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Bulbothrix bulbillosa, a presumed Galapagos endemic, is common in Rio Grande do Sul State, Brazil (Parmeliaceae, lichenized Ascomycota)

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Abstract – *Bulbothrix bulbillosa* is recorded for the first time in Brazil, Rio Grande do Sul State. The species was described recently from the Galapagos Islands, Ecuador and known only from the type locality. All specimens found belong to the gyrophoric acid chemotype, whereas material from Galapagos contains either gyrophoric or lecanoric acid.

Bulbate cilia / *Bulbothrix fungicola* / *Bulbothrix goebelii* / *Bulbothrix laevigatula* / isidia

INTRODUCTION

Currently seven isidiate *Bulbothrix* Hale species are known to contain gyrophoric acid as medullary substance: *B. fungicola* (Lynge) Hale, *B. papyrina* (Fée) Hale, *B. pseudofungicola*, Benatti & Marcelli, *B. scortella* (Nyl.) Hale, *B. sipmanii* Aptroot & Aubel, *B. subdissecta* (Nyl.) Hale, and also one chemotype of the recently described *B. bulbillosa* Benatti, Spielmann & Bungartz (Benatti, 2010, 2014; Benatti & Elix 2012; Bungartz *et al*., 2013). Of this group, only *B. bulbillosa*, *B. fungicola*, *B. pseudofungicola* and *B. sipmanii* have ciliate isidia, and just one, *B. bulbillosa*, has laminal ciliary bulbs. During examination of the *Bulbothrix* specimens of the HAS (Fundação Zoobotânica do Rio Grande do Sul) and ICN (Universidade Federal do Rio Grande do Sul) herbaria, including recent collections made in the past few years, the authors were able to locate some specimens that fit the description of *B. bulbillosa*, from localities in Rio Grande do Sul State, Southern Brazil, more than 5,000 km from the type locality, at lower latitude and within a different climate zone (temperate well below the Tropic of Capricorn versus tropical near the Equator. The identification is supported by comparison with the holotype and paratypes of *B. bulbillosa* listed in Bungartz *et al*. (2013). It is the first record of

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this species outside Galapagos, which indicates the possibility that this species might occur within a larger area in South America, because many places are still poorly collected.

