



Original Article

Leaf morphoanatomy of “mororó” (*Bauhinia* and *Schnella*, Fabaceae)

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ABSTRACT

Bauhinia L. and *Schnella* (Raddi.) Wund. are popularly known in Brazil as “mororó”. The leaves and stem bark are used in folk medicine for various purposes, especially against diabetes. Morphoanatomical studies of the leaves of *Bauhinia cheilantha* (Bong.) Steud., *B. pentandra* (Bong.) Steud., *B. unguilata* L. and *Schnella outimouta* (Aublet) Wund., tribe Cercideae, subtribe *Bauhiniinae* (Benth.) Walp., were carried out as subsidies to the quality control of their ethnodrugs and their derivatives, as well as an additional support to their taxonomy. The morphological and anatomical studies employed traditional techniques of stereo- and light microscopy. All species showed bifoliate leaves, a dorsiventral mesophyll, epidermis with a papillose abaxial surface, anomocytic stomata at the level of the epidermis, and tector trichomes. *Schnella outimouta* showed leaf characters distinctive from the three species of *Bauhinia*: indument puberulous on the abaxial surface, leaves hypostomatic, midrib with two collateral bundles, and a cylindrical petiole. The species of *Bauhinia* have a sericeous-pubescent indument, amphistomatic leaves with boat-shaped glands, midrib with a single bundle, and a canaliculate petiole with lateral projections. Our results provide leaf morphological and anatomical parameters, useful to distinguish the four species studied, which support the quality control of its ethnodrugs.

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1. Introduction

Bauhinia L. and *Schnella* (Raddi.) Wund. both belong to the subtribe *Bauhiniinae* (Benth.) Walp. of the tribe Cercideae Bronn (Caesalpinioideae), with pantropical and neotropical distributions respectively. They grow as trees, shrubs, and lianas with simple tendrils, with or without intrastipular spines, having seeds with a crescentic hilum and funicular aril lobes (Wunderlin, 2010a). *Bauhinia* and *Schnella* demonstrate morphological uniformity of their vegetative organs, making it difficult to identify sterile specimens.

Bauhinia and *Schnella* species are popularly known in Brasil, as “mororó”, “miroró”, and “pata-de-vaca” (Agra et al., 2007). The leaves and stem bark of *Bauhinia* species are used in folk medicine for various purposes, especially against diabetes (Agra et al., 2007, 2008).

Bauhinia is considered the most complex genus of the tribe Cercideae (Legume Phylogeny Working Group [LPWG], 2013), with approximately 160 species distributed in the tropical and subtropi-

cal regions of Asia, Africa, Australia, and Central and South America (Duarte-Almeida et al., 2015). A total of 61 species occur in Brazil, of which 39 are endemic (Vaz, 2015). The distinctive characteristics of the genus are its tree or shrub habit (rarely semi-scandent), sometimes with intrastipular spines, rarely with thorns, never with tendrils, calyx spathaceous or dividing the hypanthium into 2–5 lobes (Wunderlin, 2010a, 2010b). The genus shows great phenotypic plasticity, and different taxonomic treatments have been proposed: Benth (1865) and Wunderlin (1976, 1983, 2010a, 2010b).

Schnella was proposed by Raddi (1820), which was revalidated as a generic status by Wunderlin (2010a, 2010b) based on the molecular analyses of Hao et al. (2003) and Sinou et al. (2009). *Schnella* is Neotropical, with about 47 species distributed from Mexico to Argentina (Trethowan et al., 2015), with its center of diversity in Brazil (35 species, of which fourteen are endemic), according to Vaz (2015).

Interest in *Bauhinia* has intensified due to its reported anti-diabetic activity, especially in light of studies of *B. forficata* Link by Cechinel-Filho (2009), Menezes et al. (2007), and Silva and Cechinel-Filho (2002). Other potential medical uses of *Bauhinia* have been reported, including four treating ulcers (Silva and Cechinel-Filho, 2002), and its utility as an anti-oxidant (Braca et al.,

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2001; Pandey et al., 2011), anti-inflammatory, analgesic, and anti-pyretic (Gupta et al., 2005).

Metcalfe and Chalk (1979) noted that anatomical studies have great value in establishing the identities of herbaria specimens, especially with sterile material. Anatomical data have been shown to lend additional support to systematics in many taxonomic groups, acting as important criteria for interspecific and infra-generic delimitations in *Solanum* L. (Araújo et al., 2010; Nurit-Silva and Agra, 2011; Nurit-Silva et al., 2012; Sampaio et al., 2014), *Ficus* L. (Araújo et al., 2014), and *Bauhinia* (Rezende and Cardoso, 1994; Duarte and Debur, 2003; Lusa and Bona, 2009; Albert and Sharma, 2013), and also can contribute to the quality control of medicinal plants (Araújo et al., 2014; Porto et al., 2016).

This study therefore sought to characterize the leaf morphological anatomy of *Bauhinia cheilantha* (Bong.) Steud., *B. pentandra* (Bong.) Steud., *B. unguolata* L. and *Schnella outimouta* (Aublet) Wund., commonly confused in the Brazilian Northeast region, to identify distinctive characters among these species, which can provide additional support to their taxonomy, as well as to the quality control of their ethnodrugs and their derivatives.

2. Materials and methods

Morphological studies, identifications, collections, and field work

The identifications of the *Bauhinia* species were made through analyses of their reproductive and vegetative organs based on the specialized literature (Fortunato, 1986; Vaz and Tozzi, 2003a, 2005; Wunderlin, 2010a, 2010b). Additionally, comparative studies were carried out with specimens identified by specialists from collections at the Prof. Jayme de Moraes Coelho (EAN) and Prof. Lauro Pires Xavier (JPB) herbaria, both at the Federal University of Paraíba.

Botanical expeditions and field observations were conducted in Paraíba State, Brazil, to collect samples of *Bauhinia cheilantha* and *Schnella outimouta* (see Material examined). Fertile reference specimens were herborized following Bridson and Forman (1999) and were deposited in the EAN herbarium, with duplicates assigned to the JPB herbarium. Other materials were fixed in percent FAA (formalin-acetic acid-alcohol) for 48 h, and subsequently preserved in 70% alcohol (Johansen, 1940). Additional samples of dried materials of *Bauhinia pentandra* and *B. unguolata* (see Material examined) were rehydrated and used in anatomical studies. Leaf terminology was based on Van der Pijl (1952) and Lin et al. (2015). Indumentum classification follows Harris and Harris (2001).

Material examined

Bauhinia cheilantha: Brazil, Ceará: Poranga, 04°46'04" S – 40°52'58" W, Félix 14960 (EAN); Paraíba: Alagoa Grande, Rua Nova, 25-VIII-2015, Pereira 06 (EAN, JPB); Cabaceiras, Sítio Maniçoba, VI-2015, Pereira 04 (EAN, JPB); Campina Grande, INSA, 13-III-2012, Albuquerque & Ferraz sn (EAN, JPB); Esperança, Lagoa de Pedra, 17-VI-2003, Pitrez & Trajano 274 (EAN); Fagundes, Estrada para a Pedra de Santo Antônio, 21-V-15, Pereira et al., 03 (EAN, JPB); Itapororoca, Fazenda Macacos, 25-VI-2011, Félix 13600 (EAN, JPB); Mulungu, 25-VIII-2015, Pereira et al., 07 (EAN, JPB); Santa Terezinha, 18-IV-2006, Pegado & Félix 16 (EAN, JPB); São João Tigre, 24-II-2011, Félix 13477 (EAN, JPB); Sossego, Sítio São Miguel, 24-VI-2015, Pereira et al., 05 (EAN, JPB); Sousa, Sítio Lamarão, Estrada de acesso a São José da Lagoa Tapada, 27-V-1995, Moreira 25 (EAN, JPB).

