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A new species of *Anemopaegma* (Bignonieae, Bignoniaceae) from the Atlantic Forest of Brazil

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Abstract

The Atlantic Forest of Brazil includes one of the highest species diversity and endemism in the planet, representing a priority for biodiversity conservation. A new species of *Anemopaegma* from the Atlantic Forest of Brazil is here described, illustrated and compared to its closest relatives. *Anemopaegma nebulosum* Firetti-Leggieri & L.G. Lohmann has been traditionally treated as a morph of *Anemopaegma prostratum*; however, additional morphological and anatomical studies indicated that *A. nebulosum* differs significantly from *A. prostratum* and is best treated as a separate species. More specifically, *A. nebulosum* is characterized by elliptic and coriaceous leaflets (vs. ovate to orbicular and membranaceous in *A. prostratum*), smaller leaflet blades (3.6–5.5 x 2.0–3.0 cm vs. 6.7–13.0 x 4.2–8.4 cm in *A. prostratum*), orbicular prophylls of the axillary buds (vs. no prophylls in *A. prostratum*), solitary flowers (vs. multi-flowered axillary racemes in *A. prostratum*) and a gibbous corolla (vs. infundibuliform corollas in *A. prostratum*). In addition, *A. nebulosum* differs from *A. prostratum* anatomically in having thicker leaflet blades composed of two to four layers of palisade parenchyma (vs. one to three layers in *A. prostratum*), and seven to eight layers in the spongy parenchyma (vs. six to eight layers in *A. prostratum*). A key for the identification of all species of *Anemopaegma* from the Atlantic Forest of Brazil is presented.

Key words: Anatomy, Brazilian Atlantic Forest, cloud forest, morphology, tropical forests

Introduction

The Atlantic Forest of Brazil occurs in a variety of topographic and climatic conditions, encompassing humid lowland forests and coastal mountain regions as well as interior plateaus with long dry periods (Metzger 2009). This biome includes one of the highest species diversity and endemism in the planet, encompassing approximately 20,000 species of vascular plants (Myers *et al.* 2000). Despite its biological richness, the Atlantic Forest of Brazil is one the most threatened pieces of tropical forests around the world (Myers *et al*. 2000, Metzger 2009, Ribeiro *et al.* 2009), representing a priority for biodiversity conservation (Myers *et al.* 2000, Mittermeier *et al.* 2005).

Anemopaegma comprises approximately 47 species, representing the third largest genus of Tribe Bignonieae (Bignoniaceae) (Lohmann & Taylor 2014). The genus includes lianas and shrubs that are distributed throughout tropical America, where they occur in wet forests, dry forests and savannas (Lohmann 2006, Lohmann & Taylor 2014). Phylogenetic studies, based on molecular and morphological data (Lohmann 2003, 2006), showed that the genus is highly supported as monophyletic and inserted in a clade that is characterized by stems with multiples of four phloem wedges in cross section (Lohmann 2006).

Members of *Anemopaegma* have cylindrical and striated branchlets, without interpetiolar gland fields, solid or hollow piths, 2–3(–5)-foliolate leaves, with the terminal leaflet often modified into a simple or trifid tendril. The inflorescences are axillary, organized in fascicles, racemes or thyrses. The flowers are zygomorphic, with a cupular, truncate or 5-lobed calyx, usually with patelliform/cupular glandular trichomes (*sensu* Nogueira *et al.* 2013) clustered close to the margins; the corolla is infundibuliform, generally yellow and lepidote externally; the ovary is stipitate, smooth or covered by glandular trichomes; a large nectary disc surrounds the ovary. The capsule is thick, mostly compressed, elliptic and woody, bearing seeds with large hyaline-membranous wings that surround the seed body (Lohmann & Taylor 2014).

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Anemopaegma is one the most taxonomically complicated genera in the tribe, with most of its taxa presenting problematic circumscriptions (Gentry 1973). The high phenotypic variation of the genus was attributed to recurrent hybridization and introgression (Gentry 1973). This hypothesis was recently corroborated by a study on the reproductive biology of the *Anemopaegma arvense* species complex (Firetti-Leggieri 2009), in which no reproductive isolation was detected among species from the complex.

