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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).

Metcalf 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 infrageneric 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 etnodrugs 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 laminas. The apex is bifid to $\frac{1}{4}$ in *B. cheilantha*, $\frac{1}{2}$ in *B. pentandra*, $\frac{1}{4}$ *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 canaliculate 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

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