

Acta Botanica Brasilica 33(4): 716-723. October-December 2019.

doi: 10.1590/0102-33062019abb0112

A new glandular *Mimosa* species from Southern Brazil and insights about its glandular trichomes micromorphology

Fernanda Schmidt Silveira^{1*} , Wanderleia de Vargas² , Ana Paula Fortuna-Perez² and Silvia Teresinha Sfoggia Miotto¹

Received: March 31, 2019 Accepted: June 28, 2019

ABSTRACT

Mimosa baptistae is a new species presenting stalked glandular trichomes that give it a glutinous appearance, mainly with respect to the leaflets. Furthermore, it is an aculeate species, with multijugate leaves, white stamens, and fruits also covered by glandular trichomes. A herbaria survey and fieldwork only recorded this species as occurring on sandstone outcrops of the city Caçapava do Sul, Brazil. The species may be confused with other species of Mimosa sect. Batocaulon ser. Stipellares such as M. bifurca, M. sobralii and M. lepidota. However, features such as absence/presence of aculei, stamens color, habitat, and flower features are useful for distinguishing them from the new species. Here, we provide notes on morphology, geographic distribution, as well as anatomical detail of its glandular trichomes and content. Finally, we discussed the taxonomic significance of the micromorphology of glandular trichomes and their content in Mimosa ser. Stipellares as well as the potential applicability of such features to better understand the morphology of this series.

Keywords: glands, leaflets, Pampa, secretory structures, Serra do Sudeste

Introduction

Mimosa is one of the most diversified genera among angiosperms, with more than 500 species (Bessega et al. 2008; Simon et al. 2011). Most of Mimosa's diversity is found in Brazil, which is the main center of diversity and endemism of the genus (Simon et al. 2011), harboring circa 358 to 366 species (BFG 2015; Flora do Brasil 2020 (under construction) 2018). Furthermore, the diversity of Mimosa not only encompasses species richness, but also morphological and ecological diversity. For example, Mimosa has more trichomes diversity than any other genus of the mimosoid clade (subfamily Mimosoideae in Barneby 1991). In addition, the trichome type is a remarkable feature of

many species of *Mimosa*, and this feature is often reflected in their specific epithets (e.g., *Mimosa glanduliseta*, *M. glandulosa*, *M. lepidota*, and *M. verrucosa*, Santos-Silva et al. 2013).

Since 2006, many new *Mimosa* species have been described based on differences of its trichomes. For example, *M. suberosa* is differentiated from other species of *Mimosa* ser. *Piresianae* by its indumentum of short glandular setae (Atahuachi & Hughes 2006). Other example is *M. myrioglandulosa* differing from *M. maguirei* by its stems with sessile glandular trichomes (Dutra & Garcia 2013b). Additional recently published species, in which indumentum is an important characteristic used to separate taxa, are: *M. foreroana* (Santos-Silva & Tozzi 2012); *M. longistipula* (Dutra & Garcia 2013a), and *M. urandiensis* (Santos-Silva *et al.* 2013).

¹ Programa de Pós-Graduação em Botânica, Universidade Federal do Rio Grande do Sul, 91501-970, Porto Alegre, RS, Brazil

² Programa de Pós-Graduação em Botânica, Universidade Estadual Paulista Júlio de Mesquita Filho, 18618-970, Botucatu, SP, Brazil

^{*} Corresponding author: okologie_natur@hotmail.com

Trichomes in *Mimosa* can be glandular or non-glandular (Santos-Silva *et al.* 2013). Glandular trichomes are considered to be derived characteristics in the genus, being classified as sessile or stipitate (Santos-Silva *et al.* 2013). Recently, Grohar *et al.* (2016) have classified the stalked glandular trichomes in the *Dolentes-Brevipedes* complex as globose and clavate according to the head shape and number of cells of the stalk. In addition, glandular trichomes produce, store, and secrete different classes of secondary metabolites that may be associated with different ecological interactions; however, the chemical composition of these secretions is still unknown (Santos-Silva *et al.* 2013). In this work, we described a new species with glandular trichomes, illustrating them by using anatomical features, scanning electron microscopy and histochemical tests.

