

# A review of the genus *Bulbothrix* Hale: the species with medullary fatty acids or without medullary substances

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A taxonomic review of ten *Bulbothrix* (Parmeliaceae, Lichenized Fungi) species containing fatty acids or no substances in the medulla is presented here. The current species delimitations are confirmed. New characteristics are detailed, some synonyms are rejected, others confirmed, and range extensions are added.

**Key words:** Parmeliaceae, fatty acids, bulbate cilia.

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## Introduction

*Bulbothrix* Hale was proposed for a group of species called *Parmelia* Series *Bicornutae* (Lynge) Hale & Kurokawa (Hale 1974), characterized by small, lacinate and usually adnate thalli, simple to branched bulbate marginal cilia, cortical atranorin, simple to branched rhizines, smooth to coronate apothecia, hyaline unicellular ellipsoid to bicornute ascospores  $5.0\text{--}21.0 \times 4.0\text{--}12.0 \mu\text{m}$ , and bacilliform to bifusiform conidia  $5.0\text{--}10.0 \times 0.5\text{--}1.0 \mu\text{m}$  (Hale 1976, Elix 1993a). In a recent paper presenting a revised generic concept of parmelioid lichens based on molecular, morphological and chemical evidence, Crespo et al. (2010) include other diagnostic features such as a pored epicortex, lack of cortical pseudocyphellae, and presence of isolichenan in the cell walls. *Bulbothrix* is currently nested in the *Parmelina* clade and some species are grouped with

*Parmelinella*, making the genus paraphyletic (Crespo et al. 2010).

During a revision of the genus *Bulbothrix* (Benatti 2010) the type specimen and additional material of *Bulbothrix* was studied. These species have cilia with hollow basal bulbs, which contain differentiated (round) cells and a characteristic oily substance (Hale 1975, Feuerer & Marth 1997, Benatti 2011a). The first published part of Benatti's (2010) thesis concerns new combinations of four species, *Hypotrachyna tuskiformis* (Elix) Benatti & Marcelli, *Parmelinopsis pinguiacida* (Louwhoff & Elix) Marcelli & Benatti, *P. subinflata* (Hale) Benatti & Marcelli and *Parmotrema yunnanum* (Sheng L. Wang, J.B. Chen & Elix) Marcelli & Benatti, previously placed in *Bulbothrix* (Benatti and Marcelli 2010) and excluded due to the lack of true bulbate cilia. The second part treats the species containing medullary norstictic and protoce-

tricaric acids (Benatti 2012a). The third part treats the species with medullary salazinic acid that do not form isidia, soredia, lacinulae or pustules (Benatti 2012c), while the fourth part dealt with the species with salazinic acid that form isidia, soredia or pustules (Benatti 2013).

For a comprehensive understanding and easy assessment of all the data concerning this genus, which comprises ca. 60 species gathered in an unpublished review by Benatti (2010), it was planned to be divided in six parts. The different parts are as follows: (I) the species containing medullary norstictic and protocetraric acid, (II) the species containing salazinic acid lacking vegetative propagules, (III) the species containing salazinic acid with vegetative propagules (all already published, see Benatti 2012a and 2012c, 2013), (IV) the species containing fatty acids or no medullary substances (this paper), (V) the species containing the gyrophoric/lobaric/lecanoric acids lacking vegetative propagules, and (VI) the species containing the gyrophoric/lobaric/lecanoric with vegetative propagules, ultimately resulting in a synthesis of the whole genus followed by a worldwide key. A discussion concerning the motives for such an extensive and detailed treatment and the problems faced while working this review is explained in Benatti (2012c).

This paper discusses three species with medullary fatty acids [*Bulbothrix klementii* Hale, *Bulbothrix lopezii* Hale, *Bulbothrix lyngei* Benatti & Marcelli] and nine species without medullary substances [*Bulbothrix bulbochaeta* (Hale) Hale, *Bulbothrix caribensis* Marcelli & Benatti, *Bulbothrix cassa* Jungbluth, Marcelli & Elix, *Bulbothrix laeviuscula* (Räsänen) Benatti & Marcelli, *Bulbothrix pigmentacea* (Hale) Hale, *Bulbothrix queenslandica* (Elix & Stevens) Elix, *Bulbothrix semilunata* (Lyngé) Hale, *Bulbothrix subklementii* Marcelli, and *Bulbothrix viridescens* (Lyngé) Hale]. It includes species that form isidia or lacinulae, or that reproduce solely by apothecia. No species in this group are known so far that form soredia or pustules.

## Material and methods

Type material and additional species were studied from B, DUKE, G, H, ICN,

LWG, M, MEL, MSC, NY, S, SP, US and W, originating from Africa, Central America, the Caribbean and South America, as well as material collected in Brazil during the last 30 years, mainly by the author and the members of the Lichenological Study Group of the Instituto de Botânica (GEL) in Brazil.

The methodology and conventions are detailed in Benatti (2012a). Bulbs on cilia, rhizines, apothecia and other thallus parts were checked using the clarification method (Benatti 2011). Chemical constituents of the additional specimens examined were identified by thin-layer chromatography (TLC) using solvent C (Bungartz 2001), and compared with the data on labels left with the specimens. The types had their chemical constituents examined by high performance liquid chromatography (HPLC), following the methods described in Elix et al. (2003).

Fatty acids or lack of medullary substances are evidenced by overall negative K, C, KC and P spot test reactions. The presence of fatty acids can be perceived in TLC procedures as colorless spots left after finishing that can be revealed by showering distilled water on the plate, or in HPLC.

The species selected for comparisons are those who show close morphological or chemical similarities, and those most often compared by other authors due to peculiar characteristics.

## Results and discussion

The study confirmed ten species containing medullary fatty acids or that lack medullary substances (but see comments under *B. pigmentacea*). All species are described in detail and their diagnostic characters are discussed, with many previously unnoted details concerning morphology and when present secondary metabolites of each taxa being clarified.

*Bulbothrix bulbochaeta*, *B. laeviuscula*, *B. semilunata*, *B. subklementii* and *B. viridescens* reproduce only by forming apothecia. *Bulbothrix cassa*, *B. klementii*, *B. lyngei*, *B. pigmentacea*, and *B. queenslandica* are isidiate while *B. caribensis* and *B. lopezii* are lacinulate. *Bulbothrix australiensis*, *B. microscopic*, *B. pustulata*, *B. subglandulifera* and *B. subtabacina* are corticolous, while *B.*

*decurtata* and *B. subscortea* are saxicolous. *Bulbothrix imshaugii* and *B. isidiza* are predominately corticolous, rarely saxicolous.

### The species

***Bulbothrix bulbochaeta*** (Hale) Hale. *Phytologia* 28(5): 480. 1974. Figs 1–4 MB 341592

Basionym – *Parmelia bulbochaeta* Hale. *Contributions from the United States National Herbarium* 36: 138. 1964.

Holotype – India, Madurai district, down Shembaganur, Perumal coffee plantation, alt. 5300–5700 ft., on tree trunk, leg. D.D. Awasthi, 23-XII-1959 (LWG!, ex Herbarium D.D. Awasthi no. 4347; isotype at US!).

Thallus subirregular lacinate, dusky gray in herbarium, up to 5.2 cm diam., coriaceous, corticolous or rarely saxicolous, upper cortex 20.0–37.5  $\mu\text{m}$  thick, algal layer 25.0–42.5  $\mu\text{m}$  thick, medulla 50.0–100.0  $\mu\text{m}$  thick, lower cortex 20.0–25.0  $\mu\text{m}$  thick. Laciniae irregularly to anisotomously dichotomously branched, (0.8–) 1.4–2.7 mm wide, imbricate often becoming crowded, very adnate and very adpressed, with flat, subtruncate to truncate apices, margins plane, smooth to irregular, entire to incised, partially sublacinulate on older parts, axils oval to irregular. Upper surface smooth and continuous at the distal parts, becoming verrucose in parts that are densely pycnidiate or have many initial stage apothecia, somewhat irregularly cracked in some parts, laminal ciliar bulbs common, frequent to abundant. Adventitious lacinulae scarce and restrict to older parts, short, 0.2–0.5  $\times$  0.1–0.2 mm, plane, simple to rarely furcate, apices truncate, lower side concolor to the lower marginal zone. Maculae absent. Cilia black to rarely brown, apices initially absent or simple, soon turning furcate, trifurcate, subdichotomous or irregularly branched, 0.05–0.30  $\times$  ca. 0.03 mm, with semi-immersed bulbate bases 0.05–0.10 mm wide, abundant throughout the margin spaced 0.05–0.10 mm from each other to contiguous, absent or scarce at the apices of the laciniae. Soredia, Pustulae and Isidia absent. Medulla white. Lower surface black, shiny, subrugose, weakly papillate, densely rhizinate. Marginal zone brown to pale brown, attenuate,

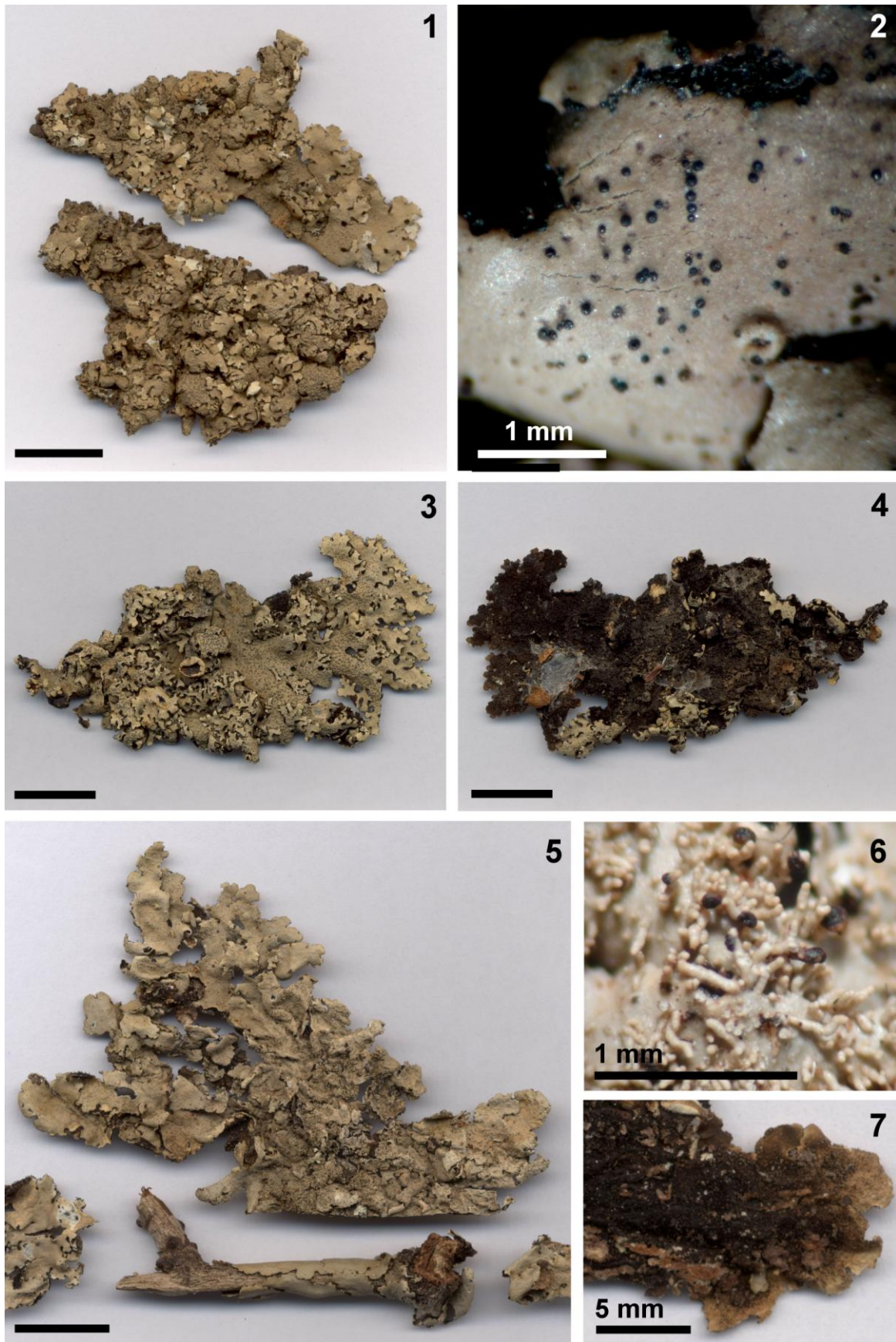
1.0–2.5 mm wide, shiny, subrugose, papillate, nude to densely rhizinate. Rhizines black, brown when close to the margins, initially simple soon turning furcate and then dichotomous or irregularly branched, without bulbate bases, 0.10–0.60  $\times$  0.03–0.05 mm, abundant, sometimes almost as a tomentum in some parts, evenly distributed. Apothecia concave, adnate to sessile, 0.2–3.2 mm diam., laminal, margin smooth becoming folded when old, somewhat irregularly coronate, amphithecia smooth, also with ciliar bulbs and occasionally with few pycnidia. Disc pale brown, epruinose, imperforate, epithecium 10.0–12.5  $\mu\text{m}$  high, hymenium 25.0–30.0  $\mu\text{m}$  high, subhymenium 25.0–42.5  $\mu\text{m}$  high. Ascospores not found (accordingly to Hale 1976 and Divakar & Upreti 2005, subrounded, 5.0  $\times$  4.0  $\mu\text{m}$ ). Pycnidia abundant, laminal over thaline wrinkles with a warty aspect or sometimes on the apothecia amphithecia, immerse, with black or brown ostioles. Conidia baciliform (3.0–) 5.0–6.0  $\times$  0.5  $\mu\text{m}$ .

