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## Two new species of *Bulbothrix* Hale

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*Bulbothrix caribensis* and *B. lyngei* are two new lichen species discovered during a systematic revision of the genus. The first species lacks medullary substances and the second species produces an undetermined fatty acid.

**Keywords:** Parmeliaceae; bulbate cilia; *Bulbothrix fungicola*

### Introduction

Hale (1974) established the genus *Bulbothrix* for species of the series *Bicornutae* (Lynge) Hale & Kurokawa of the genus *Parmelia* Ach. Species of *Bulbothrix* are easily identifiable by the small, deeply lacinate thalli with bulbate marginal cilia (the main characteristic), atranorin as cortical substance (differentiating it from *Relicina*), hyaline, unicellular, ellipsoid or bicornute ascospores and bacilliform to bifusiform conidia. The medullary chemistry is considerably variable, containing a large number of chemosyndromes including some unknown substances.

Two new species without medullary substances or with fatty acids are described in the present paper. These species were discovered during a revision of the genus *Bulbothrix* (Benatti 2010). One new species is isidiate, while the other is lacinate. Both species are corticolous and known from Latin America.

### Material and methods

Morphological characters were studied using standard stereoscopic and light microscopy. Anatomical sections, including those of apothecia and pycnidia when present, were made with a razor blade by hand. The chemical constituents were initially checked by spot tests with potassium hydroxide (K), sodium hypochlorite (C) and *para*-phenylenediamine (P), and also examined under UV light (360 nm). Subsequently, the chemical constituents were identified by thin-layer chromatography (TLC) using solvent C (Bungartz 2001), following standard methods described in Elix and Ernst-Russel (1993) and Orange et al. (2001), and cross-checked with labels containing chemical data left with the specimens by Richard C. Harris.

In the literature (e.g. Hale 1976; Elix 1994), the term lobules is often applied to any kind of secondary outgrowth morphologically and anatomically akin to thallus lobes (which also includes lacinulae and phyllidia, for example). Here, the accepted concept of lacinules is that they represent adventitious, ribbon-like secondary outgrowths from the primary lobe margins or sometimes growing from the upper cortex. Lobules are similar, but short and rounded (Marcelli et al. 2007).

At least 16 ascospores (the usual content of two full asci) were measured per apothecium, but average size ranges were obtained from 32 ascospores measured.

### The species

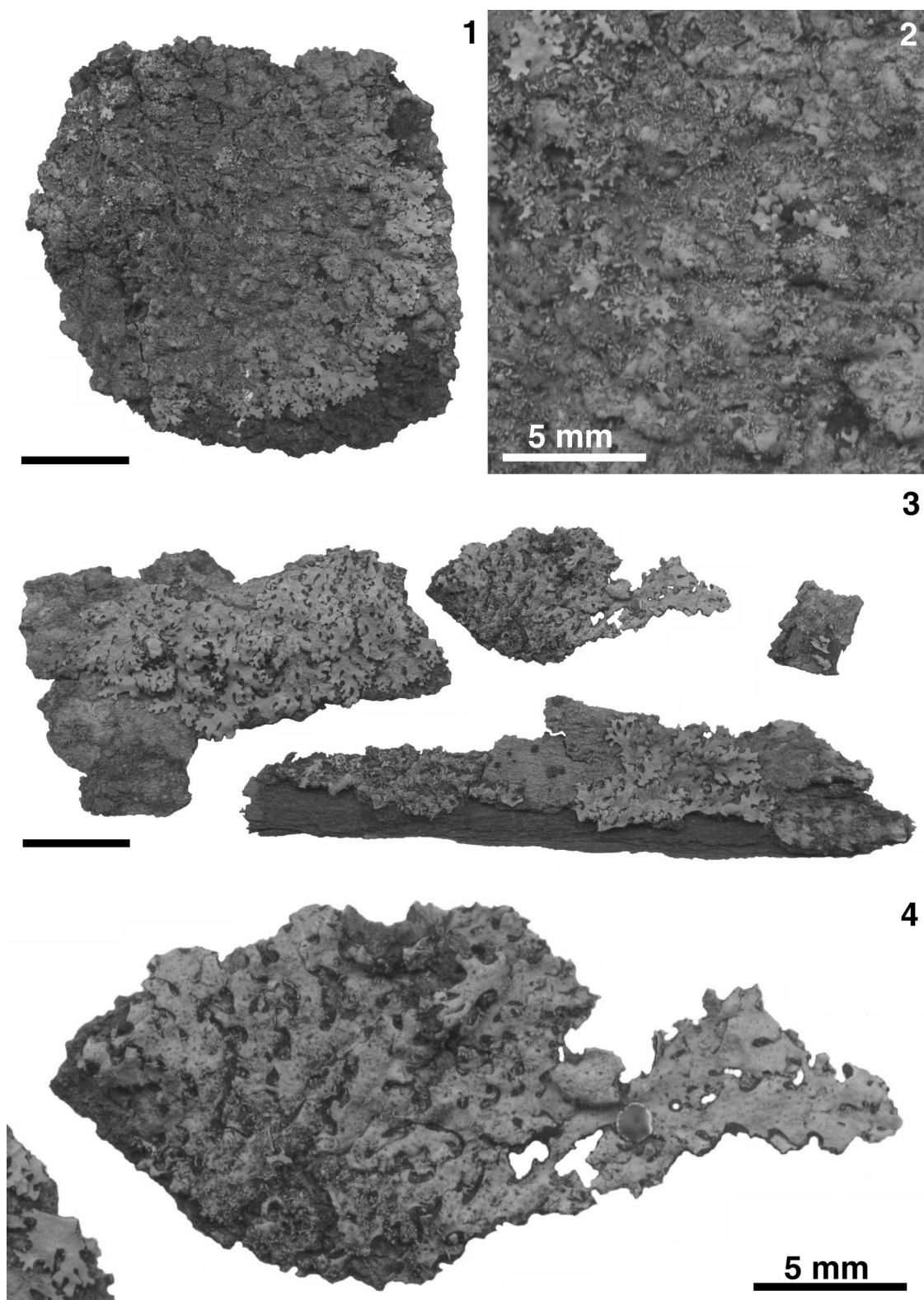
*Bulbothrix caribensis* Marcelli & Benatti, sp. nov.  
Figs. 1–2  
MycoBank: MB 519635

