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Aerial stem and leaf morphoanatomy of some species of *Smilax*

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Abstract: This study aimed to describe the morphoanatomy of the aerial vegetative organs of seven *Smilax* species, used in Brazilian folk medicine. Samples of leaves and stems were fixed with FAA 50, embedded in historesin, sectioned on a rotary microtome, stained and mounted in synthetic resin. Cuticle ornamentation was analyzed with standard scanning electron microscopy. In the frontal view, the walls of the adaxial epidermis are straight in *S. brasiliensis*, *S. cissoides*, *S. goyazana* and sinuous in the other species. The walls of the epidermis on the abaxial surface are straight in *S. brasiliensis*, *S. goyazana*, *S. rufescens*, sinuous in *S. campestris*, *S. fluminensis*, *S. oblongifolia*, and wavy in *S. cissoides*. The stomata are paracytic in *S. brasiliensis*, *S. goyazana*, *S. oblongifolia*, and *S. rufescens*, anomocytic in *S. cissoides*, *S. campestris*; anisocytic and paracytic in *S. fluminensis*. The midrib has three vascular bundles that are individually wrapped by lignified cells in *S. brasiliensis*, *S. cissoides*, and *S. fluminensis*. In the other, the three vascular bundles are surrounded by a single lignified sheath. In the stems the vascular cylinder is surrounded by a sclerenchymatous ring with the exception of *Smilax fluminensis*, which has a starch sheath and internal layers of thin-walled cells.

Introduction

According to Judd et al. (2009), Smilacaceae consists of the *Smilax* genus, which comprises 310 species that are distributed in temperate and tropical regions.

The plants are dioecious, vines or herbaceous vines, or rarely, subshrubs or shrubs. The leaves are simple, alternate, contain petioles with tendrils, primary acrodrome venation; the thickened stems are rhizophores, and the aerial stems are generally aculeated (Andreata, 1997).

According to Ferrufino-Acosta (2010), Moore et al. (2008; 2010), the description of new species of *Smilax* is based on one sex (e.g. pistillate or staminate) because the genus are dioecious plants, making it difficult to gather flowers from both types to make the correct observations of their reproductive characteristics. In addition, the same authors reported morphological phenotypic plasticity, which hinders further taxonomic study of Smilacaceae. Therefore, Moore et al. (2008; 2010) suggest that anatomical features, such as the ultrastructure of the leaf cuticle, might aid in the identification of species of *Smilax*.

Some authors have described leaf anatomy characteristics of *Smilax*: Mandarim-de-Lacerda et al. (1992) found variations in both the leaf size and biometrics of *S. rufescens*; Marquete & Pontes (1994), whose comparative anatomical study among *Smilax spicata*, *S. rufescens*, and *S. fluminensis* revealed that the only difference among the three species were that the leaves of *S. fluminensis* are amphistomatic and hypostomatic compared to the other two species; and Mandarim-de-Lacerda & Andreata (1994/1995), who conducted a comparative study between the mature leaves of *S. campestris* and *S. cognata*. Gattuso (1995) analysed *S. campestris*, Martins & Appezzato-da-Glória (2006) characterised the anatomy of the vegetative organs of *S. polyantha*, Moore et al. (2008) described the epicuticular wax of *Smilax* species from Thailand, Palhares et al. (2009) studied the leaves of *S. goyazana*, Moore et al. (2010) used features of the leaf epidermis of six *Smilax* species for a systematic analysis, and Silva et al. (2012) pointed out some diagnostic anatomical features of the aerial organs in *S. syphilitica* and *S. aff. syphilitica*.

In Brazil, the genus *Smilax* is represented by 32 species (Andreata, 1997; 2000). Species of this genus,

which is popularly known as sarsaparilla (*japecanga* in Brazil), are used in folk medicine as a tonic against rheumatism and as an anti-syphilitic (Andreato, 1997). Studies show that the aqueous extract of the rhizophore can act as a therapeutic agent in immune-inflammatory diseases, such as rheumatoid arthritis (Jiang & Xu, 2003). Leaves of *S. aspera* contain tocopherol, which is a phenolic compound known as an efficient antioxidant (Demo et al., 1998).

Despite the pharmacological activities attributed to *Smilax*, it is difficult to identify such plants taxonomically, due to the similarities in their morphology, chemical composition, and popular names (Andreato, 1997). This study aimed to describe the morphoanatomy of the aerial vegetative organs of seven *Smilax* L. species (*S. brasiliensis*, *S. campestris*, *S. cissoides*, *S. fluminensis*, *S. goyazana*, *S. oblongifolia*, and *S. rufescens*), used in Brazilian folk medicine, to characterise and define the anatomical features of each species and thereby to aid in their identification.

