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Authors: Santo, Fabio Da Silva Do Espírito, Bitencourt, Cássia, Ribeiro, Patrícia Luz, and Rapini, Alessandro

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FABIO DA SILVA DO ESPÍRITO SANTO[1*], CÁSSIA BITENCOURT[1], PATRÍCIA LUZ RIBEIRO[1,2] & ALESSANDRO RAPINI[1]

Two new species of *Marsdenia* (*Apocynaceae*) from limestone outcrops in Brazil

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Abstract: Limestone outcrops often harbour floras rich in threatened and narrowly endemic species. Still, these formations, usually associated with karst landscapes, remain floristically poorly known, and so are often neglected in conservation programmes. In Brazil, three species of *Marsdenia* (*Apocynaceae*) endemic to limestone outcrops are known: *M. breviramosa*, *M. virgultorum* and *M. zehntneri*. In this work, we describe and illustrate two new species of *Marsdenia* restricted to karst landscapes: *M. calcaria* from the state of Minas Gerais and *M. phallica* from the state of Goiás. We also include illustrations of *M. zehntneri*, the species morphologically most similar to the two new species, and in situ images of the species of *Marsdenia* from limestone formations in Brazil (except for *M. virgultorum*, which is known only from the type). A geographic distribution map and an identification key for the five species known from limestone formations are also provided.

Key words: *Apocynaceae*, *Asclepiadoideae*, Brazil, Caatinga, Cerrado, conservation, endemism, floristics, Goiás, karst, limestone, *Marsdenia*, *Marsdenieae*, Minas Gerais, new species, taxonomy, threatened species

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Introduction

Calcareous outcrops are formations of sedimentary rocks composed predominantly of calcium carbonate produced by marine organisms. After uplift, these carbonate formations are then carved by rainwater and rivers, forming karst landscapes, marked by deeply rugged terrain, often with steep columns and cliffs, deep crevices and fissures, usually associated with underground drains and caves (e.g. Vermeulen & Whitten 1999; Clements & al. 2006; De Waele & al. 2009). In these outcropping islands, the rock is either exposed or covered by shallow, poor, alkaline soils, and is situated in an environment subjected to high temperatures, intense solar incidence and strong water restriction. Plants in these landscapes tend to be specialized and tolerant of extreme conditions; on the other hand, they are uncompetitive and quite vulnerable to disturbances (Médail & Verlaque 1997). In addition, their populations are usually small and isolated; therefore, they are susceptible to local extinctions caused by stochastic events and have a lower recolonization capacity (Fischer & Stöcklin 1997).

Calcareous formations harbour a high diversity of plants, especially in terms of rare and endemic species, as well as vulnerable ones (Cowling & al. 1994; Xu 1995; Willis & al. 1996a, b; Bardot-Vaucoulon 1997; Médail & Verlaque 1997; Vermeulen & Whitten 1999; Tuyet 2001; Zhu & al. 2003; Clements & al. 2006; Hart 2007; Zhang

1 Programa de Pós-graduação em Botânica, Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana, Av. Transnordestina s/n, Novo Horizonte, Feira de Santana, Bahia 44036-900, Brazil; *e-mail: fse.santo@yahoo.com.br (author for correspondence).
2 Centro de Ciências Agrárias, Ambientais e Biológicas, Universidade Federal do Recôncavo da Bahia, Rua Rui Barbosa 710, Centro, Cruz das Almas, Bahia 44380-000, Brazil.

& al. 2017). This unique diversity is highly threatened by the cement and lime industry, the transformation of the vegetation cover, removal of wood in forested areas, tourism and climate change (Vermeulen & Whitten 1999; Clements & al. 2006; Day 2010a, b; Coelho & al. 2012; Melo & al. 2013). Human impacts on some karst landscapes have been severe and lasting (e.g. Day 2010a, b), but karst lands that are harder to access tend to be the habitat of the last remnants of natural vegetation in a region, even when close to cities; on the other hand, their flora remains poorly known and the lack of information causes its neglect in conservation programs (Vermeulen & Whitten 1999; Coelho & al. 2012).

