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## Rediscovery and new combination of *Serpocaulon demissum* (Polypodiaceae), an endangered endemic species to the Brazilian inselbergs

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### Abstract

*Serpocaulon demissum* is herein proposed as a new combination based on the study of the type specimens and protologue of *Goniophlebium demissum*, as well as the comparison with its sympatric and morphologically similar species, *S. catharinae* and *S. vacillans*, two widespread species from the Atlantic Forest. *Serpocaulon demissum* is similar to these species by the presence of patent rhizomes scales and pinnatisect laminae, but differs from them by its thick amylaceous rhizomes, larger rhizome scales, and spores without folded perine. This taxon represents an endemic species from the Brazilian inselbergs. A re-description that includes a lectotype designation, line drawings, SEM pictures of the spores, pictures of the plant in the field, information of the novel habitat and rhizome stores, distribution map, and the conservation status of the species is presented.

**Keywords:** Brazil, disjunct distribution, desiccation-tolerant fern, *Goniophlebium*, nomenclature

### Resumo

*Serpocaulon demissum* é aqui proposto como uma nova combinação baseada no estudo dos espécimes tipo e no protólogo de *Goniophlebium demissum*, assim como na comparação com as suas espécies simpátricas e morfológicamente similares, *S. catharinae* e *S. vacillans* (duas espécies amplamente distribuídas da floresta Atlântica). *Serpocaulon demissum* é similar a estas espécies pela presença de escamas dos rizomas patentes e lâminas pinnatisectas, porém, distingue-se destas espécies por ter os rizomas maiores e amiláceos, escamas maiores no rizoma e esporos sem perina dobrada. Este táxon representa uma espécie endêmica dos ecossistemas dos inselbergs brasileiros. A redescrição que inclui: a designação do lectótipo, ilustração, SEM imagens dos esporos, imagens da planta no campo, informações sobre o novo habitat e o armazenamento de amido nos rizomas, mapa de distribuição, e o estado de conservação da espécie são apresentadas.

**Palavras chave:** Brasil, distribuição disjunta, samambaia tolerante a dessecação, *Goniophlebium*, nomenclatura

### Introduction

*Serpocaulon* Smith (2006: 924) (Polypodiaceae) is a tropical American fern genus that was formerly included in *Polypodium* Linnaeus (1753: 1082) (Smith *et al.* 2006). Despite its main diversity occurring at middle elevation humid forests of the northern Andes (Sanín 2018), the Brazilian Atlantic forest is recognized as an important center for the speciation of the genus (Labiak & Prado 2008, Kreier *et al.* 2008, Schwartzburd & Smith 2013, Sanín & Salino 2018), with around 15 species. At least four of these are considered endemic to the Atlantic rain forest: *S. catharinae* (Langsdorff & Fischer 1810: 9) Smith (2006: 926), *S. glandulosissimum* (Brade 1934: 230) Labiak & Prado (2008: 147), *S. meniscifolium* (Langsdorff & Fischer 1810: 11) Smith (2006: 926), and *S. vacillans* (Link 1833: 97) Smith (2006: 929), Sanín and Salino (pers. obs.).

One of the most remarkable geological formations within the Atlantic rain forest, especially in Brazil, are the inselbergs (from the German ‘insel’ meaning island, and ‘berg’ meaning mountain) (Barthlott & Porembski 2000a),

or ‘Sugar Loaf Land’ as called by de Paula *et al.* (2016). Representing individual or groups of monolithic mountains that appear abruptly in the landscape and which consist mainly of granitic or gneissic rock outcrops (Fig. 1), forming an old landscape element from Precambrian times on crystalline continental shields (Porembski *et al.* 1997, Barthlott & Porembski 2000a). They are only sparingly covered with soil and organic matter and, because of strong spatial and ecological isolation, they are considered terrestrial island habitats (Porembski *et al.* 2000).

The largest concentration of inselbergs is located in north-eastern Brazil (Ab’Sáber 1967, Vieira *et al.* 2015), including the states of Rio de Janeiro, Espírito Santo, Minas Gerais, Bahia, and Pernambuco (Porembski & Barthlott 2000). These regions exhibit high plant species richness and elevated rates of endemism (Porembski *et al.* 1998, Safford & Martinelli 2000, Taylor & Zappi 2004, de Paula *et al.* 2017), with 416 of the 995 species of angiosperms occurring on rock outcrops within the Atlantic Forest domain considered endemic (Stehmann *et al.* 2009).

Due to the prevailing ecological filters, including lack of soil and water, high exposure to UV radiation, high temperatures and constant winds (Porembski & Barthlott 2000, Scarano 2002), the community composition on the inselbergs is clearly distinguished from the surrounding forest matrix (Porembski 2007, de Paula *et al.* 2015). The vascular plant species of these rock outcrops frequently present desiccation-tolerant traits, represented by a combination of morpho-physiological features such as pseudo-stems, carpet-like mats (especially among monocots), and succulent, coriaceous tissues that serve as water-storage organs (Porembski *et al.* 1997). Among ferns and lycophytes, the genera *Anemia* Swartz (1806: 155) (Anemiaceae), *Cheilanthes* Swartz (1806: 126), *Doryopteris* Smith (1841: 404), *Hemionitis* Linnaeus (1753: 1077), *Pellaea* Link (1841: 59) (Pteridaceae), and *Selaginella* Palisot-Beauvois (1804: 478) (Selaginellaceae) are predominant (Barthlott & Porembski 2000b, de Paula *et al.* 2017), all taxa with special adaptation and predominant records in xerophytic ecosystems (Brighigna *et al.* 2002, Grusz *et al.* 2014, Mickel 2016). In this context, the inselbergs are important habitats for desiccation-tolerant vascular plants (Gaff 1977, Meirelles *et al.* 1997).

Derived from an on-going revision of *Serpocaulon*, and the collective effort to document the vascular diversity from Brazilian inselbergs, a novel species was recognized after study of the name *Goniophlebium demissum* and the types linked to *S. catharinae*. Later this entity was found in the field locality of Garrafão, Espírito Santo State.