Material and Methods

Three adult individuals of *Smilax brasiliensis* Sprengel, *S. campestris* Grisebach, *S. cissoides* Martius ex Grisebach, *S. fluminensis* Steud., *S. goyazana* A. De Candolle, *S. oblongifolia* Pohl ex Grisebach, and *S. rufescens* Grisebach were analysed. These individuals were collected from natural populations of different regions of Brazil: Itapagipe-MG, Porto Alegre-RS, Feira de Santana-BA, Itirapina-SP, Parque Nacional da Chapada dos Veadeiros, Alto Paraíso de Goiás-GO, Ouro Preto-MG, and Parque Estadual Ilha do Cardoso, Cananéia-SP, respectively. *Smilax brasiliensis*, *S. goyazana* and *S. oblongifolia* were collected from open habitat Cerrado (tropical savanna) physiognomies; *S. campestris* in Campos (subtropical grasslands); *S. cissoides* in Caatinga; *S. rufescens* in Restinga (sandy coastal plain); and *S. fluminensis* in riparian forest vegetation.

The collected material was identified and the specimens were incorporated into the ESA herbarium under the numbers ESA 107635, ESA 107657, ESA 107656, ESA 107633, ESA 107645, ESA 107643, and ESA 107663, respectively.

In fully expanded leaves, the midrib, internervural sector, and margin at the median region of the leaf blade were analysed. The stem was analysed in the region of the third internode and the internode near the ground.

Samples were fixed in FAA 50 (1:1:18 formaldehyde:glacial acetic acid:50% ethanol (v/v)) for 48 h (Johansen, 1940). Transverse and longitudinal sections (30-60 µm thick) were made with the aid of a razor blade (leaves and stems) and sliding microtome

(stems), clarified with sodium hypochlorite, and washed in 20% distilled water. The sections were stained with safranin and astra blue (Bukatsch, 1972), dehydrated in an ethanol series and 50 and 100% butyl acetate and mounted in Entellan® synthetic resin (Merck, Darmstadt, Germany).

The chemical natures of the substances were determined using the following histochemical tests: ferric trichloride solution for phenolic compounds (Johansen, 1940), Lugol's iodine solution to identify starch (Johansen, 1940, Berlyn & Miksche, 1976), ruthenium red to identify pectins (Johansen, 1940), Sudan IV to identify lipidic compounds (Jensen, 1962), and acidic phloroglucin to detect lignin (Johansen, 1940).

The tissue dissociation technique was used to analyse epidermal and lignified mesophyll cells. This technique involves treating the leaf with chromic and nitric acids (both at a concentration of 10%), applying a dye, and mounting the blade with glycerin (Johansen, 1940).

For SEM analyses, leaf blades were fixed in Karnovsky medium (Karnovsky, 1965) for 24 h, dehydrated in a graded acetone series, and critical point-dried with CO₂ (Horridge & Tamm, 1969). Samples were attached to aluminium stubs and coated with gold (30-40 nm). The samples were examined under an LEO VP 435 scanning electron microscope at 20 kV. The ornamental patterns of the epicuticular wax were identified following the classification of Metcalfe & Chalk (1979).

The images were digitally captured with a Leica DMLB microscope (Leica™, Wetzlar, Germany) using a video camera connected to a computer and utilising IM50 (Leica™, Wetzlar, Germany) software for the image analyses.

The morphology of the vegetative organs has been documented through photographs with a digital camera or through botanical illustrations. The description of the morphology and classification of leaf venation patterns were based on the findings of Ash et al. (1999).

Results

The leaf blades of the studied *Smilax* species are long and have a leathery consistency with the exception of *S. cissoides*, which has a membranous leaf consistency. The leaf blades vary in form among species: ovate in *S. brasiliensis* (Figure 1A), ovate in *S. cissoides* (Figure 1C), cordate in *S. fluminensis* (Figure 1D), elliptic in *S. goyazana* (Figure 1E), oblong-elliptic in *S. campestris* (Figure 1B), oblong in *S. oblongifolia* (Figure 1F), and oblong-elliptic in *S. rufescens* (Figure 1G). All species have basal acrodrome venation with

three major midribs in *S. campestris*, *S. oblongifolia*, and *S. rufescens* (Figure 1B, F, G) and five major protruding midribs in *S. brasiliensis*, *S. cissoïdes*, *S. fluminensis*, and *S. goyazana* (Figure 1A, C, D, E).

Spiniform emergences (Figure 1H) are observed in the leaves of all species, except for *S. fluminensis*. These emergences occur in midrib extensions in *S. cissoïdes* and *S. goyazana*, and in the veins and leaf margins in *S. brasiliensis*, *S. campestris*, *S. oblongifolia*, and *S. rufescens*.