Materials and methods

Taxonomical analysis

Foreign and Brazilian herbaria were consulted, particularly from South America (BHCB, BOTU, CORD, CESJ, CTES, E, ESA, F, FURB, HAS, HDCF, HPBR, HUCS, HUEM, HURG, HVAT, IAC, ICN, JOI, K, MBM, MO, MPUC, MVM, NY, P, PACA, PEL, R, RB, SJRP, SMDB, SPSF, and USZ (acronyms according to Thiers 2019). However, the new species was recognized only from exsiccates at ICN, HAS, and HDCF herbaria. In addition, field collections were conducted to obtain additional data about flowering, fruiting, geographic distribution of the new species, which was delimited, based on external morphology, micromorphology of trichomes, and ecological constrains.

Anatomical methods

Samples of dried leaflets of the new species based on Schmidt-Silveira 855 and duplicates of at least two specimens were rehydrated in glycerine and serially dehydrated in ethanol (until 95 % ethanol). Leaflets were embedded in Historesin (Leica), and cross sections were prepared using a rotary microtome. Samples were stained with Toluidine pH 4.7 (Vidal 1977). Images were obtained using an Olympus digital camera linked to a light microscope in the Laboratory of Plant Anatomy of UNESP, Botucatu, SP, Brazil (LaPAV).

We performed the following histochemical tests on leaflets: Sudan IV (Pearse 1980) for lipids, NADI reagent (David & Carde 1964) for oleoresins, vanillin hydrochloric (Mace & Howell 1974) for tannin, ferric chloride (Johansen 1940) for phenolic compounds, Dragendorf (Svendsen & Verpoort 1983) for alkaloids, and cupric acetate (Ganter & Jollés 1969) for resin.

In addition, we provide images of the glandular trichomes on the branches and fruits from scanning electron microscopy (SEM) analyses, illustrating that they appear to be sessile trichomes without an anatomical approach.

The samples were submitted to critical point-dried using ${\rm CO_2}$, mounted on aluminum stubs, coated with gold, and examined using a Quanta 200 (FEI Company) scanning electron microscope at 20 kVat in the Electron Microscopy Centre of the Institute of Bioscience of UNESP, Botucatu, SP, Brazil.

Results

Taxonomic treatment

Mimosa baptistae Schmidt-Silveira & Miotto **sp. nov**. Type: BRAZIL, Rio Grande do Sul: Caçapava do Sul, estrada para Pedra do Segredo, próximo à Pedra do Leão, 30°32'40.7"S 53°32'53.2"W, 240 m elev., 07 Sep 2015 [fl+fr], Schmidt-Silveira, 855 (holotype: ICN isotypes K, MBM, NY). (Figs. 1-3).

Mimosa baptistae is an aculeate species, which has a glutinous foliage, multijugate leaves, leaflets of all pinnae are inserted alternate along pinna-rachis, having diminute leaflets ($1.5-2.6\times0.9-1.25$ mm), presence of glandular trichomes with obconical head and unisseriate stalk on leaflets; subglobose inflorescence, white stamens; and fruits covered by glandular trichomes.