TLC/HPLC – cortical atranorin, no medullary substances (see also Hale 1976, Hale & Kurokawa 1964).

Distribution – Africa: Ethiopia (Swinscow & Krog 1988). Asia: India (Hale & Kurokawa 1964, Hale 1976, Divakar & Upreti 2005, Nayaka & Upreti 2006), Mongolia (Schubert & Klement 1971) and Thailand (Ramkhamhaeng University Herbarium 2006).

Comments – *Bulbothrix bulbochaeta* is characterized by the sublinear average sized laciniae, the emaculate upper cortex often with small ciliar bulbs, absence of vegetative propagules, cilia with small bulbs without or with simple to branched apices, a black lower cortex with a brown marginal zone, branched rhizines without basal bulbs, coronate apothecia with rounded to subellipsoid small ascospores and without medullary substances.

Hale & Kurokawa (1964) believed that this species was endemic to the Indian subcontinent. Hale (1976) tried unsuccessfully to re-collect specimens in a region near the type locality believing that the species was rare, or that the continuous deforestation in the region could eventually extinguish the species; however, Schubert & Klement (1971) reported the species in Mongolia, and Swinscow &



**Figs 1–7** – 1 Holotype of *Bulbothrix bulbochaeta* (LWG). 2 Detail of the laminal ciliar bulbs on the holotype. 3 Isotype of *Bulbothrix bulbochaeta* (US). 4 Detail of the lower side of the isotype. 5 Holotype of *Bulbothrix cassa* (SP). 6 Detail of pycnidiate isidia in the holotype. 7 Detail of the lower side of the isotype. **Scale bars** = 1 cm (1,2,3,4,5), 1mm (6), and 5 mm (7).

Krog (1988) for Ethiopia. According to the Ramkhamhaeng University Herbarium (2006), the species also occurs in Thailand. Additional citations are also from India (Divakar & Upreti 2005, Nayaka & Upreti 2006).

The holotype (Fig. 1) consists of two fragments, the upper cortex with a large quantity of pycnidia that develop over several small warts apparently arising from the formation of the thaline edges of the apothecia, with an increased frequency of the warts towards the older parts. Part of the structures that appear to be pycnidia can actually be apothecia in their very initial stage of development, since they do not have paraphyses or similar structures. The only mature apothecium found has a pale brown disc without developed asci.

The isotype (Fig. 3-4) is also a fragment of the same size as the holotype, in the same conditions and with the same characteristics, and has two mature apothecia, which also unfortunately have hymenia without asci. The legend on page 10 in Hale (1976) indicates that the picture "e" is from the holotype, when in fact it is the picture of the isotype deposited in the herbarium US, as the holotype is currently in LWG.

The presence of small laminal cilia bulbs can frequently be seen in the type material (Fig. 2), ranging from frequent in some parts to abundant in others. The laminal bulbs can be differentiated from pycnidia or parasitic fungi by the more rounded and sleek aspect, like those on the marginal cilia. They are also hollow, containing idioblasts cells and an oily substance (Hale 1975, Feuerer & Marth 1997, Benatti 2011a).

The occurrence of laminal bulbs is also common in other species, such as *B. ventricosa* (Hale & Kurokawa) Hale and *B. viatica* Spielmann & Marcelli (Benatti 2010, 2012a), although in the case of *B. bulbochaeta* these structures have a pattern that is more similar to the one seen in specimens of *B. queenslandica* (Elix & Stevens) Elix (Benatti 2010).

The holotype has bulbs bordering patches of pycnidia, in places where the amphitecia margins are starting to form. Originally laminal, some of these seem to begin the formation of the corona of the apothecia. The type material also includes parasitic fungi

amidst the pycnidia and laminal ciliar bulbs.

Depending on the specimen, a variety of apical branching patterns can be observed in the cilia. Very young cilia tend not have apices (Swinscow & Krog 1988 have referred to cilia like these as "black nodules"), while those more mature tend to form simple, furcate or even subdichotomous apices.

The descriptions of *B. bulbochaeta* of Hale (1976), Hale & Kurokawa (1964) and Divakar & Upreti (2005) all indicate very small ascospores (5.0 x 4.0 µm). However, Swinscow & Krog (1988) mentioned ascospores much larger than those of the Asian specimens, measuring 8.0–10.0 × 4.5 µm. Measurements taken by the first three authors have identical sizes and all are Asian materials; they are always smaller than the measurements provided by Swinscow & Krog (1988) for the African material, which could point to a similar taxon.

Marcelli (1993) compared *B. subklementii* Marcelli (SP!) to *B. bulbochaeta*. This species differs by having very narrow lacinia (ca. 0.3–0.8 mm wide), a pale brown lower cortex with a slightly darker marginal zone, and by the less abundant rhizines that have black bulbs.

*Bulbothrix semilunata* (Lynge) Hale (S!) can be differentiated from *B. bulbochaeta* by the very narrow laciniae (ca. 0.2–0.5 mm wide), and by the ascospores, which are much larger and bicornute (12.0–23.0 × 3.0–4.0 µm), having a crescent or sigmoid shape.

Hale & Kurokawa (1964) compared *B. coronata* (Fée) Hale (G!) and *B. viridescens* (Lynge) Hale (S!, W!) to *B. bulbochaeta*. *Bulbothrix coronata* also differs by the narrower lacinia (ca. 0.5–1.0 mm wide), cilia and rhizines even more densely branched, apothecia with more regular coronation, larger ellipsoid ascospores (ca. 7.0–10.0 × 4.0–6.0 µm) and by the presence of medullary gyrophoric acid.

Due to the similarity of characteristics, *B. viridescens* is perhaps the species that could most easily be confused with *B. bulbochaeta*. However, it too differs by the narrower laciniae (ca. 0.5–1.0 mm wide), cilia and rhizines with generally simple apices, apothecia that are either ecoronate or coronate, and by never forming laminal ciliar bulbs.

*Bulbothrix meizospora* (Nylander) Hale (H-NYL!) was compared with *B. bulbochaeta* by Divakar & Upreti (2005). This species differs by the wider laciniae (ca. 2.0–6.0 mm wide) with a more rounded, sublobate aspect, cilia less frequent and usually more restricted to the lobes axils (also having larger bulbs often without apices), ecoronate apothecia containing much larger ascospores (ca. 12.0–22.0 × 8.0–12.0 μm), and by the presents of salazinic acid in the medulla.

*Bulbothrix cassa* Marcelli & Jungbluth (SP!, B!) differs by being isidiate, by the subirregular laciniae with rounded apices and crenate margins (similar in appearance of those in *B. ventricosa*), less abundant cilia which are usually restricted to the axils of crenae, and by the cilia and rhizines with generally simple apices.

*Bulbothrix laeviuscula* (Räsänen) Benatti & Marcelli (H!) is very similar to *B. bulbochaeta*, but differs by having a thinner thallus (ca. 75.0–110.0 μm thick), narrower laciniae (0.5–1.5 mm wide), usually simple cilia and rhizines, by the lack of a corona in the apothecia despite the presence of amphitecial bulbs, and by the larger ellipsoid ascospores (6.0–9.0 × 4.0–5.5 μm).

***Bulbothrix caribensis*** Marcelli & Benatti. Mycology 2(4): 255. 2011.

For a description, comments and images, see Benatti 2011b.

*Bulbothrix caribensis* is mainly characterized by the narrow sublinear lacinia, emaculate upper cortex, the formation of frequent to abundant laminal lacinulae often covering parts of the upper cortex, cilia with simple to sparsely branched apices, a black lower cortex with pale brown margins, simple to little sparsely branched rhizines with basal or displaced bulbs, and by the absence of medullary acids.

***Bulbothrix cassa*** Jungbluth, Marcelli & Elix. Mycotaxon 104: 52. 2008. Figs 5–7 MB 511166

Holotype – Brazil, São Paulo State, Itirapina Municipality, Estação Experimental de Itirapina, 22°15'S 47°49'W, 770 m alt., cerrado sensu stricto inside the João Batista de Arruda Prison area, on shrub twig, leg. P.

Jungbluth, A. A. Spielmann, L. S. Canêz & J. C. Sfair 840, 24-III-2004 (SP!, isotype at B!).

Thallus subirregular sublaciniate, dusky gray in herbarium, fragments up to 5.8 cm diam., submembranaceous, corticolous, upper cortex 12.5–17.5 μm thick, algal layer 20.0–32.5 (–50.0) μm thick, medulla 62.5–80.0 μm thick, lower cortex 5.0–12.5 μm thick. Laciniae irregularly to anisotomously dichotomously branched, (0.9–) 1.7–3.1 (–3.5) mm wide, contiguous to slightly imbricate, adnate but slightly adpressed, with slightly involute, subrounded to occasionally subtruncate apices, the margins plain, smooth and sinuous to crenate or subirregular, entire, not lacinulate, the axils oval. Upper surface smooth to subrugose, continuous, laminal ciliar bulbs absent. Lacinulae absent, not even adventitious marginal ones. Maculae weak to distinct, punctiform, laminal, usually hard to perceive due the scars left by the broken off isidia. Cilia black, without apices or with simple and partially bent downward apices, 0.05–0.30 × ca. 0.03 mm, with sessile bulbate bases 0.05–0.15 (–0.30) mm wide, frequently along the margins, solitary or in small groups in the crenes and the axils spaced 0.05–0.15 mm from each other to very rarely contiguous, often extended, reniform and withered, becoming absent or scarce at the apices of the laciniae and some random parts. Soredia and pustulae absent. Isidia frequent to abundant, laminal and sometimes arranged in clusters, granular to smooth cylindrical, straight to slightly tortuous, 0.05–0.30 (–0.50) × ca. 0.05 (–0.10) mm, simple to sparsely branched, erect, commonly caducous, concolor, eciliate, the more developed ones partially pycnidiate. Medulla white. Lower surface mostly black, turning partially dark brown at some random parts and at the transition to the margins, opaque to slightly shiny, subrugose to venate, moderately rhizinate. Marginal zone pale brown, attenuate, 0.5–3.5 mm wide, opaque to shiny, smooth to subrugose, papillate, nude gradually turning darker and rhizinate following the center. Rhizines black to partially brown, with whitish apices when near the margins of at the transition to the center, simple to occasionally furcate, partially with dark basal or dislocate bulbs, 0.10–0.60 ×

0.03–0.05 mm, frequent, evenly distributed. Apothecia plane to subplane, adnate, 0.3–0.9 mm diam., laminal, ecoronate, margin smooth to subcrenate, amphithecia smooth without ornamentations (possibly isidiate when more developed). Disc pale brown, epruinose, imperforate, epithecium 10.0–12.5  $\mu\text{m}$  high, hymenium 20.0–25.0  $\mu\text{m}$  high (only incompletely formed seen), subhymenium 37.5–42.5  $\mu\text{m}$  high. Ascospores not found (hymenia without asci). Pycnidia laminal to submarginal, frequent, immerse, with brown or black ostioles. Conidia weakly bifusiform 5.0–7.0  $\times$  1.0  $\mu\text{m}$ .

TLC/HPLC – cortical atranorin, no medullary substances (see also Jungbluth *et al.* 2008).

Distribution – South America: Brazil – São Paulo (Jungbluth 2006, Jungbluth *et al.* 2008).

Additional specimens examined – Brazil, São Paulo State, Mogi-Guaçu Municipality, Reserva Biológica de Mogi-Guaçu, Fazenda Campininha, 22°22'S, 46°56'W, 590 m alt., orchard surrounded by cerrado and gallery forest at the edge of the Goiabeiras Stream, at small tree branch, leg. M. P. Marcelli & M. Falco 33160, 02-IV-1999 (SP). Idem, São Carlos Municipality, Campus of the Universidade Federal de São Carlos, UFSCar, streets and vicinities of the south gate, of the rectory building and the neighboring buildings, 22°1'S, 47°53'W, alt. 855 m, over eucalyptus trunk, leg. M. N. Benatti & G. G. Batista 2068, 03-IX-2006 (HUFSCar).

Comments – *Bulbothrix cassa* is characterized by the subirregular laciniae, maculate upper cortex, cilia with or without simple apices restricted to the laciniae crenes and axils, simple to branched eciliate but partly pycnidiate isidia, a black to dark brown lower cortex with pale brown margins, simple rhizines partialy with basal bulbs, ecoronate apothecia, and lack of medullary substances.

The holotype (Fig. 5) consists of a larger fragment and some smaller ones, all in good condition, generally free of substrate and not glued to the vouchers, which made the lower cortex analysis easier. The isotype (Fig. 7) is a fragment, in the same condition as the holotype. There are no mature apothecia on

either the holotype or the isotype. There are pycnidia with conidia on the material. Isidia are quite frequent with a very peculiar habitus.