Differences in morphology, like the thick rhizome with lanceolate concolorous rhizome scales that present marginal projections and spores with verrucae, contrast with the mentioned species that presents thinner rhizomes, with subulate to ovate-lanceolate rhizome scales lacking projection and spores with folded perine, supporting its recognition as part of the genus *Serpocaulon* and the subsequent need for a new combination. We present an illustration, a comparison with its relatives, a distribution map, its conservation status, and evidence that the rhizome stores starch.

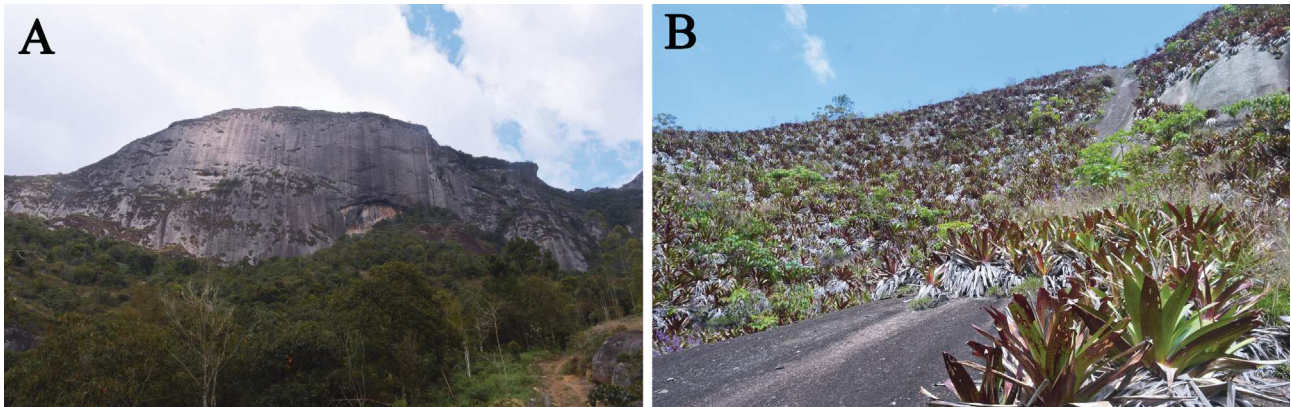


FIGURE 1. Brazilian inselbergs locality where *Serpocaulon demissum* occurs: A, B., Pedra do Garrafão, Espírito Santo.

## Material and methods

Herbarium specimens, and digital images including the types from B, BHCB, BM, CESJ, LE, MBM, P, R, RB, SP, UFP, UPCB, URM, and VIC (Thiers 2018) were studied (in total 118 specimens, 81 of *S. catharinae*, 19 of *S. vacillans*, and 18 of *Goniophlebium demissum* misidentified as *S. catharinae*). In addition, observations of species in the field were made in the State of Espírito Santo. Following Rödl-Linder (1990), Moran (1995), and Lellinger (2002), 58 morphological characters were selected and examined.

Scanning electron microscope (SEM) images of spores were obtained following Ramírez-Valencia *et al.* (2013) and Ramírez-Valencia & Sanín (2016). Using a FEI Quanta 200 SEM, with accelerating voltage of 20 kV, in the Centre for Microscopy at the Universidade Federal de Minas Gerais.

The conservation status of the species was assessed by applying the IUCN Red List Categories and Criteria (IUCN 2017). It was accurate with the extent of occurrence (EOO) and area of occupancy (AOO) (IUCN 2017) that were determined using the Geospatial Conservation Assessment Tool (GeoCat) with a default cell width of 2 km (Bachman *et al.* 2011).

Rhizome starch accumulation is the principal marker to recognize storage of carbohydrates (Bessho-Uehara *et al.* 2018), and because it corresponds closely to the diameter of this organ (Lemoine *et al.* 2013), we used Johansen's test (Johansen 1940) in order to evaluate the presence of starch in the species and its relatives. This test involves application of Lugol reagent (Iodine, potassium iodate and distillate water) to slide-mounted transverse hand sections of five rhizomes per taxon. If the tissue stains dark-brown to dark-blue after five minutes, it indicates the presence of starch (Johansen 1940).