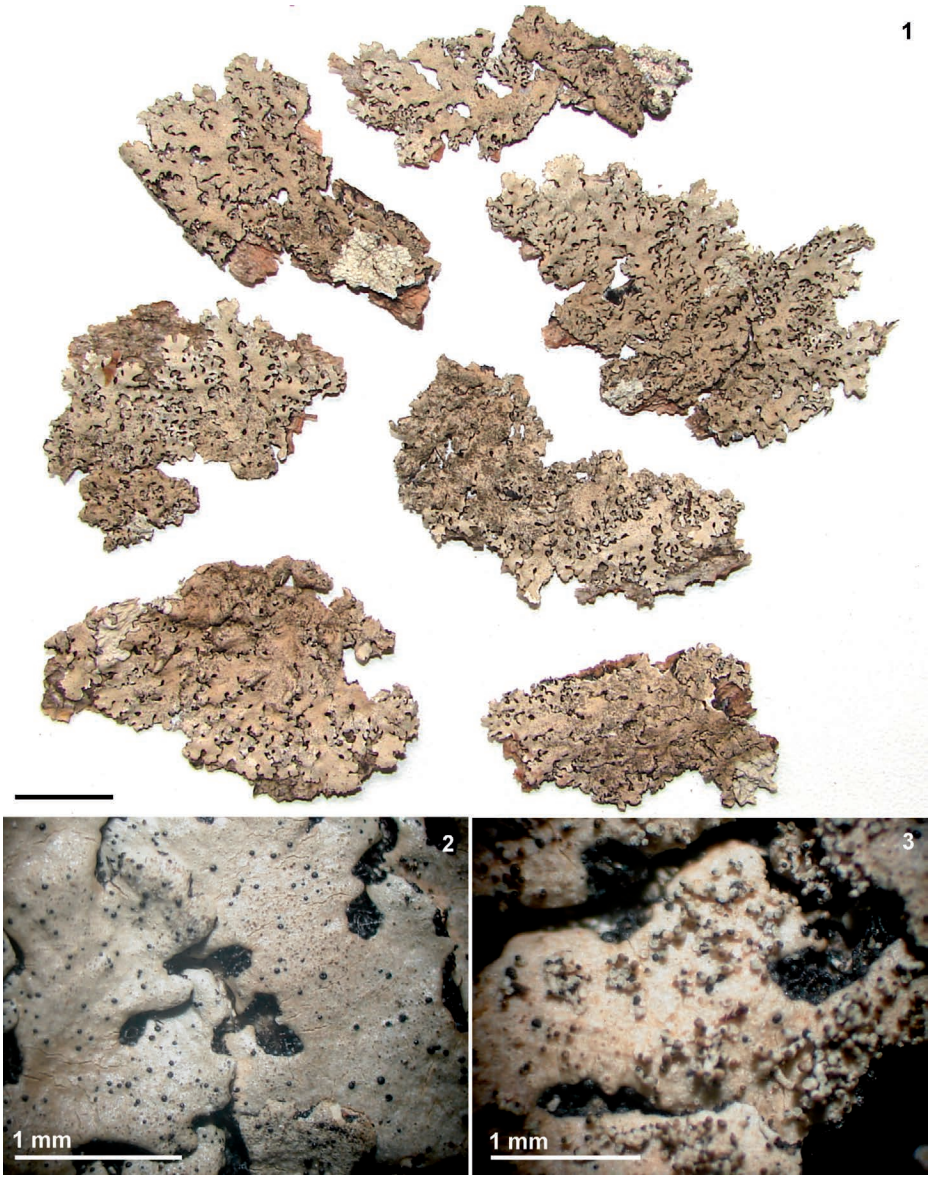
MATERIAL AND METHODS

Morphological and anatomical characters of the specimens were analyzed using standard methods with dissecting and compound microscopes. Anatomical sections, including those of pycnidia when present, were made with a razor blade by hand (only a single specimen with apothecia was found). Chemical constituents were checked by spot tests with potassium hydroxide (K), sodium hypochlorite (C) and para-phenylenediamine (P), and also examined under UV light (360 nm), by thin-layer chromatography (TLC). The extracts were prepared using acetone (exhaustive extraction) and chromatographed in silica gel plates F_{254} (Macherey-Nagel) using toluene: acetic acid 85:15 (v/v). The revelation was done under $UV_{254\ light}$ and using methanol/sulfuric acid at 10% followed by heating. After this, it was applied a solution of *p*-anisaldehyde/sulfuric acid and heating. Microcrystal was done using GAW (glycerin: ethanol: water 1:1:1 v/v/v), GE-31 (glycerin: acetic acid 3:1 v/v) and GE-13 (glycerin: acetic acid 1:3 v/v).

RESULTS

Bulbothrix bulbillosa Benatti, Spielmann & Bungartz. *The Bryologist* 116: 361. 2013. **Figs.1-3**

Thallus up to 6.0 cm wide, subcoriaceous, corticolous (also on twigs), greenish gray becoming dusky gray in the herbarium, sublinear-laciniate. Laciniae 0.3-1.2 (-2.2) mm wide, dichotomously anisotomously or trichotomously branched, contiguous, adnate, strongly attached; apices plane, truncate to subtruncate; margin plane, smooth to sinuous or subirregular, entire, ciliate, axils oval. Upper surface continuous to occasionally cracked on older parts, smooth, with numerous laminal ciliar bulbs; Maculae absent, sometimes with scars visible, left by fallen isidia. Adventive lacinules generally rare and sparse, very short, randomly distributed along the margins (or upper cortex, see comments), simple, flat, 0.1-0.6 × ca. 0.1-0.2 mm, truncate or acute, underside concolorous with the lower margin. Cilia black, initially simple eventually becoming furcate and then a few slightly dichotomously or irregularly branched, 0.05-0.35 × ca. 0.02 mm, with semi-immersed to emersed basal bulbs ca. 0.05 (-0.10) mm wide, abundant along the margins, spaced ca. 0.05 (-0.10) mm from each other to contiguous, absent only at the apices of the laciniae. Medulla white. Soredia and pustulae absent. Isidia scarce to common, laminal, granular to smooth cylindrical, straight to slightly tortuous, 0.05-0.30 (-0.40) × ca. 0.05 mm, simple to rarely little branched, erect, firmly attached to caducous, concolorous with the thallus, ciliate. *Lower surface* black, ±shiny, smooth to subrugose, moderately to densely rhizinate; Marginal zone shiny, attenuate, light brown turning darker at the transition to the center, smooth, papillate, 0.5-4.0 mm wide, occasionally with few rhizinae; Rhizines light to dark brown or blackish, initially simple, soon becoming dichotomously or



Figs 1-3. **1.** A specimen of *Bulbothrix bulbillosa* (Fleig 2527). **2.** Detail of the laminal bulbs. **3.** Detail of the ciliate isidia. Bars = 1 cm (1), 1 mm (2, 3).

irregularly branched, partially with subtle basal bulbs, 0.10-0.50 × ca. 0.03 mm, frequent to abundant, equally distributed. Apothecia laminal, adnate, flat, 0.2-1.2 mm wide, margins smooth to subrugose, coronate, amphithecia smooth, with ciliar bulbs; discs light brown, epruinose, imperforate; ascospores not found (hymenia without asci or with a few immature ones). Pycnidia very scarce, laminal, with black ostioles; conidia not found.