Shrubs or treelets attaining 2.5–5 m tall. Branches reddish-brown, cylindrical to subquadrangular with grooves, glabrous, covered with a colorless and bright substance such as a resin, armed with straight or semi-recurved aculei of 3-6 mm long. **Leaves** bipinnate 20-68 mm, (4)5-8(10) jugate, pinnae subopposed to alternate of (7)15-28(33) mm long, (8)11–19(25) pairs of leaflets. **Spicule** present. **Paraphyllidia** reniform, $0.4-0.5 \times 0.2-0.3$ mm, glandular. **Petioles** developed, 7.5–12(19) mm long, glabrous, with glandular trichomes when young, cylindrical to sulcate. **Leaflets** oblong, $1.5-2.6 \times 0.9-1.25$ mm, alternate arranged along the pinna, base asymmetric and semi-hastate, apex rounded, apparently veinless in both faces, visible orange-brown punctuations, margin glabrous and smooth, glandular trichomes with obconical head and unisseriate stalk on leaflets, lipid-secreting. **Stipules** tiny, retrorse, deciduous, brownish, triangular, 0.4-0.8× 0.3-0.6 mm, without veins, margin glabrous. **Sinflorescence** racemose, pseudoracemose or paniculate. **Inflorescence** capituliform, suglobose with peduncles of (4)9-12 mm long, glabrous and covered with a colorless and bright substance such as a resin. **Bracts** spatulated, $0.5-0.6 \times 0.14-0.19$ mm, glabrous, including the margin, before anthesis smaller than corolla. Calyx campanulate, glabrous, glandular, reddish-brown, 0.5–0.65 mm long, covering ¼ of corolla length. **Corolla** subtubular or vase-shaped, 2-2.25 × 0.7-0.9 mm, lobes vinaceous, glandular. Ovary oblong, glabrous, glandular, 0.6-0.8 mm long, stipite 0.35-0.5mm long. **Stamens** eight, exserted 1.6-3.0 mm long, free, but united below ovary, filaments white. Fruits oblong, plane-compressed, moniliform, covered with a colorless and bright substance

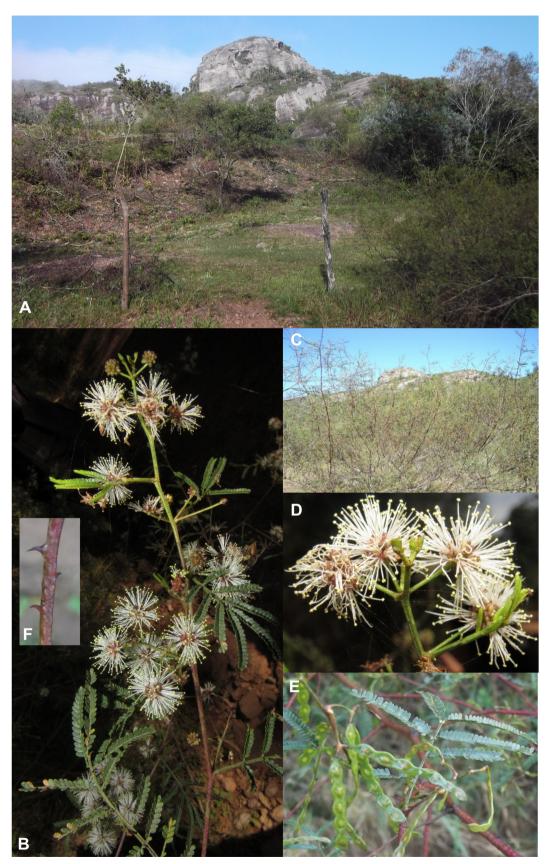


Figure 1. *Mimosa baptistae*: **A.** Habitat at Serra do Sudeste, Pampa biome, Brazil. **B.** Detail of a shrub individual; **C.** Population density. **D.** Arrangement of inflorescence, also showing the white stamens. **E.** Fruits. **F.** Detail of branches covered by a colorless and bright substance, resembling a resin. (Photos B and D from G.P. Coelho; Photos A, C, E, F from F. Schmidt-Silveira).