## Taxonomic treatment

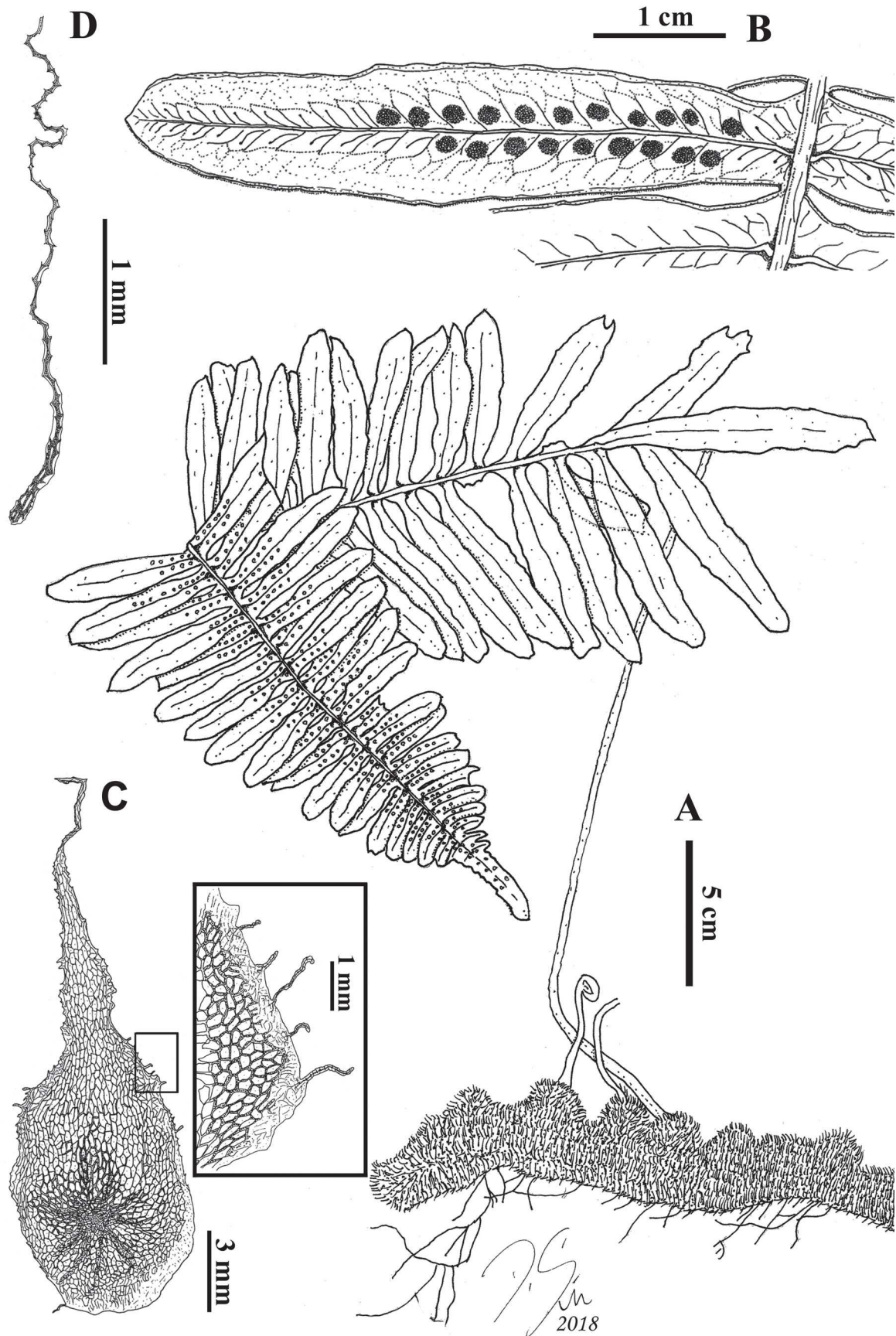
*Serpocaulon demissum* (Fée) D. Sanín, **comb. nov.** (Fig. 2, 3, 4, 5B–C, 6C–D, 7B). *Goniophlebium demissum* Fée, *Cryp. Vasc. Brésil* 2: 63. 1872. *Polypodium catharinae* var. *rotundatum* Christ, *Bull. Herb. Boissier ser. 2*, 2: 372 (1902). *Polypodium demissum* (Fée) C. Chr. *Index Fil.* 325. 1906. Type:—BRAZIL. Rio de Janeiro: Jurujuba, Santa Cruz [Santa Cruz da Barra], 7 April 1872, *A. Glaziou 5651* (lectotype P [P171187], here designated; isolectotypes P [P171187], P [P00624706], P [P00624705]).

**Etymology:**—The Latin epithet *demissus* means hanging down, related to the pendulous laminae.

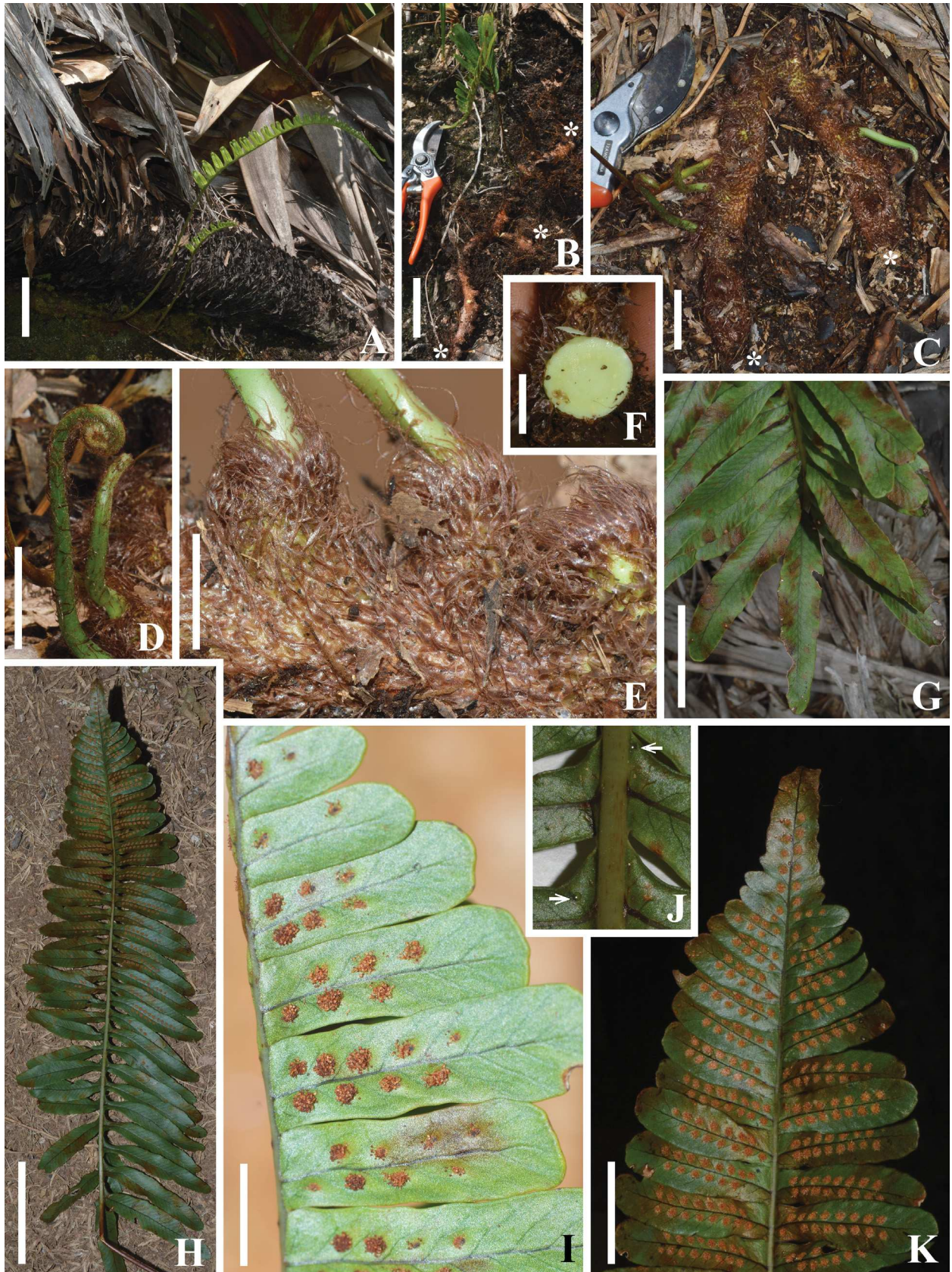
Plants rupicolous (growing in leaf litter over rocks). Rhizomes 5–12 mm in diameter, short-creeping, light to dark brown, moderately pruinose. Scales dense along rhizomes, 4–9 × 2–3 mm, concolorous, light brown, lanceolate, subpeltate, insertion radially folding, patent, bases rounded, apices long-acuminate, caudate, with scattered pale marginal projections 1–2 mm long, especially at the base. Phyllopodia 1.0–1.8 cm apart, 2–5 mm long. Fronds 19.2–62.0 cm long, erect. Petioles 6.5–29.0 cm, light brown, sinuate at the base, proximally and distally subterete. Laminae 12–34 × 4–14 cm, pinnatisect, lanceolate, truncate proximally, apex gradually reduced to a short-attenuate segment. Segments in 26–38 pairs, chartaceous, slightly revolute, proximal surcurrent and reflexed, medial decurrent, and apical pinnatisect, glabrous or sparsely pubescent with light brown to hyaline hairs, 3–18 cells long, catenate, strigose, scattered, mainly in axils of segments and rachis; scales bicolorous, dark brown at center, hyaline at margins, 1.0–3.2 × 0.15–0.24 mm, 10–26 × 2 cells wide, linear, subpeltate, sinuate, rounded and appressed at the base, each with a long-acuminate patent apex, dentate, mainly in axils of segments and rachis. Medial segments 2–6.5 × 0.4–0.9 cm. Veins forming 13–25 rows along and 1–2 rows of areoles between the costa and the margin of the segment, fertile veinlets usually inconspicuous. Sori in 6–23 × 1 (rarely 2) rows per segment, not reaching apices of segments. Spores 52–56 × 30–35 µm, monolete, ellipsoid to plano-convex, with verrucate laesurae 23–29 µm long (Fig. 6C–D).