**Diagnosis:** Species cum thallo similis *Bulbothrici fungicolae* sed lacinulatis non isidiatis, lacinulis planis et stricto laminalibus, ciliis et rhizinis magis ramosis et acidis lichenum medulla absentia differt.

**Holotype:** Puerto Rico, Distr. Mayagüez, Maricao State Forest, just NE of Universidad Católica Estación Biológica, along Hwy 120 at km 15.8, along trail on hill top, ca. 700 m semi-dry forest and open area around towers, leg. R.C. Harris 22294, 5-VI-1988 (NY).

**Thallus** up to 3.7 cm wide, submembranaceous, corticolous, grayish green with dark parts in the herbarium, sublinearly lacinate; upper cortex 10.0–12.5 µm thick, algal layer 15.0–20.0 µm thick, medulla 20.5–30.0 µm thick, lower cortex 17.5–27.5 µm thick. *Laciniae* dichotomously, anisotomically to irregularly branched, 0.3–0.9 mm wide,

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Figures 1–2. *Bulbothrix caribensis*. 1. Holotype. 2. Detail showing the laminal lacinulae.  
 Figures 3–4. *Bulbothrix lyngei*. 3. Holotype. 4. Detail of a fragment containing a mature ecoronate apothecia. Scale bars: 1, 3–4 = 1 cm; 2 = 5 mm.

contiguous to  $\pm$  imbricate at older parts, very adnate, strongly attached; *Apices* plane, truncate to subtruncate. *Margin* flat, smooth, sinuous to subcrenate or subirregular, entire to slightly incised, partially lacinulate, ciliate, axils oval to irregular. *Upper surface* continuous, smooth, partially hidden by the lacinules, without laminal ciliar bulbs; *maculae* absent; *lacinules* short, generally spreading on the upper surface, rarely marginal, originating as isidia-like propagules with the exception of a few adventitious marginal ones, frequent becoming abundant at older thallus parts, the laminal ones erect to procumbent, commonly simple then furcate or sometimes irregularly branched, flat and dorsiventral from the beginning of their development,  $0.05\text{--}0.35 \times 0.05\text{--}0.10\text{--}0.20$  mm, truncate or acute, ciliate, underside light brown, sometimes with a few rhizinae. *Soralia*, *pustules* and *isidia* absent (see lacinules). *Cilia* black, sometimes brown, with simple, furcate, double to occasionally subdichotomously or irregularly branched apices,  $0.05\text{--}0.20 \times \text{ca. } 0.03$  mm, with semi-immersed basal bulbs ca.  $0.05$  mm wide, abundant along the margins, spaced ca.  $0.05$  mm from each other to contiguous, scarce or absent only at the laciniae apices. *Medulla* white. *Lower surface* black, shiny, smooth, unevenly papillate, moderately rhizinate. *Marginal zone* shiny, pale brown, smooth, unevenly papillate, briefly attenuated ca.  $0.5$  mm wide, with few rhizinae. *Rhizines* brown, initially simple or furcated becoming subdichotomous or irregularly branched, with blackened basal or displaced bulbs,  $0.05\text{--}0.30 \times \text{ca. } 0.03$  mm, frequent and equally distributed, becoming scarcer at some spots. *Apothecia* and *pycnidia* not found.