Globally, carbonate karst is estimated to occur over approximately 10–15% of the land surface (Ford & Williams 2007). However, it can be commoner in particular regions; for instance, karst occurs on more than half of the Caribbean land area, and is mostly concentrated (90%) in the Greater Antilles (Day 2010a, b). In contrast, carbonate areas cover probably less than 2% of the South American land surface, and most of them are concentrated in Central Brazil, with a few areas scattered along the Andes (Auler 2004). In Brazil, the karst area corresponds to 5–7% of the country, totalling between 425 000 and 600 000 km^2, most of which is from the Upper Proterozoic and Lower Cambrian, belonging to the Bambuí and Una groups, in the states of Minas Gerais, Bahia and Goiás, Central Brazil (Auler & Farrant 1996, in which see fig. 1 for the distribution of karst areas in Brazil).

Recently, a new species of *Allamanda* L. (*A. calcicola* Souza-Silva & Rapini, *Apocynaceae*), a genus widely used as an ornamental due to its large and abundant flowers, has been recognized, occurring exclusively in karst landscapes in the São Francisco Basin (Souza-Silva & Rapini 2009). In Serra do Ramalho, SW Bahia, along with *A. calcicola*, another new species of *Apocynaceae* was found, *Marsdenia breviramosa* Rapini & Fontella (Rapini & Fontella-Pereira 2011). Among the 40 species of *Marsdenia* in Brazil, only two others occur exclusively in limestone outcrops: *M. virgultorum* (E. Fourn.) Malme, described in the 19th century (Fournier 1885) and known only from the type material, collected in Lagoa Santa, Minas Gerais, and *M. zehntneri* Fontella, which, although occurring in the states of Minas Gerais, Bahia and Goiás, was only described in the 1960s (Fontella-Pereira 1965).

During the preparation of the taxonomic treatment of *Marsdenia* (*Apocynaceae*: *Asclepiadoideae*: *Marsdenieae*) from Brazil, two other new species, endemic to limestone outcrops and previously confused with *M. zehntneri*, were recognized. They are described below as *M. calcaria*, from N Minas Gerais, and *M. phallica*, from Goiás. The five *Marsdenia* shrub species endemic to karst landscapes in Brazil can be readily separated based on the key below. However, the morphological similarity between them suggests a phylogenetic proximity, and this complex of species in *Marsdenia* could be an excellent group for biogeographic investigations in limestone outcrops.

Material and methods

For the treatment of Brazilian *Marsdenia*, we examined specimens at more than 30 herbaria in Brazil, Europe and the United States: ALCB, B, CEN, CEPEC, CVRD, HB, HRB, HST, HUEFS, IAN, IBGE, INPA, IPA, JPB, K, MBM, MBML, MG, MO, NY, P, PEUFR, R, RB, SPF, UB, UFP, UFRN, UPCB, US, VIES and W. Besides, in the last 20 years, neotropical asclepiads have also been examined in other herbaria (e.g. BHCB, BM, BR, C, F, G, LE, M and S; herbarium codes according to Thiers 2018+). We consulted floristic inventories and floras of *Apocynaceae* from the neotropics, with special attention to those from South America (e.g. Rothe 1915; Morillo 1978; Fuentes & Morales 2004). We used the Taxonomic Species Concept (Rapini 2004) and the morphological terminology in Radford & al. (1974) and Hewson (1988). For fruits, we followed Spjut (1994), according to whom fruits of *Apocynaceae* that derive from two carpels are schizocarpic, and each of their two fruitlets (monocarps) is a follicarium (plural: follicaria).

Key to shrubby species of *Marsdenia* from limestone outcrops in Brazil

1. Leaves with lamina elliptic; corolla adaxially purple, lobes straight . . . . . ***M. virgultorum***
– Leaves with lamina filiform, lanceolate, narrowly elliptic or linear; corolla adaxially greenish cream or greenish, with or without a vinaceous tinge at basal portion of lobes, lobes patent or recurved **2**
2. Leaves with lamina filiform, arranged in brachyblasts . . . . . ***M. breviramosa***
– Leaves with lamina lanceolate, narrowly elliptic or linear, opposite or irregularly arranged in nodes with up to 4 leaves, but not in brachyblasts **3**
3. Corolla tube < 2.3 mm long, lobes greenish with a central vinaceous tinge at basal portion of lobes, throat pilose; corona lobes recurved or occasionally nearly straight . . . . . ***M. phallica***
– Corolla tube > 2.4 mm long, lobes uniformly greenish, throat pubescent or glabrous; corona lobes incurved **4**
4. Corolla throat glabrous, tube > 4.9 mm long, lobes > 5.9 × 3.9 mm; corona lobes > 6.7 mm long, lower portion orbicular and concave; corpusculum > 0.4 mm long, pollinia > 0.49 × 0.32 mm . . . . . ***M. zehntneri***
– Corolla throat pubescent, tube < 2.9 mm long, lobes < 4.7 × 3.1 mm; corona lobes < 3.1 mm long, lower portion trullate and invaginated; corpusculum < 0.31 mm long, pollinia < 0.38 × 0.25 mm . . . . . ***M. calcaria***

