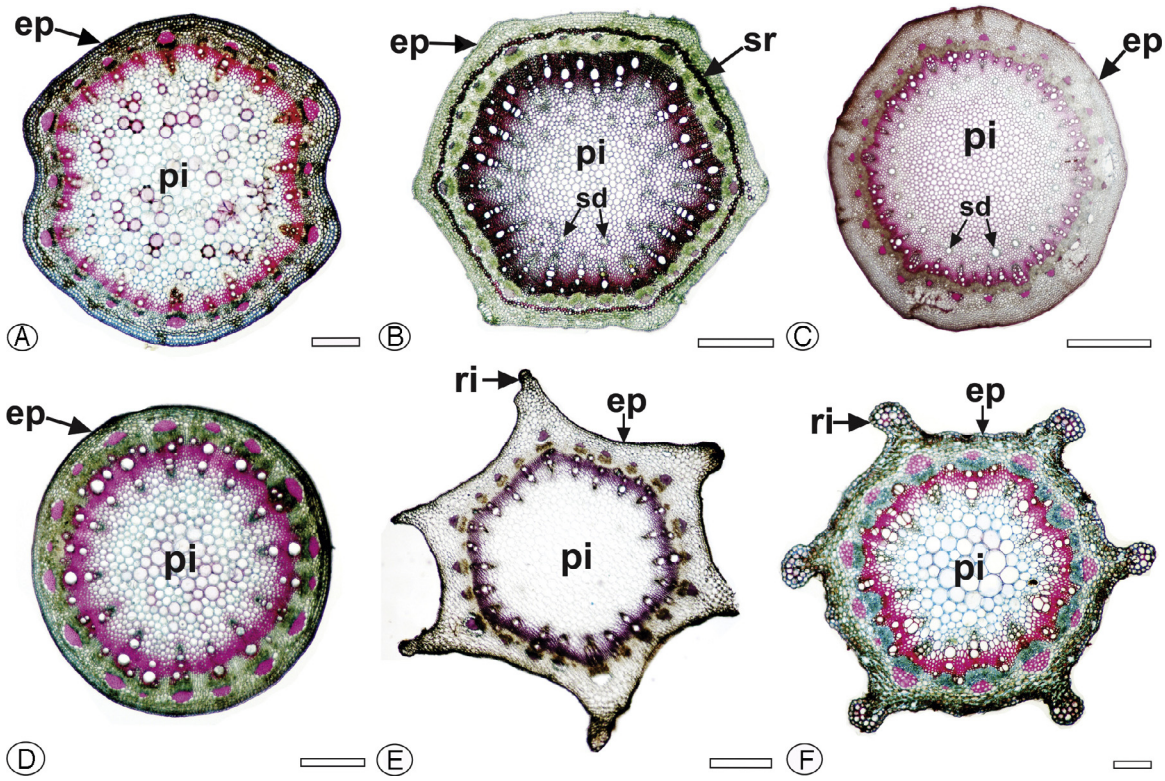

Fig. 8. *Mikania* spp. General appearance of stem in cross-section, showing the epidermis (ep), pith (pi), ribs (ri), sclerenchymatous ring (sr). A. *M. campanulata*, B. *M. cordifolia*, C. *M. glomerata*, D. *M. hastato-cordata*, E. *M. microptera*, F. *M. sessilifolia*. Scale bar = 200 μm.

A circular stem shape seems to be the pattern in *Mikania* (Ritter and Mioto, 2005), although *M. malacolepsis* (Rodrigues et al., 1996) and *M. micrantha* (Araújo et al., 2015) had a hexagonal shape.

The stem epidermis was uniseriate and possessed anomocytic stomata, as well as a thin and slightly striated cuticle (Fig. 9A–C, G, I) which reacted positively with Sudan III in the histochemical tests (Fig. 9C). However, the stomata were located above the other epidermal cells in *M. cordifolia* (as previous described for the midrib). Glandular and non-glandular trichomes (as described in the leaves) were also encountered in the stem. Additionally, the stem of *M. sessilifolia* showed several capitate glandular trichomes (Fig. 9I, L). Under the epidermis there were 1–3 continuous layers of angular collenchyma in all the studied species (Fig. 9A–C, G, K).

In the present study it was observed that the stem shape, the presence of a sclerenchymatous ring in the cortex, and the presence of secretory ducts, sclerenchymatous cells, and calcium oxalate crystals in the pith were very important means of differentiating *Mikania* spp. as seen in Box 3.