In the frontal view (Figure 2 A-F), the adaxial epidermis walls are straight in *S. brasiliensis*, *S. cissoïdes*, and *S. goyazana* and sinuous in the other species. The epidermis walls are straight on the abaxial surface in *S. brasiliensis*, *S. goyazana*, and *S. rufescens*, sinuous in *S. campestris*, *S. fluminensis*, and *S. oblongifolia*, and wavy in *S. cissoïdes*. The leaf is amphistomatic in *S. brasiliensis* and *S. goyazana* and hypostomatic in the other species. In *S. cissoïdes*, *S. fluminensis*, and *S. rufescens*, there are few stomata on the adaxial surface. The stomata are paracytic in *S. brasiliensis* (Figure 2A), *S. goyazana* (Figure 2E), *S. oblongifolia*, and *S. rufescens*, anomocytic in *S. cissoïdes* (Figure 2C) and *S. campestris* (Figure 2B), and anisocytic and paracytic in *S. fluminensis* (Figure 2D).

The ultrastructural analysis revealed distinct types of wax deposition and ornamentation of the leaf cuticle (Figure 2 G-L). The cuticle showed wax crystals forming crusted protuberances on the adaxial side in *S. brasiliensis* (Figure 2G), whereas papillose wax deposition occurred on the cuticle of the abaxial surface, similar to both sides of *S. campestris* (Figure 2H), and on the abaxial side of *S. oblongifolia*. Granular cuticle deposition was an additional type that was found among the species and was observed on both sides of the leaves of *S. cissoïdes* (Figure 2I) as minute granules, on both sides of the leaves of *S. rufescens* (Figure 2L) as verrucose granules, and on both sides of the leaves of *S. fluminensis* (Figure 2J) as globular granules. A third deposition type, membranous platelets, was found on both sides of the leaves of *S. goyazana* (Figure 2K) and on the adaxial surface of the leaves of *S. oblongifolia*.

The transverse sections (Figure 3A-D) indicate that the epidermis is uniseriate on both sides of the leaf, and parietal thickening is present.

The leaf margins of all of the species showed the same pattern (Figure 3A): non-stratified epidermis with smaller and more rounded cells than in the rest of the leaf blade and thick cuticles. The layers of cells beneath the epidermis have narrow lumen and thick, lignified walls. Other cells on the margin can have phenolic content and idioblasts containing raphides.

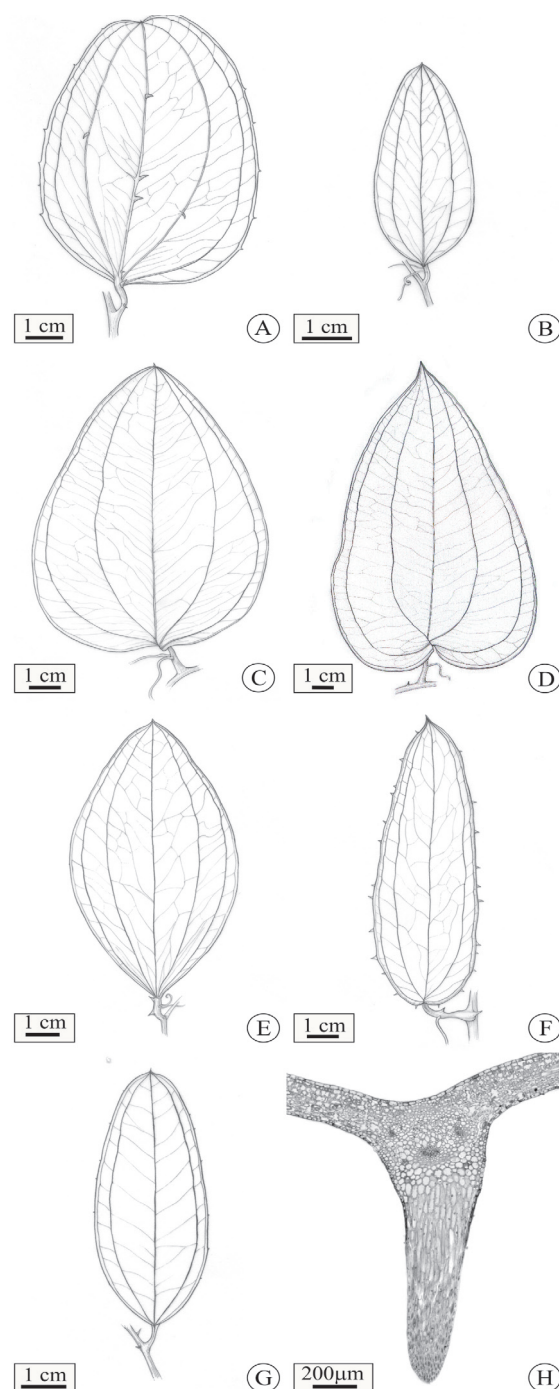


Figure 1. Details of the external leaf morphologies of *Smilax brasiliensis* (A), *S. campestris* (B), *S. cissoïdes* (C), *S. fluminensis* (D), *S. goyazana* (E), *S. oblongifolia* (F), and *S. rufescens* (G). H. Spiniform emergence in the midrib of *S. cissoïdes*.