One of the most uncommon characteristics of *B. cassa* is the occasional formation of dark, round swellings in the isidia, which Jungbluth (2006) and Jungbluth *et al.* (2008) interpreted as bulbs of the same type seen in the marginal cilia. As seen under the microscope, these structures are actually pycnidia (Fig. 6), with conidia and conidiogenous hyphae instead of idioblasts cells and oily substances.

Unlike the ciliary bulbs that are smaller and more sessile, normally seen in species such as *B. fungicola* (Lyngé) Hale (S!) and *B. sipmanii* Aptroot & Aabel (U!, B!, TNS!, US!), the pycnidia are large (often larger than the bulbs), have a characteristic ostiole region, and as here are also immersed in isidia. At first glance, they could even be mistaken for parasites. Pycnidia formed on isidia occur in only one other species, *B. papyrina* (Fée) Hale.

The venations in the lower cortex cited by Jungbluth *et al.* (2008) are not very prominent, although they are perceptible. Specimens of other *Bulbothrix* species form more prominent venations when compared directly, such as seen in specimens of *B. isidiza* (Nylander) Hale.

In agreement with the authors, most rhizines actually arise from the venations, especially at the margins where the surface is smoother, although they are not restricted only to these parts. The bulbate bases of the rhizines are more easily noticeable in the younger and clearer ones, as mentioned by Jungbluth (2006).

Although Jungbluth *et al.* (2008) mentioned that the marginal bulbs fuse when they are at the axils of the crenes, what was observed is that the more developed cilia acquire a reniform aspect as the lacinia separate. No evidence of the merging bulbs was found, nor even multiple apices in a same bulb. Several specimens of different species in this genus showed a higher probability that a bulb can often stretch and develop more than one apice, rather than the fusion of adjacent bulbs.

*Bulbothrix ventricosa* (Hale & Kurokawa) Hale (TUR-V!) was compared to *B. cassa* by Jungbluth (2006) and Jungbluth *et al.*

(2008). It is quite similar in appearance to *B. cassa*, but differs by the isidia without formation of pycnidia; constant presence of laminal ciliar bulbs; the variable, usually mixed shades of brown coloration of the lower cortex; and by the presence of medullary norstictic acid.

Other morphologically similar species compared to *B. cassa* by Jungbluth (2006) and Jungbluth *et al.* (2008) were *B. isidiza* (Nylander) Hale (H-Nyl!), *B. tabacina* (Montagne & Bosch) Hale (L!, PC!) and *B. bulbochaeta* (Hale) Hale (LWG!, US!).

*Bulbothrix isidiza* differs by the non-pycnidiate isidia, the pale brown lower cortex, and by having medullary salazinic acid. *Bulbothrix tabacina* is similar to *B. isidiza* by the non-pycnidiate isidia and medullary salazinic acid, but differs because it has a black lower cortex.

Although commented by the authors, the differences cited for the length of the cilia apices and of the conidia sizes proved to be small and inexpressive after comparing materials of the three species. Characteristics such as these need careful treatment as they can indeed be of importance in separating some species while in others they represent a variability between specimens or might even be noted in different parts of a single specimen.

*Bulbothrix klementii* Hale (M!, US!) differs by narrower sublinear laciniae (ca. 0.5–1.0 mm wide), the emaculate upper cortex, more abundant cilia with branched apices, a very pale brown lower cortex with dichotomously branched rhizines, and by having medullary conlensoinic acid (detectable by chromatography). The isidia of *B. klementii* may rarely form small ciliar bulbs but not pycnidia as those seen in *B. cassa*.

*Bulbothrix pigmentacea* (Hale) Hale (US!) differs from *B. cassa* by the much narrower sublinear laciniae (0.2–0.7 mm wide) emaculate upper cortex, abundant cilia with branched apices, simple isidia without pycnidia, a black lower cortex with black or brown margins, abundant and branched rhizines, and by the presence of a K– reddish pigment in the medulla, lower cortex and rhizines. The medulla of this species probably contains small concentrations of gyrophoric acid, detectable only by TLC/HPLC.

*Bulbothrix queenslandica* (Elix & Stevens) Elix (MEL!) differs by the narrower sublinear laciniae (ca. 0.5–1.5 mm large), an upper cortex frequently with laminal ciliar bulbs, cilia with furcate or trifurcate apices which are not restricted to the axils of laciniae and crenes, ciliate isidia, a black lower cortex with pale brown margins, dichotomously branched rhizines, and coronate apothecia.

*Bulbothrix klementii* Hale. Smithsonian Contributions to Botany 32: 18. 1976.

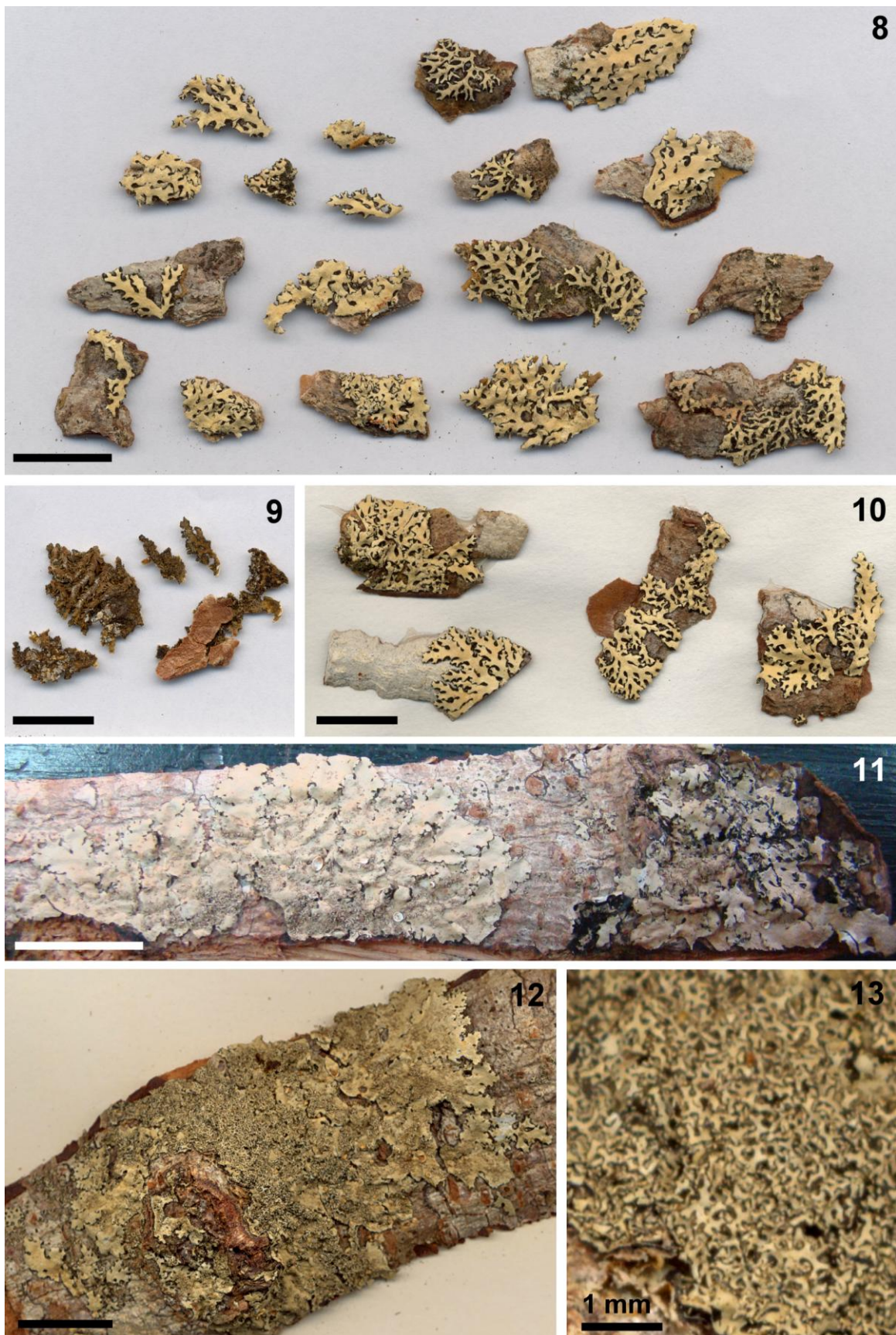
Figs 8–10

MB 341603

Holotype – Venezuela, Rio Atabapo, Cerro Pavón, “auf Rinde”, 110 m alt., 13-II-1958, leg. K. Mägdefrau 286 (M!, isotype in US!).

Thallus sublinear to almost linear lacinate, light dusky gray in herbarium, fragments up to 2.4 cm diam., submembranaceous and fragile, corticolous, upper cortex 10.0–12.5 µm thick, algal layer 12.5–15.0 µm thick, medulla 80.0–95.0 µm thick, lower cortex 10.0–12.5 µm thick. Laciniae dichotomously to trichotomously branched to occasionally irregularly ramified, 0.3–1.1 mm wide, contiguous to sometimes slightly imbricate, strongly adnate and adpressed, with flat, truncate to subtruncate apices, the margins flat, smooth to sinuous or subirregular, entire, rarely sublacinulate, the axils oval. Upper cortex continuous and smooth, occasionally with transversal irregular cracks on older or random parts, laminal ciliar bulbs absent. Adventitious lacinulae scarce on random parts of the margins, short, 0.10–0.50 × 0.05–0.20 mm, plane, simple, apices truncate or acute, lower side concolor to the lower marginal zone. Maculae absent. Cilia black, brown or occasionally pale brown, apices initially simple, soon turning furcate and then subdichotomous, 0.05–0.30 × ca. 0.03 mm, usually with semi-immersed to sessile bulbate bases 0.05–0.10 (–0.15) mm wide, abundant along the margins spaced 0.05 mm from each other to contiguous, usually scarce in the apices of the laciniae. Soredia and pustulae absent. Isidia infrequent to scarce, arranged in small laminal clusters, granular to smooth cylindrical, straight to slightly tortuous,





**Figs 8–13** – 8 Holotype of *Bulbothrix klementii* (M). 9 Detail of the lower side of the holotype. 10 Isotype of *Bulbothrix klementii* (US). 11 Holotype of *Bulbothrix lopezii* (photo: J. Hernandez). 12 Part of the Isotype of *Bulbothrix lopezii*. 13 Detail of the laminal dichotomous laciniae of the isotype. **Scale bars** = 1 cm (8, 9, 10, 11, 12), and 1 mm (13).

0.05–0.30 × ca. 0.05 mm, simple, erect to partially procumbent, firm to caducous, concolor or with pale brown apices, usually eciliate, but some can occasionally have ciliar bulbs. Medulla white. Lower surface pale brown to cream colored, shiny, smooth, densely rhizinate up to the edges of the margins. Marginal zone indistinct from the center, pale brown, shiny, smooth, rhizinate. Rhizines pale brown, usually clearer than the cortex or even occasionally whitish, initially simple soon turning dichotomously branched, partially with blackish bulbate bases or dislocated bulbs, 0.10–0.30 × ca. 0.03 mm, frequently almost like a tomentum, evenly distributed. Apothecia not found. Pycnidia laminal, scarce, immerse, with black ostioles. Conidia not found, only a few conidiogenous hyphae.

TLC/HPLC – cortical atranorin, medullary conlesoinic acid (see also Hale 1976).

Distribution – South America: Venezuela (Hale 1976) and Oceania: Australia (Elix 1995). Here it is cited for the first time for Brazil.

Additional specimen examined – Brazil, Iguape Municipality, restinga woods, unknown substrate, leg. M.P. Marcelli & O. Yano 6657, 17-VII-1989 (SP).

Comments – *Bulbothrix klementii* is characterized by the narrow sublinear laciniae, emaculate upper cortex, abundant cilia with branched apices, simple laminal isidia with rarely ciliar bulbs, a very pale brown lower cortex with an indistinct marginal zone, branched rhizines with dark basal or displaced bulbs, and the presence of medullary colensoinic acid.

The holotype (Fig. 8–9) consists of dozens of small fragments around 0.5 to 2.5 cm in diameter, containing a small amount of isidia in scattered groups on part of the laciniae. The material is in good condition, despite being small, and is on tree bark. The isotype (Fig. 10) consists of four small fragments in the same conditions as the holotype, but they are glued to the exsiccate voucher.

This is one of the *Bulbothrix* species with the clearest lower cortex, similar to *B.*

*thomasiana* Benatti & Marcelli (Marcelli *et al.* 2011). The color is practically homogeneous across the entire lower surface. The rhizines are even lighter than the lower cortex, except for their bulbs, which are blackish. Despite the rhizines covering the cortex, they are so clear and translucent that they do not obstruct the view due their transparency.

As noted by Hale (1976), the entangled cilia form a dense mat along the margins, often intertwining with the rhizines, but they can be readily distinguished by color, usually being darker. This is quite common in *Bulbothrix* species with very dichotomously branched cilia and rhizines, and may be one of the reasons why I found that several studied specimens and even some of the type materials were originally confused with species of the genus *Hypotrachyna* (Vainio) Hale (Benatti 2010).