Chemical reactions: cortical atranorin (K+ yellow, UV–), medullary gyrophoric acid (K–, C+ rose to reddish rose, KC+ rose to reddish rose, P–, UV–).

Holotype: ECUADOR. Galapagos. Isabela, Volcán Darwin, southwestern slope, above Tagus Cove, 0º 13'57.9"S, 91º20'5.1"W, 613 m, transition zone, SW-exposed lava flow of weathered AA-lava with scarce vegetation (*Macraea laricifolia*, *Croton scouleri*, *Dodonaea viscosa*, *Scalesia microcephala*, *Cordia revoluta* and *Opuntia insularis*), on bark of branches of *Croton scouleri*; sunny, wind- and rain-exposed, 11-Dec-2007, Bungartz 7393 (holotype: CDS 37880).

Specimens examined: Brazil, Rio Grande do Sul State, Municipality of Itapuã, Praia de Fora, dunes, corticolous, 18-VII-2005, A. Lemos & S. Martins 2038 (HAS); idem, Municipality of Triunfo, Braskem, on *Myrsine umbellata* Mart., 04-VII-2003, S.M. Mazzitelli & M. Käffer 834 (HAS); idem, Municipality of Viamão, Itapuãn, on *Mimosa bimucronata*, 20-X-1984, M. Fleig 2383 (ICN); idem, Municipality of São Sepé, Passo dos Freire, corticolous, 300 m alt., 10-II-1985, M. Fleig 2527 (ICN); idem, corticolous, 06-II-1988, M. Fleig 3214a (ICN); idem, Municipality of Osório, alt. 1 m, 101.5 km N of Morro Alto, corticolous, 09-IV-1993, M. Fleig 5116 (ICN); idem, Municipality of São Gabriel, 290.5 km W of Vila Nova, corticolous, 07-V-1993, M. Fleig 5532 (ICN); idem, Municipality of Quaraí, Cerro do Jaraú, open pasture field, corticolous, 03-XI-1995, M. Fleig 7166 (ICN).

Comments: As this is the second time the species is found, and this at more than 5,000 km from the type locality on the Galapagos Islands, we have provided a detailed description of our specimens which may reveal to be important when comparing with future collections. The type locality (Galapagos Islands, Ecuador) is located ca. 1000 km from Ecuador in the Pacific Ocean, crossed by the Equator. As explained in Bungartz *et al*. (2013) the archipelago comprises over 123 islands, islets and large rocks emerged from the sea due to volcanic activity, with fourteen islands commonly recognized as the main ones because of their size (Snell *et al*. 1995, 1996).

The climate in Galapagos is unusually dry, with a hot and cool season with prevailing winds from the south and southeast (Trueman & d'Ozouville 2010). There are five main vegetation zones that can be distinguished: coastal, dry, transition, humid and high altitude dry zone (Bungartz *et al*. 2009; Tye *et al*. 2002). On the other hand, the habitats where the specimens were collected in Brazil are more variable, consisting of vegetation types and climates quite different from those in the Galapagos (especially restinga wood, grasslands and forests), often on well-exposed places that receive lots of light. The samples collected near Triunfo, Viamão and Osório have a subtropical, humid climate, whereas those collected near São Gabriel and Quaraí grow in a subtropical and hot climate. The annual average temperature in the state of Rio Grande do Sul is 19.4°C, the average relative humidity is 76% and the average rainfall varies from 1,324 mm to 1,500 mm $year^{-1}$ (Livi, 1998; Backes 2012). The plant cover is much diversified, especially due to determinant environmental factors, such as temperature, precipitation, humidity, water availability, and include not only coastal restinga forests, but also granite hills, a very distinct formation in which generally forests and grasslands occur with a great variety of physiognomic-floristic types. Around some rocky outcrops small tree-shrub groups occur and the vegetation is strongly influenced by the Atlantic Rainforest (Martins & Marcelli, 2011). In the other regions of southern Brazil, the vegetation type is grassland, but along the rivers there are also riparian forests or remnants of slope forest, as well as some forest patches (Andrade, 2013).