*Spot reactions*: upper cortex K+ yellow, UV–; medulla K–, C–, KC–, P–, UV–.

*TLC*: cortical atranorin and chloroatranorin; medullary acids absent (in agreement with a label from R.C. Harris on the holotype).

*Additional specimen examined*: Venezuela, Amazonas, Depto. Rio Negro, Cerro de la Neblina, NE end of NW plateau, summit camp #10, 1690 m,  $00^{\circ}54'30''$  N,  $66^{\circ}02'30''$  W, 12.5 km NW of Pico Phelps, 16.5 km NE of Base camp, *Heliophora-Neblinaria* savanna with *Euterpe* along drainage streams, leg. W.R. Buck 12908, 12/13-II-1985 (NY).

*Comments*: *Bulbothrix caribensis* is characterized by the sublinear, narrow laciniae, emaculate upper surface, frequent to abundant, ciliate, laminal lacinulae that sometimes cover parts of the laciniae, simple to variably branched cilia and rhizinae, a black lower cortex with a light brown marginal zone, rhizinae with basal or displaced bulbs, and absence of medullar substances.

At first, it was thought that these specimens would belong to *B. lopezii* Hale, as this is the only other lacinulate species with negative medullary spot tests. However,

comparisons with the isotype (US!) and other specimens of *B. lopezii* showed that the latter is different: it has wider laciniae ( $1.0\text{--}3.0$  mm wide), lacinulae with an evident regularly to irregularly dichotomous branching pattern (which becomes clearly evident when specimens of both species are seen side-by-side), more strongly branched cilia and rhizinae (including those on the lacinules), and it contains medullary fatty acids detected by TLC.

The type specimen of *B. caribensis* was originally interpreted by R.C. Harris (on a label dated 1988) as an “acid deficient” specimen of *B. suffixa* (Stirt.) Hale (BM! lectotype, GLAM! duplicate). In fact, its morphology closely resembles Hale’s concept of the latter species (Hale 1976), and the overall appearance of *B. lacinulata* Jungbluth, Marcelli & Elix (SP!, holotype). The problem regarding *B. suffixa* and *B. lacinulata* is that I also discovered that Hale’s concept of *B. suffixa* was not correct, because the type specimen is isidiate. The lacinulate material that fits that concept now corresponds to the name *B. lacinulata*. The new species is, therefore, not really similar to *B. suffixa*, but to *B. lacinulata*, which differs by its medullary gyrophoric acid chemistry, in addition to some morphological features: in *B. caribensis* the lacinulae are mostly simple, always plane and almost strictly laminally distributed (rarely occurring at the margins), and both the cilia and rhizinae are variably ramified. Specimens of *B. lacinulata* have marginal, usually subcanaliculate or semi-cylindrical lacinulae, that eventually become laminal, while the cilia and rhizinae varies from simple to furcate.

All other known *Bulbothrix* species with medullar fatty acids or without medullar substances that form propagules are isidiate [e.g., *Bulbothrix cassa* Jungbluth, Marcelli & Elix (SP! holotype, B! isotype), *B. klementii* Hale (M!, holotype US! isotype), *B. pigmentacea* (Hale) Hale (US! holotype), *B. queenslandica* (Elix & Stevens) Elix (MEL! holotype), and also the other species described here, *B. lyngei* Benatti & Marcelli].

The specimens found are corticicolous and, so far, only known from somewhat open areas between 700–1500 m altitudes of Porto Rico Island and the Venezuelan Gran Savannah.