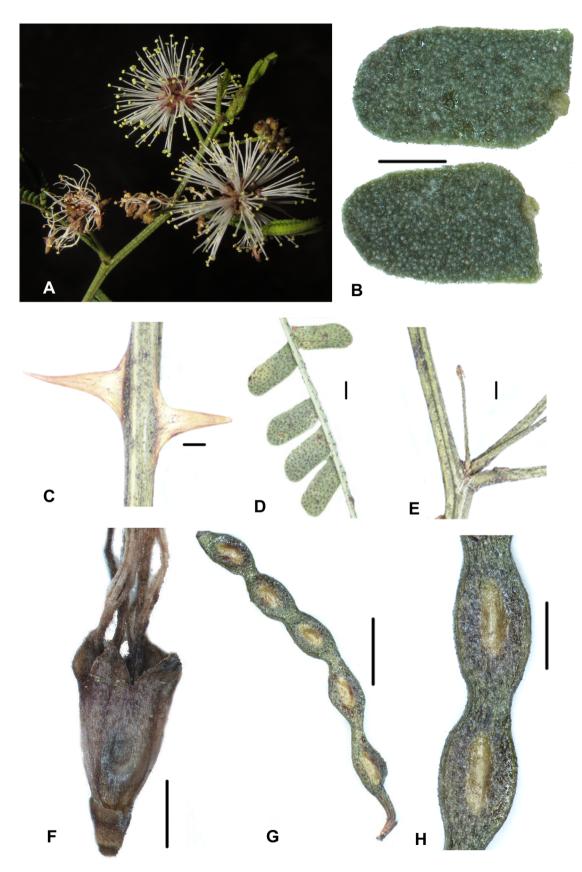


Figure 2. *Mimosa baptistae* morphological details. **A.** Capituliform subglobose inflorescence **B.** Leaflets C. Aculei. **D.** leaflets punctuations **E.** Subquadrangular branches with grooves. **F.** Calyx and corolla **G.** Fruit. **H.** Articles covered by a colorless and bright substance, resembling a resin. Scale bars of 1mm. (A–H from the holotype, photo A from G. P. Coelho; photos B–H from F. Schmidt-Silveira).