**Distribution & habitat:**—*Serpocaulon demissum* is endemic to the Brazilian inselbergs of the Atlantic Forest ecosystem (Fig. 1, 4), between [200]560–1850 m, known from Bahia, Espírito Santo (Fig. 1), Minas Gerais, and Rio de Janeiro States.

**Additional specimens examined:**—BRAZIL. Bahia: Rio de Contas, Pico das Almas, vertente leste, vale acima da Fazenda Silvina, 1450–1500 m, 29 November 1988, *R.M. Harley & B.L. Stannard 26677* (K); Rio de Contas, Pico das Almas, 25 km da cidade em direção ao Campo do Queiroz, 1850 m, 13°33'S, 41°57'W, 26 February 2006, *F.B. Matos et al. 1049* (RB). Espírito Santo: Castelo, Parque Estadual de Forno Grande, Trilha para o Forno, 1100 m, 20°30'58" S, 41°05'01" W, 12 February 2008, *P.H. Labiak et al. 4566* (RB); Castelo, Parque Estadual de Forno Grande, 1300 m, 20°30'58" S, 41°05'20" W, 26 June 2008, *A. Salino et al. 13607* (BHCB); Pancas, Pedra da Colina, 745 m, 19°13'52" S, 40°52'36" W, 11 March 2016, *L.F.A. de Paula 894* (RB); Santa Maria do Jetibá, Garrafão, Pedra do Garrafão, 1081 m, 20°10'24.5" S, 40°55'06.8" W, 28 August 2009, *A. Salino et al. 14539* (BHCB); Santa Maria do Jetibá, Pedra do Garrafão, depois da cultura do morango, 20°10'24.5" S, 40°55'06.6" W, 1079 m, 22 September 2018, *D. Sanín et al. 7229* (BHCB, CESJ, F, HUA); Santa Teresa, Pedra da Onça, 1 February 2000, *V. Demuner et al. 688* (BHCB). Minas Gerais: Dolores de Guanhanes, Serra do Caraça, 700 m, 19°04'14.3" S, 42°52'23.2" W, 15 August 2005, *T.E. Almeida et al. 82* (BHCB); Teófilo Otoni, afloramento rochoso lado esquerdo da MG 418, cerca de 30 km