The species is named after the Caribbean Sea, which borders the countries where the specimens analyzed were originally collected.

*Bulbothrix lyngei* Benatti & Marcelli, sp. nov. Figs. 3–4  
MycoBank: MB 519636

*Diagnosis*: Similis *Bulbothrici fungicolae* sed ciliis et rhizinis dichotome ramosis, apotheciis ecoronatis, ascosporis minoribus et rotundatis, acidis gyrophorici absentibus et acidi aliphatici medulla continens differt.

*Holotype*: Brasil, Pará, Serra do Cachimbo, Aeroporto Cachimbo, ca. 20 km N of the border with Mato Grosso on Cuiabá-Santarém highway (BR163) ca.  $9^{\circ}22'$ ,  $54^{\circ}54'$ W,



ca. 430–480 m, gallery forest along the Rio Formiga, 27-IV-1983, leg. L. Brako & M. J. Dibben 6129 (NY).

*Thallus* fragments up to 3.7 cm wide, subcoriaceous, corticolous, light dusky gray in the herbarium, sublinear-laciniate, upper cortex 15.0–20.0  $\mu\text{m}$  thick, algal layer 15.0–17.5  $\mu\text{m}$  thick, medulla 35.0–47.5  $\mu\text{m}$  thick, lower cortex 12.5–20.0  $\mu\text{m}$  thick. *Laciniae* 0.3–0.7 (–0.9) mm wide, dichotomously or trichotomously or anisotomously branched, contiguous to  $\pm$  crowded, adnate, strongly attached. *Apices* plane, truncate to subtruncate. *Marginal zone* plane, smooth to sinuous or subirregular, entire, rarely sublacinulate, ciliate, axils oval. *Upper surface* continuous, smooth, without laminal ciliar bulbs. *Maculae* absent, scars visible, left by fallen isidia. Adventive *lacinules* generally sparse, very short, randomly distributed along the margins, simple, flat, 0.1–0.2  $\times$  ca. 0.1 mm, truncate or acute, underside concolorous with the lower margin. *Cilia* black, initially simple, soon becoming furcate and then slightly dichotomously or irregularly branched, 0.05–0.25 (–0.35)  $\times$  ca. 0.02 mm, with semi-immersed to emerged basal bulbs ca. 0.05 (–0.10) mm wide, abundant along the margins, spaced ca. 0.05 mm from each other to sometimes contiguous, absent only at the apices of the laciniae. *Medulla* white. *Soredia* and *pustulae* absent. *Isidia* scarce to abundant, laminal, granular to smooth cylindrical, straight to slightly tortuous, 0.05–0.30  $\times$  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, smooth, slightly papillate, 0.2–1.0 mm wide, with few rhizinae. *Rhizines* light to dark brown, initially simple, soon becoming dichotomously or irregularly branched, partially with subtle basal or more frequently displaced bulbs, 0.05–0.35  $\times$  ca. 0.02 mm, frequent to abundant but less common in some parts, equally distributed. *Apothecia* (only one mature in the holotype) laminal, adnate, flat, 1.3 mm wide, margins subrugose, amphithecia smooth, ecoronate; discs light brown, epruinose, imperforate, epithecium 5.0–7.5  $\mu\text{m}$ , hymenium 25.0–30.0  $\mu\text{m}$ , subhymenium 12.5–17.5  $\mu\text{m}$ ; *Ascospores* rounded to subrounded 4.0–5.0 (–6.0)  $\times$  3.5–5.0  $\mu\text{m}$ , epispore ca. 0.75  $\mu\text{m}$  thick. *Pycnidia* not found.

*Spot reactions*: upper cortex K+ yellow, UV–; medulla K–, C–, KC–, P–, UV–.

*TLC*: cortical atranorin and chloroatranorin; traces of an unknown medullary fatty acid (in agreement with a label from R.C. Harris with the holotype), found around  $R_f$  40 in solvent C.