Bulbothrix bulbillosa is recognized by the presence of ciliary bulbs that are present everywhere on the thallus: besides the margins and rhizines (the only

bulbs with apical portions), the bulbs occur also on the upper surface (Fig. 2), the apothecia (full amphithecia and rim), and the isidia (Fig. 3). Some few specimens (e.g., *Lemos* 2038) are quite small on thin twigs and have few isidia in early development stages, these without bulbs or with a few ones yet, besides the upper cortex being covered with bulbs – an indication that they might have different trigger conditions of development or maybe an ontogenic variation. Only a single fertile specimen was confirmed (*Fleig* 3214a), with coronate apothecia also with bulbs on the amphithecium. One specimen (*Fleig* 2383) has a few laminal lacinulae amidst the isidia on its oldest parts; it is uncertain whether these are adventive or developed from isidia, but they are otherwise identical to the other specimens examined here, including medullary substance (no traces of different substances found together with it). Most of the materials found were originally identified with different names according to their labels, such as *B. fungicola* (Lynge) Hale, *B. goebelii* (Zenker) Hale, or *B. laevigatula*, (Nyl.) Hale and some specimens had remained unidentified. *Bulbothrix fungicola* does not develop laminal ciliary bulbs and has simple to furcate cilia and rhizines (Benatti, 2014), *B. goebelii* is a non-isidiate species continuing lobaric acid instead of gyrophoric acid (Benatti & Elix, 2012), and *B. laevigatula* does not develop bulbs neither on isidia nor on its upper surface, always containing lecanoric acid as medullary substance (Benatti, 2014; Bungartz *et al*., 2013).Very few pycnidia were found (they can be easily confused with the laminal bulbs which are quite numerous), however all of them without conidia.

All specimens found in Rio Grande do Sul State belong to the gyrophoric chemotype of *Bulbothrix bulbillosa*. The type material and several other specimens examined contain lecanoric acid, whereas further specimens contain gyrophoric acid, but otherwise the chemotypes are hardly distinguishable (Bungartz *et al*., 2013). It is possible that the chemotypes could represent two very morphologically similar species, since there are several cases in Parmeliaceae of morphologically similar species distinguished apparently only by these chemical components (as confirmed by molecular analysis), but unfortunately more material is yet needed to clarify this possibility, including fresh samples for molecular studies.

The material from Rio Grande do Sul State differs from the Galapagos specimens apparently only by the narrower laciniae (the Galapagos specimens have an average width of 1-2 mm, about twice the width commonly found here), otherwise being very similar. It is yet not ascertained if this characteristic variation might occur only on the gyrophoric chemotype because no specimens were collected so far with lecanoric chemotype from Rio Grande do Sul State for a sustainable statistical comparison (and no materials are known so far from other localities), although the Galapagos materials (both those containing lecanoric or gyrophoric) all have similar lobe widths. There is a possibility that with fresh materials that could be found useful for molecular studies this chemotype could be eventually ascertained as different taxa. All specimens examined present rose to reddish rose reactions, while the type and additional specimens from Galapagos curiously react always reddish, both lecanoric and gyrophoric chemotypes. It is the only currently know species with this variation in the genus, as all others react either bright red (lecanoric) or pale rose (gyrophoric), as noticed by Hale (1976) and Benatti (2013, 2014). Comparing the chromatographic substances migration of the constituents present in the analyzed extracts and that of the patterns of gyrophoric and lecanoric acids, it was observed that all samples showed presence only of gyrophoric acid, no extract containing lecanoric acid. All extracts also showed atranorin and some spots above the gyrophoric acid that indicate what could possibly be traces of steroids/

triterpenoids. The microcrystal extracts did not show structures that indicate gyrophoric acid, however (Neli K. Honda, pers. comm.) crystalline structures exhibit distortions possibly due to the presence of other substances (non-phenolic) that affect crystallization, and some substances of this group, such as hiascic and crustinic acids have one more phenolic hydroxyl, relative to gyrophoric acid, and due to this migrate a little less in chromatography (when compared with same eluent). Other substances in the gyrophoric acid group are methylated products, and because of this they migrate slightly more than the gyrophoric acid. Because of this and the color of the spot tests, even that the microcrystal tests were not able to crystallize the substance properly (possibly to small concentrations), the chromatographic profile of the analyzed extracts indicates that besides atranorin, the only other phenolic component present is the gyrophoric acid.

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