The only description for *B. klementii* that exists is the original one in Latin (Hale 1976). The only other study found citing this species was from Elix (1995), which has no description or comments, only citing two specimens as *B. klementii* (Elix & Stevens) Hale. According to a label with the holotype, Hale had previously named the species as *Bulbothrix conlesoinica*. Despite having validly published the species with another epithet, he did not add a new label with the material with the published name *B. klementii*. According to Hale (1976) the final name was given in honor of Dr. Oscar Klement, because he had seen the material at M and correctly recognized the specimen as a Parmeliaceae species. However, Klement had originally identified the specimen as *Anzia colpodes* (Acharius) Stizenberg.

In his comments, Hale (1976) drew attention to the presence of medullary colensoinic acid, the only occurrence of this chemical substance in the genus, known only to the genera *Stereocaulon* and *Hypotrachyna*. Hale also reported that the substance was confirmed by chromatography together with material of *Hypotrachyna livida* (Taylor) Hale.

In his monograph of genus the *Bulbothrix*, Hale (1976) placed *B. klementii* in the key to species without vegetative propagation, although having described it with isidia. In fact, as in the description, *B. klementii* is indeed isidiate, although not all the

fragments of the holotype and isotype have isidia, as they are sparse and usually form small groups.

In his key, Hale (1976) placed *B. klementii* next to *B. viridescens* (Lynge) Hale (S!, W!), which has no medullary substances or isidia, but has a black lower cortex with dark brown margins. The cilia and rhizines of *B. viridescens* also differ because they are usually simple to occasionally furcate.

Marcelli (1993) described *B. subklementii* Marcelli (SP!) realizing an echivoque of Hale (1976) in the identification key, considering the description of *B. klementii* being valid in which the formation of isidia were depicted. *Bulbothrix subklementii* differs by the narrow lacinae (ca. 0.2–0.5 mm wide) and the usually simple or furcate cilia, and by not forming vegetative propagules. This species also has no medullary chemical substances.

*Bulbothrix scortella* (Nylander) Hale, previously regarded as a synonym of *B. goebelii* (Zenker) Hale by Hale (1976), is somewhat similar to *B. klementii* but differs by a darker brown lower cortex and by the presence of medullary gyrophoric acid. The isidia are also always eciliate in this species, and the rhizines are usually black or dark brown.

For a comparison of *Bulbothrix klementii* and *B. cassa* see treatment section of *B. cassa* in this manuscript.

*Bulbothrix queenslandica* (Elix & Stevens) Elix (MEL!) is similar to *B. klementii*, but differs by the maculate upper cortex, constant presence of small laminal ciliar bulbs, cilia with simple to furcate apices, simple to little branched and usually ciliate isidia, a black lower cortex with pale brown margins, and by the absence of medullary substances.

***Bulbothrix laeviuscula*** (Räsänen) Benatti & Marcelli. Mycosphere 3(1): 47. 2012.

For a description, comments and images, see Benatti 2012b.

*Bulbothrix laeviuscula* is characterized by the narrow sublinear lacinae, emaculate upper cortex commonly with small laminal ciliar bulbs, cilia with absent or simple apices, not forming vegetative propagules, a black lower cortex with brown margins, simple to

slightly irregularly branched rhizines without basal bulbs, apothecia with irregular corona and bulbs in the amphitecia containing small subellipsoid ascospores, and the lack of medullary substances.

***Bulbothrix lopezii*** Hale. Mycotaxon 25(1): 85. 1986. Figs 11–13  
MB 104076

Holotype – Venezuela, Mérida, Sierra Nevada de Mérida, quebrada de Fafoy, cercanías de El Carrizal, 1400 m alt., leg. López & Hale 20087, 4-IV-1979 (MERF n.v., isotype at US!).

Thallus sublinear lacinate, dusky gray in herbarium, fragments up to 5.0 cm diam., submembranaceous and fragile, corticolous, upper cortex 10.0–12.5 µm thick, algal layer 10.0–15.0 µm thick, medulla 25.0–37.5 µm thick, lower cortex 7.5–10.0 µm thick. Lacinae irregularly to anisotomously dichotomous branched, 1.0–1.6 (–3.2) mm wide, contiguous to imbricate or laterally overlapped, adnate and adpressed, with flat, truncate to subtruncate apices, the margins flat, smooth to sinuous, crenate or occasionally subirregular, entire to slightly incised and occasionally sublacinulate, the axils oval. Upper cortex continuous and smooth with few occasional irregular cracks partially occluded by the laminal lacinulae except at the distal parts, laminal ciliar bulbs absent. Lacinulae abundant covering much of the upper cortex, laminal or occasionally marginal, originated in a similar manner as isidia to function as vegetative propagules, or partially adventitious, short, flat and dorsiventral from the beginning on, 0.10–1.10 × 0.05–0.20 mm, initially simple soon turning dichotomous or occasionally irregularly branched, procumbent, truncate or acute, ciliate, underside concolorous with the lower marginal zone. Maculae weak, punctiform, laminal, scarce and restricted to the younger parts of the lacinae. Cilia black, with initially simple to furcate apices, soon turning subdichotomous or irregularly branched, 0.05–0.20 (–0.30) × ca. 0.03 mm, with semi immerse to sessile bulbate bases ca. 0.05–0.10 mm wide, frequently along the margins spaced 0.5–0.10 mm from each other turning contiguous at the crenes and axils, usually absent or scarce on the apices of

the laciniae. Soredia, pustulae and isidia absent (see lacinulae). Medulla white. Lower surface black, shiny, smooth to subrugose, densely rhizinate. Marginal zone attenuate, pale brown, shiny, 0.5–1.0 mm wide, smooth, papillate, slightly rhizinate. Rhizines pale brown, initially simple or furcate turning dichotomous or irregularly branched, mostly with dark basal or displaced bulbs, 0.05–0.30 (–0.40) × ca. 0.03–0.05 mm, abundant almost like a tomentum, evenly distributed. Apothecia subconcave, adnate, 0.3–1.2 mm diam., laminal to submarginal, ecoronate, margin and amphitecia smooth. Disc pale brown, epruinose, imperforate, epithecium 7.5–10.0 µm high, hymenium 25.0–30.0 µm high, subhymenium 15.0–20.0 µm high. Ascospores ellipsoid to oval, 5.0–7.0 × 3.0–5.0 µm, epispore ca. 1.0 µm. Pycnidia not found.

TLC/HPLC – cortical atranorin, traces of unknown medullary substances, probably undetermined fatty acids (see also Hale 1986). There are some occasional spots of an unknown K– reddish pigment in the thalli.

Distribution – South America: Venezuela (Hale 1986, López-Figueiras 1986, Marcano *et al.* 1996). Here it is cited for the first time to Panama.

Additional specimens examined – Panama, Chiriquí, Boquete, mountain slope above the town, on *Cupressus sempervirens* trees used as a hedge between two cultivated fields, ca. 1650 m elev., leg. W. L. Culberson & C. F. Culberson 19505, 16/VII/1983 (DUKE). Venezuela, Merida, La Solada along Via La Azulita, rocky open pasture, ca. 900 m, leg. M. E. Hale 42753, 03-II-1974 (US). *Idem*, Sierra Nevada de Merida, quebrada de fafoy, cercanías de El Carrizal, 1400 m, corticícola, leg. M. López Figueiras & M.E. Hale 20103, 04-IV-1979 (US). *Idem*, leg. M. López Figueiras & M. E. Hale 20141, 04-IV-1979 (US). *Idem*, en el camino entre El Carrizal y El Filo de Agua Fria, 1400 m, corticícola, leg. M. López Figueiras & M.E. Hale 20349, 05-IV-1979 (US).

Comments – *Bulbothrix lopezii* is characterized by median sublinear laciniae, weakly maculate upper cortex covered by plane, branched ciliate lacinulae, abundant and slightly branched cilia, a black lower cortex

with pale brown margins, branched pale brown rhizines with basal or displaced dark bulbs, ecoronate apothecia containing small or rounded ellipsoid ascospores for the genus standards, and by the lack of medullary substances.

The holotype could not be examined, but images were provided for examination by courtesy of Jesus Hernandez, curator of the herbarium VEN, who had obtained them during a visit to MERF where the holotype is located.

The holotype material (Fig. 11), which is apparently a small fragment of a thallus, shows no significant differences to the isotype and the other specimens studied. The images show the laminal lacinulae, although they appear less abundant than those seen in the isotype.

The isotype (Fig. 12) is a large fragment (almost an entire small thallus) in good condition, on tree bark glued to the voucher. There are some fragments of another *Bulbothrix* species attached to the bark, which are isidiate and very damaged. The species is possibly *B. scortella* (Nylander) Hale, judging by the brown lower cortex color and the C + and KC + rose medullary reactions, probably gyrophoric acid.

The upper cortex of the isotype is practically covered with laminal lacinulae, and there is a small amount of apothecia containing ascospores in good condition, although they are apparently not very developed. No pycnidia were found in the material.

All material studied is consistent with the description of Hale (1986). The weak maculation of the thalli is probably due to the large amount of lacinulae, as the maculae are usually visible only in young parts where the lacinulae are less numerous, being still absent, or are in their initial stages. Although Hale (1986) has described the laciniae as being 1.0–3.0 mm wide, the most common size range is 1.0–1.5 mm, as few were observed to go beyond these measures and rarely reach 3.0 mm.

The laminal lacinulae are plane, generally dichotomous (Fig. 13), and have small bulbate cilia. They have a regular cut, and are flattened with a dorsiventral differentiation, rather than resemble cleaved

isidia, as commonly occurs in *B. pseudocoronata* (Gyelnik) Benatti & Marcelli (G!, M!, W!). There are also some marginal lacinulae, but these are mostly adventitious.

The marginal cilia of *B. lopezii* are initially simple, gradually turning more branched towards the axils of the laciniae, especially the larger and more developed ones. Likewise, the cilia of the lacinulae range from simple and very short with tiny little bulbs to slightly dichotomous (with less frequency than the marginal ones). The rhizines are very branched and pale brown, contrasting with the lower cortex and with the cilia that are both black, which is unusual for the commonly found to the genus.

Hale (1986) compared *B. viridescens* (Lynge) Hale (S!, W!) to *B. lopezii*. Both species do not have any medullary substances, and have very small ascospores, similarly in size and shape, but *B. viridescens* differs by the cilia with simple or absent apices, rhizines with simple apices and no basal bulbs. It does not form lacinulae or any other propagules, and has partially coronate apothecia.

Interestingly, Hale did not compare *B. suffixa* (Stirton) Hale (BM!, GLAM!), which according to his concept (Hale 1976) was a similar lichen, differing only by the medullary substance (gyrophoric acid). As was checked (Benatti 2012b), the concept of Hale for *B. suffixa* correctly applies to the type material of *B. pseudocoronata*. The type material of *B. suffixa* has only rare marginal adventitious lacinulae, and what appear to be the initial stages of laminal isidia with brownish apices. The type material of *B. suffixa*, however, is unfortunately too immature to make a sufficient statement.

*Bulbothrix pseudocoronata* has very narrow laciniae (ca. 0.2–0.5 mm wide), forms simple to furcate cilia and rhizines, marginal to laminal lacinulae (a contrary origin pattern of *B. lopezii*, which are laminal to marginal) that are simple or irregularly branched, flat, semi-cylindrical or subcanaliculate, has coronate apothecia and medullary gyrophoric acid.

***Bulbothrix lyngei*** Benatti & Marcelli, *Mycology* 2(4): 257. 2011.

For a description, comments and images about this taxon, see Benatti 2011b.

*Bulbothrix lyngei* is characterized by the narrow sublinear laciniae, emaculate upper cortex, simple isidia with small ciliar bulbs, cilia with branched apices, a black lower cortex with pale brown margins, brown branched rhizines with small bulbs, ecoronae apothecia containing small and rounded ascospores and by the presence of medullary fatty acids.

***Bulbothrix pigmentacea*** (Hale) Hale. *Phytologia* 28(5): 480. 1974. Fig. 14 MB 341607

Basionym – *Parmelia pigmentacea* Hale. *Journal of Japanese Botany* 43: 325. 1968.

Holotype – Philippines, Luzon, Quezon Province, Sierra Madre, about 15 km east of Pagbilao, Chuan logging area, virgin dipterocarp forest, elev. about 300 m, VII/VIII-1964, M. E. Hale & J. Banaag 26895 (US!).