Thirty-four species of *Anemopaegma* occur in Brazil, seven of which are found in the Atlantic Forest: (i) *A. chamberlaynii* (Sims) Bureau & K. Schum., (ii) *A. citrinum* Mart. ex DC., (iii) *A. floridum* Mart. ex DC., (iv) *A. gracile* Bureau & K. Schum., (v) *A. hilarianum* Bureau & K. Schum., (vi) *A. prostratum* DC. and, (vii) *A. setilobum* A.H. Gentry. An additional species of *Anemopaegma* was recently identified during the preparation of a taxonomic revision of the genus by Firetti-Leggieri & Lohmann (unpublished data). This new species is here described and compared to the other species from the Atlantic Forest of Brazil.

Material & Methods

The morphological description of *A. nebulosum* was based on herbarium specimens and followed the terminology of Radford *et al.* (1974) and Weberling (1989). Comparisons to the closest relatives of *A. nebulosum* were based on specimens from the following herbaria: ESA, INPA, MBM, MO, R, RB, SP, SPF & UEC (herbarium acronyms follow Thiers 2013). Conservation assessment for the new species was based on the geographical distribution of each species, applying the IUCN red list category criteria (IUCN 2014).

Anatomical studies of leaflet blades of *Anemopaegma nebulosum* and *A. prostratum* were conducted in order to identify additional morphological features to separate these taxa. Leaf sections were based on herbarium specimens, all of which were subjected to the reversal of herbarium specimen process (Smith & Smith 1942), subsequently washed with distilled water and dehydrated in an increasing ethanol series. Leaflet fragments were included in historesin (Gerrits 1991), and cross sections of 14–16 m were obtained using a Leica rotary microtome. Sections were stained with toluidine blue 0.05% (O' Brien *et al.* 1964). Digital photographs were taken using a digital camera attached to a light microscope. In order to obtain information about the thickness of the leaflets, 50 regions of the leaflets of *Anemopaegma nebulosum* and of *A. prostratum* were measured. A complete list of specimens used in this study is presented in Table 1.

TABLE 1. List of *Anemopaegma* specimens sampled in the anatomical study.

Species	Voucher
Anemopaegma nebulosum Firetti-Leggieri & L.G. Lohmann, sp. nov.	J. Cordeiro *et al.* 1672 (SPF 185.478)
	J. Cordeiro & C.B. Poliquesi 909 (SPF 185.479)
	J.M. Silva *et al*. 1527 (SPF 103.981)
	J.M. Silva *et al.* 2647 (SPF 185.481)
Anemopaegma prostratum DC.	W. Hoehne 823 (SPF 10.823)
	F. França *et al*. 899 (SPF 137.270)
	O. Handro s.n. (SPF 144.373)

Taxonomic Treatment

Anemopaegma nebulosum Firetti-Leggieri & L.G. Lohmann, *sp. nov.*

Anemopaegma nebulosum is similar to *A. prostratum* DC., but differs by the orbicular prophylls, elliptic and coriaceous leaflet blades, and solitary gibbous flowers present in the axils of leaves (Fig. 1)

Type:—BRAZIL. Paraná: Guaratuba, Serra do Araçatuba, 15 Sep 1995 (fl), *J.M. Silva et al. 1527* (Holotype: SPF-103981!, Isotype: ESA-048739!)