The mesophyll tends to be dorsiventral in these species with the exception of *S. fluminensis* (Figure 3D), which exhibited a homogeneous mesophyll with cells that had a thickened pectin wall (data not shown). The

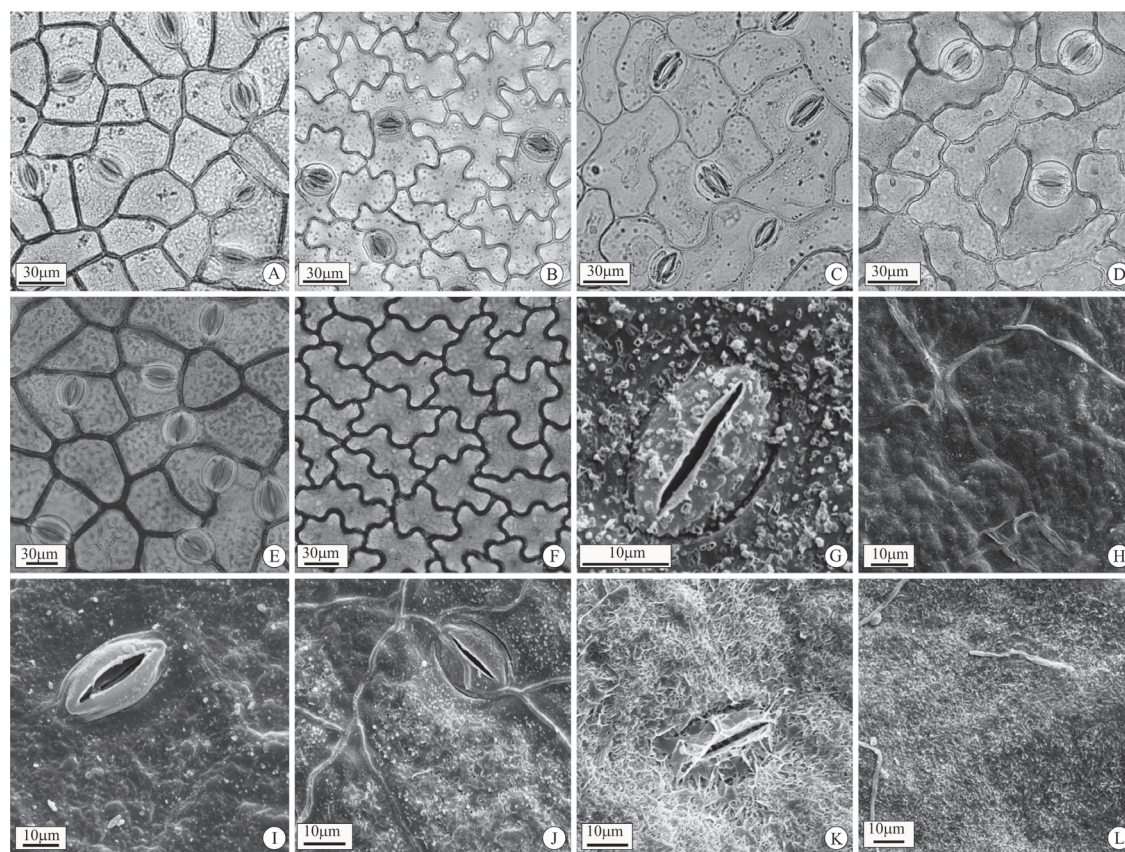


Figure 2. Frontal view of the epidermis. A. *Smilax brasiliensis*; B. *S. campestris*; C. *S. cissooides*; D. *S. fluminensis*; E. *S. goyazana*; F. *S. oblongifolia*. A-E. Epidermis of the leaf abaxial face. F. Epidermis of the leaf adaxial face. G-L. Electron micrographs of the leaf surface, showing different patterns of deposited epicuticular wax. G. *Smilax brasiliensis*, with wax crystals forming crusted protuberances. H. *S. campestris*, with deposited papillose wax; I. *S. cissooides*, with small granular depositions; J. *S. fluminensis*, deposited globose granules; K. *S. goyazana*; L. *S. rufescens*, with verrucose (adaxial face) and papillose (abaxial face) granules. G, H, J, K, L. Epidermis of the leaf adaxial face. I. Epidermis of the leaf abaxial face.

mesophyll that tends to be dorsiventral is characterised by 1-4 layers of short "M"-shaped cells (Figure 3B, arrow) in a juxtaposed arrangement toward the adaxial side (Figure 3B-C), where the remaining mesophyll layers consist of brachiform cells (Figure 3E), leaving wide intercellular spaces.