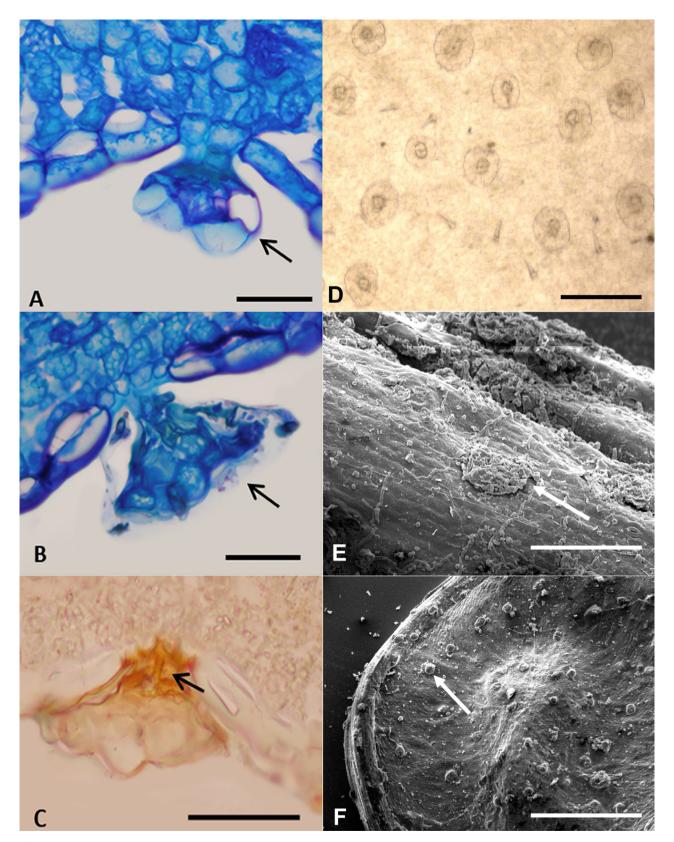


Figure 3. Optical micrographs (**A-D**) of glandular trichomes on the abaxial surface of *Mimosa baptistae* and SEM images (**E-F**). **A-B.** Glandular trichomes with unisseriate stalk before (**A**) and after secretion (**B**) (black arrow). **C.** Sudan IV positive histochemistry test showing lipids (black arrow). **D.** Overview of the surface of leaflets treated with sodium hydroxide, showing the glandular trichomes. **E.** Overview of a branch surface showing glandular trichome (white arrow). **F.** Glandular trichome on the fruit surface (white arrow). Scale bars: $50\mu m$ (A-C); $100 \mu m$ (D); (E) $200 \mu m$, (F) 2.0 mm.

such as a resin, $13-44 \times 2.8-3.5$ mm, short-stipitate, 1.7-3.0 mm long, (1) 3-5(7) articulate, apex cuspidate, valves and replum glabrous and glandular. **Seeds** immature.

Flowering and fruiting—August-January.

Distribution and ecology—*Mimosa baptistae* may be considered an endemic species up to now for Caçapava do Sul at the hills of Pedra do Segredo, Pedra do Leão and their neighborhood, in Serra do Sudeste (Rio Grande do Sul State), where it grows on sandstone outcrops, shrublands (vassoural), and rocky grasslands, at approximately 200 to 500 m elevation. Caçapava do Sul belongs to the physiographic region of Serra do Sudeste (Fortes 1959), which is highlighted as a high-level priority area for conservation (CNCFlora 2014) in the Pampa biome. Furthermore, Pedra do Segredo and Pedra do Leão harbor other species endemic and rare such as *Pavonia secreta* Grings & Krapov, *Petunia secreta* Stehmann & Semir, *Petunia exserta* Stehmann, and *Croton pygmaeus* L.R. Lima (Grings *et al.* 2011).

Etymology—The specific epithet is in honor of Dr. Luís Rios de Moura Baptista, who studied many groups of plants from the Flora of Rio Grande do Sul, including the *Mimosa* genus.

Comments— *Mimosa baptistae* resembles some species of *Mimosa* section *Batocaulon* ser. *Stipellares* such as *M. bifurca* Benth., *M. lepidota* Herzog, *M. sobralii* Grings & Ribas by its glutinous foliage and the alternate insertion of the leaflets. However, the most outstanding external morphological difference among all these species is the presence of aculei only in *M. baptistae*. From *M. bifurca*, the new species also differs by the color of the stamens (white *vs.* pink), as well as its restricted range and habitat (only known to Caçapava do Sul, Brazil *vs.* widespread in Argentina, Brazil, Paraguay and Uruguay). Whereas *M. lepidota* inhabits tropical-montane valleys in the Bolivian Andes, at 1800 to 2350 m altitude, *Mimosa baptistae* is restricted to low altitudes in subtropical grasslands in Brazil, and it is an

armed species with deciduous and non-cochleately stipules besides shorter measures (e.g., peduncles (4) 9–12 mm long vs. 15–30mm long; calyx 0.5–0.65mm long vs. 1.2–1.4 mm long). From *M. sobralii, M. baptistae* differs by the white stamens (vs. yellow), longer corolla (2–2.5 mm vs. 1.3–1.5 mm), and type of habitat (vassoural and rocky grasslands of Pampa biome vs. forest edges and rocky formations of Araucaria forests in Mata Atlantica biome).

Although M. baptistae seems to be closer to M. intricata Benth. in our adapted key, these species are readily distinguished by the habit (treelets vs. prostrate subshrubs) and color of their stamens (white vs. pink). Nevertheless, we found some exsiccates of *M. baptistae* identified as *M*. intricata. For example, Machado & Marchiori (2017) showed some wood anatomical features for *M. baptistae*, which was mistakenly identified as M. intricata. Nonetheless, their work also supports the circumscription of the new species in Mimosa ser. Stipellares due to the presence of vessels in large clusters, a feature apparently restricted to species of this series (Machado & Marchiori 2017). On the other hand, Mimosa baptistae may be easily confused as an armed version of Mimosa bifurca when flowers are absent. This similarity to M. bifurca has been reported for many other species of Mimosa ser. Stipellares (e.g., M. glycyrrhizoides Barneby and M. sobralii).