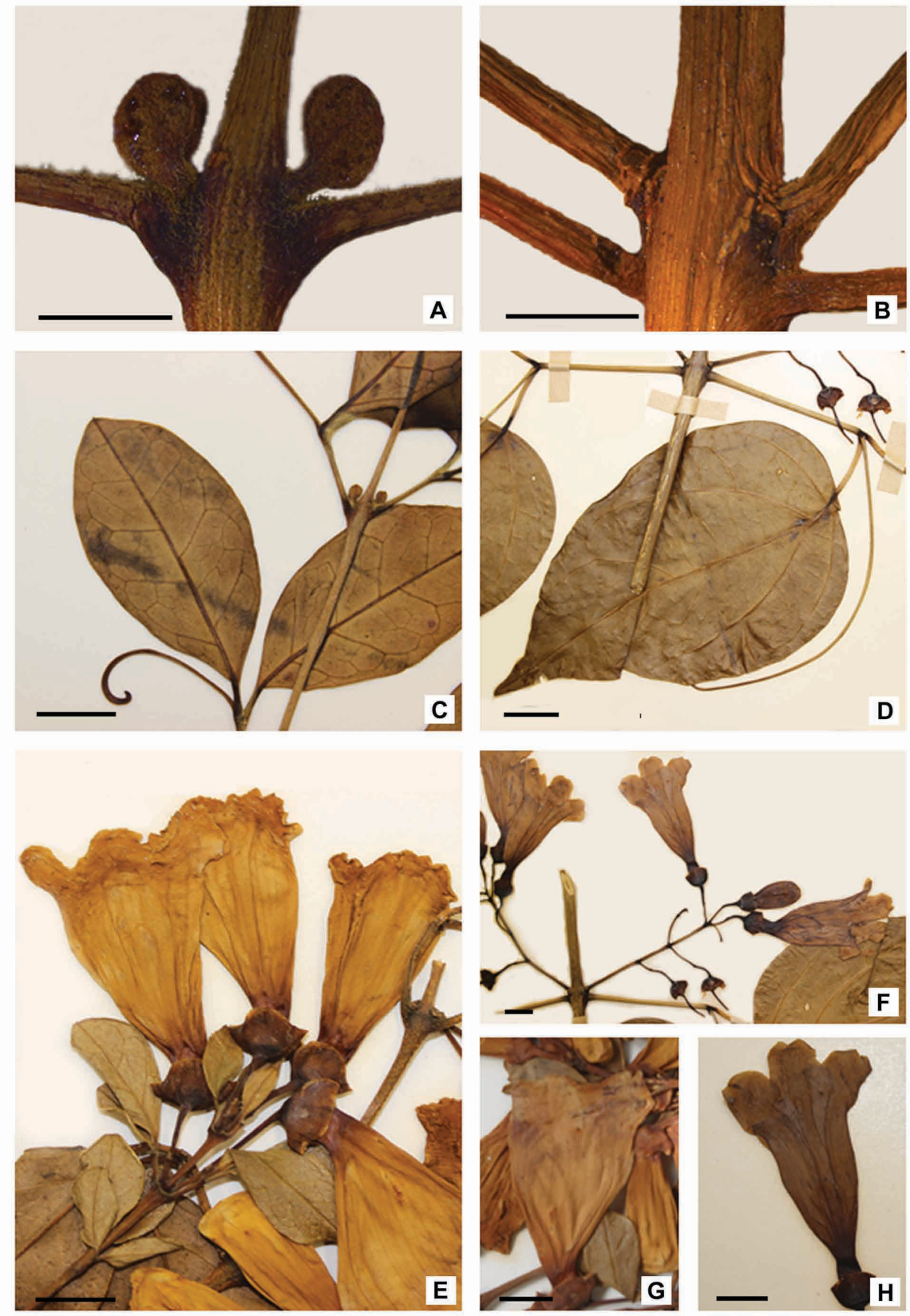

FIGURE 1. Morphological comparison between *Anemopaegma nebulosum* and *A. prostratum*. **A**. Orbicular prophylls in *A. nebulosum*. **B**. Prophylls lacking in *A. prostratum.* **C**. Elliptic and coriaceous leaflets in *A. nebulosum*. **D**. Ovate and membranaceous leaflets in *A. prostratum*. **E**. Solitary flowers in the axil of each leaf in *A. nebulosum*. **F**. Flowers arranged in lax racemes in *A. prostratum*. **G**. Gibbous flower in *A. nebulosum*. **H**. Infundibuliform flower in *A. prostratum*. (Figs. A and C from J. Cordeiro & C.B. Poliquesi 909; Fig. B from P.H. Miyagi 253; Figs. D, F and H from SPF-84375; Figs. E and G from J.M. Silva *et al*. 1527). Scale bars = 0.8 mm (Figs. A and B); 1 cm (Figs. C, D, E, F, G, H).