Idioblasts containing phenolic compounds and idioblasts with raphides were found in the mesophyll. The lateral vascular bundles in all seven species can vary in size but are always surrounded by lignified sclereid cells and fibres. Sclereid types can vary among the species (Figure 3F-H, Chart 1), and the following was found: fibriform (Figure 3H) and columnar sclereids (Figure 3F) in *S. brasiliensis*, *S. campestris*, *S. cissooides*, and *S. rufescens*, columnar sclereids in *S. oblongifolia*, astrosclereids in *S. goyazana* (Figure 3G), and astrosclereids, and columnar sclereids in *S. fluminensis*.

The species midrib (Figure 4A-B) in this study has three similarly-sized central vascular bundles that

are individually wrapped by lignified cells (Figure 4A) in *S. brasiliensis*, *S. cissooides*, and *S. fluminensis*, while they are surrounded by a single continuous sheath (Figure 4B) in *S. campestris*, *S. oblongifolia*, *S. goyazana*, and *S. rufescens*.

Morphoanatomy of the aerial stem

The stems of all species have several spiny structures in the internode region, which is rare in the nodal region, with the exception of *S. fluminensis* in which these spiny structures are common. The spiny structures from both the internodal and nodal regions have no vasculature.

In the studied species, the third internode stem region (Figure 4C-E), the aerial region close to the ground (Figure 4F) have thick cuticles, uniseriate epidermis, stomata and uniseriate hypodermis with lignified cells. Idioblasts containing raphides are found in the cortical region. The endoderm is not thickened

Chart 1. Leaf features that allow separation of the studied *Smilax* species.

Leaf Features/species	<i>S. brasiliensis</i>	<i>S. campestris</i>	<i>S. cissoides</i>	<i>S. fluminensis</i>	<i>S. goyazana</i>	<i>S. oblongifolia</i>	<i>S. rufescens</i>
External morphology	Ovate (Figure 1A)	Oblong-elliptic (Figure 1B)	Ovate (Figure 1C)	Cordate (Figure 1D)	Elliptic (Figure 1E)	Oblong (Figure 1F)	Oblong-elliptic (Figure 1G)
Number of protruding midribs	5 (Figure 1A)	3 (Figure 1B)	5 (Figure 1C)	5 (Figure 1D)	5 (Figure 1E)	3 (Figure 1F)	3 (Figure 1G)
Epidermal cell walls. Adaxial face front view	Straight	Sinuuous	Straight	Sinuuous	Straight	Sinuuous (Figure 2F)	Sinuuous
Epidermal cell walls. Abaxial face front view	Straight (Figure 2A)	Sinuuous (Figure 2B)	Wavy (Figure 2C)	Sinuuous (Figure 2D)	Straight (Figure 2E)	Sinuuous	Straight
Stomata types	Paracytic (Figure 2A)	Anomocytic	Anomocytic	Paracytic and Anisocytic (Figure 2D)	Paracytic	Paracytic	Paracytic
Stomata occurrence	Amphistomatic	Hypostomatic (Figure 3C)	Hypostomatic, with rare stomata on the adaxial face	Hypostomatic, with rare stomata on the adaxial face (Figure 3D)	Amphistomatic (Figure 3A)	Hypostomatic	Hypostomatic, with rare stomata on the adaxial face
Cuticular deposition pattern on the adaxial face	Crusty protuberances (Figure 2G)	Papillose (Figure 2H)	Minute granules	Globular granules (Figure 2J)	Membranous platelets (Figure 2K)	Membranous platelets	Verrucose granules (Figure 2L)
Cuticular deposition pattern on the abaxial face	Papillose	Papillose	Minute granules (Figure 2I)	Globular granules	Membranous platelets	Papillose	Verrucose granules
Sclereid type	Fibriform and columnar	Fibriform and columnar	Fibriform and columnar	Astrosclereids and columnar	Columnar, astrosclereids and fibriform (Figure 3F-H)	Columnar	Fibriform and columnar
Midrib	Three separate bundles	Three bundles in a common lignified sheath	Three separate bundles (Figure 4A)	Three separate bundles	Three bundles in a common lignified sheath (Figure 4B)	Three bundles in a common lignified sheath	Three bundles in a common lignified sheath