Bauhinia pentandra: Brazil. Paraíba: Itaporanga, Caminho para a Serra Água Branca, 1993, Rocha et al., 1695 (JPB); Pombal, Fazenda Nova Canaã, 18-I-1952, Carneiro 1650 (JPB); Sousa Sítio Lamarão, 27-V-1995, Moreira 23 (JPB); Sousa, Fazenda Jangada, 17-IV-98, Gadelha Neto 424 (JPB); Sousa, Fazenda Jangada, IX-93, Gadelha Neto

61 (JPB); Sousa, Sítio Lamarão, Estrada de acesso São José da Lagoa Tapada, 06-IX-1994, Moreira 7 (JPB).

Bauhinia unguolata: Brazil, Ceará: Poranga, 04°44'49" S – 40°52'11" W, Félix 14947 (EAN).

Schnella outimouta: Brazil, Paraíba: Areia, Estrada para o Sítio Mineiro, 16-I-2015, Pereira 01 (EAN); Areia, Estrada para Pilões, 01-XII-2015, Félix et al. sn (EAN).

Anatomical studies

Leaf samples from the second to fifth nodes were used in the anatomical studies. Paradermic sections of the adaxial and abaxial surfaces, and transverse sections were performed on leaves by free hand using commercial razor blades. Transverse sections were made with adult leaves of the leaf blades, petioles, and pulvini.

All sections were cleared using 2% sodium hypochlorite, rinsed in distilled water, and neutralized with 1% acetic acid. The paradermic sections were stained with Safranin with 1% solution in 50% alcohol, according to Franklin (1945). The transverse cross sections were stained with Astra blue and Safranin, modified by Bukatsch (1972).

The sections were mounted under coverslips with glycerol (50%) and subsequently analyzed and photomicrographed using a Qwin system and video camera (Leica ICC50 HD) coupled to an optical microscope (Leica DM 750) for capturing images.

Characterizations of the cell walls of the epidermis and mesophyll are based on Fahn (1990). Classifications of the stomata follow Metcalfe and Chalk (1979), while leaf venation patterns follow Hickey (1973).

3. Results

Leaf morphology

All of the species of *Bauhinia* (*B. cheilantha*, *B. pentandra*, *B. unguolata*) and *S. outimouta* studied showed alternate and bilobed leaves with fused lobes and entire margins. The leaves of all of the *Bauhinia* species observed had chartaceous consistencies, while those of *S. outimouta* were coriaceous. Oval-oblong leaf blades were predominant, except in *B. pentandra*, which showed somewhat hastate leaves, with open and acute divaricate lobes.

All of the species showed symmetrical or slightly asymmetrical bilobed laminae. The apex is bifid to $\frac{1}{4}$ in *B. cheilantha*, $\frac{1}{2}$ in *B. pentandra*, $\frac{1}{4}$ in *B. unguolata*, and $\frac{3}{4}$ in *S. outimouta*. The base is cordate in *B. cheilantha* and *S. outimouta*, truncate in *B. unguolata*, and somewhat cordate-hastate in *B. pentandra*.

The adaxial leaf surface is glabrous in *B. cheilantha*, *B. pentandra*, and *S. outimouta* (Fig. 1A, C, G 3), and glabrescent in *B. unguolata*, with small trichomes on the midrib (Fig. 1E). All *Bauhinia* species show navicular glands occur on the abaxial surface, and the indument is sericeous-pubescent with simple and multicelled trichomes (Fig. 1B, D, F). The indument is puberulous-ferruginous with short, simple, eglandular trichomes; navicular glands are absent in *S. outimouta* (Fig. 1H).

The petiole is canalliculate and pubescent, with both simple, unicellular and multicellular trichomes, with navicular glands in *B. cheilantha*, *B. pentandra*, and *B. unguolata*. However, *S. outimouta* has a cylindrical and puberulent petiole, with short, ferruginous trichomes.

Two pulvini were observed on the petioles of all of species: one proximal and inserted on the stem, and the other distal and inserted at the base of the leaf blade. A motile cushion is present, from which emerge 9 to 13 main veins that are palminervous in *Bauhinia*, and acrodromous in *S. outimouta*. *B. cheilantha* showed axillary gemma at the base of the proximal pulvinus (Fig. 2A). The pulvinus of

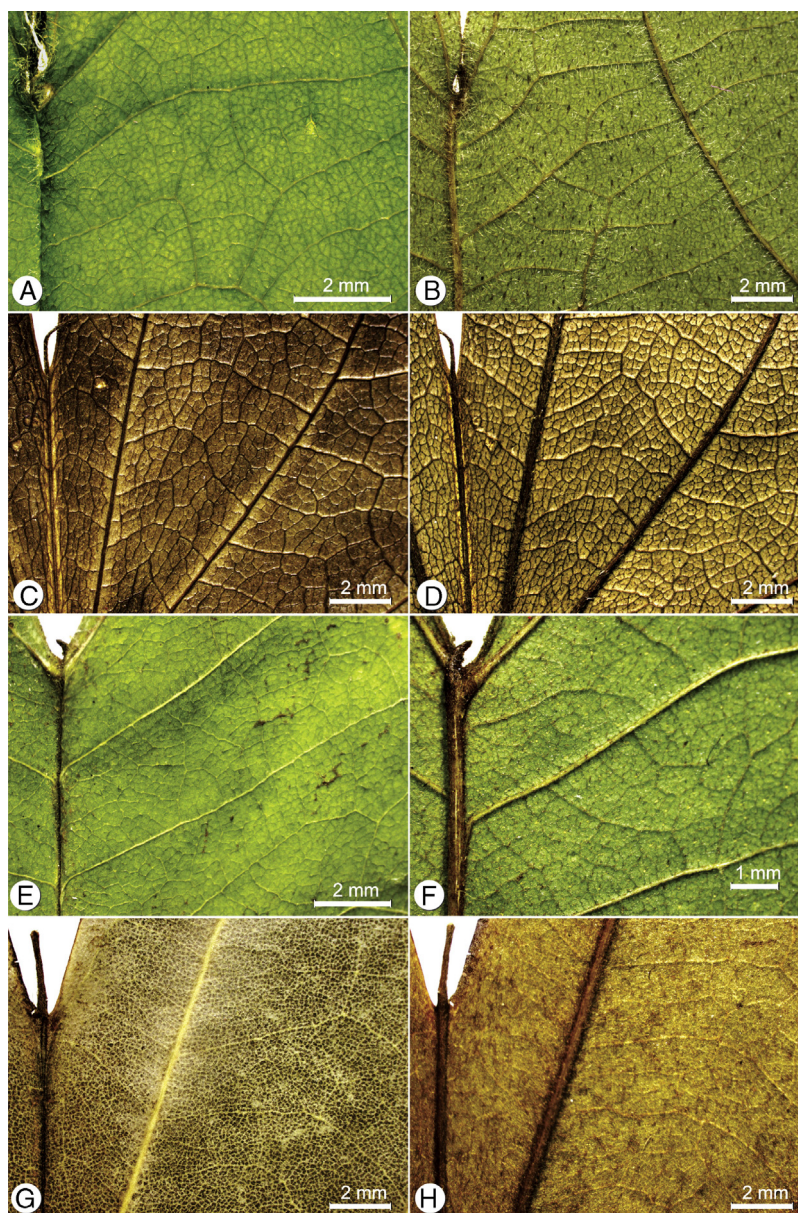


Figure 1. In frontal view. (A and B) *Bauhinia cheilantha*, adaxial and abaxial surfaces; (C and D) *Bauhinia pentandra*: adaxial and abaxial surfaces; (E and F) *Bauhinia unguolata*: adaxial and abaxial surfaces; *Schnella outimouta*: (G and H) adaxial and abaxial surfaces.

B. pentandra is inserted on the stem, between the geminate aculeus and the axillary gemma (Fig. 2D). A linear stipule and an extrafloral glandular nectary were observed at the base of the pulvinus in *B. unguolata* (Fig. 2G). The proximal pulvinus of *S. outimouta* is inserted between a pair of rounded stipules (Fig. 2J).