Additional specimens examined. BRAZIL. Rio Grande do Sul: Caçapava do Sul, Pedra do Segredo, 07. Sep 2015 [fl], F. Schmidt-Silveira 856 (ICN); idem, 00 Jan 1999 [fr], M. Sobral & J. Larocca 8715 (ICN); idem 00 Aug 1998 [fl], M. Sobral, J. Larocca & D. Guadagnin 9548 (ICN); idem, 12 Nov 1980 [fr], J. Mattos 21591 (HAS); idem, 15 Aug 1998 [fl], S. Bordignon, B. Irgang et al. 1426 (HAS); idem 13 Aug 1986 [fr], J.N.C Marchiori 486 (HDCF); idem 4 Sep 1986 [fr], J.N.C Marchiori 509 (HDCF); 30°S36'45.4", 53°W31'32.7", 408 m elev., 07.09.2015 [fr], F. Schmidt-Silveira 857 (ICN).

Key to Mimosa ser. Stipellares with leaflets arranged alternate along the pinna-rachis (adapted from Barneby, 1991).

1. Plants unarmed	2
1'. Plants armed with straight aculei	5
2. Fruits hispid, forming a unit; inflorescence oblong	M. strobiliflora
2'. Fruits glabrous, clearly individualized, not forming a unit; inflorescence globose	3
3. Purple or pink stamens; wide range and habitats (from eastern Paraguay through northeastern	Argentina to interior
Uruguay and Southern Brazil (RS, SC, PR, SP)	M. bifurca
3'. White stamens; restricted range and habitats (see below for more detail)	4
4. Shrubs or trees up to 8 m tall; plants growing on forest edges and rocky formations on m	ountain slopes from
northeastern Rio Grande do Sul state (Brazil), at 400 to 900 m altitude	M. sobralii
4'. Shrubs attaining 2–2.5 m tall; plants growing on woodlands of Tropical-montane valleys in the Bo	olivian Andes, at 1800
to 2350 m altitude	M. lepidota
5. Inflorescence spiciform; branches setose, covered by glandular-setulose trichomes	
5'. Inflorescence globose; branches glabrous, covered only by glandular trichomes	6
6. Pods denselv hispid: leaflets 2–3-nerved at dorsal face	M. ølvcvrrhizoides

- Anatomical and histochemical features

We only found a morphotype of glandular trichomes in the leaflets of *M. baptistae*, which exhibits an obconical head and unisseriate stalk (Fig. 2A-D). This trichome presents a variable number of cells in the head and stalk with one or two cells in front view, and usually occurs on the abaxial surface of the leaflet. Regarding this characteristic, we classified it as a stipitate trichome. Nevertheless, this trichome morphology in SEM images resembles a sessile trichome (Fig. 2E-F). Furthermore, these glandular trichomes secreted lipids, observed for the Sudan IV histochemistry test (Fig. 2C, Tab.1).

Table 1. Histochemical tests performed with leaflets of *Mimosa baptistae*.