Marsdenia calcaria F. Esp. Santo, **sp. nov.** – Fig. 1A–G, 2A–C.

Holotype: Brazil, Minas Gerais, Bocaiúva, Engenheiro Dolabela, on the road from Engenheiro Dolabela to BR-

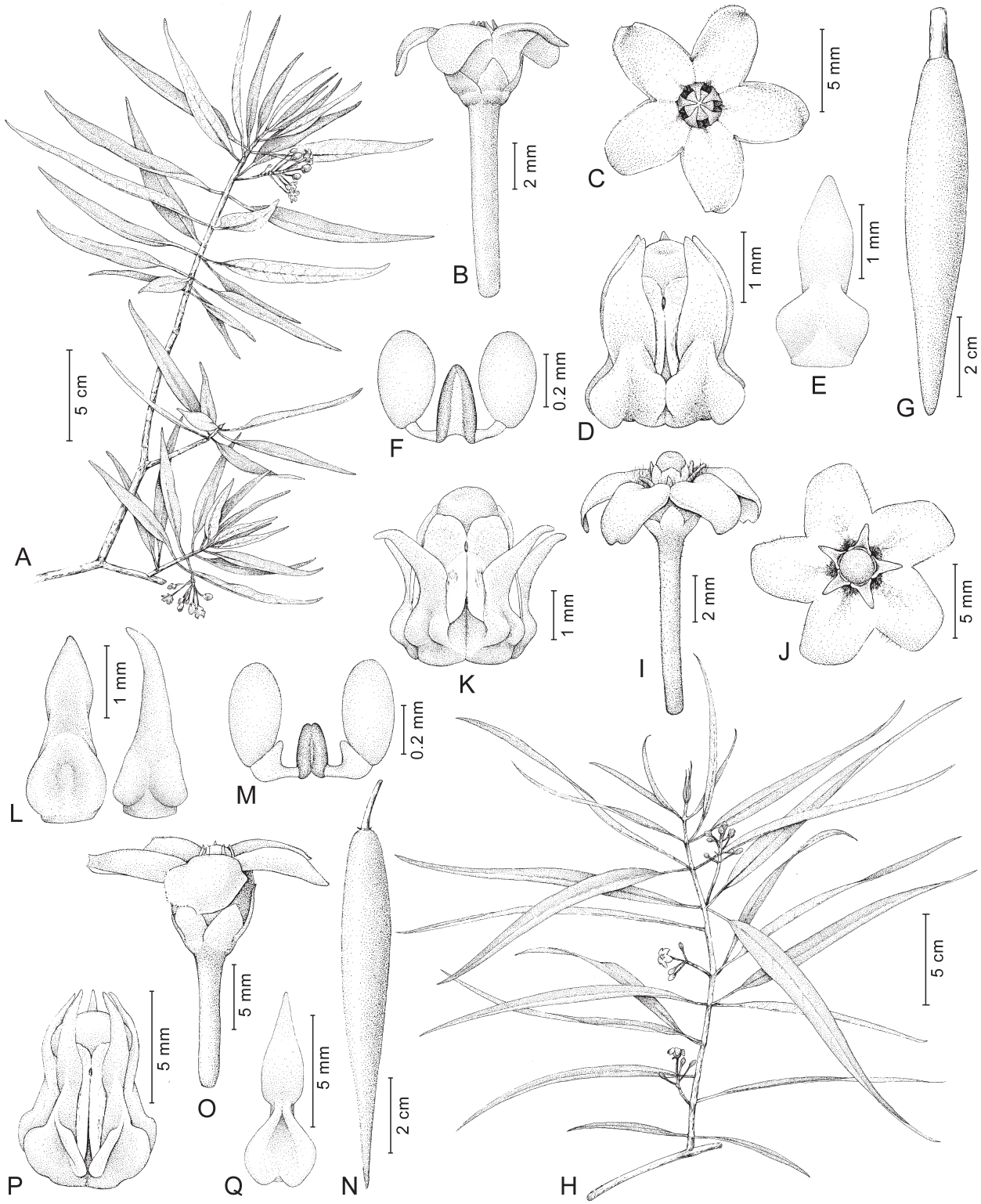

Fig. 1. *Marsdenia calcaria*: A: branch with leaves and inflorescences; B: flower, lateral view; C: flower, apical view; D: gynostegium; E: corona lobe, abaxial view; F: pollinarium; G: follicarium. – *Marsdenia phallica*: H: branch with leaves and inflorescences; I: flower, lateral view; J: flower, apical view; K: gynostegium; L: corona lobes, abaxial view (showing variability); M: pollinarium; N: follicarium. – *Marsdenia zehntneri*: O: flower, lateral view; P: gynostegium; Q: corona lobe, frontal view. – Drawn from: *M. calcaria*: A–F from the holotype, G from *J. R. Pirani & al. 4399*; *M. phallica*: H–M from the holotype, N from *A. C. Sevilha & al. 2138*; *M. zehntneri*: O–Q from *B. J. Dias & al. 120*. – All drawn by Gustavo Surlo.

Fig. 2. *Marsdenia calcaria*: A: habit; B, C: inflorescence with buds and open flowers, lateral view. – *Marsdenia phallica*: D: habit; E: flower, apical view; F: flower, lateral view. – *Marsdenia breviramosa*: G: branch with leaves and inflorescences. – *Marsdenia zehntneri*: H: habit; I: inflorescence with buds and open flowers, lateral view. – Photographs: A, C by F. S. Espírito Santo; B, D–F by D. J. L. Sousa; G–I by A. Rapini.

135, 17°27'37.7"S, 44°00'57.2"W, 600 m, 22 Nov 2016 (fl.), *F. S. Espírito Santo & al. 233* (HUEFS!; isotypes: K!, NY!, RB!, SPF!).

Diagnosis — *Marsdenia calcaria* resembles *M. zehntneri*, differing by the adaxially pubescent (vs glabrous) corolla throat and especially by the smaller flowers: corolla tube < 2.9 mm long (vs > 4.9 mm long) and lobes < 4.7 × 3.1 mm (vs > 5.9 × 3.9 mm), and corona lobes < 3.1 mm long (vs > 6.7 mm long), with trullate, auriculate and invaginated lower portion (vs orbicular and concave).