Thallus sublinear lacinate, light gray in herbarium, up to 3.5 cm diam., submembranaceous and fragile, corticolous, upper cortex 10.0–12.5 µm thick, algal layer 12.5–25.0 µm thick, medulla 15.0–22.5 µm thick, lower cortex 7.5–10.0 µm thick. Laciniae anisotomic dichotomously or trichotomously to irregularly branched, 0.2–0.7 mm wide, contiguous, adnate and adpressed, with flat, truncate to subtruncate apices, the margins flat, smooth to sinuous or irregular, entire to slightly incised, occasionally sublacinulate, the axils oval to irregular. Upper cortex smooth and continuous, with few occasional irregular fissures, laminal ciliar bulbs absent. Adventitious marginal lacinulae scarce on random parts, short, 0.1–0.3 × ca. 0.05 mm, plane, simple, apices truncate, lower side concolor with the lower marginal zone. Maculae absent. Cilia black, apices initially simple soon turning furcate and then dichotomously branched, 0.05–0.25 × ca. 0.03 mm, with semi-immersed to sessile bulbate bases ca. 0.05 mm wide, abundant along the margins, spaced up to 0.05 mm from each other to contiguous, becoming absent or scarce at the apices of the laciniae. Soredia and pustulae absent. Isidia scarce to frequent, laminal, granular to very short, smooth, cylindrical, straight, 0.05–0.10 × ca. 0.05 mm, simple, erect, firm, concolor or with brownish apices, eciliate. Medulla white, randomly

spotted with a red K- pigment which is also visible in parts of the upper and lower cortices, some of the cilia and rhizines, and which gets darker when soaked with the reagent. Lower cortex black, occasionally with dark brown spots, shiny, smooth to subrugose, densely rhizinate. Marginal zone black and indistinct from the center to brown and attenuate, up to 0.5 mm wide, shiny, smooth, rhizinate almost to the edges of the margins. Rhizines black, initially simple soon turning furcate and then regularly dichotomously branched, with blackish, sometimes subtle bulbate bases, 0.10–0.50 (–0.80) × 0.03–0.10 mm, abundant almost like a tomentum, evenly distributed. Apothecia and pycnidia not found.

TLC/HPLC – cortical atranorin. There are reddish spots of an unknown pigment in the medulla (see also Hale 1968, Hale 1976). The medullary spot tests C and KC are usually negative but occasionally they appear to be + weakly rose. The species might probably contain trace amounts of medullary gyrophoric acid that are difficult to ascertain.

Distribution – Asia: Philippines, Malaysia (Hale 1968, 1976), Thailand (Wolseley & Aguirre-Hudson 1997). Here it is cited for the first time to Papua New Guinea.

Additional specimens examined – Papua New Guinea, leg. S. L. Thrower 2812, 30-VI-1976 (US). Australia, Queensland, Edmund Kennedy National Park, on Cilft Road, NW of Cardwell, sea level, leg. M. E. Hale 65885, 27-VII-1983 (US). Philippines, Surigao del Sur, Mindanao, about 40 km NW of Lianga, virgin dipterocarp forest, Lianga Bay Lumber Co. logging area, ca. 350 m, leg. M. E. Hale & J. Banaag 24699, VII/VIII-1964 (US). Idem, Quezon Prov., Quezon National Park, leg. M. E. Hale 26983, VII-1964 (US).

Comments – *Bulbothrix pigmentacea* is characterized by the very narrow sublinear laciniae, emaculate upper cortex, cilia with branched apices, simple eciliate laminal isidia, a black lower cortex with black or brown margins, branched rhizines with basal bulbs, and by the presence of a reddish K- pigment randomly scattered in the medulla and visible at many parts of the thallus.

The holotype (Fig. 14) consists of two thalli with less than 2 cm in diameter and some

small fragments, close together on rough tree bark, glued to the voucher. They are in good condition, although the upper cortex was much damaged, maybe by Hale (?) who might have been searching for parts containing the red pigmentation in the medulla.

The type material is not very isidiate, and there is no sign of the formation of apothecia or pycnidia in the sample. The red pigment can be seen in several parts where the medulla was exposed, and is also readily apparent in transverse sections under a light microscope in all the studied material, as mentioned by Hale (1976).

Hale (1968) studied a few specimens (only five) collected in the Philippines and Malaysia. From the characters he mentioned, most are in agreement with the type material and other specimens studied here, only the laciniae width is confirmed to be a bit narrower than his description (0.2–0.7 vs. 0.5–1.0 mm wide.).

Hale (1968) compared *B. pigmentacea* to *B. subdissecta* (Nylander) Hale (H-NYL!, BM!), a species which he later accepted as a synonym of *B. goebellii*, due to the branched cilia and the presence of isidia. He cited that both species were common in dipterocarp forests in the lowlands of Southeast Asia.

Following the same logic of his comparison with *B. subdissecta*, Hale (1976) distinguished *B. goebellii* from *B. pigmentacea*, by the narrower laciniae and the medullary unknown pigment of the latter. Hale (1968) also differentiated the species through a lack of C reaction and the presence of an unidentified red pigment in the lower cortex and rhizinae of *B. pigmentacea*. He also mentioned that *B. pigmentacea* was smaller and more fragile than *B. subdissecta*.

All the small thalli studied here, including the holotype, are in fact similar to the thalli of *B. subdissecta*, but they are indeed smaller and more fragile looking. However, after the careful examination of the material, there are more results to be considered.

Several analyzed specimens of *B. pigmentacea* reacted variably to the C test, including some specimens which were identified by Hale himself. The results of these specimens varied from C- and KC- to C +, and KC + weakly rose, and conferring yet in all

the morphological characteristics with the type material.

Based on the obtained data and the comparisons, it is possible that gyrophoric acid could be present in trace concentrations in the thin medulla of the thalli of *B. pigmentacea*, and that the substance could have been unnoticed by Hale, or even that the author had perhaps considered it as traces of a contaminant, which was an idea I had first when studying the type before studying more specimens.

In fact, the characteristics seen in *B. pigmentacea* are typical for a species of this genus that usually contains medullary gyrophoric, lecanoric or lobaric acids and has narrow laciniae with truncated apices, densely ciliate and rhizinate thalli, with cilia and rhizines containing small basal bulbs and dichotomous branched apices.

As noted, the strong red K– pigment of *B. pigmentacea* can be seen scattered randomly throughout the medulla, often being visible at the upper or lower cortices, frequently spotting some of the cilia and the rhizines. At a first glance the pigment could be overlooked as perhaps a spot coming from some external source. Despite this it can indeed be found in the medulla, which is mostly white, but due to its thin layer it can be easily destroyed after the removal of the cortex, making it difficult to locate the red pigment spots.

*Bulbothrix subdissecta* differs by the larger width of the laciniae (ca. 0.5–1.5 mm wide.), a less fragile thallus, maculate upper cortex, larger isidia, and by the very evident presence of medullary gyrophoric acid (combined with lobaric acid), and the absence of any medullary pigmentation.

*Bulbothrix laevigatula* (Nylander) Hale (H-Nyl!, PC!) also differs by the broader laciniae (ca. 0.5–2.5 mm wide), larger isidia, and by the presence of medullary lecanoric acid and absence of medullary pigments. The thalli of this species are more robust when compared directly with each other.

*Bulbothrix fungicola* (Lynge) Hale (S!) differs by the maculate upper cortex, frequently ciliate isidia, simple to partially furcate cilia and rhizines, and by the presence of gyrophoric acid in the medulla, which is also not pigmented.

*Bulbothrix apophysata* (Hale & Kurokawa) Hale differs by the broader laciniae (ca. 0.5–1.5 mm wide), frequently fissured upper cortex, larger isidia, presence of medullary lobaric acid, and the absence of pigments in the medulla.

*Bulbothrix queenslandica* (Elix & Stevens) Elix. Mycotaxon 47: 126. 1993. Fig. 15 MB 360132

Basionym – *Parmelia queenslandica* Elix & Stevens. Australian Journal of Botany 27: 873. 1979.

Holotype – On trunk of Forest tree, Burleigh Heads National Park, Moreton District, Queensland, Australia, 28°04'S, 153°27'E, alt. 6 m, leg. J. A. Elix 1145, 30-VIII-1975 (MEL!).

Thallus sublinear to linear lacinate, dusky green in herbarium, fragments up to 3.8 cm diam., subcoriaceous, corticolous, upper cortex 7.5–15.0 µm thick, algal layer 17.5–22.5 µm thick, medulla 42.5–60.0 µm thick, lower cortex 10.0–15.0 µm thick. Laciniae anisotomously dichotomously or trichotomously to occasionally irregularly branched, 0.5–1.1 (–1.4) mm wide, slightly imbricate, adnate and adpressed, with flat to slightly involute, truncate to subtruncate apices, the margins flat, slightly sinuous to crenate or irregular, incised, occasionally sublacinulate, the axils oval to irregular. Upper cortex smooth and continuous, transversal fissures common only on older parts, laminal ciliar bulbs common, abundant. Adventitious marginal lacinulae scarce on random parts, short, 0.05–0.50 × 0.10–0.20 mm, plane, simple to furcate or irregularly branched, apices truncate, lower side concolor with the lower marginal zone. Maculae weak to distinct, punctiform, laminal, more evident at distal parts of the thallus. Cilia black or brown, apices simple to furcate or trifurcate, 0.05–0.30 × ca. 0.03 mm, with semi-immersed to sessile bulbate bases 0.05–0.10 mm wide, abundant along the margins spaced ca. 0.05 mm from each other to eventually contiguous in the crenes and axils, becoming absent or scarce at the apices of the laciniae. Soredia and Pustulae absent. Isidia scarce to frequent, usually grouped at some parts, laminal, granular to smooth cylindrical, straight to tortuous, 0.05–0.25 (–0.40) × ca.

0.05 (–0.10) mm, simple to slightly ramified and sometimes very juxtaposed seeming to be coralloid, erect, firm to caducous, concolor, ciliate. Medulla white. Lower cortex black, shiny, smooth to subrugose, moderately to densely rhizinate, with some open parts. Marginal zone pale brown, attenuate, ca. 0.5–1.5 mm wide, shiny, smooth, papillate, rhizinate. Rhizines pale brown close to the marginal zone, blackening towards the center, initially simple soon turning furcate and then dichotomously branched, with bulbate bases or dislocate bulbs (easily seen on the brown ones), 0.10–0.40 × ca. 0.03 mm, frequent to abundant, evenly distributed. Apothecia (none on the type) subplane, adnate, 1.0 mm diam., laminal, coronate, margins and amphitecia smooth, the amphitecia with ciliar bulbs. Disc brown, epruinose, imperforate, hymenia very poorly developed. Ascospores not found (hymenia without asci). Pycnidia not found.

TLC/HPLC – cortical atranorin and chloroatranorin, no medullary substances (see also Elix 1993b, 1994, Elix & Stevens 1979).

Distribution: Asia – Thailand (Pooprang *et al.* 1999). Oceania: Australia (Elix & Stevens 1979, Elix 1993b, Elix 1994).

Additional specimens examined – Australia, Queensland, Moreton District, South Eastern Queensland, West Mt. Cotton Road, Mt. Cotton, on *E. drepanophylla*, 27°37'S, 153°13'E, leg. R. Rogers & C. Scarlet 7233, 16-XII-1975 (MEL). Idem, Westlake, Horizon Road, on *Acacia* sp., 27°32'S, 152°54'E, leg. R. Rogers & C. Scarlett 6179, 09-XII-1975 (MEL). Idem, Moreton Bay, Mud Island, on coastal mangrove, leg. N. Stevens 2272 (US).

Comments – *Bulbothrix queenslandica* is characterized by the sublinear narrow laciniae, maculate upper cortex commonly with laminal ciliar bulbs, bulbate cilia with simple to furcate apices, simple to slightly branched ciliate isidia, a black lower cortex with pale brown margins, dichotomously branched rhizines with basal or displaced bulbs, and by the absence of medullary substances.

No apothecia were found on the type material; only a single mature apothecium was found on another specimen, which was coronate as mentioned by Elix & Stevens (1979) and also has amphitecial bulbs.

The holotype (Fig. 15) consists of four fragments in excellent condition, but all glued to the voucher. One has the lower cortex facing upwards. In all of them the presence of laminal bulbs can be easily observed, even amidst the isidia which are scarce on the material. This pattern is similar in the types and other specimens of *B. ventricosa* (Hale & Kurokawa) Hale (TUR-V!) and *B. laeviuscula* (Räsänen) Benatti & Marcelli (H!).

It is particularly easy to use the light brightness of a light microscope to visualize cells (idioblasts) and the oily substance in this species (Hale 1975, Feuerer & Marth 1997, Benatti 2011a), which are within the internal cavities of the laminal bulbs.

Due to the dark wall of the bulbs in *Bulbothrix* of *Relicina*, the typical oily substance and idioblast cells can be made visible by using a method of clearing with sodium hypochlorite (Benatti & Marcelli 2010, Benatti 2011a) or by crushing the bulb; however, due to the thinner wall of the ciliar bulbs in *B. queenslandica*, the clearing technique can be omitted.

This species was described by Elix & Stevens (1979) as *Parmelia queenslandica*, five years after Hale had already proposed the genus *Bulbothrix*. From the comments it is clear that at that time the authors were not sure about the genus. Elix (1993b) subsequently accepted the species and recombined it in *Bulbothrix*.

The marginal cilia of *B. queenslandica* have the same pattern as *B. fungicola* (Lyngé) Hale (S!), with simple to furcate or trifurcate apices. In *B. queenslandica* the rhizines are more branched than the cilia and normally turn very soon dichotomous even before the transition from the marginal zone to the center.