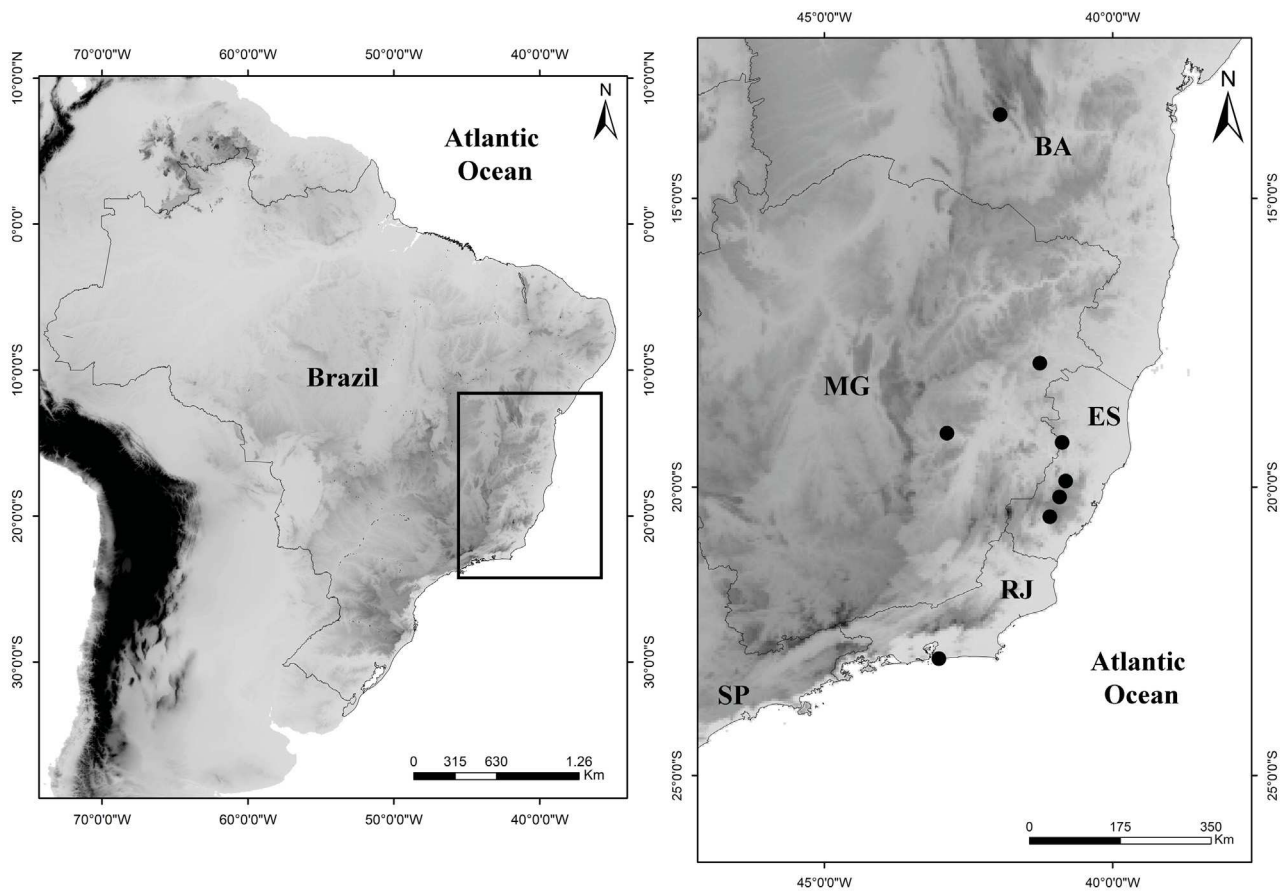


**FIGURE 2.** *Serpocaulon demissum*: A. Habit. B. Detail of segments. C. Rhizome scale. D. Laminar scale. From: *D. Sanin et al. 7229* (BHCB).



**FIGURE 3.** *Serpocaulon demissum*: A. Habit, scale bar: 10 cm. B. Rhizome with size perspective, scale bar: 5 cm. C. Rhizome branching, scale bar: 2 cm. D. Fiddlehead, scale bar: 4 cm. E. Phyllopodia detail, scale bar: 2 cm. F. Rhizome cross-section, scale bar: 2 cm. G. Base of the lamina with falcate and reflexed pinnae, scale bar: 3 cm. H. Abaxial view of the fertile lamina, scale bar: 5 cm. I. Middle fertile segment detail, scale bar: 1.5 cm. J. Proximal segments with nectaries. K. Apex of the lamina, scale bar: 2 cm. All from *D. Sanín et al.* 7229 (BHCB). The asterisk indicates the rhizome apex and the arrows indicate the location of nectaries.

norte Teófilo Otoni, em ilha de vegetação de afloramento granítico, 560 m, 17°51'22" S, 41°15'39" W, 27 January 2014, *L.F.A. de Paula et al.* 686 (BHCB); Teófilo Otoni, afloramento rochoso lado esquerdo da MG 418, cerca de 30 km norte Teófilo Otoni, em ilha de vegetação de afloramento granítico, 560 m, 17°51'22" S, 41°15'39" W, 16 April 2011, *L.F.A. de Paula et al.* 334 (BHCB); Teófilo Otoni Afloramento rochoso lado esquerdo da MG 418, cerca de 30 km norte de Teófilo, 560 m, 17°51'22" S, 41°15'39" W, 16 April 2011, *L.F.A. de Paula et al.* 336 (RB). Rio de Janeiro: Niterói, Parque Estadual da Serra da Tiririca, Pedra de Itacoatiara, 3 December 1994, *M.G. Santos & F.C. Pinheiro* 62 (RB); Niterói, Parque Estadual da Serra da Tiririca, Pedra de Itacoatiara, 20 March 1994, *M.G. Santos & F.C. Pinheiro* 51 (RB); Niterói, Morro do Tucum, Itacoatiara, 7 July 2012, *L.P. Mauad* 306 (BHCB, RB).