Description — *Shrub* erect, up to 3 m tall. *Stems* glabrous, with colleters at nodes and petiole insertion points. *Leaves* opposite; *petiole* 0.9–2.4 cm long, glabrous, occasionally with sparse trichomes; *lamina* lanceolate, narrowly elliptic or linear, 5.2–11.9 × 0.4–1.5 cm, glabrous, adaxially with (0–)2–5 colleters at base of main vein, base cuneate to attenuate, margin entire, revolute, apex attenuate to acute. *Inflorescences* subaxillary, corymbose, 6–18-flowered; *peduncle* 0.35–1.6 cm long, puberulent or glabrous; *bracts* ovate or lanceolate, 0.8–1.8 × 0.3–1.1 mm, margin ciliate. *Pedicel* 0.6–1.2 cm long, puberulent. *Sepals* ovate, 2.2–3 × 1.7–2.3 mm, puberulent, glabrescent, adaxially with 5 alternisepalous colleters, margin ciliate, apex obtuse to acute. *Corolla* greenish, shortly campanulate, abaxially glabrous, adaxially pubescent on throat, trichomes reaching distal half of tube and proximal half of lobes; *tube* 2.5–2.8 mm long; *lobes* oblong, recurved, 4–4.6 × 2.3–3 mm, margin ciliate, apex irregularly emarginate. *Corona lobes* 5, 2.2–3 × 1.1–1.65 mm; *lower portion* fused to dorsal side of anther, trullate, auriculate, invaginated; *upper portion* free from anther, lanceolate, apex acute, incurved over style head. *Anthers* c. 0.4 × 0.22 mm, with narrowly falcate lateral wings; *apical membranous appendix* transversely elliptic, 0.6–0.8 × 0.9–1.1 mm, not exceeding apex of style head. *Corpusculum* ovoid, 0.28–0.30 × 0.15–0.17 mm, apically rounded. *Caudicles* 0.15–0.18 mm long. *Pollinia* elliptic, 0.34–0.37 × 0.20–0.24 mm. *Gynostegium* subsessile, 3–3.8 mm long; *style head* subglobose, c. 1 × 1.5 mm, exserted from corolla tube. *Follicaria* fusiform, 6.1–14.1 × 1.4–1.8 cm, glabrous. *Seeds* not seen.