Lianas. Branchlets terete, striated, lenticelated with peltate glandular trichomes, puberulous with simple and multicelular non-glandular trichomes, without interpetiolar gland fields; prophylls of the axillary buds stipitate, orbicular, stipe 0.07–0.11 cm, blade 0.21–0.38 × 0.22–0.4 cm, with non-glandular and glandular (peltate and patelliform/cupular) trichomes, inconspicuous venation. Leaves 3–2-foliolate with the terminal leaflet modified into a simple tendril, pubescent; petioles 1.1–1.6 cm, semicircular, with the upper surface canaliculated, densely pubescent on the upper surface and glabrous on the lower surface in young leaves, trichomes restricted to the base in adult leaves; petiolules 0.3–0.7 cm; blades 3.5–5.5 × 1.8–3.0 cm, coriaceous, elliptic, apex acute to acuminate and mucronulate, base shortly atenuate, margin entire and slightly revolute, simple and multicellular non-glandular trichomes on the midrib of the abaxial surface, peltate glandular trichomes distributed on both sides; venation brochidodromous. Flowers solitary in the axil of each leaf, pedicellated; pedicel 1.2–1.45 cm, with a pair of bracts in the middle of the pedicel; bracts 1.0 mm, simple, sessile, with rounded apex; calyx 0.7–0.91 × 1.0–1.3 cm, cupular, 5-costated, truncate or shortly dentate (lacinia 0.02–0.03 cm), glabrous or with non-glandular trichomes sparsely distributed, margin ciliate, persistent until fruit ripening, patelliform/cupular glandular trichomes clustered close to margins; corolla pale yellow outside and yellow inside, gibbous, 3.82–4.9 cm long, base 0.7–1.1 × 0.5–0.8 cm usually covered by the calyx, 5-lobed, lobes 0.6–1.1 × 1.0–1.6 cm, rounded, ciliate, externally lepidote (with peltate glandular trichomes), without patelliform/cupular glandular trichomes in the upper portion of the tube and lobes, glabrous internally, except at the region of insertion of the stamens in which capitate glandular trichomes with multicellular and non-secretory stalk and multicellular glandular heads are found; androecium with one staminode 0.6 cm long, cream and glabrous, fertile stamens attached at the same height, filaments cream with non-glandular trichomes sparsely distributed, longer filaments 3.0 cm long, shorter filaments 1.8 cm long, anthers cream, glabrous, thecae 0.3–0.4 cm, pollen grains in monads; gynoecium with pistil 4.2–4.3 cm long, ovary ca. 0.3 × 0.1 cm, cylindric, with ca. 24 ovules, with 2–4 series of ovules per locule, peltate glandular trichomes covering externally the ovary, stipe not evident, style 3.5–3.6 cm long, cream and glabrous, stigma rhomboid, lobes 0.4 cm, cream and glabrous; nectary 0.2 cm long, annular, in the base of the ovary. Fruit not seen; seeds 3.0 × 3.3 cm, winged, hyaline wings involving the whole embryo, embryo 0.9 × 1.1 cm, cordate. Fig. 2.

Distribution and ecology: *Anemopaegma nebulosum* occurs in tropical montane cloud forests of southern Brazil, in the States of Paraná and Santa Catarina. This species blooms from September to December. No fruits have been observed to date.

Etymology: The epithet refers to the nebular forests of southern Brazil in which this species is distributed. Nebular forests, also called upper montane rain forests or cloud forests, are a type of vegetation found in the upper mountains, in several parts of the world, occurring in approximately 60 countries and several oceanic islands of the tropics (Aldrich *et al.* 2000). In Brazil, this vegetation type is associated with the high mountains of the Serra do Mar (Paraná, Rio Grande do Sul, Santa Catarina and São Paulo), Serra da Mantiqueira (Minas Gerais, Rio de Janeiro and São Paulo; Bertoncello *et al.* 2011), Parque Nacional do Caparaó (Espírito Santo and Minas Gerais) and Parque Nacional do Itatiaia (Minas Gerais and Rio de Janeiro; Pompeu 2015). It occurs in a very wet environment, with average temperatures below 15˚C. In the upper mountains, the canopy is uniform and homogeneously composed of a dense and low woody vegetation, usually with small and sclerophyllous leaves and thin, tortuous and branched trunks, completely covered with mosses and epiphytes (Portes *et al.* 2001). Some genera of plants are diagnostic of neotropical cloud forests, in particular *Ilex* (Aquifoliaceae), *Drymis* (Winteraceae), *Podocarpus* (Podocarpaceae), *Miconia* (Melastomataceae) and *Clethra* (Clethraceae) (Koehler *et al.* 2002). Apart from presenting high biological endemism, cloud forests are responsible for important environmental functions such as the regulation of water supply due to the ability to trap the moisture from clouds, and stabilize the soils of high slopes (Koehler *et al.* 2002; Sheer and Mocochinski 2009).