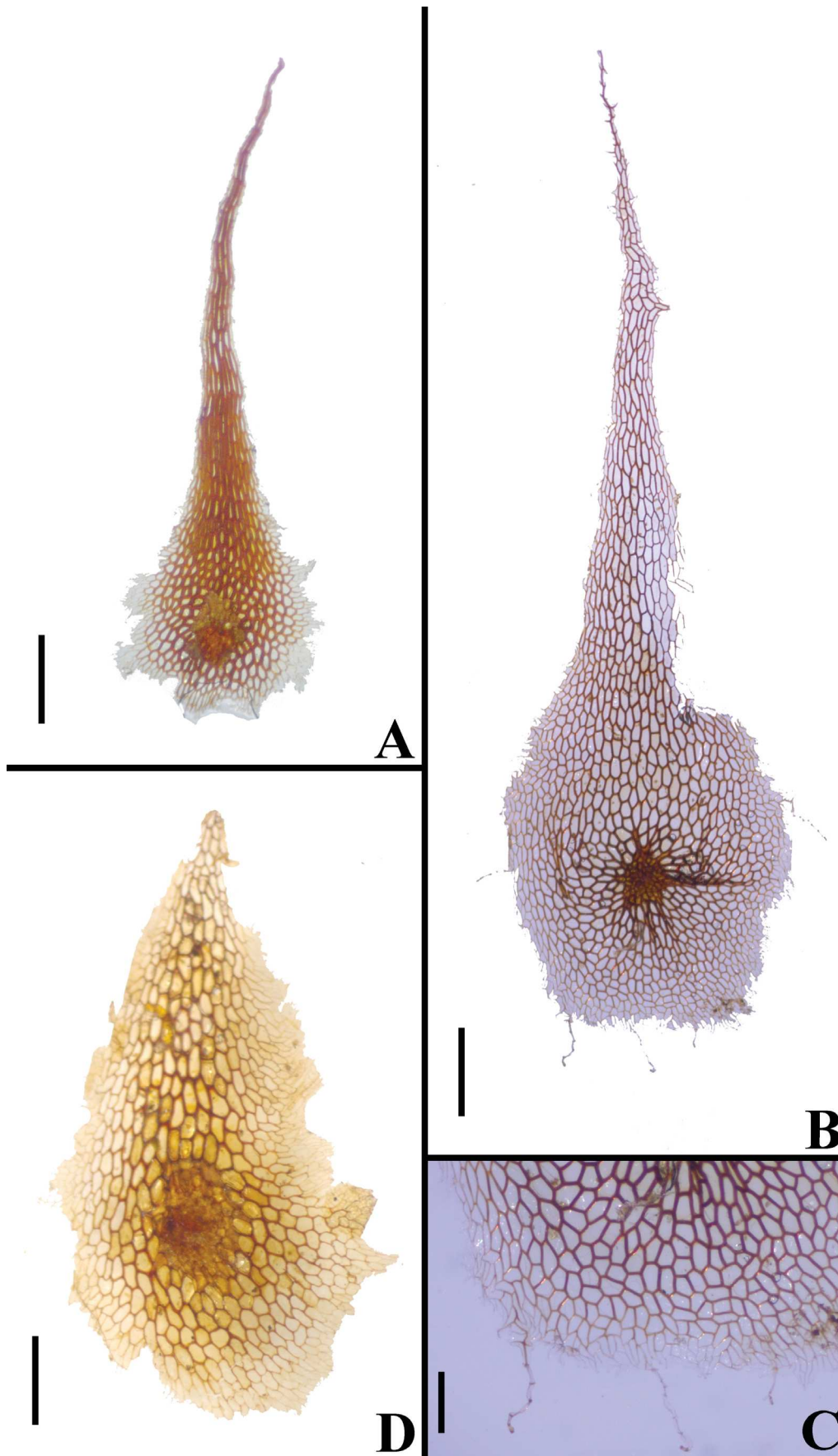


**FIGURE 4.** Distribution of *Serpocaulon demissum*. Black dots represent collections. Conventions: ES, Espírito Santo; MG, Minas Gerais; RJ, Rio de Janeiro, and SP, São Paulo States.

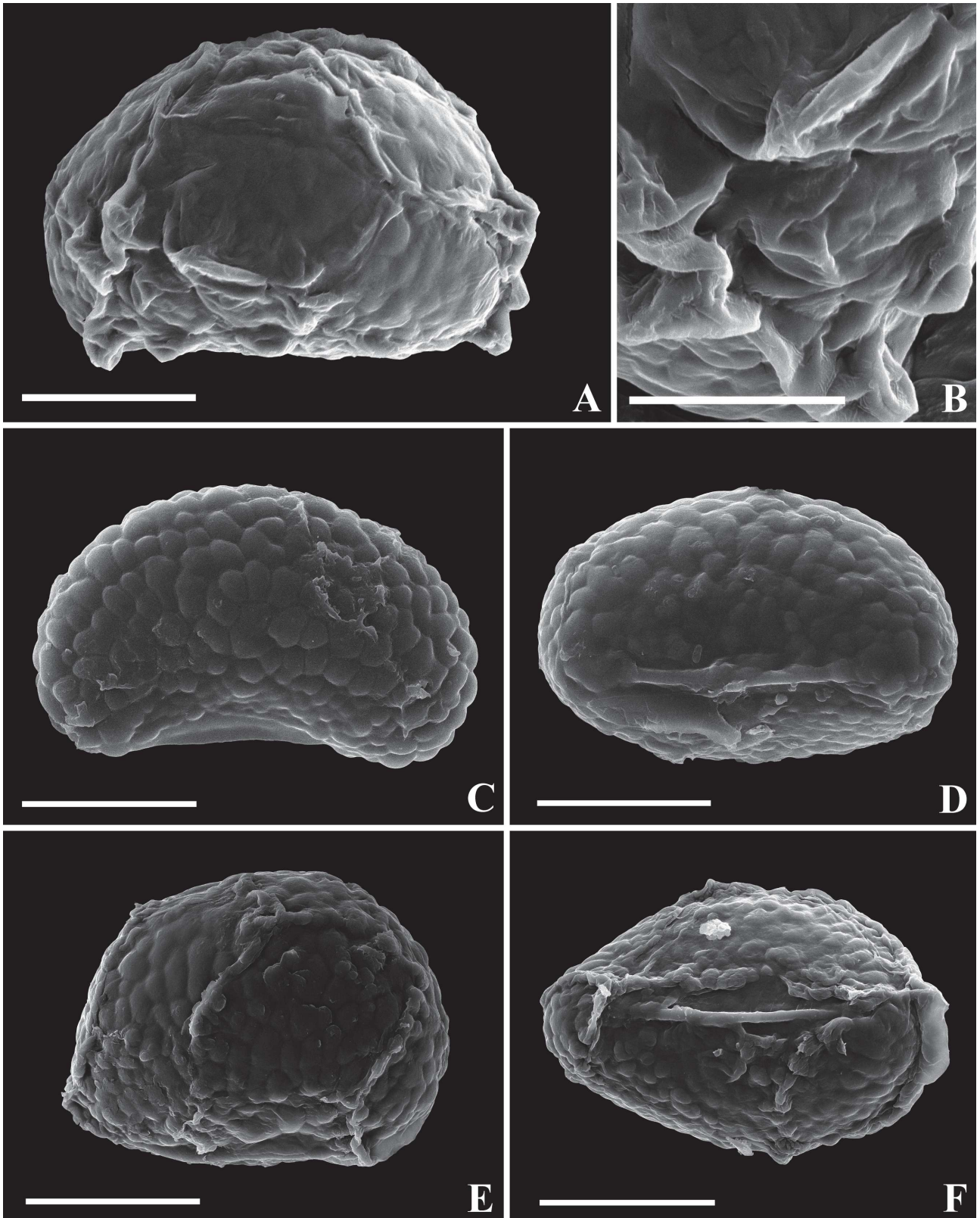
**Discussion:**—The distribution of *Serpocaulon demissum* is similar to described inselbergs endemics species: *Mandevilla fistulosa* Ferreira de Sales (2006: 115), *M. grazielae* Ferreira de Sales (2006: 117), and *M. harleyi* Ferreira de Sales (2006: 121), (Apocynaceae) (Ferreira de Sales *et al.* 2006), and *Bradea borreroides* (Oliveira & Sobrado (2016: 84) (Rubiaceae). Its altitudinal distribution is similar to *Nanuzia plicata* Martius (1824: 16) Smith & Ayensu (1976: 38) (Velloziaceae), which colonizes rocky substrates from the seacoast to more than 1500 m.a.s.l. (Porembski & Barthlott 2000). Remarkably, this area corresponds to a recently recognized phytogeographical region for endemic bromeliads in the Atlantic Forest in Brazil (de Paula *et al.* 2016) from the states of Bahia to Rio de Janeiro.