All species showed a motile cushion at the apex of the distal pulvinus, this being prominent and somewhat rounded on the adaxial surface of *B. cheilantha* (Fig. 2B), with a central groove on the abaxial surface (Fig. 2C). The motile cushion of *B. pentandra* is semicircular on the adaxial surface (Fig. 2E), but inconspicuous on the abaxial face (Fig. 2F). The motile region of *B. unguolata* is large-elliptical on the adaxial (Fig. 2H) and inflated on the abaxial surface (Fig. 2I). *S. outimouta* demonstrated circular motile cushions on the adaxial surface (Fig. 2K) that were inconspicuous on the abaxial surface (Fig. 2L).

Leaf anatomy

Bauhinia cheilantha (Fig. 3A), *B. pentandra* (Fig. 3C), and *B. unguolata* (Fig. 3E) showed straight, polygonal, anticlinal epidermal walls, slightly curved on the adaxial surface in front view, but sinuous in *S. outimouta* (Fig. 3G). The epidermis on the abaxial surface of *B. cheilantha* and *B. pentandra* showed sinuous anticlinal walls (Fig. 3B, D), but curved and somewhat papillose walls in *B. unguolata* (Fig. 3F), with elongated papillae in *S. outimouta* (Fig. 3H). All species showed a uniseriate and papillose leaf epidermis on the abaxial surface in cross-section (Fig. 4A, C, E, G), with a thick cuticle in *B. cheilantha* and *B. unguolata*, but a thin cuticle in *B. pentandra* and *S. outimouta*.

The species of *Bauhinia* studied showed amphistomatic leaves with both anomocytic and anisocytic stomata occurring on both surfaces (Fig. 3A–F). Paracytic type stomata were also observed on

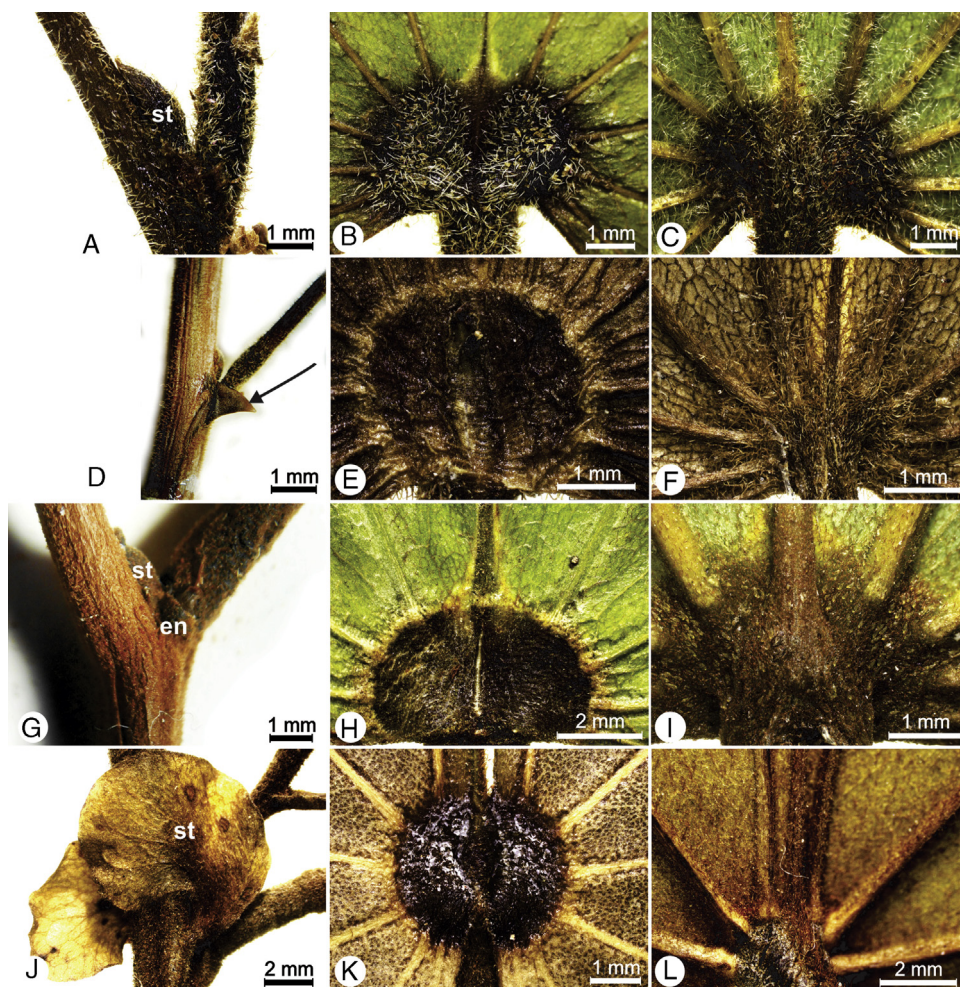


Figure 2. Insertion of the proximal and distal pulvinus, and motile region. (A–C) *Bauhinia cheilantha*; (D–F) *Bauhinia pentandra*; (G–I) *Bauhinia unguolata*. (J–L) *Schnellia outimouta*. Legend: (arrow) axillary bud. (st) stipule. (en) extrafloral nectary.

both surfaces of *B. pentandra* (Fig. 3C) and *B. unguolata* (Fig. 3F). *S. outimouta* demonstrated a hypostomatic pattern, with anisocytic and anomocytic stomata (Fig. 3H). The stomata of the four species were at the level of epidermis.

The mesophyll of *B. unguolata* is dorsiventral in cross section, with uniseriate palisade parenchyma in (Fig. 4E), being biseriate in the other species (Fig. 4A, C, G), with drusiferous idioblasts often being observed (Fig. 4C). The spongy parenchyma showed 2–4 layers in *B. cheilantha* and *B. pentandra* (Fig. 4A–C), with smaller cells than the other species and small intercellular spaces. *B. unguolata* and *S. outimouta* show 4–5-seriate spongy parenchyma, with large intercellular spaces and more elongated cells, tending toward brachiform (Fig. 4E, G). Idioblasts of prismatic crystals were observed in the vascular systems of secondary bundles, mainly in *B. pentandra* (Fig. 4D).

The leaf margins appeared as very distinctive characteristics among the four species, being: obtuse and slightly reflexed in *B. cheilantha*, (Fig. 4B); obtuse with a collateral vascular bundle with a sclerenchymal sheath in *B. pentandra* (Fig. 4D); acute in *B. unguolata* (Fig. 4F); rounded in *S. outimouta* (Fig. 4H); filled by sclerenchyma in *B. pentandra* and *B. unguolata*.

The midrib of *Bauhinia* is plane-convex in cross section, with collateral vascular bundles that are delimited by two strands of sclerenchyma, with a crystaliferous sheath and a collenchymatous cortex with sparse drusiferous idioblasts. The median portion of the midrib of *B. cheilantha* has a central arc-shaped vascular bundle (Fig. 5A), V-shaped in *B. pentandra* (Fig. 5B), and U-shaped in

B. unguolata (Fig. 5C). The median portion of the midrib of *S. outimouta* is concave-convex, with a cortex of angular collenchyma and drusiferous idioblasts; two semicircular vascular bundles were observed in the central portion (the main vascular bundle being amphicribal while the accessory bundle is collateral), with sclerenchymal sheaths surrounding both (Fig. 5D).

The proximal pulvinus was circular to subcircular in cross section in all of the species studied (Fig. 6A, D, G, J), with a conspicuous cortex and a sheath of drusiferous idioblasts surrounding the collenchyma, external to the vascular system (Fig. 7A). There were large numbers of cells near the petiole in the process of lignification. *B. cheilantha* has two vascular bundle systems: one larger and partially concentric cortical bundle, and a smaller medullar bundle, both collateral (Fig. 6A). The vascular system of *B. pentandra* showed only one vascular bundle, rounded to semicircular, bicollateral, surrounded by 3–5 thin layers of collenchyma (Figs. 6D and 7A). Two central vascular bundles were observed in *B. unguolata*, a larger amphicribal bundle surrounded by 3–5 layers of collenchyma, and a minor medullar collateral bundle (Fig. 6G). The vascular system of the proximal pulvinus in *S. outimouta* showed a discontinuous ring of collateral bundles surrounded by a collenchyma sheath, while the medullar region showed a bicollateral bundle (Fig. 6J).