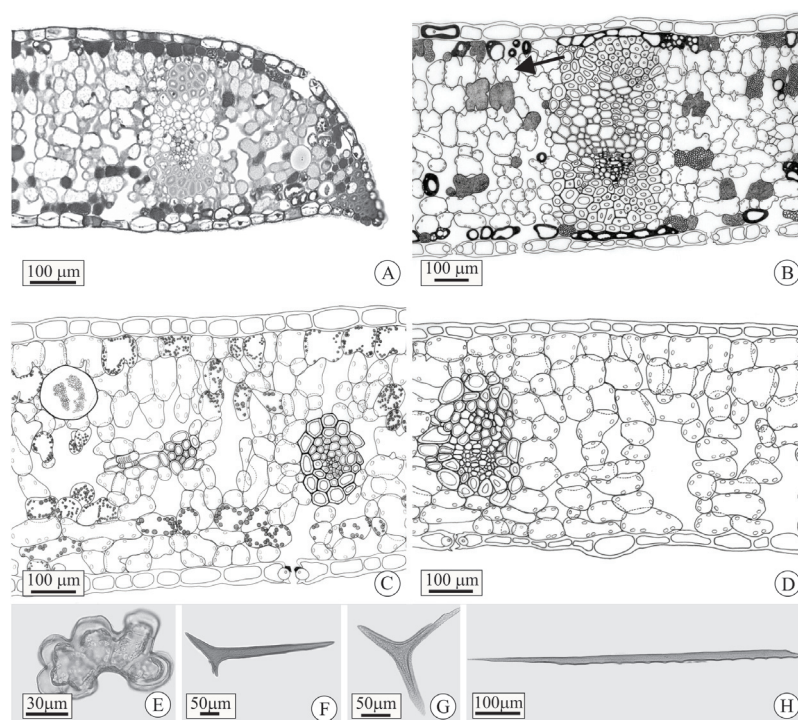


Figure 3. Transverse sections of the leaf margin (A) and internervural sector (B-D) of *S. goyazana* (A-B), *S. campestris* (C), *S. fluminensis* (E), the arrow indicates the “M-shaped” cells. E-H. Dissociated mesophyll cells of *S. goyazana*. E. Braciform cells; F. Sclereid columnar; G. Astrosclereid; H. Sclereid fibriform.

and differentiated, but *S. fluminensis* has a starch sheath in the third internode region (Figure 4E).

The vascular cylinder is an atactostele with collateral bundles in all studied species, although there is a variation in the degree of cell wall lignification depending on the portion analysed (Figure 4C-F).

In the third internode region of the aerial stem, a continuous sclerenchymatous ring surrounds the vascular cylinder in almost all of the species (Figure 4C) with the exception of *S. fluminensis*, which has cells with thin walls and larger lumens in the starch sheath (Figure 4D-E).

The structure of the internode near the ground is similar to that of the third internode. However, the continuous sclerenchymatous ring throughout the stem is only found in *S. brasiliensis*, *S. cissooides*, and *S. rufescens*. The other species have discontinuous sclerenchymatous ring (Figure 4F).

Peripheral bundles in the cortex are present in the portions of the stem near the ground (Figure 4F) and in the portion of the third internode of the stem of *S. fluminensis*.

Starch grains are found only around the vascular bundles in the cylinder of *S. brasiliensis* in both internodes regions and in the vascular cylinder of the internode near the ground in *S. campestris*.

Discussion

Regarding to leaf morphology, the number of protruding midribs can be a useful feature in characterizing *Smilax* species. *S. brasiliensis*, *S. cissooides*, *S. fluminensis*, and *S. goyazana* can be distinguished from the other studied species because they have five prominent midribs and would be grouped with *S. quinquenervia* and *S. subsessiliflora*, which have been studied by Guimarães (2009).

Guaglianone & Gattuso (1991) find a diversity of leaf forms for *S. campestris* specimens collected from different environments. According to the authors, the leaf form varies between ovate, ovate-lanceolate, elliptic, oblong, and cordate, with high variations in length and width. Although there is some variation in leaf shape and size of the seven species studied, the