*Additional specimens examined*: Costa Rica, Prov. Cartago, on the road Limón-Turrialba, halfway between Siquirres and Chitaría, fence poles between pasture and road, ca. 700 m alt., leg. H. Sipman 12371 p. min. p., 9-I-1979

(NY); Porto Rico, leg. B. Fink 1916 (S); Idem, Manati, on Mayagua, leg. B. Fink s.n., 14-I-1916 (NY); Idem, leg. B. Fink 1411, 14-I-1916 (US). Brasil, São Paulo, Municipality of Ibiúna, SKY site, on tree branch, leg. M.P. Marcelli 12486, 12-XI-1991 (SP). Idem, on palm trunk, leg. M.P. Marcelli 11355, 10-III-1991 (SP). Idem, on branches and trunks of peach trees, leg. M.P. Marcelli 13319, 07-III-1992 (SP). Idem, on fallen twigs, leg. M.P. Marcelli 13320, 05-VII-1992 (SP).

*Comments*: *Bulbothrix lyngei* is characterized by the narrow sublinear laciniae, emaculate upper cortex, mostly simple isidia with small ciliar bulbs, dichotomously branched cilia, black underside with light brown margins, brown dichotomous rhizinae with subtle bulbs, and ecoronate apothecia containing small, rounded ascospores. The material studied showed only trace amounts of an unknown fatty acid detected by TLC.

This species is similar to *B. fungicola* (Lynge) Hale (S!, lectotype), which differs by the less ramified (dichotomous) cilia and rhizinae, coronate apothecia, larger ascospores, and the presence of gyrophoric acid in the medulla. Another constant difference is that the rhizinae are always brown (normally light brown) in *B. lyngei*, whereas they are black in *B. fungicola*.

*Bulbothrix queenslandica* (Elix & Stevens) Elix (MEL!, holotype) is similar to *B. lyngei* in laciniae morphology, ciliate isidia, absence of medullary spot test reactions and medullary substances. However, it is strongly maculate, commonly has laminal ciliary bulbs, the cilia and rhizinae are less branched much like those of *B. fungicola* (simple to furcate), the rhizinae are black and the apothecia coronate. Laciniae in *B. queenslandica* are also slightly but constantly larger (0.3–0.9  $\times$  0.5–1.4 mm wide) than those seen in *B. lyngei*.

*Bulbothrix klementii* Hale (M! holotype, US! isotype) differs by forming isidia that are only occasionally ciliate [in the manner of some specimens of *Parmelinopsis minarum* (Vainio) Elix & Hale], a uniform light brown underside, and by the presence of medullary colensoinic acid.

*Bulbothrix pigmentacea* (Hale) Hale (US!, holotype) does not show any medullary spot test reaction, containing trace amounts of gyrophoric acid detected only by TLC/HPLC; however, it differs by having always eciliate isidia, and by constantly presenting K– reddish pigment spots in the medulla, lower cortex and rhizinae. This species has also a less densely rhizinate lower cortex, with black rhizinae.

*Bulbothrix cassa* Jungbluth, Marcelli & Elix (SP! holotype, B! isotype) differs by the larger (1.5–3.0 mm wide) and subirregular laciniae with rounded apices, simple cilia and rhizinae (cilia partially without apices) and occasionally pycnidiate, but not ciliate, isidia. Cilia in this species are typically axillary. It also does not have fatty acids.

Other isidiate species with dichotomously ramified cilia and rhizinae, viz. *B. laevigatula* (Nylander) Hale (H-Nyl!, lectotype), *B. subdissecta* (Nylander) Hale (H-Nyl!, lectotype), and *B. apophysata* (Hale & Kurokawa) Hale (US! holotype, TNS! isotype), all differ by having eciliate isidia, larger, ellipsoid ascospores (ca.  $6.0\text{--}10.0 \times 4.0\text{--}7.0$   $\mu\text{m}$ ) and medullary lecanoric, gyrophoric and/or lobaric acids, respectively.

*Bulbothrix thomasiana* Benatti & Marcelli (Marcelli et al. 2011) has isidia with well developed ciliate apices and a very light brown underside, as in the new species, but presents medullary lobaric acid. It also has larger, ellipsoid ascospores.

*Bulbothrix scortella* (Nyl.) Hale (FH-Tuck!, lectotype) differs by the uniformly brown lower cortex with a marginal zone sometimes darker than the center, eciliate isidia, larger ascospores, and by the presence of medullary gyrophoric acid.

All specimens found are corticolous. They were found so far on Southeastern Brazil, and some Central American and Caribbean countries, between 400 and 700 m altitudes.

The new species is named after the Norwegian lichenologist Bernt Lynge, who first saw similarities between species of *Parmelia* s.lat. with bulbate cilia, then establishing section *Bicornuta*, on which the genus *Bulbothrix* Hale was eventually based.

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