In the present study, only *M. cordifolia* had a sclerenchymatous ring in the cortex (Figs. 8B and 9B) and this reacted with hydrochloric phloroglucin in the histochemical tests (Fig. 9B). The sclerenchymatous ring in the cortex was mentioned in *M. hirsutissima* (Oliveira and Akisue, 2005) and *M. malacolepsis* (Rodrigues et al., 1996).

In all the *Mikania* species, the endodermis bound the cortex internally (Fig. 9A, B, E, G) and contained starch grains, which reacted with iodine-iodide (Fig. 10E). Perivascular fiber caps could be observed next to the phloem (Fig. 9A, B, E, G, K) and these reacted with hydrochloric phloroglucin (Fig. 9B). A cambial zone was evident and formed more xylem than phloem (Fig. 9G, K).

In the present study, these secretory ducts were observed in the pith of *M. cordifolia* (Fig. 8B) and *M. glomerata* (Figs. 8C and 9F). Secretory ducts in the pith were observed in *M. conferta* (Oliveira et al., 1999), *M. glomerata* (Neves and Sá, 1991) and in *M. laevigata* (Budel et al., 2009).

In all the species, the pith was well-developed and comprised isodiametric thin-walled parenchymatic cells (Figs. 8A–F and 9J), but *M. campanulata* showed sclerenchymatous cells in the pith (Fig. 8A) that reacted with hydrochloric phloroglucin in the histochemical tests (Fig. 9D).

Prismatic crystals were only observed in the medullary region of *M. cordifolia* (Fig. 9H). The presence or absence of crystals, and their type, can be characterized as taxonomic features (Meric, 2009) and crystals have not been cited in the *Mikania* genus (Oliveira et al., 1994, 1999, 2000; Rodrigues et al., 1996; Budel et al., 2009; Amorin et al., 2014; Araújo et al., 2015).

In the present study, the crystals were analyzed for their elemental composition and the spectra showed prominent peaks for calcium (26.9%), carbon, (31.4%), oxygen (25.6%) and sulfur (16.1%), as can be seen in Fig. 10, indicating these crystals were possible complex compounds of which calcium oxalate was the main compound and calcium sulfate was the minor component.

The occurrence of crystals is common in plants, even though their size and number are responsive to changes in the concentration of calcium in the environment (Nakata, 2003). Excess calcium is frequently precipitated in calcium salts such as oxalate, carbonate, silicate, sulfate, phosphate, citrate and malate (Weiner and Dove, 2003). Crystals have been identified in several studies as calcium oxalate by using EDS (Lersten and Horner, 2011; He et al., 2012; Raman et al., 2014). However, a small number of studies have indicated crystals formed by calcium sulfate (Storey and Thomson, 1994; He et al., 2012).

The morphological features of *Mikania* spp. leaves supported the differentiation of the species. However, when the plants were fragmented or pulverized, the anatomical features of the leaves and