The distal pulvinus was circular in cross-section in all of the species studied, although sometimes slightly compressed on one surface. The parenchymatous cortex is conspicuous, with a sheath of drusiferous idioblasts surrounding the vascular system (Fig. 7B). The proximal pulvinus showed four parallel collateral bundles,

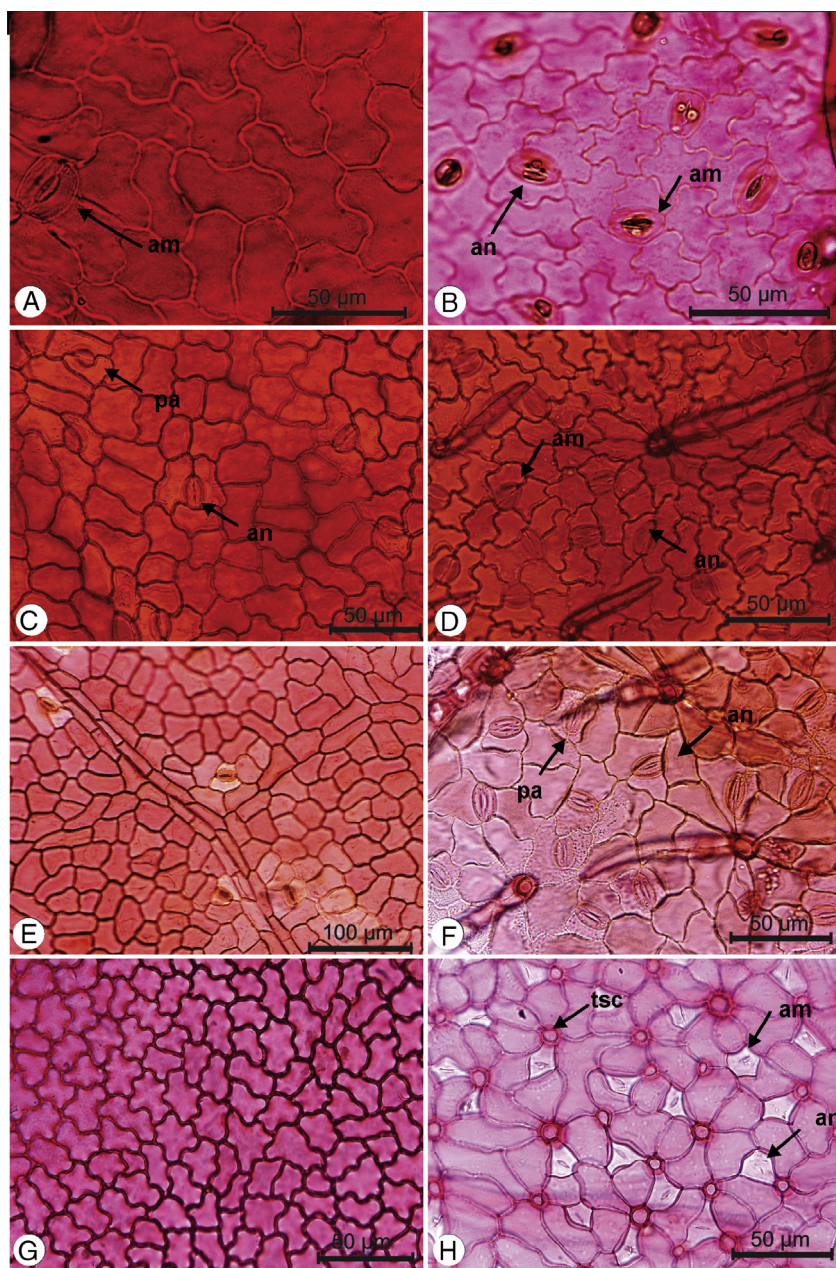


Figure 3. Epidermis on the adaxial (left side) and abaxial (right side) surfaces. (A and B) *Bauhinia cheilantha*; (C and D) *Bauhinia pentandra*. (E and F) *Bauhinia unguolata*. (G and H) *Schnellia outimouta*. Legends: (am) anomocytic; (an) anisocytic; (pa) paracytic; (tsc) trichome scar.

and was wrapped with collenchyma (Fig. 6B, E, H, K). Smaller, central bundles have phloem facing to the cortex of the major bundles.

The median portion of the petiole is somewhat U-shaped in cross-section in all *Bauhinia* species (*B. cheilantha* [Fig. 6C], *B. pentandra* [Fig. 6F], and *B. unguolata* [Fig. 6I]) due to two lateral projections on the adaxial surface; the petiole of *S. outimouta* is rounded (Fig. 6L). The cortex of all of the species is filled with parenchyma, with areas of collenchymatic tissue (Fig. 7D) and drusiferous idioblasts (Fig. 7A).

The vascular system is surrounded by a sclerenchymatic ring, which is delimited by a crystaliferous sheath (Fig. 7C). The vascular system in *B. cheilantha* is formed by four bundles, two of them being central with a larger amphicrival bundle in the cortical portion; a smaller amphivasal bundle is present in the medullary portion. There are also two accessory collateral bundles that can be seen

in lateral projections (Fig. 6C). *B. pentandra* has three bundles, two collateral in lateral projections, with a large amphicrival bundle occupying most of the cortex (Fig. 6F). *B. unguolata* showed four bundles, two of which are amphicrival, parallel to the central region of the cortex, and two are collateral in lateral projections (Fig. 6I). The arrangements of the central vascular bundles of *S. outimouta* are similar to those observed in *B. cheilantha* (Fig. 6L). The distinctive morphological and anatomical characters of *B. cheilantha*, *B. pentandra*, *B. unguolata* and *S. outimouta* are presented in Boxes 1 and 2.

4. Discussion

Species of *Bauhinia* constitute a difficult group to identify considering only leaf morphology and, in the absence of fertile material, their morphologies are usually considered insufficient to support