The rhizines are brown at the younger parts of the laciniae and it is easy to visualize the dark basal or displaced bulbs in this stage. When they darken at mature stages, the bulbs become difficult to be perceived, diminishing their evidence due the gradual thickening of the rhizines, especially when they adglutinate. As observed, this also happens in specimens of other species which share the same characteristic.

The isidia are usually infrequent and scattered in clusters, occasionally concentrated



at certain parts, sometimes giving the false impression of being coralloid due to the junction of the bases of the more branched ones. As seen in the isidia of *B. fungicola* and *B. sipmanii* Aptroot & Aabel (U!, B!, TNS!, US!), the isidia of *B. queenslandica* also have cilia, usually composed by small bulbs without apices or with very subtle apices.

Elix & Stevens (1979) compared *B. queenslandica* to *B. apophysata* (Nylander) Hale (US!, TNS!). This species differs by the more branched cilia, absence of laminal ciliar bulbs, always simple and eciliate isidia, and by the presence of medullary lobaric acid.

*Bulbothrix pigmentacea* (Hale) Hale (US!) was another species compared by the authors. This species can be distinguished by the more delicate and membranous thalli, the absence of cortical maculae, eciliate isidia, more branched cilia, and by constant formation of a reddish pigment in the lower portion of the medulla that also stains the lower cortex and the rhizines.

*Bulbothrix fungicola* differs by the much narrower laciniae (ca. 0.2–0.7 mm wide); ciliate isidia which are usually more abundant, always simple and very short; absence of laminal ciliar bulbs; less branched, usually simple to furcate rhizines; and by the presence of medullary gyrophoric acid.

For a comparison with *Bulbothrix cassa* see treatment *B. cassa* in this manuscript.

*Bulbothrix bulbochaeta* (Hale) Hale (LWG!, US!) is similar to *B. queenslandica* by also presenting formation of laminal ciliar bulbs, cilia and rhizines with a similar branching pattern and by the absence of medullary acids, but has broader laciniae (1.5–2.5 mm wide), an emaculate upper cortex and the absence of isidia.

*Bulbothrix laeviuscula* (Räsänen) Benatti & Marcelli (H!) also differs from *B. queenslandica* by the emaculate thallus without formation of isidia, although it is similar in the width of the laciniae, the thickness of the thalli and the presence of laminal ciliar bulbs.

*Bulbothrix laevigatula* (Nylander) Hale (H-NYL!, PC!) differs by the emaculate upper cortex, much broader laciniae (ca. 1.0–2.5 mm wide), eciliate isidia, absence of laminal ciliar bulbs, ecoronate apothecia and by the presence of medullary lecanoric acid.

*Bulbothrix semilunata* (Lynge) Hale. Phytologia 28(5): 479. 1974. Figs 16–19 MB 341611

Basionym – *Parmelia semilunata* Lynge. Arkiv för Botanik 13(13): 23. 1914.

Holotype – Brasiliae civit. Matto Grosso, Serra da Chapada, Buriti, ad corticem arboris Malpighiaceae, 19-VI-1894, leg. Malme s. n. (S!).

Thallus linear to sublinear laciniate, light greenish gray in herbarium, up to 2.5 cm diam., subcoriaceous, corticolous or ramulicolous, upper cortex 17.5–25.0 µm thick, algal layer 15.0–22.5 µm thick, medulla 35.0–47.5 µm thick, lower cortex 15.0–22.5 µm thick. Laciniae isotomously to anisotomously dichotomously branched, contiguous to occasionally slightly imbricate, 0.2–0.5 (–0.6) mm wide, strongly adnate and very adpressed, with flat, truncate to partially acute apices, the margins flat, smooth to sinuous or subirregular, entire to slightly incised, occasionally sublacinulate, the axils oval or irregular. Upper cortex smooth and continuous, laminal ciliar bulbs absent. Adventitious marginal lacinulae scarce on older parts, short, 0.2–0.3 × 0.1–0.2 mm, plane, simple to furcate or irregularly branched, apices truncate or acute, lower side concolor with the lower marginal zone. Maculae absent. Cilia black, apices initially simple or occasionally double, short, soon turning furcate, trifurcate and then subdichotomous or subirregular, 0.05–0.15 × ca. 0.03 mm, with semi-immersed to sessile bulbate bases (sometimes with a tapered appearance) ca. 0.05 mm wide, contiguous along the margins, becoming absent or scarce only at the apices of the laciniae. Soredia, pustulae and isidia absent. Medulla white. Lower surface black, shiny, smooth, densely rhizinate. Marginal zone dark brown almost indistinct from the center, slightly attenuate, up to 0.5 mm wide, shiny, smooth, slightly less rhizinate than the center. Rhizines black to dark brown, furcate, subdichotomous or irregularly branched, without bulbate bases, 0.50–0.20 × 0.03–0.05 mm, abundant almost like a tomentum, very intertwined, evenly distributed. Apothecia subconcave to plane, adnate to sessile, 0.3–2.3 mm diam., laminal to submarginal, coronate,

margin smooth to subcrenate partially interrupted by the clear bulbs, amphitecia smooth, occasionally dark and also with ciliar bulbs. Disc pale brown, epruinose, imperforate, epithecium 7.5–15.0  $\mu\text{m}$  high, hymenium 50.0–65.0  $\mu\text{m}$  high, subhymenium 17.5–20.0  $\mu\text{m}$  high. Ascospores bicornute, crescent to sigmoid, the curvature closed to open, thicker at the apices restricting the lumen to the central portion, 10.0–20.0 (–23.0)  $\times$  3.0–4.5  $\mu\text{m}$ , epispore ca. 0.5  $\mu\text{m}$ . Pycnidia laminal, frequent, immerse, with black ostioles. Conidia baciliform to weakly bifusiform 5.0–7.5  $\times$  0.75  $\mu\text{m}$ .

TLC/HPLC – cortical atranorin, no medullary substances (see also Hale 1976).

Distribution – South America: Brasil: MG (Ribeiro 1998), MT (Lynge 1914, Hale 1976, Marcelli 1993). Ribeiro (1998) also mentioned MS, Mato Grosso do Sul State; however, a reference to this citation was not found. Here it is cited for the first time to the northern Brazilian State of Pará.

Additional specimens examined – Brazil, Pará State, Serra do Cachimbo, Base Aérea do Cachimbo, ca. 20 km N of the boarder with Mato Grosso on Cuiabá-Santarém highway (BR-163), ca. 9°22'S, 54°54'W, ca. 430–480m, broad, sandy level plain along Rio Braço de Norte with sandstone exposure, low ridges and valleys to the N & S, leg. Brako & Dibben 5804c, 25-IV-1983 (NY, p.min.p. of the type material of *B. oliveirae*). Idem, Mato Grosso State, Serra da Chapada, Buriti, 800–1100 m, leg. G.O. Malme s.n., 10-IV-1894 (US). Idem, between Jaciara and São Vicente, ca. 100 km ESSE of Cuiabá, 750m alt., cerradão. on thin twig, leg. M. P. Marcelli 8444a, 2-VII-1980 (SP). Idem, Minas Gerais State, Lima Duarte Municipality, Parque Estadual do Ibitipoca, on the trunk of *Plathymenia foliolosa* Benth. on the main road, leg. M.P. Marcelli, C.H. Ribeiro & A.E. Luchi 27630, 21-III-1995 (SP). Idem, sobre on the trunk of *Plathymenia foliolosa* Benth. isolated behind the cafeteria, near the gallery forest, leg. M.P. Marcelli, C.H. Ribeiro & A.E. Luchi 27748, 22-III-1995 (SP).

Comments – *Bulbothrix semilunata* is characterized by the very narrow linear laciniae, emaculate upper cortex, contiguous

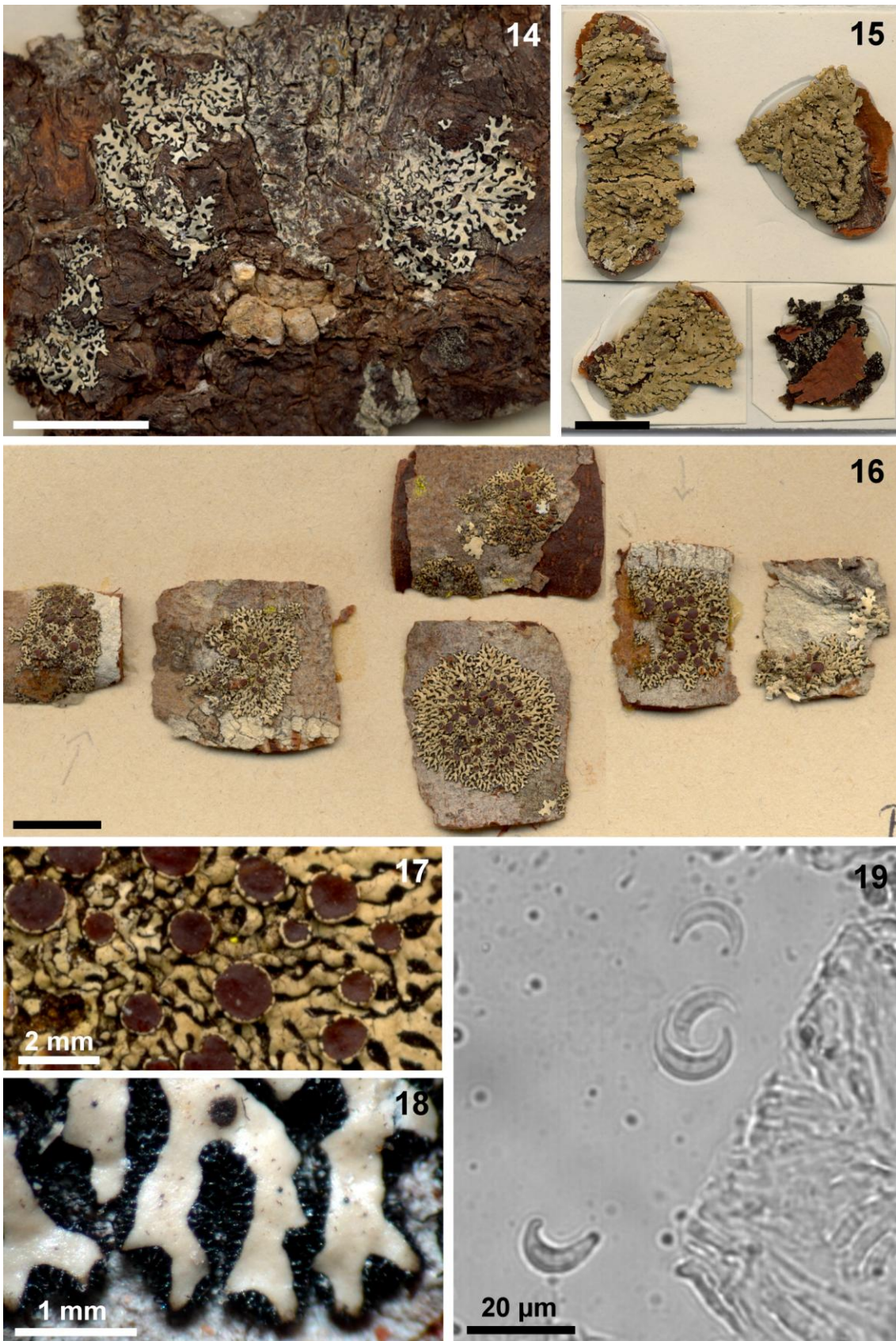
small bulbate cilia with simple, furcate or subdichotomously branched apices, a black lower cortex with brown margins, furcate to subdichotomous or irregularly branched rhizines without basal bulbs, coronate apothecia containing large bicornute ascospores with a crescent or sigmoid shape, and the by the lack of medullary substances.

This is the type species of Section *Bicornutae* Lynge (1914), and subsequently the type species of the genus *Bulbothrix* Hale (1974). It is also the only species with bicornute ascospores not to contain medullary substances and one of the species with the smallest laciniae width (rarely exceeding 0.5 mm), which are linear and dichotomously divided.

The holotype (Fig. 16) consists of five small thalli, generally intact and in good condition. The ascospores are unique in shape compared to those seen in other species of *Parmeliaceae*, presenting a very peculiar comma or crescent shape (Fig. 19), unknown to species within the family other than *B. bicornuta* (Müller Argoviensis) Hale (G!, BM!), *B. glandulifera* (Fée) Benatti & Marcelli (G!) and more recently *B. sipmanii* Aptroot & Aabel (U!, B!, TNS!, US!).

Due to their shape, the positioning of the ascospores within the asci takes on a spiral aspect, the whole of them somewhat resembles the shape of a coil, which also occurs with the three other species with the same type of ascospores.

Although Lynge (1914) has pointed out that the apothecia were ornated with pycnidia, it actually was confirmed that they are small ciliar bulbs, which appear on the margin of the apothecia surrounding the disc (Fig. 17). This is also true for the other species of the genus with coronate apothecia. None of these structures showed any conidia or conidiogenous hyphae after they were examined under a microscope. They did, however, show the typical oily substance and idioblasts (Hale 1975, Feuerer & Marth 1997, Benatti 2011a) which can be found in the bulbs of marginal cilia. The author also mentioned



**Figs 14–19** – 14 Holotype of *Bulbothrix pigmentacea* (US). 15 Holotype of *Bulbothrix queenslandica* (MEL). 16 Holotype of *Bulbothrix semilunata* (S). 17 Detail of the coronate apothecia of the holotype. 18 Detail of the marginal contiguous cilia of the holotype. 19 Bicornute ascospores of *Bulbothrix semilunata*. Scale=1 cm (14, 15, 16), 2 mm (17), 1 mm (18), and 20 µm (19)

the lack of spot test reactions but cited the presence of the orange pigment esquirine, which was not found in any of the specimens studied here.