Conservation status:—This species is considered Data Deficient (DD) according to IUCN Standards and Petitions Subcommittee (2014) since this taxon is only known from very few specimens, with little information about its distribution and abundance. Further field studies are needed so that its conservation status can be properly documented.

Additional material examined:—BRAZIL. Paraná: Guaratuba, Serra do Araçatuba, Mata nebular, 1250 m de altitude, 22 Nov 1959 (fl), *G. Hatschbach 6495* (MBM). Guaratuba, Serra do Araçatuba, Mata nebular, 1,200 m de altitude, 19 Nov 1971 (fl), *G. Hatschbach 28106* (MBM). Guaratuba, Serra do Araçatuba, 15 Sep 1995 (fl), *J.M. Silva et al. 1527* (SPF). Guaratuba, Serra do Araçatuba, Matinha nebular, 1 Dec 1998 (fl), *J.M. Silva et al. 2647* (SPF). Tijucas do Sul, Serra Papanduva, 4 Dec 2003 (fl), *E. Barbosa & J.M. Silva 821* (ESA, MBM). Santa Catarina: Campo Alegre, Serra do Iqueriri, 19 Nov 1992 (fl), *J. Cordeiro & C.B. Poliquesi 909* (SPF). Subida à Serra do Quiriri, 28 Dec 1999 (fl), *J. Cordeiro et al. 1672* (SPF). Guaruva, Serra do Quiriri, 16 Oct 2004 (fl), *J.M. Silva et al. 4143* (SPF).