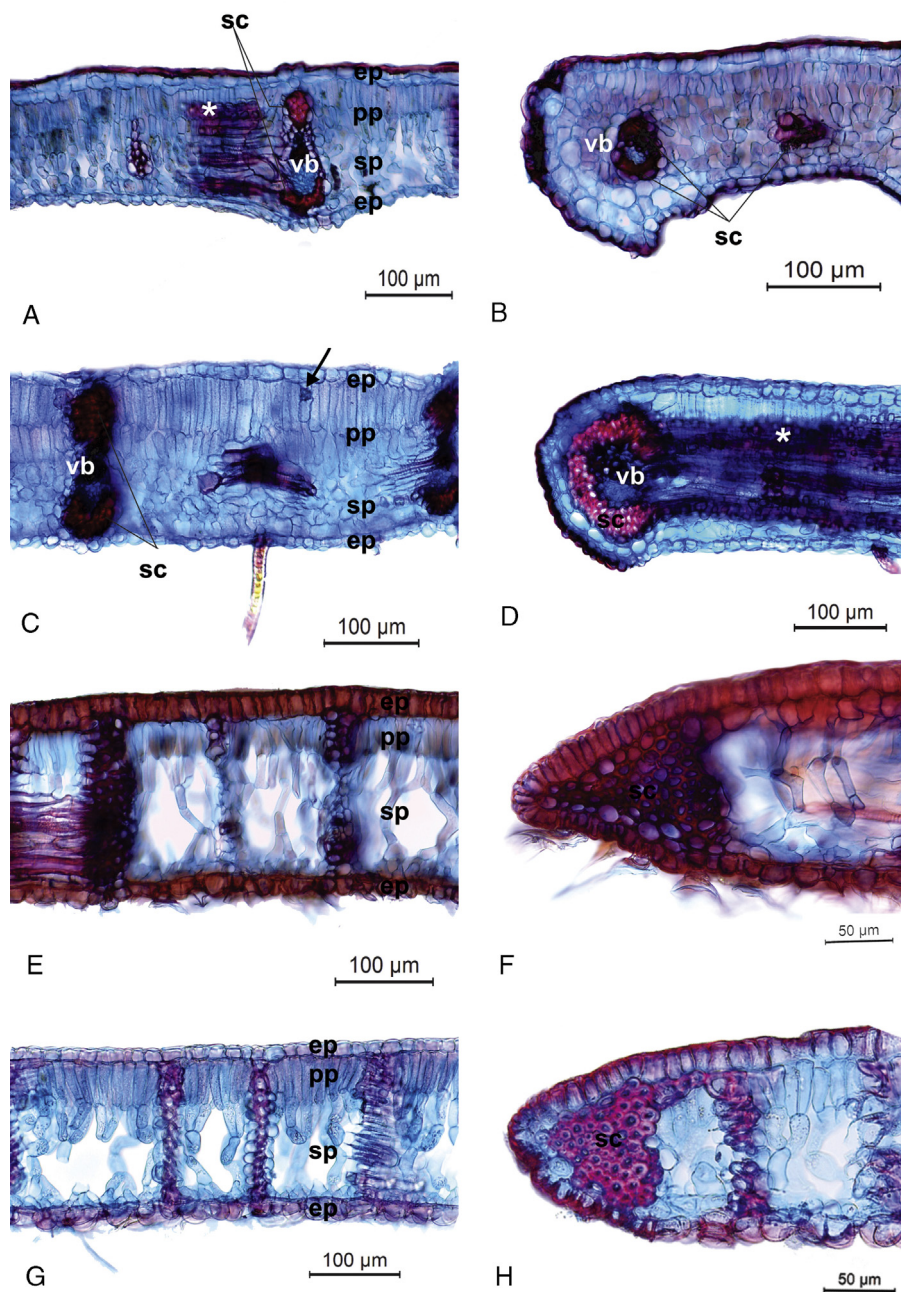


Figure 4. Mesophyll and leaf blade margins: (A and B) *Bauhinia cheilantha*; (C and D) *Bauhinia pentandra*; (E and F) *Bauhinia unguolata*; (G and H) *Schnella outimouta*. Legends: (arrow) Druse, (*) prismatic crystals, (ep) epidermis, (pp) palisade parenchyma, (sp) spongy parenchyma, (sc) sclerenchyma.

the identification of taxa (as was observed in the present work with fresh samples and herbarium specimens of *B. cheilantha* and *B. unguolata*). Their leaf shapes overlap and may exhibit transitional forms of mature and young leaves – so that collections of those species have become mixed, corroborating the observations of Wunderlin (1983).

Bauhinia cheilantha, *B. pentandra*, *B. unguolata*, and *S. outimouta* showed leaf patterns characteristic of tribe Cercideae (Chen and Zhang, 2005), although *B. pentandra* differed from the others by the shape of its leaves and by having navicular glands restricted to the leaf margins (corroborating the findings of Duarte-Almeida et al., 2015). The presence of navicular glands was a common character among the studied species of *Bauhinia* (*B. cheilantha*, *B. pentandra*

and *B. unguolata*), and was noted by Metcalfe and Chalk (1979), differentiating them in relation to the genus *Schnella*. These observations are likewise consistent with those of Duarte-Almeida et al. (2015) who found that type of gland only in species of *Bauhinia* (and their absence in *Schnella*) in their analysis of 79 species of the Cercideae tribe using that character as a subsidy for the taxonomy of the group.

Pulvini are structures that aid in leaf flexibility and foliar movements (Rodrigues and Machado, 2006), especially in the motile region, when the articulation between the base of the leaf and the apex of the distal pulvinus is responsible for nictinastic movements (Vaz and Tozzi, 2003b, 2005; Rodrigues and Machado, 2006; Lusa and Bona, 2009). The shape of the distal pulvinus and the motile

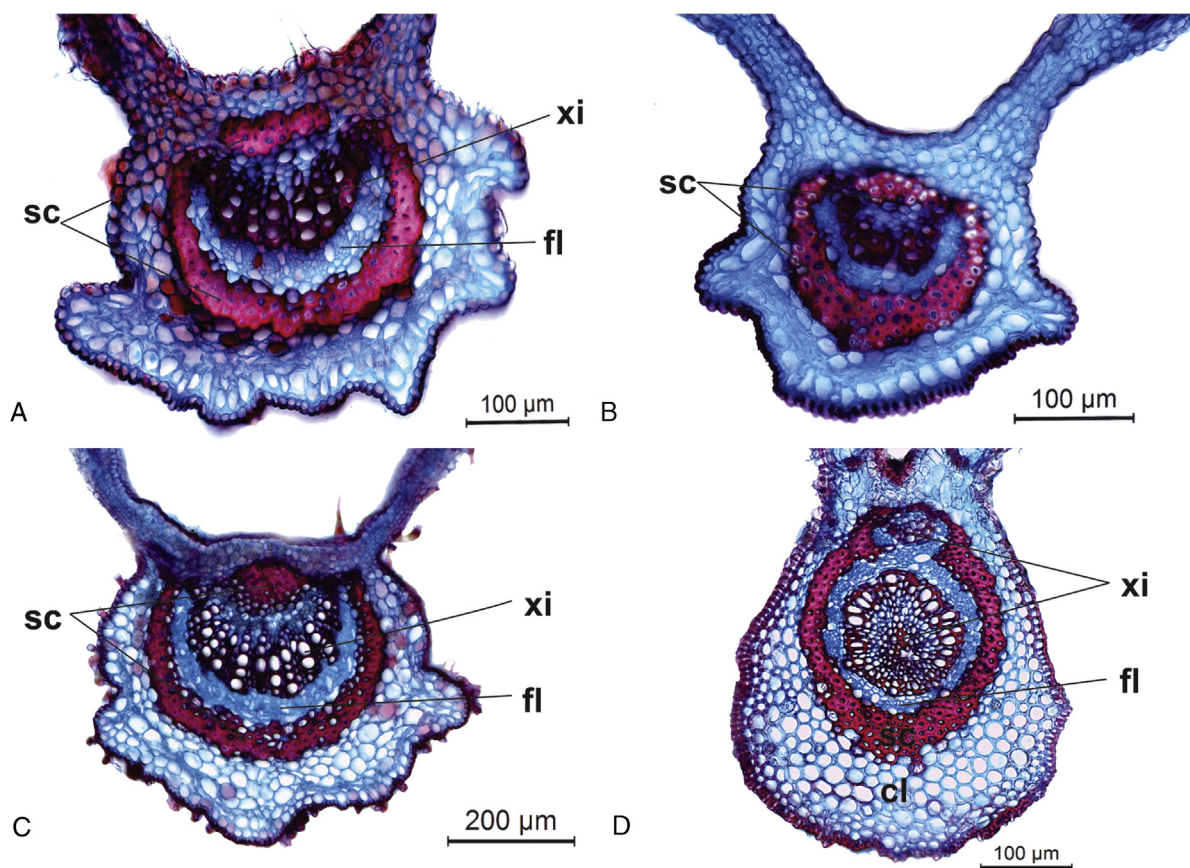


Figure 5. (A) *Bauhinia cheilantha*; (B) *Bauhinia pentandra*; (C) *Bauhinia unguolata*; (D) *Schnella outimouta*. Legends: (cl) angular collenchyma; (sc) sclerenchyma; (ph) phloem, (xy) xylem.

region were important characters that supported the delimitations of the four species studied here.

In terms of anatomical studies, analyses of the epidermis revealed a straight to curved pattern of the epidermises of species of *Bauhinia*, as was likewise reported for other species of the genus by [Albert and Sharma \(2013\)](#), [Lusa and Bona \(2009\)](#), and [Rezende and Cardoso \(1994\)](#). As that curved pattern is a common feature in species of that group, it was not useful as a diagnostic feature to separate the *Bauhinia* species studied here. It did differ from the pattern observed in *S. outimouta* (sinuous type) and that recorded for *S. microstachya* Raddi by [Duarte and Debur \(2003\)](#), thus serving as a distinctive feature for separating those genera.