Marcelli (1993) and Ribeiro (1998) also collected specimens of *B. semilunata*. Marcelli (1993) commented the fact that the species is poorly collected, probably due to the ramulicolous habit and its small size, sometimes being found mixed with samples of other species. Hale (1976) also cited additional material of *B. semilunata* found under these same conditions.

Ribeiro (1998) described rhizines which are partly bulbate and have pale brown apices. This was not confirmed in the specimens studied. The ascospores in his material are in agreement with those seen in the holotype, and as described by the author, the cilia indeed vary from simple or furcate to slightly branched. Other authors (Lynge 1914, Hale 1976, Marcelli 1993) just mention that the apices are branched.

There are differences in the ascospore sizes cited in the literature. Lynge (1914) and Marcelli (1993) cited measurements close to or equivalent to those found here (including to the holotype), respectively  $13.0\text{--}18.0$  ( $-21.0$ )  $\times$   $3.0\text{--}4.0$   $\mu\text{m}$  and  $16.2\text{--}20.7$   $\times$   $1.8\text{--}3.6$   $\mu\text{m}$ . Hale (1976) and Ribeiro (1998) cited smaller measurements, respectively  $9.0\text{--}12.0$   $\times$   $2.0\text{--}3.0$   $\mu\text{m}$  and  $10.0\text{--}15.0$   $\times$   $5.0$   $\mu\text{m}$ , which are more similar to those found in *B. glandulifera* (Benatti 2012b).

Hale (1960) commented that *B. semilunata* could easily be mistaken as one of the group of *Parmelia coronata* Fée (G!) [= *Bulbothrix coronata* (Fée) Hale] due to the small grayish thalli and the coronate apothecia, although in his monograph (Hale 1976) he did not specify if they were coronate. Aside from the difference in shape and size of ascospores which are ellipsoidal and smaller ( $5.0\text{--}10.0$   $\times$   $4.0\text{--}6.0$   $\mu\text{m}$ ) in *B. coronata*, this species has much broader laciniae ( $0.5\text{--}2.0$  mm wide), more dichotomously branched cilia and rhizines, and contains medullary gyrophoric acid.

Hale (1976) also compared *B. semilunata* to *B. schiffneri* (Zahlbruckner) Hale (W!) a synonym of *B. glandulifera* (Benatti 2012b). This species differs by the cilia and

rhizines which are more dichotomously branched, usually smaller bicornute ascospores (ca.  $8.0\text{--}13.0$   $\times$   $3.0\text{--}4.0$  mm) with a more accentuate crescent aspect, and by the presence of medullary gyrophoric acid.

The other non-isidiate species with bicornute ascospores, *B. bicornuta*, differs by the much broader laciniae ( $0.5\text{--}2.5$  mm wide), the more dichotomously branched cilia and rhizines, ecoronate apothecia, and by the presence of medullary lecanoric acid.

For a comparison between *Bulbothrix semilunata* and *B. bulbochaeta* see treatment of *B. bulbochaeta* in this manuscript.

***Bulbothrix subklementii*** Marcelli. Acta Botanica Brasilica 7(2): 48. 1993. Fig. 20 MB 459291

Holotype – Brazil, Mato Grosso do Sul State, between Rio Verde de Mato Grosso and Coxim, km 629,5 of the BR-163 highway, shrubs at the edge of the cerrado, on thin branch, leg. M.P. Marcelli 8495, 28-VI-1980 (SP!).

Thallus sublinear laciniate, light dusky in herbarium, up to 2.4 cm diam., submembranaceous, ramulicolous, upper cortex  $27.5\text{--}35.0$   $\mu\text{m}$  thick, algal layer  $20.0\text{--}25.5$   $\mu\text{m}$  thick, medulla  $10.0\text{--}15.0$   $\mu\text{m}$  thick, lower cortex  $15.0\text{--}17.5$   $\mu\text{m}$  thick. Laciniae anisotomously dichotomously to irregularly branched, ( $0.2\text{--}$ )  $0.3\text{--}0.5$  mm wide, contiguous to occasionally slightly imbricate, adnate and loosely adpressed, with flat, truncate to subtruncate apices, the margins flat, smooth to slightly sinuous or subirregular, entire, rarely sublacinulate, axils oval to irregular. Upper cortex smooth and continuous, laminal ciliar bulbs absent. Adventitious marginal lacinulae scarce on older parts, short,  $0.10\text{--}0.50$   $\times$   $0.05\text{--}0.30$  mm, plane, simple, apices truncate or acute, lower side concolorous with the lower marginal zone. Maculae absent. Cilia black to occasionally brown, apices generally simple to furcate or occasionally irregularly branched,  $0.05\text{--}0.25$   $\times$  ca.  $0.03$  mm, with semi-immersed to sessiled bulbate bases  $0.05\text{--}0.10$  ( $-0.15$ ) mm wide, abundant, along the margins spaced ca.  $0.05$  mm from each other to contiguous, becoming absent or scarce at the apices of the laciniae and some random parts of the margins.

Soredia, pustulae and isidia absent. Medulla white. Lower surface pale brown to cream, shiny, smooth, moderately rhizinate. Marginal zone brown, slightly darker and almost indistinct from the center, shiny, smooth slightly papillate, rhizinate. Rhizines pale brown, frequently clearer than the cortex, initially simple turning furcate and then dichotomous or partially irregularly branched, frequently with blackish basal or displaced bulbs,  $0.10\text{--}0.30 \times 0.03$  mm, commonly agglutinated, frequent turning more abundant at some parts, evenly distributed. Apothecia plane turning subconcaave when mature, adnate to subpedicelate, 0.6–2.2 mm diam., laminal, margins smooth to subcrenate, coronate, partially interrupted by the ciliar bulbs, amphithecia smooth, generally also with many ciliar bulbs, occasionally with few pycnidia. Disc brown, epruinose, imperforate, epithecium 10.0–15.0  $\mu\text{m}$  high, hymenium 15.0–25.0  $\mu\text{m}$  high, subhymenium 30.0–37.5  $\mu\text{m}$  high. Ascospores not found (hymenia without asci). Pycnidia laminal to submarginal, frequent, immerse, with black ostioles. Conidia not found.

TLC/HPLC – cortical atranorin and chloroatranorin, no medullary substances (see also Marcelli 1993).

Distribution – South America: Brasil – Mato Grosso do Sul (Marcelli 1993).

Comments – *Bulbothrix subklementii* is characterized by the sublinear narrow laciniae, emaculate upper cortex, cilia with simple to furcate apices, absence of vegetative propagules, a very pale brown lower cortex with margins occasionally darker than the center, branched rhizines with dark basal or displaced bulbs, coronate apothecia and by the absence of medullary substances.

The holotype (Fig. 20) consists of two small thalli 2 and 3 cm in diameter. Part of the upper cortex and of the apothecia was devoured by insects, and the material is unfortunately damaged. There is no evidence of the formation of vegetative propagules in the intact parts. There are several apothecia and pycnidia, although the hymenia are without asci and the pycnidia without conidia. The thalli are on pieces of thin branches of shrubs.

This species has a very pale brown lower cortex similarly to the color of coffee diluted with milk, with the exception of the marginal zone that is always of a darker shade than the center. The rhizines are usually clearer than the lower cortex and similar to those of *B. klementii* Hale (M!, US!), except for the basal or displaced bulbs, which are blackish in color.

The rhizines of *B. subklementii* are clear and translucent to the point of not obstructing the view of the lower brownish cortex, even in those parts where they are most dense and intertwined.

The characters described by Marcelli (1993) are in accordance with those being observed in the type material; however, the black dots in the rhizines were found to not be pycnidia and the interior spherical cells not being conidia. These structures are bulbs, similar to those of the cilia and amphithecia, and these cells were actually idioblasts (Feuerer & Marth 1997), which produce the oily substance.

The cilia in *B. subklementii* tend to be less branched than the rhiziness (cilia simple to furcate while the rhizines are furcated to dichotomous), similar to what can be found in *B. queenslandica* (Elix & Stevens) Elix (MEL!).

*Bulbothrix klementii* differs from *B. subklementii* by the broader laciniae (0.3–1.0 vs. 0.2–0.5 mm wide), by more furcate to dichotomously branched cilia, formation of laminal isidia, and by the presence of medullary colensoinic acid, which is detectable in thin layer chromatography. *Bulbothrix klementii* is in fact isidiate, although in the key in Hale (1976) it is among the species that do not form isidia.

Marcelli (1993) was correct in assuming that Hale (1976) had equivocally placed *B. klementii* among the species without isidia in his key, and that the description, where these structures appear, would to be correct. Based on this assumption, the author described the non isidiate *B. subklementii*, which is similar to *B. klementii* in the morphology and spot tests reactions due the absence of medullary substances.

Marcelli (1993) compared *B. semilu-*

*nata* (Lyngé) Hale (S!) to *B. subklementii*. The former species differs by the more branched cilia, a black lower cortex, black or very dark brown rhizines, and by the bicornute instead of ellipsoid ascospores, which are also larger (12.0–23.0 × 3.0–4.0 µm.) than those of *B. subklementii*.

*Bulbothrix viridescens* (Lyngé) Hale (S!, W!) differs from *B. subklementii* by the broader laciniae (ca. 0.5–1.5 mm), a black lower cortex with dark brown margins, simple cilia and rhizines without bulbate bases and by the apothecia that are partly coronate and ecoronate, without amphithecial bulbs.

*Bulbothrix cassa* Jungbluth, Marcelli & Elix (SP!, B!) differs from *B. subklementii* by the much broader laciniae (ca. 2.0–3.0 mm wide) with rounded apices, usually axillary simple cilia, simple rhizines, formation of isidia that are partially pycnidiate, and by the lower cortex of variable color, which is a mix of black and brown.

*Bulbothrix queenslandica* also forms simple to branched cilia and has no medullary substances as *B. subklementii*, but it differs by the maculate upper cortex commonly with laminal ciliar bulbs, formation of simple to slightly branched and usually ciliate isidia, and by the black lower cortex with pale brown margins.

***Bulbothrix viridescens*** (Lyngé) Hale. *Phytologia* 28(5): 481. 1974. Figs 21–23 MB 341621

Basionym – *Parmelia viridescens* Lyngé. *Arkiv för Botanik* 13(13): 117. 1914.

Lectotype – Brasiliae civit. Matto Grosso, Santa Anna da Chapada, in margine silvulae, ad corticem, leg. G.O. Malme 2453, 28-II-1894 (S!, isolectotype at W!).

Thallus sublinear laciniate, grayish olive green in herbarium, fragments up to 6.4 cm diam., subcoriaceous, corticolous, upper cortex 12.5–17.5 µm thick, algal layer 15.0–30.0 µm thick, medulla 47.5–80.0 µm thick, lower cortex 12.5–17.5 µm thick. Laciniae irregularly to partially anisotomously dichotomously branched, 0.5–1.1 (–2.0) mm wide, contiguous, adnate and loosely adpressed, with flat to partially involute, truncate to subtruncate apices, the margins flat to involute, subcrenate to crenate or irregular,

entire to partially slightly incised, commonly sublacinulate, axils oval. Upper cortex smooth and continuous, turning slightly transversely fissured in random parts, laminal ciliar bulbs absent. Adventitious marginal lacinulae frequent, short, 0.2–0.8 × 0.1–0.4 mm, flat, simple to irregularly branched, apices truncate or acute, lower side concolorous with the lower marginal zone. Maculae absent. Cilia black, apices absent or simple, short and curved, frequently bent downwards, 0.05–0.15 (–0.25) × ca. 0.03 mm, with semi-immersed to sessile bulbate bases 0.05–0.10 (–0.15) mm wide, abundant along the margins, spaced 0.05–0.10 mm from each other turning contiguous at the axils and becoming scarce at the apices of the laciniae. Soredia, pustulae and isidia absent. Medulla white. Lower surface black, shiny, smooth to subrugose, slightly papillate, moderately rhizinate. Marginal zone dark brown, almost indistinct from the center, shiny, ca. 0.5 mm wide, smooth, slightly rhizinate. Rhizines black, simple or occasionally slightly irregularly branched, without bulbate bases, 0.10–0.40 × ca. 0.04 mm, frequent to sometimes more abundant at some parts, evenly distributed. Apothecia subconcave to plane or subconvex, adnate to sessile, 0.3–2.1 mm diam., laminal to submarginal, margins smooth to irregularly crenate, ranging from ecoronate (generally the younger and part of the mature ones), to coronate (most of the mature ones), amphithecia smooth, very rarely with some scarce clear bulbs. Disc pale brown, epruinose, imperforate, epithecium 5.0–12.5 µm high, hymenium 40.0–52.5 µm high, subhymenium 15.0–22.5 µm high. Ascospores rounded to subspheric or subellipsoid, 4.5–6.5 (–7.5) × 4.0–5.0 µm, epispore ca. 0.5 µm. Pycnidia scarcer, laminal, immerse, with dark brown ostioles. Conidia baciliform to weakly bifusiform 5.0–8.0 (–10.0) × ca. 0.75 µm.