Histochemistry tests	Compounds	Reaction
Cupric acetate	Resins	Negative
Dragendorf	Alkaloids	Negative
Ferric Chloride	Phenolic Compounds	Negative
Nadi reagent	Oleoresins	Negative
Sudan IV	Lipids	Positive
Vanillin hydrochloric	Tannin	Negative

Discussion

Mimosa baptistae is morphologically related to representatives of Mimosa sect. Batocaulon ser. Stipellares, which is recognized by the dilated paraphyllidia (Barneby 1991) and is considered a monophyletic series referred to as clade K in the *Mimosa* phylogeny (Simon *et al.* 2011). This series has ca. 21 species, occurring in open fields, and is practically restricted to the south of South America distributed in Argentina, Brazil, Paraguay, and Uruguay, except Mimosa lepidota that occurs in valleys of the Bolivian Andes. Furthermore, this series is divided into two circles of affinity based on leaflet distribution along pinna rachis and glandular trichomes, designated as stalked and sessile glands (Barneby 1991). Nevertheless, these affinities were proposed without an anatomical comparative framework of glandular trichomes; such a framework may be useful to support differences among species in this series. Thus, future studies are required to test whether the micromorphology of glandular trichomes would be a useful feature to distinguish species in Mimosa ser. Stipellares.

In *Mimosa*, trichomes have been used in the revision of taxonomic complexes (*e.g.*, Grohar *et al.* 2016) and in the description of new taxa, particularly in *Mimosa* sect. *Mimosa* (*e.g.* Morales *et al.* 2013; Morales & Fortunato 2013). Furthermore, Santos-Silva *et al.* (2013) pointed out that trichome morphology in *Mimosa* offers a set of characteristics that can be used for differentiating species and species

groups in combination. However, most of these works have considered only the macromorphological point of view, in other words, only the external morphology of trichomes and without anatomical details. In our work, we showed that Mimosa baptistae has stalked glandular trichomes by micromorphological analyses (optical micrographs), although it appears to have sessile glandular trichomes under a macromorphological view (SEM images). Our results suggest that the sessile or impressed glands described for this group in Mimosa ser. Stipellares should be reviewed concerning the micromorphological view (anatomical analyses) due to these glands being misidentified as sessile glandular trichomes despite being stalked glandular trichomes or not being macromorphologically distinguished (e.g., glandular content). Moreover, these findings may have implications on the delimitation of groups of affinity inside of Mimosa ser. Stipellares.

This study reports for the first time the chemical content of glandular trichomes for the *Mimosa* genus, showing that this new species secretes a lipophilic substance. However, further research is required to identify the chemical nature of this substance and whether it is the same substance that covers all the structures of *Mimosa baptistae*. Differences in secretory content have distinct ecological functions and may be a good source for elucidating the diversification in *Mimosa* ser. *Stipellares*. Furthermore, secretory structures such as glandular trichomes may synthetize and/or accumulate metabolites of high phytochemical and pharmacological value (Fahn 1979), representing a field still unexplored in *Mimosa*.

Acknowledgements

We gratefully acknowledge the Electron Microscopy Center of the Institute of Bioscience of the UNESP (Botucatu) for their assistance with sample preparation and Silvia Rodrigues Machado for suggestions. We also wish to thank Marcos Sobral for his help and Guilherme Peres Coelho for the photos provided, the Department of Zoology UFRGS for allowing the use of photo equipment, and CNPq for financial support.

References

Atahuachi MB, Hughes CE. 2006. Two new species of *Mimosa* (Fabaceae) endemic to Bolivia. Brittonia 58: 59-65.

Barneby RC. 1991. *Sensitivae censitae*: a description of the genus *Mimosa* Linnaeus, (Mimosoideae) in the New World. Memoirs of the New York Botanical Garden 65: 1-835.

Bessega C, Hopp HE, Fortunato RH. 2008. Toward a phylogeny of *Mimosa* (Leguminosae: Mimosoideae): a preliminary analysis of Southern South American species based on chloroplast DNA. Annals of the Missouri Botanical Garden 95: 567-579.