Phenology — Collected with flowers in November and with fruits in May.

Distribution and ecology — *Marsdenia calcaria* is known exclusively from a limestone outcrop, growing directly on rocks, in Engenheiro Dolabela, state of Minas Gerais, Brazil (Fig. 3), in remnants of deciduous forest, where individuals of *Myracrodruon urundeuva* Allemão (*Anarcardiaceae*) and *Encholirium* sp. (*Bromeliaceae*) are common.

Conservation status — The species is known only from the type locality, situated about 100 m from a minor road, 800 m from the BR-131 highway and 1.2 km from the village. The surroundings were changed by agricultural and livestock activities, causing the population of *Marsdenia calcaria* to become even more isolated. Two other similar calcareous outcrops look suitable to this species, one 270 m and other 1.1 km to the north; together, they form a strip smaller than 0.5 km^2. Found in a single locality, with a small area of occurrence and occupation, under strong anthropogenic pressure, this species should be considered Critically Endangered, CR B1ab(iii)+2ab(iii), according to IUCN (2016) criteria.

Etymology — The epithet of the new species is a reference to its occurrence exclusively on limestone outcrops.

Remarks — *Marsdenia calcaria* is characterized by its erect, shrubby habit, lanceolate, narrowly elliptic or linear leaves, corymbose inflorescences, with small flowers compared to those of *M. zehntneri*, corolla with pubescent throat and corona lobes formed by a trullate and auriculate lower portion, and a lanceolate, apically acute upper portion. Among the shrubby species of *Marsdenia* that occur on limestone outcrops in Brazil, *M. calcaria* is more similar to *M. phallica* and *M. zehntneri*.

Additional specimens examined — Brazil: Minas Gerais, Bocaiúva, Engenheiro Dolabela: Granjas Reunidas, 3 May 1963, *A. P. Duarte 7763* (HB, RB); ibid., 17°28'S, 44°01'W, 4 Nov 1988, *R. M. Harley & al. 25506* (CEPEC, HB, K, MO, NY, RB, SPF); ibid., 17°30'S, 44°00'W, 17 May 1998, *J. R. Pirani & al. 4399* (SPF).

Marsdenia phallica F. Esp. Santo, **sp. nov.** – Fig. 1H–N, 2D–F.

Holotype: Brazil, Goiás, Guarani de Goiás, fazenda Forquilha, 13°26'36.9"S, 46°19'40.1"W, 475 m, 26 Nov 2016, *F. S. Espírito Santo & al. 234* (HUEFS!; isotypes: K!, NY!, RB!, SPF!).

Diagnosis — *Marsdenia phallica* resembles *M. calcaria* and *M. zehntneri*, differing by the corolla lobes adaxially with a central vinaceous tinge at the basal portion (vs uniformly greenish to greenish cream), throat pilose (vs pubescent in *M. calcaria* and glabrous in *M. zehntneri*) and corona lobes recurved or occasionally nearly straight at the apex (vs incurved).

Description — *Shrub* erect, up to 2 m tall. *Stems* glabrous, with colleters at nodes and petiole insertion points. *Leaves* opposite; *petiole* 0.4–2.3 cm long, glabrous or puberulent; *lamina* lanceolate, narrowly elliptic or linear, 4.1–19.1 × 0.15–2 cm, glabrous, adaxially with (0–)2–6 colleters at base of main vein, base cuneate to attenuate, margin entire, revolute, apex attenuate to acute. *Inflorescences* subaxillary, corymbose, 5–15-flowered; *peduncle* 0.3–2.7 cm long, glabrous or sparsely puberulent; *bracts* ovate or lanceolate, 0.74–2.7 × 0.42–1.6 mm, margin