TLC/HPLC – cortical atranorin, no medullary substances (see also Hale 1976).

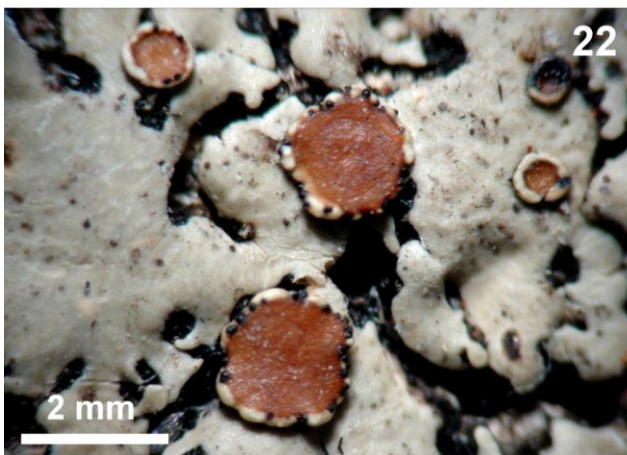
Distribution – South America: Argentina (Ferraro 1981, Adler 1988, 1992), Uruguay (Hale 1976, Osório 1992) and Brazil – Mato Grosso (Lyngé 1914, Kalb 1982, Marcelli 1993), Mato Grosso do Sul (Fleig & Riquelme 1991, Marcelli 1993), and Pará (Brako *et al.* 1985).



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23

**Figs 20–23** – 20 Holotype of *Bulbothrix subklementii* (SP). 21 Lectotype of *Bulbothrix viridescens* (S). 22 Detail of irregularly coronate apothecia on different maturity stages. 23 Isolectotype of *Bulbothrix viridescens* (W). **Scale bars** = 1 cm (20, 21, 23), 1 mm (15), and 2 mm (22).

Additional specimens examined – Brazil, Mato Grosso State, Buriti Municipality, reserva biológica do Colégio Evangélico de Buriti, 600-650 m alt., more or less illuminated hillside woods, leg. M. P. Marcelli 8076a, 8-VII-1980 (SP). Idem, Mato Grosso do Sul State, Aquidauana Municipality, Vila Piraputanga, 20° 27'S, 55° 29'W, 200 m alt., hilltop with ca. 50 m, on shrub cortex, leg. M. Fleig & I. Riquelme 125, 31-V-1990 (ICN). Idem, middle part of the hill, on shrub cortex, leg. M. Fleig & I. Riquelme 147, 31-V-1990 (ICN).

Comments – *Bulbothrix viridescens* is characterized by the narrow sublinear and often sublacinulate laciniae, emaculate upper cortex, cilia with absent, simple or furcate apices, a black lower cortex with brown margins, simple to irregularly branched rhizines without basal bulbs, mixed ecoronate and coronate apothecia containing small rounded or subellipsoid ascospores, and by the absence of medullary substances.

The lectotype (Fig. 21) consists of three fragments in excellent state of collection and preservation, with several apothecia in various stages of maturation. The material is on tree bark that is glued to the voucher. Pycnidia are few and scarce. The isolectotype (Fig. 23) is a fragment slightly smaller than that of the holotype, but it has all the same basic characteristics, and only differs slightly in the measurements of a few characters, and has even fewer pycnidia.

Lynge (1914) mentioned apothecia "that sometimes were apparently a little pycnidiate" (*interdum conceptacle pycnoconidiorum incospicuis*). What was verified in the material was the appearance of ciliar bulbs at the margins of some of the apothecia, formed from a slow process of coronation, in which only some of the younger and in general those mature tend to have coronate margins (Fig. 22). This was the only species which had both ecoronate and coronate apothecia on a same thallus (Benatti 2010).

The irregular coronation of apothecia of *B. viridescens* was commented on only by Marcelli (1993) who mentioned that Hale (1976) said that "the pycnidia in the corona of the apothecia present a more irregular distribution than in *B. coronata* (Fée) Hale"

(G!), which actually features a coronation usually more dense and with a regular format.

As described by Lynge (1914), the conidia are bacilliform or weakly bifusiformes subtle (he was the first author to notice this feature in a species of *Bulbothrix*). The ascospores and conidia measurements found in the type material and the other specimens studied are equivalent to those mentioned by the author.

Hale (1960, 1976) mentioned that a K+ reaction occurs in the medulla. This reaction was occasionally observed, and as mentioned by Lynge (1914), it is likely that the K+ yellow reaction is caused by the presence of atranorin on the upper parts of the medulla, since no other substance can be detected by chromatography.

Specimens cited by Ferraro (1981) and Adler (1988) attributed to *B. viridescens* from Argentina can possibly in truth be specimens of *B. laeviuscula* (Räsänen) Benatti & Marcelli (H!), since the descriptions of these specimens differs from the characteristics of *B. viridescens* at various points.

The material examined by Adler (1988) differs from *B. viridescens* presenting larger laciniae on average (ca. 1.0– 2.0 mm wide), the upper surface with frequent ciliar bulbs, dichotomously branched rhizines with basal bulbs, apothecia commonly with ciliar bulbs on the amphithecia and larger ascospores (6.0–10.0 × 4.0–7.0 µm).

Due the similarity with the Uruguayan specimens of *B. laeviuscula* and the characteristics observed in the type material, it is possible that the specimens mentioned by Adler (1988) in her doctoral as *B. viridescens* are in truth *B. laeviuscula*. This can be based on the laminal bulbs which were observed by Adler (1988). Dr. Adler informed that she was not able to locate the material again due to changes in the herbarium of the University of Buenos Aires.

According to the description of Ferraro (1981), her material has broader laciniae (1.0–3.0 mm wide) with rounded apices, frequently furcate cilia, which are often more abundant in the axils of the laciniae, although the apothecia were cited as urceolate and coronate. The author also described the thallus as yellowish, and dark when herborized, while



the specimens examined here have grayish olive green, and are clear when herborized.

Marcelli (1993) noted that *B. viridescens* is often found in thin twigs. The author cited a slightly larger ascospore size ( $6.3\text{--}7.2 \times 3.6\text{--}4.5 \mu\text{m}$ ) than that found on the holotype, and although these are only occasionally slightly larger, there are no other significant differences between the specimens.

For a comparison with *Bulbothrix bulbochaeta* see treatment under *B. bulbochaeta* in this manuscript.

*Bulbothrix laeviuscula* differs by the constant presence of laminal and amphithecial ciliar bulbs, although the apothecia do not present a regular corona in the margins of the amphithecia, and by the ellipsoid and larger ascospores ( $5.0\text{--}9.0 \times 4.0\text{--}5.5 \mu\text{m}$ ).

*Bulbothrix coronata* was compared to *B. viridescens* by Marcelli (1993). It can be distinguished by the branched cilia and rhizines, larger ascospores of similar size to those of *B. laeviuscula* ( $6.0\text{--}10.0 \times 4.0\text{--}5.0 \mu\text{m}$ ), regularly coronate apothecia, and by the presence of medullary gyrophoric acid. Marcelli (1993) also commented on *B. viridescens* have a more adpressed, delicate thallus, also with a more irregular branching pattern, compared to *B. coronata*.

*Bulbothrix semilunata* (Lynge) Hale (S!) differs from *B. viridescens* by the very narrow laciniae (ca. 0.2–0.5 mm wide), more branched cilia and rhizines, and by the formation of always coronate apothecia, containing much larger ( $12.0\text{--}23.0 \times 3.0\text{--}4.0 \mu\text{m}$ ) and bicornute ascospores, with a crescent or sigmoid shape.

### *Nomen Inquirendum*

*Parmelia stenophyllizans* Zahlbruckner, Catalogus Lichenum Universalis vol. 6: 75. 1930. Fig. 24  
MB 398302

Synonym – *Parmelia stenophylla* Müller Argoviensis. Bulletin de la Société royale de Botanique de Belgique 32: 128. 1893. [The name given by Müller Arg. was a homonym of *Parmelia stenophylla* (Acharius) Heugel, nowadays *Xanthoparmelia stenophylla* (Acharius) Ahti & Hawksworth].

Holotype – Costa Rica, Boruca, leg. Pittier 5434 (G!).

Thallus linear laciniate, pale greenish grey in the herbarium, fragments up to 0.8 cm wide, subcoriaceous, corticolous (anatomic data not taken due the condition of the specimen). Laciniae isotomically to anisotomically dichotomously branched, 0.1–0.3 (–0.4) mm wide, contiguous, very adnate and strongly adpressed, with flat, truncate or acute apices, the margin flat, smooth to subirregular, entire, slightly sublacinulate, axils oval to rounded. Upper surface smooth and continuous, laminal ciliary bulbs absent. Adventitious marginal to submarginal lacinules scarce on older parts, short, simple, flat,  $0.05\text{--}0.30 \times 0.05\text{--}0.10 \text{ mm}$ , truncate or acute, lower side concolor with the lower marginal zone. Maculae absent. Cilia black, initially simple or double, soon turning dichotomously or irregularly branched,  $0.05\text{--}0.20$  (–0.30)  $\times$  ca. 0.03 mm, with semi-immersed to sessile basal bulbs ca. 0.05–0.10 mm wide, abundant along the margins and contiguous, to absent or scarce on the laciniae apices. Medulla white. Soredia, Pustulae and Isidia absent. Lower surface black, shiny, smooth, densely rhizinate. Marginal zone dark brown almost indistinct from the center, attenuated, ca. 0.1–0.2 mm wide, shiny, smooth, rhizinate. Rhizines black to dark brown, initially furcate, soon turning subdichotomously or irregularly branched, usually with subtle basal bulbs,  $0.10\text{--}0.20 \times 0.03\text{--}0.05 \text{ mm}$ , abundant almost like a tomentum, evenly distributed. Apothecia and Pycnidia not found.

TLC/HPLC – cortical atranorina (K+ yellow), no medullary substances (see also Hale 1976).

Distribution – Central America: Costa Rica (Müller Argoviensis 1893, Zahlbruckner 1930, Hale 1976).

Comments – Due to the very bad conditions of the material is virtually impossible to know to what species it belongs. The specimen is a very little developed thallus, without apothecia, pycnidia and appears that was beginning to form some uncertain kind of propagule (maybe isidia or lacinulae). Although very rare, there are some adventitious submarginal and marginal lacinulae. When



**Fig 24** – Holotype of *Parmelia stenophyllizans* (G). **Scale bars** = 1 cm.

young they might look like isidia, but they already have a differentiated lower cortex.

It is a similar case like the type material of *B. suffixa* (Stirton) Hale (BM! GLAM!), which is another meager and problematic specimen (Jungbluth et al. 2008). The type material of *P. stenophyllizans* seems to be quite small lichen, such as the species with bicornute ascospores, but despite the aspect might be of a lacinulate *Bulbothrix* species lacking medullary substances.

In agreement with the comments of Hale (1976) and those left by him in the exsicata, all spot tests result negative. A written label (from Hale, perhaps?) says KC and P negative, no crystals. In this paper he mentioned that C and KC reactions also do not occur. I did not detect K reaction, and even if there were one, it would be impossible to not assign it to traces of atranorin at the upper portion of the medulla, due the thallus being very thin.

According to his comments, Hale (1976) accepted *P. stenophyllizans* as a “*nomen inquerendum*”, due to the impossibility of certification of the specimen. The author stated only that the type material was fragmented and sterile, but had branched cilia with bulbate bases, what was confirmed here. Hale (1976) suspected that *P. stenophyllizans* probably would be a close species or even *B. schiffneri* (medullary gyrophoric acid) or *B. semilunata* (no medullary substances), but could not confirm the identification since there is no apothecia in the material. However, both species do not form any type of propagule, and Hale (1976) apparently did not notice the lacinulae in *P. stenophyllizans*.

The description from Müller Argoviensis (1893) is very short and only gives dimensions and shape of the thalli, with some few comments. He described small rosettes ca. 1 cm wide, with dichotomously branched whitish laciniae ca. 0.5 mm wide, obtuse to acute apices, being slightly adpressed, flat, smooth and shiny, with a densely rhizinate lower cortex and without apothecia. Müller Argoviensis (1893) commented on the light color and the aspect of the laciniae be as those of *Parmelia coronata* Fée [= *B. coronata* (Fée) Hale], believing they were somehow related. Unfortunatelly much data is missing in the description and the material lack characteristics for a better comparison with *B. coronata*, which is a larger lichen and has medullary gyrophoric acid (see comments under this species).

Due to a handwrite note by Müller Argoviensis left with the type material, there are reasons to suspect that the collector of this material was Adolfo Tonduz, who collected botanical material in Costa Rica between the years 1891-92. However, there is no more information about it, and Hale (1976) used the name and collector number of the article from Müller Argoviensis (1893), although these do not appear on the labels of the type material.

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