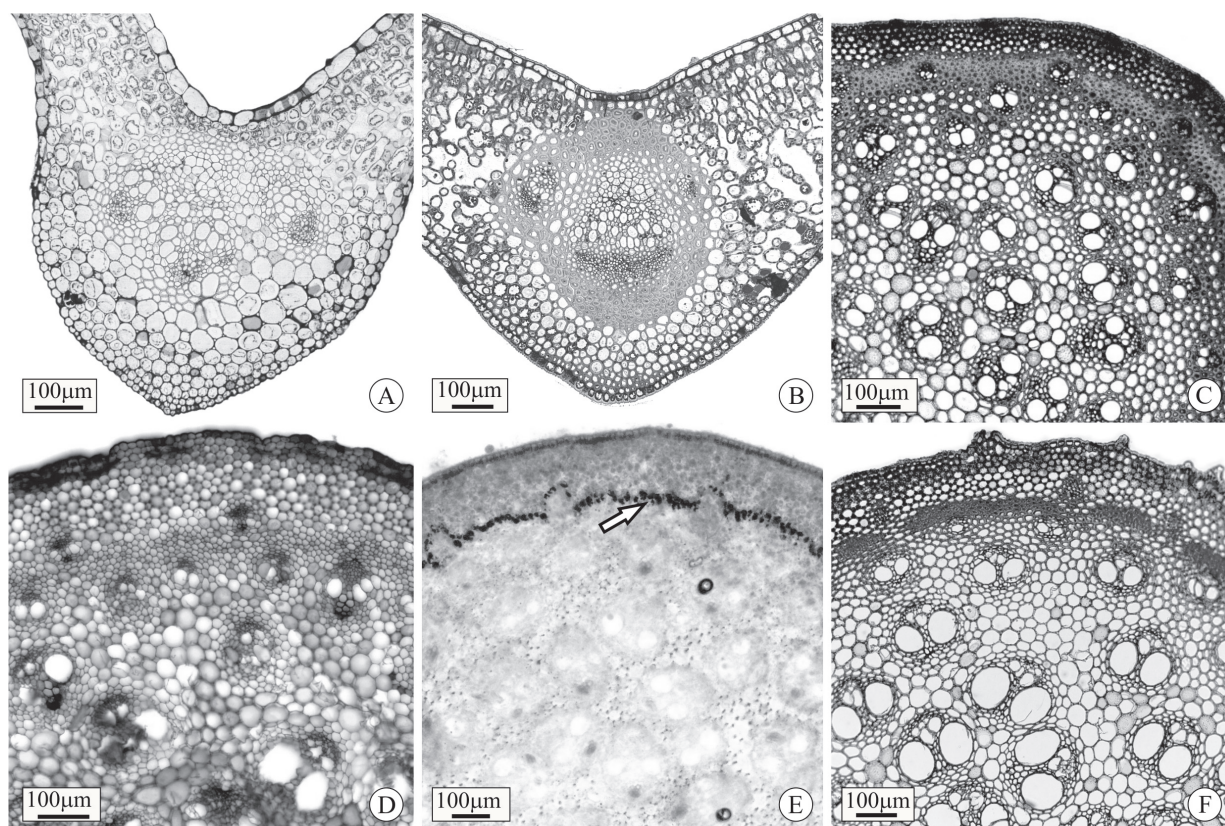


Figure 4. A-B. Transverse sections of the leaf blade in the midrib region. A. *S. cissooides*, vascular bundles individually wrapped by a sheath; B. *S. goyazana*, vascular bundles surrounded by a single continuous sheath; C-F. Transverse sections of the non-thickened stem; C-E. Stem in third internode region; F. Stem region near the ground; C. *S. brasiliensis*, sclerenchymatous continuous ring in the vascular cylinder; D-E. *S. fluminensis*. Absence of sclerenchymatous ring (D) and starch sheath (arrow) (E). F. *S. campestris*. Discontinuous sclerenchymatous ring (F).

features described here reflect the patterns found at these sampling sites.

Among the leaf structural features analysed in this study, the epidermis in a frontal view was essential for separating the species into three groups: anticlinal walls on both sides (*S. brasiliensis* and *S. goyazana*), sinuous walls on both sides (*S. campestris*, *S. fluminensis*, and *S. oblongifolia*), and a different pattern for each of the two sides (*S. cissoides* and *S. rufescens*). Considering these groups, *S. polyantha* (Martins & Appezzato-da-Glória, 2006), *S. stenophylla*, and *S. quinquenervia* (Guimarães et al., 2010) would be included in the third group, and *S. subsessiliflora* (Guimarães et al., 2010) would be included in the second group. The sinuosity of the anticlinal walls of the epidermic cells increases contact among adjacent cells, and may help to maintain leaf structure under mechanical stress (Haberlandt, 1928). According to Silva et al. (2012), variation in the degree of sinuosity in epidermal cell walls observed in *S. syphilitica* is related to stress during leaf differentiation (Avery, 1933), the cuticle hardening process (Watson, 1942).

The cuticle also differs among species, even between two sides in the same species. Martins & Appezzato-da-Glória (2006) described the cuticular ornamentation of both sides in *S. polyantha* as scale-like. Guimarães (2009), based on Guaglianone & Gattuso (1991), observed a "rough-microtuberculate" cuticle on the leaf surface in *S. quinquenervia*, whereas this type refers to a papillose pattern, which is adopted here for *S. campestris*. Guimarães et al. (2010) observed a striated cuticle on the leaf surface of both sides in *S. subsessiliflora*. Moore et al. (2008, 2010) use the ornamentation features of the epicuticular waxes as taxonomic characteristics to distinguish species of the *Smilax* genus found in Thailand.

As demonstrated in this study, stomata can vary among *Smilax* species. The stomata of *S. campestris* and *S. cissoides* are classified as anomocytic, as described for the species studied by Guaglianone & Gattuso (1991) and Gattuso (1995). For the other species analysed, the stomata are classified as paracytic, as in *S. polyantha* (Martins & Appezzato-da-Glória, 2006), *S. quinquenervia*, and *S. subsessiliflora* (Guimarães, 2009).