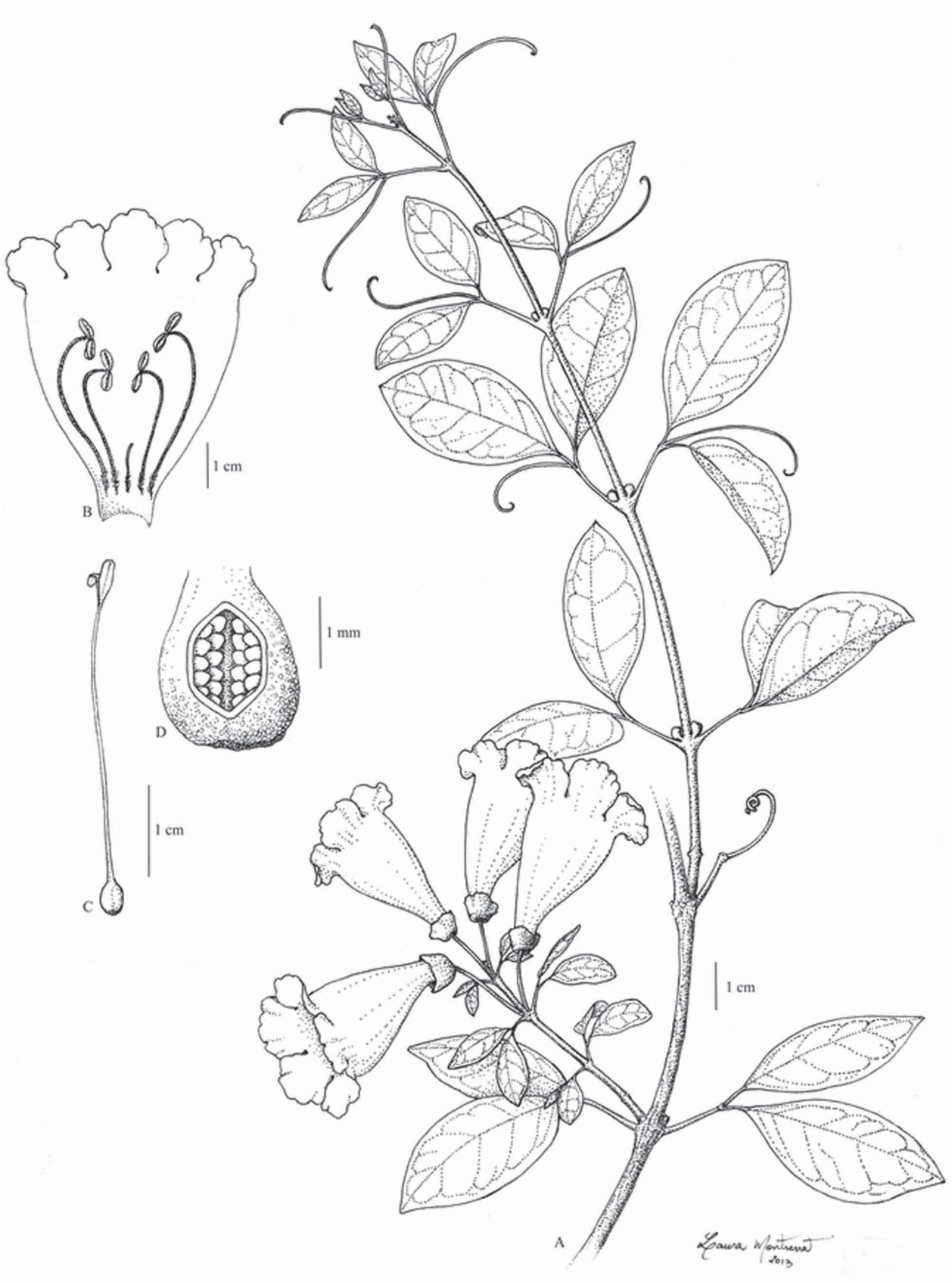

FIGURE 2. *Anemopaegma nebulosum*. **A**. Branchlet with leaves, prophylls, tendrils and flowers. **B**. Open flower showing stamens and staminodes. **C**. Pistil. **D**. Ovary showing the ovules arrangements. (Drawm from J. Cordeiro & C.B. Poliquesi 909).