*Serpocaulon demissum* was found at the margins of monocotyledonous mats containing principally species of Bromeliaceae and Velloziaceae. These mats are fundamental for providing establishment sites for other plant life-forms (Porembski *et al.* 2000), because they accumulate organic matter between their roots (Porembski & Barthlott 2000).

Recent botanical exploration in the inselbergs localities of Linhares (Espírito Santo) and Teófilo Otoni (Minas Gerais), culminated in the description of two new endemic species of monocots (Viana & de Paula 2013, Gonçalves & de Paula 2016), and four eudicots (Ferreira de Sales *et al.* 2006, Oliveira & Sobrado 2016, Gouvêa *et al.* 2018). These discoveries show that these outcrop ecosystems are highly diverse, and still relatively unexplored (Giulietti & Pirani 1988, Porembski & Barthlott 2000, Porembski *et al.* 2000, de Paula *et al.* 2016, 2017).



**FIGURE 5.** Comparison between rhizome scales of the related species of *Serpocaulon*: A. *S. catharinae* (from *D. Sanín et al.* 6834, BHCB). B, C. *S. demissum* (from *D. Sanín et al.* 7229, BHCB). D. *S. vacillans* (from *D. Sanín et al.* 6855, BHCB). Scale bars: A, B & D: 1 mm, C: 0.5 mm.



**FIGURE 6.** Spore comparisons between the related species of *Serpocaulon*. A. Lateral view of *S. catharinae*. B. Detail from folded perine of *S. catharinae* (A, B from *D. Sanín et al. 6771*, BHCB). C, D. Lateral and proximal view of *S. demissum* (from *A. Salino et al. 14539*, BHCB). E, F. Lateral and proximal view of *S. vacillans* (from *D. Sanín et al. 6855*, BHCB). Scale bars: A, C–F: 20  $\mu\text{m}$ ; B: 10  $\mu\text{m}$ .



*Similar species and nomenclature*:—*Serpocaulon demissum* resembles *S. catharinae* and *S. vacillans* (Table 1, Figs. 5–7), by sharing short-creeping rhizomes (except for *S. vacillans*, which has long-creeping rhizomes) and pinnatisect laminae with segments having predominantly one row of sori between the costae and the segment margins. It possesses moderately pruinose and rugose rhizomes as in *S. catharinae* and has sinuate petiole bases like *S. vacillans*.

**TABLE 1.** Comparison of morphological features of Brazilian Atlantic Forest related species of *Serpocaulon*.

Character/species	<i>S. catharinae</i> (n= 81)	<i>S. demissum</i> (n= 18)	<i>S. vacillans</i> (n= 19)
Habit	Terrestrial, rupicolous and epiphytic	Rupicolous (over litter matter)	Terrestrial, subterranean (rarely rupicolous)
Rhizome width (mm)	3.3–8.4	5–12	3.2–7.5
Rhizome glaucous	Glaucous	Sparsely glaucous	Non-glaucous
Scale length (mm)	1–7	4–9	2–5
Scale color	Bicolorous	Concolorous	Concolorous
Scale shape	Subulate	Lanceolate	Triangular to ovate-lanceolate
Scale margins	Dentate to crenate	With marginal projections	Dentate to crenate
Petiole bases	Straight	Sinuate	Sinuate
Laminar pubescence	Sparse	Sparse	Dense
Spore perispore	Prominent folds	No folds	Scarcely folded

*Serpocaulon demissum* differs from *S. catharinae* by having concolorous, larger (4–9 × 2–3 mm vs. 2.1–3.7(–7.9) × 0.4–1.9 mm) lanceolate rhizome scales (Fig. 5B and C), bullate at insertion, with scattered pale marginal prolongations of the margin cells 1–2 mm long, especially at the bases (Fig. 5C), petioles proximally sinuate (Fig. 3D), inconspicuous veinlets (Fig. 3I), and spore ornamentation lacking folded perine (Fig. 6C & D) (vs. bicolorous subulate rhizome scales (Fig. 5A) with dentate margins, straight petiole bases, conspicuous veinlets, and spore ornamentation with folded perine (Fig. 6A, B)).