- BFG The Brazil Flora Group. 2015. Growing knowledge: an overview of Seed Plant diversity in Brazil. Rodriguésia 66: 1085-1113.
- CNCFlora Centro Nacional de Conservação da Flora. 2014. Áreas Prioritárias para conservação e uso sustentável da Flora Brasileira ameaçada de extinção. Rio de Janeiro, Instituto de Pesquisas Jardim Botânico do Rio de Janeiro.
- David R, Carde JP. 1964. Coloration differentielle dês inclusion lipidique et terpeniques dês pseudophylles du *Pin maritime* au moyen du reactif Nadi. C.R. Académie des Sciences Paris série D 258: 1338 –1340.
- Dutra VF, Garcia CP. 2013a. Three new species of *Mimosa* (Leguminosae) from Minas Gerais, Brazil. Systematic Botany 38: 398-405.
- Dutra VF, Garcia CP. 2013b. Two new species and one new variety of *Mimosa* sect. *Habbasia* (Leguminosae: Mimosoideae) from Central Brazil. Kew Bulletin 68: 163-171.
- Fahn A. 1979. Secretory tissues in plants. London, Academic Press.
- Flora do Brasil 2020 (under construction). 2018. *Mimosa*. Jardim Botânico do Rio de Janeiro. http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB23084. 20 Nov. 2018.
- Fortes AB. 1959. Geografia física do Rio Grande do Sul. Porto Alegre, Globo. Ganter P, Jollés G. 1969. Histologie normale et pathologique. Paris, Gauthier-Villars.
- Grings M, Krapovickas A, Boldrini II. 2011. A new species of *Pavonia* (Malvaceae) from Southern Brazil. Systematic Botany 36: 419-423.
- Grohar M, Rosenfeldt S, Morales M. 2016. Leaflet trichome micromorphology in the Dolentes-Brevipedes taxonomic complex (Mimosa L., Mimosoideae). Turkish Journal of Botany 40: 45-58.
- Johansen DA. 1940. Plant microtechnique. New York, McGraw- Hill Book. Mace ME, Howell CR. 1974. Histochemistry and identifications of condensed tannin precursor in roots of cotton seedlings. Canadian Journal of Botany 52: 2423-2426.

- Machado PFS, Marchiori JNC. 2017. Anatomia dos lenhos de *Mimosa intricata* Benth. e *Mimosa taimbensis* Burkart (Fabaceae). Balduinia 57: 19-29
- Morales M, Santos-Silva J, Ribas OS. 2013. A new species of *Mimosa* sect. *Mimosa* (Leguminosae, Mimosoideae) from Southern Brazil. Brittonia 65: 148-153.
- Morales M, Fortunato RH. 2010. Novedades taxonómicas y nomenclaturales en *Mimosa* L. subser. *Mimosa* (Leguminosae) para Sudamérica Austral. Candollea 65: 170-84.
- Pearse AGE. 1980. Histochemistry theoretical and applied. 4th. edn. London, Churchill Livingstone
- Santos-Silva J, Tozzi AMGA. 2012. Mimosa foreroana (Leguminosae, Mimosoideae), a new species from Nariño, Colombia. Systematic Botany 37: 437-441.
- Santos-Silva J, Tozzi AMGA, Simon MF, Urquiza NG, Morales M. 2013. Evolution of trichome morphology in *Mimosa* (Leguminosae-Mimosoideae). Phytotaxa 119: 1-20.
- Simon MF, Grether R, Queiroz LP, Särkinen TE, Dutra VF, Hughes CE. 2011. The evolutionary history of *Mimosa* (Leguminosae): toward a phylogeny of the sensitive plants. American Journal of Botany 98: 1201-1221.
- Svendsen AB, Verpoort R. 1983. Chromatography of alkaloids. New York, Elsevier Scientific Publishing Company.
- Thiers B. [continuously updated]. 2019. Index Herbariorum: A global directory of public herbaria and associated staff. New York, New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg. org/science/ih/. 20 Nov. 2018.
- Vidal BC. 1977. Acid glycosaminoglycans and endochondral ossification: microespectrophotometric evaluation and macromolecular orientation. Cellular and Molecular Biology 22: 45-64.