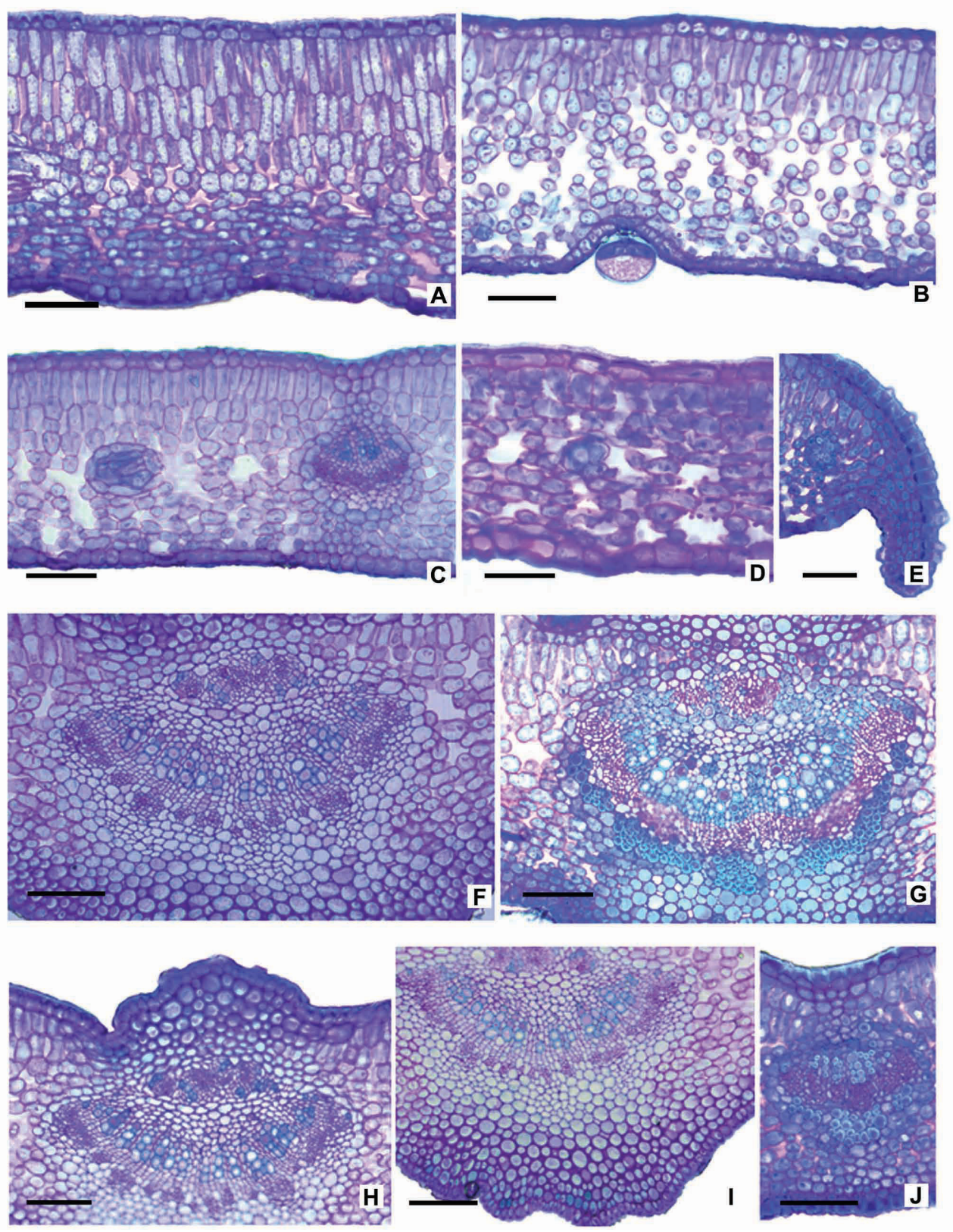

FIGURE 3. Cross-sections of leaflet blades of *Anemopaegma nebulosum* and *A. prostratum*. **A**. Dorsiventral mesophyll of *A. nebulosum*. **B–D**. Mesophyll of *Anemopaegma prostratum*. **B**. Uniseriate epidermis of the abaxial surface showing stomata and peltate glandular trichome. **C**. Dorsiventral mesophyll with two to three layers of palisade parenchyma. **D**. Dorsiventral mesophyll with one layer of palisade parenchyma. **E**. Leaflet margin of *A. nebulosum*. **F–G**. Cross sections of the midrib showing vascular systems. **F**. *Anemopaegma prostratum*. **G**. *Anemopaegma nebulosum*. **H–I**. Midrib of *A. prostratum* showing collenchyma between the adaxial epidermis and the vascular system (H) as well as between the abaxial epidermis and the vascular system (I). **J**. Vascular bundle of leaflet blade of *A. prostratum* with sheath extension. Scale bars = 100 µm (Figs. A, B, C, E, F, G, H, I, J), 50 µm (Fig. D).

Discussion

Collections of *Anemopaegma nebulosum* have been identified in herbaria as *Anemopaegma prostratum* "forma matinha nebular." Sandwith & Hunt (1974) treated this taxon as a morph of *A. prostratum* in the *Flora Ilustrada Catarinense* but mentioned that it might represent a new species. Even though *Anemopaegma nebulosum* is closely related to *A. prostratum*, it differs by elliptic and coriaceous leaflets (vs. ovate to orbicular and membranaceous leaflets in *A. prostratum*), orbicular prophylls of the axillary buds (vs. absent prophylls in *A. prostratum*), solitary flowers (vs. multi-flowered axillary racemes in *A. prostratum*), and gibbous corollas (vs. infundibuliform corollas in *A. prostratum*).

This new taxon is vegetatively similar to *A. brevipes* S. Moore, a species from the "cerrado" areas of Brazil. Apart from being geographically separated, *A. nebulosum* can also be separated morphologically by the orbicular prophylls of the axillary buds (vs. elliptic in *A. brevipes*), solitary flowers (vs. axillary racemes in *A. brevipes*) and gibbous corollas (vs. infundibuliform corollas in *A. brevipes*).