An amphistomatic pattern of stomata distribution was common to the species of *Bauhinia* studied here, corroborating [Metcalf and Chalk \(1979\)](#) who reported the stomata on the adaxial face as being sparse or restricted to the ribs; this same situation was recorded for *B. blakeana* Dunn by [Albert and Sharma \(2013\)](#), and for *B. forficata* Link. and *B. variegata* L. by [Lusa and Bona \(2009\)](#). The hypostomatic pattern reported by [Albert and Sharma \(2013\)](#) for *B. tomentosa* L., *B. purpurea* L., *B. racemosa* Lam., and *B. malabarica* Roxb. was not observed by us. The pattern of stomata distribution was hypostomatic in *S. outimouta*, and has also been reported for *S. microstachya* by [Duarte and Debur \(2003\)](#).

Anomocytic, anisocytic, and paracytic stomata were observed in all of the species studied. Those features were likewise previously reported for other species of *Bauhinia* and *Schnella* by [Albert and Sharma \(2013\)](#), [Duarte and Debur \(2003\)](#), [Lusa and Bona \(2009\)](#), and [Rezende and Cardoso \(1994\)](#), and corroborated by [Metcalf](#)

[and Chalk \(1979\)](#) who noted that different arrangements of subsidiary cells can be seen within the same species, or even a single leaf.

The dorsiventral mesophyll pattern observed in all of the species studied corroborated the dorsiventral pattern observed in other genera and species of the subfamily Caesalpinioideae ([Metcalf and Chalk, 1979](#)). The anatomy of the leaf margins, however, proved to be a distinctive character among the four species studied here, similar to reports for other species of *Bauhinia* ([Lusa and Bona, 2009](#)).

Regarding the anatomy of the pulvinus, the patterns observed here were similar to those reported by [Rodrigues and Machado \(2006\)](#) for the structures of the proximal pulvini of some species of the Leguminosae. Those structures are different in terms of the distributions of the vascular tissue observed in the *Bauhinia* species and *S. outimouta* studied here, but are not taxonomically informative – as the proximal pulvinus is consistently positioned in a transitional area between the stem and leaf tissues.

The canaliculate petiole shape, in cross-section, showed two vascularized lateral adaxial projections in species of *Bauhinia*, which was also reported for *B. forficata* and *B. variegata* ([Lusa and Bona, 2009](#)), and for *B. curvula* Benth ([Rezende and Cardoso, 1994](#)). The rounded shape observed in *S. outimouta*, on the other hand, is similar to that of *S. microstachya* ([Duarte and Debur, 2003](#)). The presence of a sclerenchymatous ring and a crystaliferous sheath surrounding the central vascular system in *Bauhinia*, also reported by [Metcalf and Chalk \(1979\)](#) was likewise observed in *S. outimouta* – and therefore does not constitute a differential character between the genera or species in the present work.

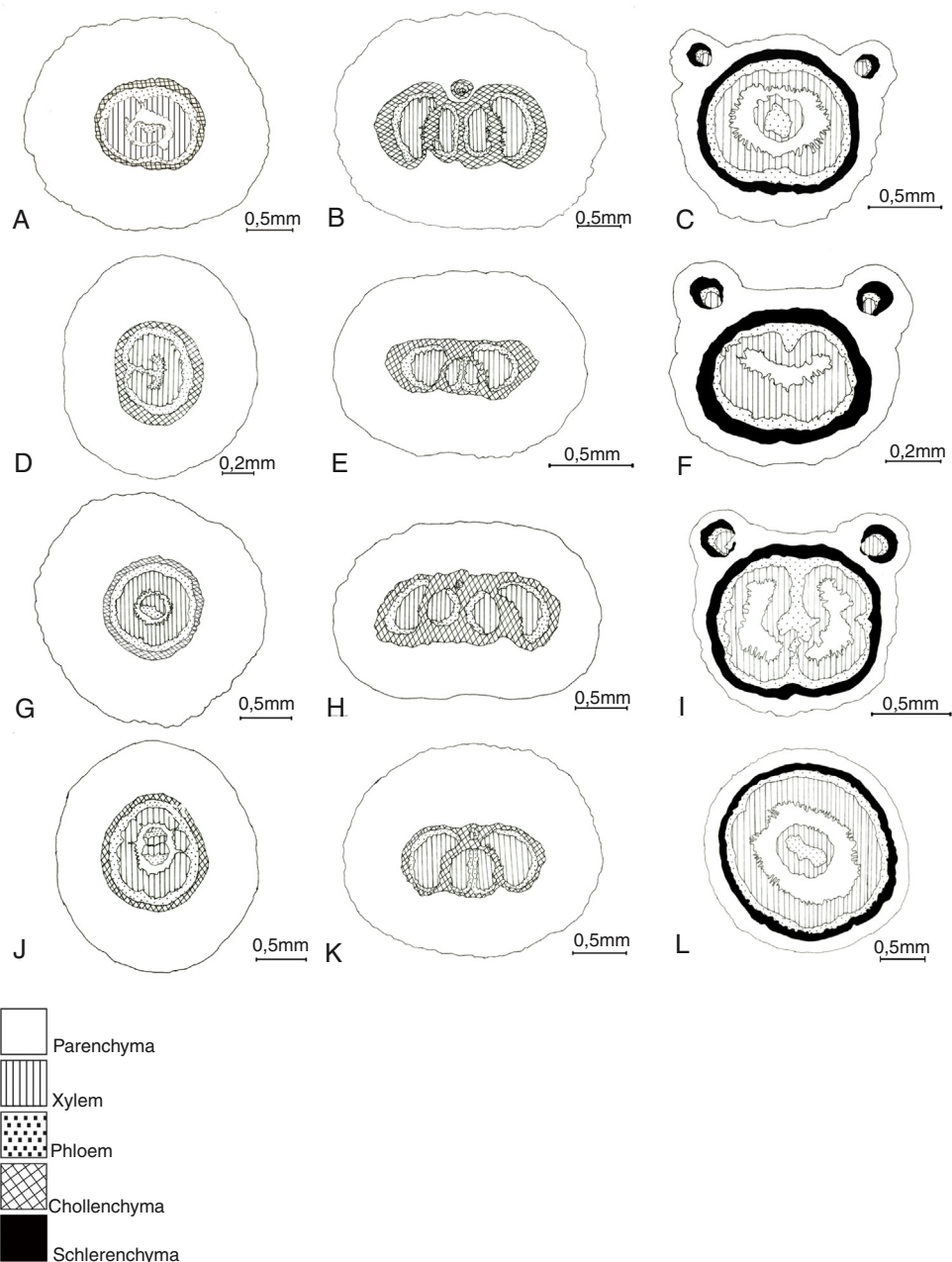


Figure 6. Vascularization of the middle portion of the proximal (left) and distal (middle) pulvinus, and petiole (right). (A–C) *Bauhinia cheilantha*; (D–F) *Bauhinia pentandra*; (G–I) *Bauhinia unguolata*; (J–L) *Schnella outimouta*.

Box 1

Morphological characters of *Bauhinia* and *Schnella* species.