From *Serpocaulon vacillans*, *S. demissum* differs by the superficially rupicolous rhizomes (Fig 3B, C), lanceolate rhizome scales (Fig. 5B), laminae glabrous or with sparse trichomes 3–18 cells long, scales distributed mainly in the axils of the segments, and spore ornamentation lacking folded perine (Fig. 6C & D) (vs. subterranean rhizomes, deltate to ovate-lanceolate rhizome scales (Fig. 5D), dense laminar pubescence with hairs 2–3 cells long, with scarce (if any) linear scales in the base of segments, and spore ornamentation with folded perine (Fig. 6 E & F)).

Hensen (1990) presented the name *Goniophlebium demissum* Fée as a new synonym for *Polypodium catharinae*. However, the author did not present information of the spores and the rhizome scales to support his conclusions. This information is presented here, and along with its distribution it is possible to recognize it as part of *Serpocaulon*'s diversity.

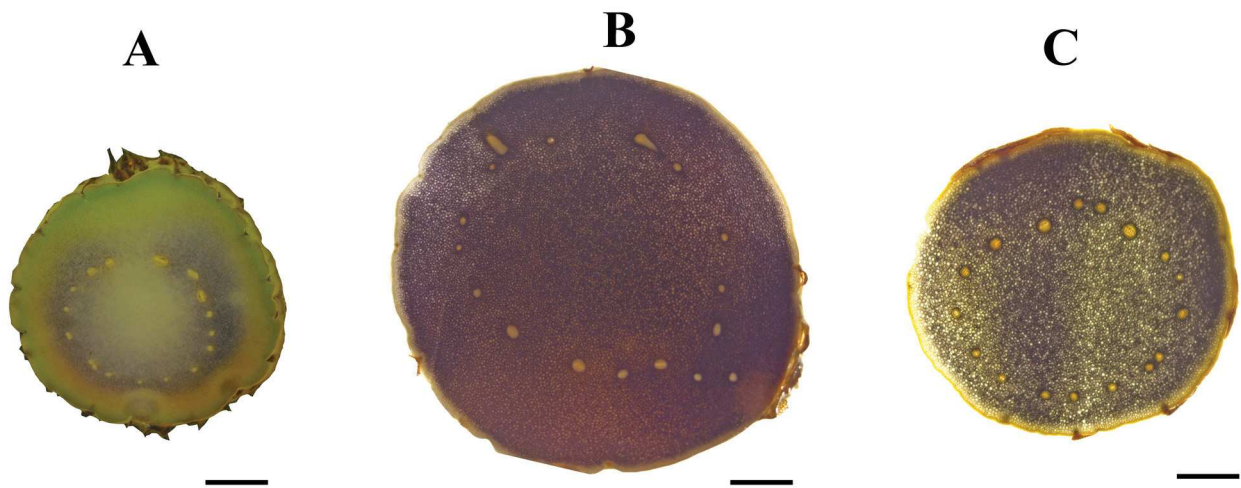
*Rhizome starch accumulation*:—The presence of thick rhizomes supports that *S. demissum* developed a specialized system to store nutrients and water. Similar adaptations have been noted for several taxonomical groups in Brazilian inselbergs (Barthlott & Porembski 2000b, Conceição & Pirani 2005, Alves & Kolbek 2010, Porembski *et al.* 2000).

In a comparison with its relatives, all the species evaluated contain starch in their rhizome. However, *S. demissum* differs of *S. catharinae* because it presents starch in the whole rhizome (Fig. 7B) (vs. only around the vascular bundles (Fig. 7A)). It shares a similar starch distribution with *S. vacillans* but this species differs in the lack of a dense cuticle and lower amount of starch (Fig. 7C). Hovenkamp *et al.* (2016) evaluated the seasonal accumulation of starch in trophopods of the temperate fern *Matteuccia struthiopteris* (Linnaeus 1753: 1066) Todaro (1866: 235) (Onocleaceae) explained their results with the production of seasonal leaves, which was correlated with the temperate seasonality. We believe that the thick rhizomes of *S. demissum* similarly represents a functional trait that provides advantage in this harsh environment.

**Conservation assessment**:—*Serpocaulon demissum* is considered eligible for IUCN listing as Endangered (EN) (IUCN 2017) on the basis of its Area of Occupancy (AOO): 36 km<sup>2</sup>, which is far less than the 500 km<sup>2</sup> as it is recommended by the Geospatial Conservation Assessment Tool (GeoCat) (Bachman *et al.* 2011). According to the World Database of Key Biodiversity Areas (<http://www.keybiodiversityareas.org/home>), the population from Castello locality is the only one in a protected area (Parque Estadual de Forno Grande, Espírito Santo State). The global population is at risk from human impacts such as granite mining in inselbergs, installation of telecommunication

towers, the use of outcrops for cultivation, the invasion of exotic grasses, and tourism (Safford & Martinelli 2000, Viana & de Paula 2013, Gonçalves & de Paula 2016, de Paula *et al.* 2016, 2017, Oliveira & Sobrado 2016).

Studies on rock-dwelling populations of rock ferns (Schneller & Holderegger 1996, Vogel *et al.* 1999, Suter *et al.* 2000) attributed their population structure to multiple events of single-spore colonization via intra-gametophytic selfing, promoted by its long-distance dispersion capacity (Perrie & Brownsey 2007, De Groot *et al.* 2012, Bauret *et al.* 2017). In this context, the low gene flow registered between populations of angiosperms from different inselbergs (Barbará *et al.* 2007, Palma-Silva *et al.* 2011) pointed that each inselberg might host genetically isolated populations (de Paula *et al.* 2016, Hmeljievski *et al.* 2017). Stressing that those species are more susceptible of extinction by small alterations. Because of the small population size, severe fragmentation of the already isolated populations resulting from its rocky outcrop distribution, this species should be a priority candidate for conservation efforts.



**FIGURE 7.** Comparison of Lugol starch test (Johansen 1940), on the rhizome of *Serpocaulon demissum* and its relatives. A. *S. catharinae* (from D. Sanin *et al.* 7209, BHCB). B. *S. demissum* (from D. Sanin *et al.* 7229, BHCB). C. *S. vacillans* (from D. Sanin *et al.* 7164, BHCB). Scale bars: A–C: 1 mm.

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