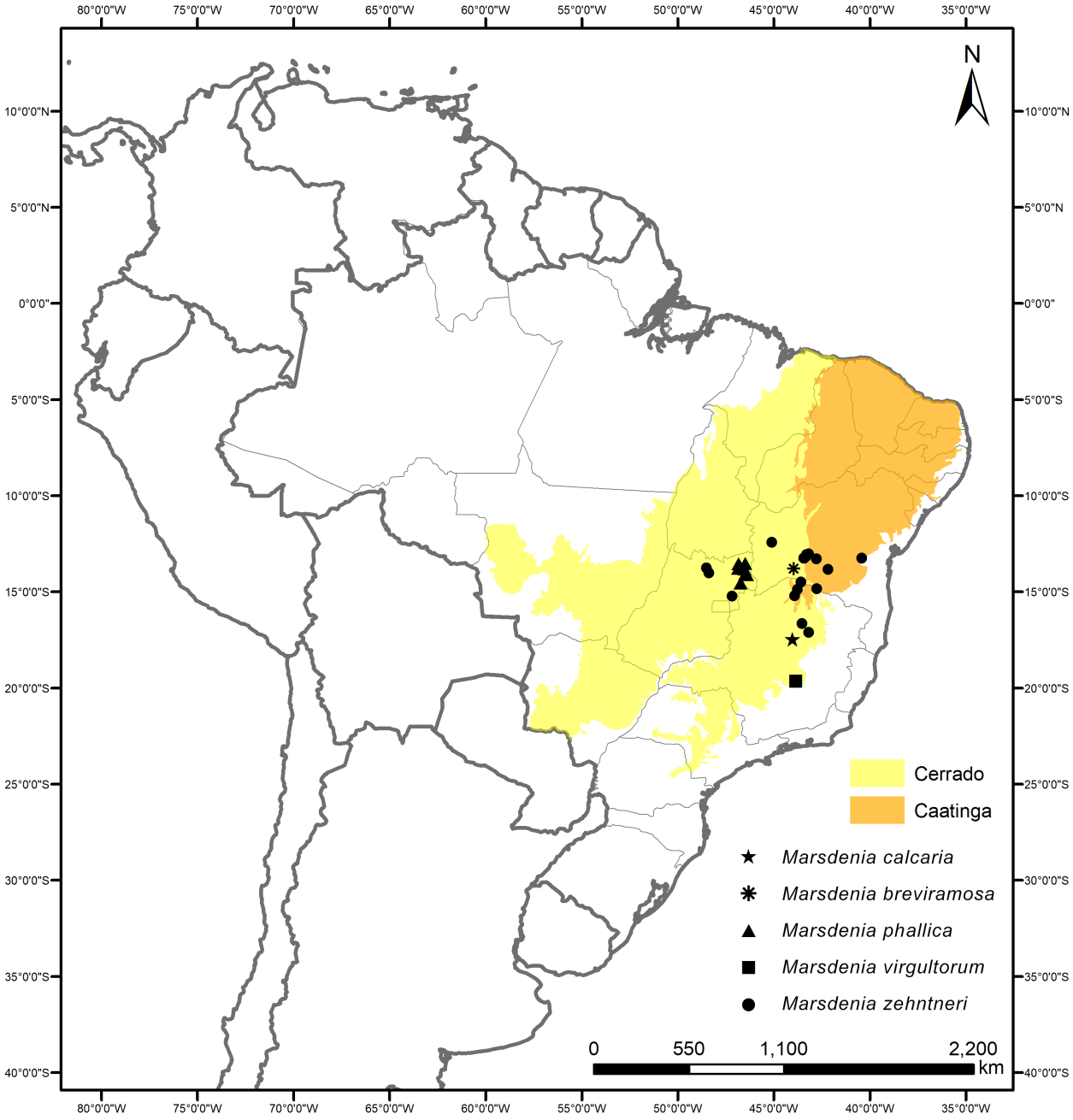

Fig. 3. Distribution map of shrubby species of *Marsdenia* (*M. breviramosa, M. calcaria*, *M. phallica*, *M. virgultorum* and *M. zehntneri*) endemic to limestone outcrops in Brazil, showing the Cerrado and Caatinga domains.