Leaf characters		Species			
		<i>B. cheilantha</i>	<i>B. pentandra</i>	<i>B. unguolata</i>	<i>S. outimouta</i>
Leaf blade	Consistency	Chartaceous	Chartaceous	Chartaceous	Leathery
	Shape	Oval-oblong	Cordate-hastate	Oval-oblong	Oval-oblong
	Level of bifidness of the leaf blade	1/4	1/2	1/4	3/4
	Base of blade	Cordate	Cordate-hastate	Truncate	Cordate
	Apex of blade	Rotund	Acute	Acute	Acute
	Abaxial indument	Sericeous-pubescent	Sericeous-pubescent	Sericeous-pubescent	Puberulous
	Glands on the abaxial surface	Present	Present	Present	Absent
	Venation	Palminervous	Palminervous	Palminervous	Acrodromous
Petiole and pulvinus	Petiole shape	Canaliculate	Canaliculate	Canaliculate	Cylindrical
	Insertion in the proximal pulvinus	Axillary gemma	Between geminate aculeos	Between extrafloral nectaries	Between rounded stipules
	Indument of motile region	Pubescent	Glabrous	Glabrous	Puberulous

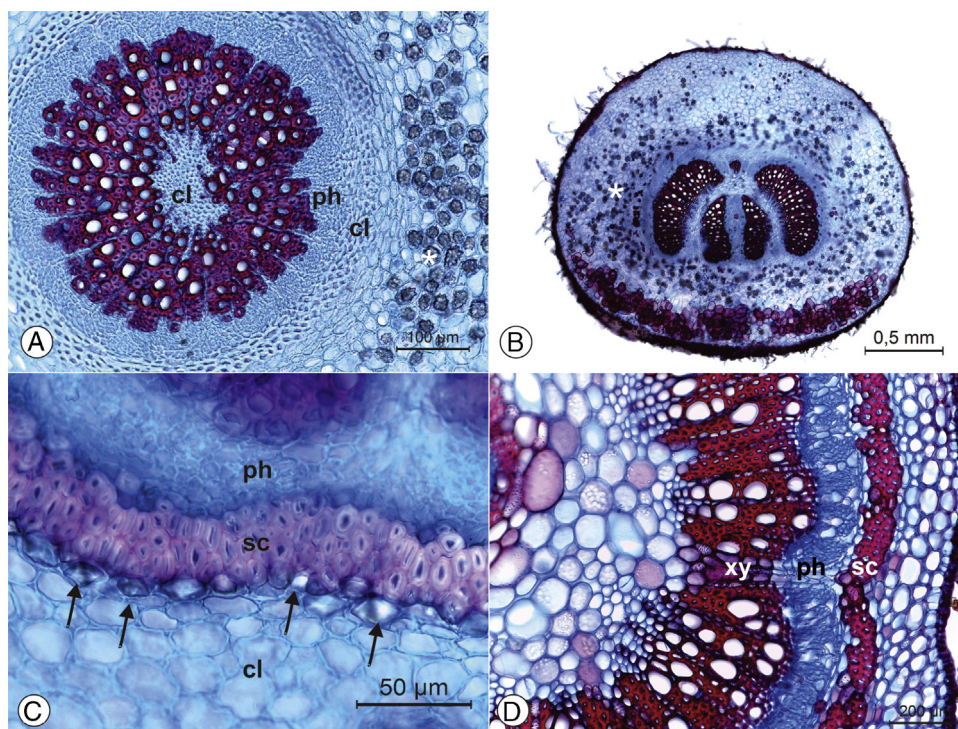


Figure 7. Details of the pulvinus and petiole: (A) Proximal pulvinus of *Bauhinia pentandra*; (B) distal pulvinus of *Schnella outimouta*, with druse idioblasts; (C) petiole of *B. pentandra*; (D) petiole of *S. outimouta*. Legends: (cl) collenchyma; (xy) xylem; (ph) phloem; (sc). Sclerenchyma: (*) druse; (arrow) crystals.

Box 2

Anatomical characters of *Bauhinia* and *Schnella* species.

Character		Species				
		<i>B. cheilantha</i>	<i>B. pentandra</i>	<i>B. unguolata</i>	<i>S. outimouta</i>	
Leaf blade	Anticlinal walls	Adaxial	Straight-curved	Straight-curved	Straight-curved	Sinuous
		Abaxial	Sinuous	Sinuous	Curved	Curved
	Stomata	Distribution	Amphistomatic	Amphistomatic	Amphistomatic	Hypostomatic
		Type	Anomocytic	Anomocytic	Anomocytic	Anomocytic
Margin	Shape		Obtuse and reflexed	Obtuse	Acute	Rounded
		Vascular bundle	1-collateral	1-collateral	Absent	Absent
	Presence of sclerenchyma	In the vascular bundle	In the vascular bundle	Filling the entire margin	Filling the entire margin	
	Midrib	Shape	Plane-convex	Plane-convex	Plane-convex	Concave-convex
Petiole and pulvinus	Proximal pulvinus	Vascular bundles	1-collateral	1-collateral	1-collateral	2-amphicribal
		Vascular system	2-collateral	1-bicollateral	1-amphicribal + 1-collateral	colateral + 1-bicollateral
	Petiole	Shape	U-shaped	U-shaped	U-shaped	Circular
		Lateral bundles	2-lateral	2-lateral	2-lateral	Absent
	Collateral bundle	2-collateral	2-collateral	2-collateral	Absent	

5. Conclusions

The morphology of the petiole, motile region, and the structures present in the insertion area of the pulvinus were one of most relevant to the separation of *Bauhinia* species from *Schnella*. The anatomy of the leaf epidermis and its auxiliary structures (stomata and navicular glands), as well as the petiole vascularization, were diagnostic for separating the species of *Bauhinia* and *Schnella*, characterizing them as distinct species.

6. Authors' contributions

LBSP (Master student) contributed in collecting plant samples, identification, and running the laboratory work (preparing herbaria samples and plant anatomy studies), analysis of the data and preparation of the paper. RCS contributed with support to the anatomical studies. LPF contributed in collecting and identification of plant samples. MFA designed the study with *Bauhinia*, and supervised all laboratory and field work, as well as contributed to

critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

7. Conflicts of interest

The authors declare no conflicts of interest.