Yates & Duncan (1970) have studied the leaf anatomy of *S. auriculata*, *S. bona-nox*, *S. glauca*, *S. laurifolia*, *S. rotundifolia*, *S. smallii*, *S. tamnoides*, and *S. walteri*. They describe only dorsiventral mesophyll. Gattuso (1995) also uses dorsiventral classification to determine mesophyll type in *S. campestris*. A mesophyll that tends to be dorsiventral is present in *S. polyantha* (Martins & Appezzato-da-Glória, 2006), because the palisade parenchyma in the adult leaf presents "M-shaped" cells. *Smilax fluminensis* exhibits a homogeneous mesophyll in the present study

in accordance with the description of Guaglianone & Gattuso (1991), and the other species exhibit the same mesophyll type that has been described for *S. polyantha* (Martins & Appezzato-da-Glória, 2006).

The taxonomic significance of foliar sclereids was emphasized by many botanists among them Rajanna & Ramakrishnan (2010) who observed that the type and pattern of distribution of sclereids were not influenced by environmental factors, so they were useful in distinguishing clones of *Camellia*. The type of foliar sclereids also varied in the studied species and none of the species presented osteosclereids as described for *S. polyantha* (Martins & Appezzato-da-Glória, 2006), so this feature associated with other anatomical features could be useful in distinguishing *Smilax* species. The occurrence of abundant sclereids in the vascular bundles, as verified in the studied species, could offer an effective protection against collapse after severe dehydration (Turner, 1994) and could have a light-guiding function as suggested by Karabourniotis (1998) who studied the anatomy and orientation of the foliar sclereids of the evergreen sclerophyll *Phillyrea latifolia*.

Collateral vascular bundles, which are found in the studied *Smilax* species, are also reported for *S. spicata* (Marquete & Pontes, 1994), *S. polyantha* (Martins & Appezzato-da-Glória, 2006), and *S. campestris* (Gattuso, 1995). The midrib has three vascular bundles that are individually wrapped by lignified cells in *S. brasiliensis*, *S. cissoides*, and *S. fluminensis*, as reported for *S. quinquenervia* and *S. subsessiliflora* by Guimarães et al. (2010). In the other species studied, the three vascular bundles are surrounded by a single lignified sheath, as observed by Guaglianone & Gattuso (1991) for *S. cognata*, *S. pilcomayensis*, *S. assumptionis*, and *S. campestris*.

Spiniform emergences in the leaves and/or aerial stems, which are present in the studied species, are not considered thorns because there are no vasculature in their projections. According to Guimarães (2009), there are spiniform emergences in the node and internode regions of the aerial stem branches, where such emergences are vascularised in *S. quinquenervia* and non-vascularised in *S. subsessiliflora*.

Cells with sclerification on the leaf margin, which are found in the studied species, have also been reported by Yates & Duncan (1970), Marquete & Pontes (1994), and Martins & Appezzato-da-Glória (2006).

Caponetti & Quimby (1956) and Van Fleet (1942) claim that the endodermis cannot be differentiated in the younger portions of the stems of *Smilax* species and are only thickened and differentiated in the portions near the ground. In the present study, it is not possible to distinguish the thickened endoderm in both portions of the internode in all studied species, but *S. fluminensis* has starch sheath

in the third internode region.

Peripheral bundles are observed in the cortex of the seven studied species in the internode near the ground, as well as in the third internode region in *S. fluminensis*. Guimarães (2009) considers such bundles as cortical vascular bundles, and they are found in both *S. quinquenervia* and *S. subsessiliflora* (rare occurrence) in the portions of the aerial stem branch. Guaglianone & Gattuso (1991), analysing *S. fluminensis*, *S. pilcomayensis*, *S. cognata*, *S. assumptionis*, and *S. campestris*, also note series (one or two) of peripheral vascular bundles outside the aerial stem sclerenchymatous ring.

In the seven *Smilax* species analysed in the present study and species analysed by Caponetti & Quimby (1956) and Ervin & Evert (1967), the aerial stem has an outer portion of the vascular cylinder, forming a sclerenchymatous ring, and in the inner portion, the dispersed bundles in the parenchyma have an individual sclerenchyma sheath. However, as observed for *S. polyantha* (Martins & Appezzato-da-Glória, 2006), the lignification of cells surrounding the vascular bundles in the vascular cylinder varies according to stem region. The third internode region is not lignified in *S. polyantha* (Martins & Appezzato-da-Glória, 2006), while in the seven studied species, this region and the internode near the ground have a sclerified ring surrounding the entire vascular cylinder.

Difficulty in identifying species from the *Smilax* genus is attributed to dioecy (Andreata, 1997) as well as the morphological similarities among their flowers (Moore et al., 2010). Therefore, the set of leaf features presented in this study, including the number of protruding midribs, epidermal cell walls in frontal view, stomata type, and arrangement of collateral bundles in the midrib, together with other vegetative features, can aid in the taxonomic distinction of *Smilax* species.

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Authors contributions

ARM (PhD student), ABB, ANS (Master student), contributed in collecting plant material, running the laboratory work, analysis the data and drafted the paper. ARM and BAG designed the study, supervised the laboratory work, contributed to critical reading and final editing of the manuscript. All the authors have read the final manuscript and approved the submission.

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