Anemopaegma nebulosum and *A. prostratum* have uniseriated epidermis in both surfaces (Figs. 3A, 3C–D), hypostomatic leaflets, i.e., with stomata restricted to abaxial surface (Figs. 3A–C), and peltate glandular trichomes (Fig. 3B) on both leaflet sides. While the mesophyll is dorsiventral in the leaflet blade of both species (Figs. 3A, 3C–D), the number of layers of the palisade parenchyma is variable, ranging from two to four in *A. nebulosum* (Fig. 3A) and from one to three in *A. prostratum* (Figs. 3C–D). The spongy parenchyma also shows a variable number of layers, ranging from seven to eight in *A. nebulosum* and from six to eight in *A. prostratum*.

The leaflet margin of both species is rounded with a mesophyll composed of a variable number of layers of collenchyma, photosynthetic parenchyma and vascular bundles (Fig. 3E). The midrib region is well defined, with a vascular system composed of a U-shaped collateral vascular bundle plus phloem strands with fibers toward the adaxial surface of the midrib (Figs 3F–G); collenchyma cells are found between the vascular system, the adaxial and abaxial sides of the epidermis (Figs. 3H–I). The lateral veins are composed of collateral vascular bundles that are surrounded by a uniseriate parenchymatous sheath; the larger vascular bundle presents sheath extensions (Fig. 3J). The vascular bundle of *A. nebulosum* and *A. prostratum* does not have the fiber cap upon the xylem found in members of the *Anemopaegma arvense* species complex, from the cerrado areas of Brazil (see Firetti-Leggieri *et al.* 2013).

In sum, anatomical characters found in transverse sections of the leaflet blades of *A. nebulosum* are similar to those of *A. prostratum* (Table 2; Figs. 3A–J), as well as to species from the *Anemopaegma arvense* species complex (Firetti-Leggieri *et al*. 2013). However, *A. nebulosum* and *A. prostratum* differ in the leaflet blade thickness (Table 2), with a palisade parenchyma with two to four layers in *A. nebulosum* (vs. one to three layers in *A. prostratum*) and a spongy parenchyma with seven to eight layers in *A. nebulosum* (vs. six to eight layers in *A. prostratum*).

TABLE 2. Anatomical and morphological comparison between *Anemopaegma nebulosum* and *Anemopaegma prostratum.*

	Anemopaegma nebulosum	*Anemopaegma prostratum*
Prophylls	Orbicular	Absent
Leaflet	Elliptic/Coriaceous	Ovate to orbicular/Membranous
Petiole (cm)	1.1–1.6	3.2–6.5
Petiolule (cm)	0.3–0.7	1.6–2.6
Blade (cm)	3.5–5.5 x 1.8–3.0	6.7–13.0 x 4.2–8.4
Inflorescence	Solitary flower	Raceme
Flower	Gibbous	Infundibuliform
Leaflet blade thickness (μm)	327.8–422.2 (375.4±27.7)	122.2–300 (245.4±65.9)
Layers of palisade parenchyma	2–4	1–3
Layers of spongy parenchyma	7–8	6–8

Key to the species of *Anemopaegma* from the Brazilian Atlantic Forest

1. A single flower present in the leaf axils; corolla gibbous ..... ***A. nebulosum***
1'. Multiple flowers present in the leaf axils; corolla infundibuliform **2**
2. Inflorescences in fascicles or racemes **3**
2'. Inflorescences in thyrses **4**
3. Inflorescences in two-flowered fascicles **5**
3'. Inflorescences in multi-flowered racemes **6**
4. Calyces with long lobes (3.0–5.0 mm long) ..... ***A. setilobum***
4'. Calyces with short lobes (0.4–0.6 mm long) ..... ***A. hilarianum***
5. Calyx cupular, with patelliform/cupular glandular trichomes clustered next to the margins ..... ***A. citrinum***
5'. Calyx campanulate, aglandular ..... ***A. gracile***

6. Flowers arranged in contracted racemes, with clustered fowers (flowers ca. 0.1–0.2 cm from each other) ..... ***A. floridum***
6'. Flowers arranged in lax racemes, with scattered flowers (flowers ca. 1.2–3.5 cm from each other) **7**
7. Prophylls foliaceous ..... ***A. chamberlaynii***
7'. Prophylls lacking ..... ***A. prostratum***

Acknowledgments

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