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References

- Agra, M.F., Baracho, G.S., Nurit, K., Basílio, I.J.L.D., Coelho, V.P.M., 2007. Medicinal and poisonous diversity of the flora of "Cariri Paraibano", Brazil. *J. Ethnopharmacol.* 111, 383–395.
- Agra, M.F., Silva, K.N., Basílio, I.J.L.D., Freitas, P.F., Barbosa-Filho, J.M., 2008. Survey of medicinal plants used in the region Northeast of Brazil. *Rev. Bras. Farmacogn.* 18, 472–508.
- Albert, S., Sharma, B., 2013. Comparative foliar micromorphological studies of some *Bauhinia* (Leguminosae) species. *Turk. J. Bot.* 37, 276–281.
- Araújo, N.D., Coelho, V.P.M., Agra, M.F., 2010. The pharmacobotanical comparative study of leaves of *Solanum crinitum* Lam., *Solanum gomphodes* Dunal and *Solanum lycocarpum* A. St-Hil. (Solanaceae). *Rev. Bras. Farmacogn.* 20, 666–674.
- Araújo, N.D., Coelho, V.P.M., Ventrella, M.C., Agra, M.F., 2014. Leaf anatomy and histochemistry of three species of *Ficus* sect. *Americanae* supported by light and electron microscopy. *Microsc. Microanal.* 20, 296–304.
- Bentham, G., 1865. Leguminosae. In: Bentham, G., Hooker, J.D. (Eds.), *Genera Plantarum*. V. 1 pt 2. Reeve & Co., London, pp. 575–577, 460 pp.
- Braca, A., Tommasi, N., Bari, L., Pizzi, C., Politi, M., Morelli, I., 2001. Antioxidant principles from *Bauhinia tarapotensis*. *J. Nat. Prod.* 64, 892–895.
- Bridson, D., Forman, L., 1999. *The Herbarium Handbook*, 3rd ed. Royal Botanic Gardens, Kew Includes, Specimen storage.
- Bukatsch, F., 1972. *Azul de Astra e Safranina*. In: Kraus, J., Arduin, M. (Eds.), *Manual Básico de Métodos em Morfologia Vegetal*. Edur, Seropédica, Rio de Janeiro, p. 26.
- Cechinel Filho, V., 2009. Chemical composition and biological potential of plants from the genus *Bauhinia*. *Phytother. Res.* 23, 1347–1354.
- Chen, Y.F., Zhang, D.X., 2005. *Bauhinia larsenii*, a fossil legume from Guangxi, China. *Bot. J. Linn. Soc.* 147, 437–440.
- Duarte, M., Debur, M., 2003. Caracteres morfo-anatômicos de folha e caule de *Bauhinia microstachya* (Raddi) JF Macbr. (Fabaceae). *Rev. Bras. Farmacogn.* 13, 7–15.
- Duarte-Almeida, J.M., Clemente, M.S., Arruda, R.C., Vaz, A.M., Salatino, A., 2015. Glands on the foliar surfaces of tribe Cercideae (Caesalpinioideae, Leguminosae): distribution and taxonomic significance. *An. Acad. Bras. Cienc.* 87, 787–796.
- Fahn, A., 1990. *Plant Anatomy*. Pergamon Press, Oxford.
- Fortunato, R.H., 1986. Revision del genero *Bauhinia* (Cercideae, Caesalpinioidea, Fabaceae) para La Argentina. *Darwiniana* 4, 527–557.
- Franklin, G.L., 1945. Preparation of thin sections of synthetic resins and wood-resin composites, and a new macerating method for wood. *Nature* 155, 51.
- Gupta, M., Mazumder, U.K., Kumar, R.S., Gomathi, P., Rajeshwar, Y., Kakoti, B.B.Y., Selven, V.T., 2005. Anti-inflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models. *J. Ethnopharmacol.* 98, 267–273.
- Hao, G., Zhang, D.X., Zhang, M.Y., Guo, L.X., Li, S.J., 2003. Phylogenetics of *Bauhinia* subgenus *Phanera* (Leguminosae: Caesalpinioideae) based on ITS sequences of nuclear ribosomal DNA. *Bot. Bull. Acad. Sin.* 44, 223–228 <https://ejournal.sinica.edu.tw/bbas/content/2003/3/bot443-07.pdf>.
- Harris, J.G., Harris, M.W., 2001. *Plant Identification Terminology: An Illustrated Glossary*, 2nd ed. Spring Lake Publishing, Spring Lake, UT.
- Hickey, L.J., 1973. Classification of the architecture of dicotyledonous leaves. *Am. J. Bot.* 60, 17–33.
- Johansen, D.A., 1940. *Plant Microtechnique*. McGraw-Hill, New York.
- Lin, Y., Wong, W.O., Shi, G., Shen, S., Li, Z., 2015. Bilobate leaves of *Bauhinia* (Leguminosae, Caesalpinioideae, Cercideae) from the middle Miocene of Fujian Province, southeastern China and their biogeographic implications. *BMC Evol. Biol.* 15, <http://dx.doi.org/10.1186/s12862-015-0540-9>.
- Legume Phylogeny Working Group [LPWG], 2013. Legume phylogeny and classification in the 21st century: progress, prospects and lessons for other species-rich clades Legume phylogeny and classification in the 21st century: progress, prospects and lessons for other species-rich clades. *Taxon* 62, 217–248, <http://www.jstor.org/stable/taxon.62.2.217>.
- Lusa, M.G., Bona, C., 2009. Análise morfoanatómica comparativa da folha de *Bauhinia forficata* Link e *B. variegata* Linn. (Leguminosae, Caesalpinioideae). *Act. Bot. Bras.* 23, 196–211.
- Menezes, F.S., Minto, A.B.M., Ruela, H.S., Kuster, R.M., Sheridan, H., Frankish, N., 2007. Hypoglycemic activity of two Brazilian *Bauhinia* species: *Bauhinia forficata* L. and *Bauhinia monandra* Kurz. *Rev. Bras. Farmacogn.* 17, 8–13.
- Metcalfe, C.R., Chalk, L., 1979. *Anatomy of the Dicotyledons*, 2nd ed. Clarendon Press, Oxford, UK.
- Nurit-Silva, K., Agra, M.F., 2011. Leaf epidermal characters of *Solanum* sect. *Polytrichum* (Solanaceae) as taxonomic evidence. *Microsc. Res. Tech.* 74, 1186–1191.
- Nurit-Silva, K., Costa-Silva, R., Basílio, I.J., Agra, M.F., 2012. Leaf epidermal characters of Brazilian species of *Solanum* section *Torva* as taxonomic evidence. *Botany* 90, 806–814.
- Pandey, A.K., Ojha, V., Yadav, S., Sahu, S.K., 2011. Phytochemical evaluation and radical scavenging activity of *Bauhinia variegata*. *Saraca asoca* and *Terminalia arjuna* Barks. *Res. J. Phytochem.* 5, 89–97.
- Porto, N.M., Barros, Y.L., Basílio, I.J.L.D., Agra, M.F., 2016. Microscopic and UV/Vis spectrophotometric characterization of *Cissampelos pareira* of Brazil and Africa. *Rev. Bras. Farmacogn.* 26, 135–146.
- Raddi, G., 1820. *Quaranta piante nuove del Brasile*. Presso la Società Tipografica, Modena, pp. 1–35.
- Rezende, M.H., Cardoso, L.A., 1994. Morfologia e anatomia foliar de *Bauhinia curvula* Benth. (Leguminosae – Caesalpinioideae). *Act. Bot. Bras.* 8, 19–34.
- Rodrigues, T.M., Machado, S.R., 2006. Anatomia comparada do pulvino primário de leguminosas com diferentes velocidades de movimento foliar. *Rev. Bras. Bot.* 29, 709–720.
- Sampaio, V.S., Araújo, N.D., Agra, M.F., 2014. Characters of leaf epidermis in *Solanum* (clade *Brevantherum*) species from Atlantic Forest of Northeastern Brazil. *S. Afr. J. Bot.* 94, 108–113.
- Silva, K.L., Cechinel Filho, V., 2002. Plantas do Gênero *Bauhinia*: composição química e potencial farmacológico. *Quim. Nova* 25, 449–454.
- Sinou, C., Forest, F., Lewis, G.P., Bruneau, A., 2009. The genus *Bauhinia* s.l. (Leguminosae): a phylogeny based on the plastid trn L–trn F region. *Botany* 87, 947–960.
- Trethowan, L.A., Clark, R.P., Mackinder, B.A., 2015. A synopsis of the neotropical genus *Schnella* (Cercideae: Caesalpinioideae: Leguminosae) including 12 new combinations. *Phytotaxa* 204, 237–252.
- Van der Pijl, L., 1952. The leaf of *Bauhinia*. *Act. Bot. Neerl.* 1, 287–309.
- Vaz, A.M.S.F., 2015. *Bauhinia* in: Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. BFG, <http://dx.doi.org/10.1590/2175-7860201566411> <http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB22811> (accessed 12.11.17).
- Vaz, A.M.S.F., Tozzi, A.M.G.A., 2003a. *Bauhinia* ser. *Cansenia* (Leguminosae: Caesalpinioideae) no Brasil. *Rodriguésia* 54, 55–143.
- Vaz, A.M.S.F., Tozzi, A.M.G.A., 2003b. *Aculeatae*, a new series in *Bauhinia* section *Pauletia* (Leguminosae, Caesalpinioideae, Cercideae). *Novon* 13, 141–144.
- Vaz, A.M.S.F., Tozzi, A.M.G.A., 2005. Sinopse de *Bauhinia* sect. *Pauletia* (Cav.) DC. (Leguminosae: Caesalpinioideae: Cercideae) no Brasil. *Rev. Bras. Bot.* 28, 477–491.
- Wunderlin, R.P., 1976. The Panamanian species of *Bauhinia* (Leguminosae). *Ann. Missouri Bot. Gard.* 63, 346–354.
- Wunderlin, R.P., 1983. Revision of the arborescent *Bauhinias* (Fabaceae: Caesalpinioideae: Cercideae) native to Middle America. *Ann. Missouri Bot. Gard.* 70, 95–127.
- Wunderlin, R.P., 2010a. Reorganization of the Cercideae (Fabaceae: Caesalpinioideae). *Phytoneuron* 48, 1–5 <http://www.phytoneuron.net/PhytoN-Cercideae.pdf>.
- Wunderlin, R.P., 2010b. New combinations in *Schnella* (Fabaceae: Caesalpinioideae: Cercideae). *Phytoneuron* 49, 1–5 <http://www.phytoneuron.net/PhytoN-Schnella.pdf>.