ciliate. *Pedicel* 0.5–1.2 cm long, puberulent, glabrescent. *Sepals* ovate, 1.75–2.6 × 1.5–2.6 mm, puberulent, glabrescent, adaxially with 5 or 10 alternisepalous colleters, margin ciliate, apex obtuse. *Corolla* greenish, adaxially with a central vinaceous tinge at basal portion of lobes, shortly campanulate, abaxially glabrous, adaxially pilose on throat, trichomes occasionally reaching tinge range of lobes; *tube* 1.9–2.2 mm long; *lobes* ovate, patent to slightly recurved, 3.1–5 × 2.3–4 mm, margin sparsely ciliate, apex irregularly emarginate. *Corona lobes* 5, 2.1–3 × 0.65–1.2 mm; *lower portion* attached to dorsal side of anther, laterally bulging, occasionally orbicular and concave; *upper portion* free from anther, narrowly lanceolate, apex acute and recurved, occasionally nearly straight. *Anthers* c. 0.5 × 0.25 mm, with narrowly falcate lateral wings; *apical membranous appendix* ovate, 0.75–0.9 × 0.95–1.1 mm, below apex of style head. *Corpusculum* ovate, 0.23–0.27 × 0.13–0.15 mm, apically rounded. *Caudicles* 0.19–0.29 mm long. *Pollinia* elliptic, 0.40–0.47 × 0.22–0.31 mm. *Gynostegium* subsessile, 3–3.5 mm long; *style head* subglobose, c. 1.1 × 1.55 mm, exserted from corolla tube. *Follicaria* fusiform, 9.5–9.9 × 1.3–2.2 mm, glabrous. *Seeds* not seen.

Phenology — Collected with flowers from October to December and with fruits in March, April, July and August.

Distribution and ecology — Found in deciduous seasonal forests, on limestone outcrops, exclusively in the state of Goiás (Fig. 3).

Conservation Status — Among the areas of occurrence of *Marsdenia phallica*, only the environmental conditions of the type locality are known. It is situated on a roadside, in an area whose vegetative matrix has been replaced by pasture areas. However, estimates of the extent of occurrence (3780 km²) and area of occupancy (32 km²) indicate that *M. phallica* should be considered Endangered, EN B1ab(i,ii,iii)+2ab(i,ii,iii), according to IUCN (2016) criteria.

Etymology — The epithet of the species refers to the peculiar shape of the corona lobe, which resembles a phallus.

Remarks — *Marsdenia phallica* can be recognized by the shrubby, erect habit, lanceolate, narrowly elliptic or linear leaves, flowers with a shortly campanulate corolla, adaxially with a central vinaceous tinge at the basal portion of the lobes, pilose on the throat, and by the corona lobes usually formed by a laterally bulging lower portion and a narrowly lanceolate upper portion, acute and recurved at the apex.

Additional specimens examined — Brazil: Goiás: Alvorada do Norte, 14°32'21"S, 46°43'26"W, 25 Aug 2003, *A. C. Sevilha & al. 3088* (CEN, HUEFS); Guarani de Goiás, 13°48'12"S, 46°31'48"W, 18 Oct 2001, *R. C. Mendonça & al. 4478* (CEN, IBGE, SPF); ibid., 5 Mar 2001, *M. L. Fonseca & al. 2405* (IBGE, K n.v.); Nova Roma, 13°46'S, 46°51'W, 30 Jul 2000, *R. C. Forzza & al. 1581* (SPF); ibid., 13°31'32"S, 46°49'39"W, 25 Jul 2007, *R. C. Forzza & al. 4674* (RB); Posse, 14°03'53"S, 46°29'15"W, 3 Nov 2000, *M. A. Silva 4644* (CEN, IBGE, K, RB, SPF); ibid., 14°06'25"S, 46°23'51"W, 4 Dec 2003, *R. Mello-Silva & al. 2293* (SPF); São Domingos, 13°41'16"S, 46°44'20"W, 9 Nov 2004, *A. C. Sevilha & al. 4138* (CEN); ibid., 13°41'16"S, 46°44'20"W, 15 Apr 2002, *A. C. Sevilha & al. 2138* (CEN); ibid., 13°31'02"S, 46°28'59"W, 9 Mar 2004, *A. A. Santos 2220* (CEN, HUEFS).

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