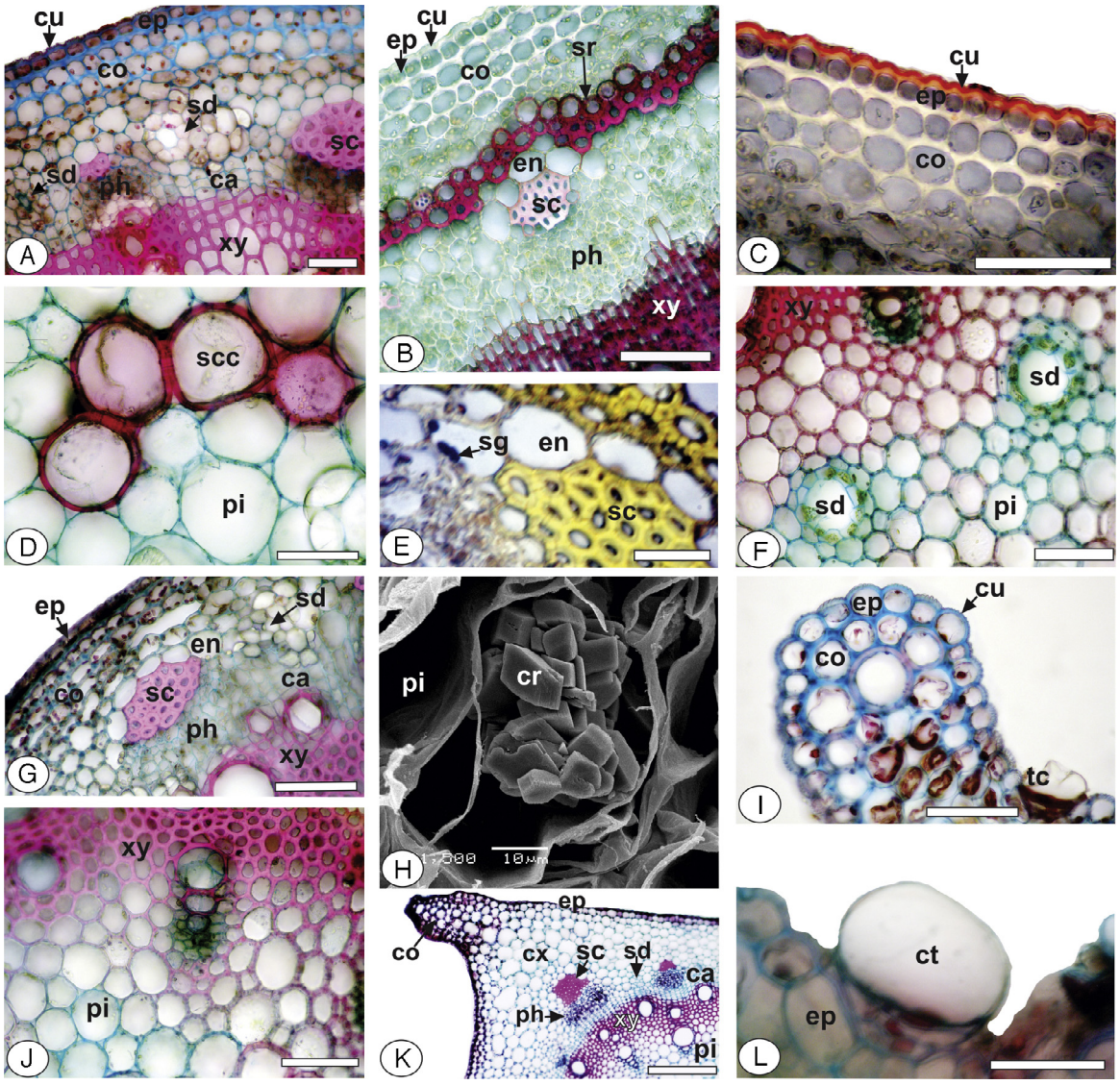

Fig. 9. *Mikania* spp. A, D. *M. campanulata*, B, E, H. *M. cordifolia*, C, F. *M. glomerata*, G, J. *M. hastato-cordata*, K. *M. microptera*, I, L. *M. campanulata*. Details of the parts of the stem, showing ca, cambia; ct, capitate glandular tricome; co, collenchyma; cu, cuticle; cx, cortex; cr, crystals of calcium oxalate; en, endodermis; ep, epidermis; pi, pith; ph, phloem; sc, sclerenchymatous sheath; scc, sclerenchymatous cells; sd, secretory ducts; sg, starch grain; and xy, xylem. Scale bar = 50 μm (D, E, G, I, J, L), 100 μm (A–C, F, K).

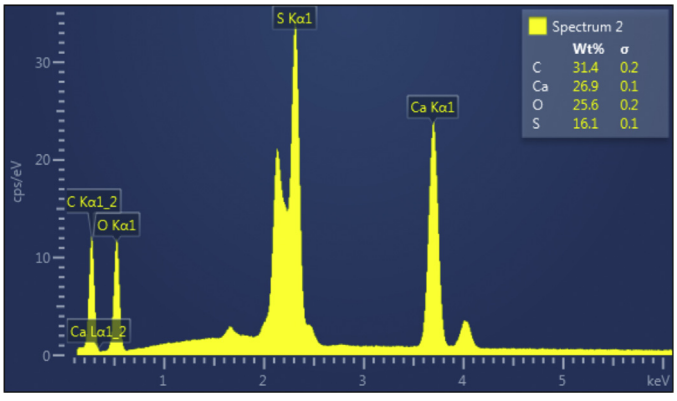

Fig. 10. EDS (energy-dispersive X-ray spectroscopy) spectra of crystals of *M. coridifolia.*

stems supplied additional helpful data for differentiating between them.

The way in which the tissues, elements and cells are organized within a plant organ allows the diagnostic fingerprint for purposes of identification (American Herbal Pharmacopeia, 2011). In the present study, the most important features were occurrence of hypodermis and lens shaped epidermal cells, set of trichomes; midrib, petiole and stem shape and vascular pattern; sclerenchymatous ring in the cortex, sclerenchymatous cells and secretory ducts in the pith.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

VPA, AAH, PAR, BEM and BJ assisted in carrying out the laboratory work. VLPS and CRCF helped in conducting the scanning electron microscopy (SEM) analysis. JPP and PVF provided a critical reading of the manuscript. JMB planned the project, collected the plant material and was responsible for its identification; she also supervised the laboratory work, and wrote the paper. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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