

## Revision of the genus *Bulbothrix* (*Parmeliaceae*, lichenized Ascomycota) in NE Argentina, with a key to the species

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**Abstract** – Eight species belonging to *Bulbothrix* from NE Argentina were studied. Among them, *Bulbothrix affixa* (Hale & Kurok.) Hale is a new continental record, and five species, viz. *B. cassa* Jungbluth, Marcelli & Elix, *B. coronata* (Fée) Hale, *B. isidiza* (Nyl.) Hale, *B. laeviuscula* (Räsänen) Benatti & Marcelli, and *B. tabacina* (Mont. & Bosch) Hale, are new citations for the country. The specimens are described, commented, and illustrated. A key for species identification is also presented.

**Bulbata cilia / isidia / gyrophoric acid / norstictic acid / salazinic acid**

**Résumé** – Huit espèces appartenant au genre *Bulbothrix* Hale du nord-est de l'Argentine ont été étudiées. Parmi ces espèces, *Bulbothrix affixa* (Hale & Kurok.) Hale représente un nouveau record continental, et cinq espèces, viz. *B. cassa* Jungbluth, Marcelli & Elix, *B. coronata* (Fée) Hale, *B. isidiza* (Nyl.) Hale, *B. laeviuscula* (Räsänen) Benatti & Marcelli, et *B. tabacina* (Mont. & Bosch) Hale, sont de nouveaux records pour le pays. Les spécimens ont été décrits, examinés et illustrés. Une clé pour l'identification des espèces est aussi présentée.

**Bulbata cils / isidies / acide gyrophorique / acide norstictique / acide salazinique**

### INTRODUCTION

*Bulbothrix* Hale is a tropical-subtropical to temperate genus, with currently 60 known accepted names (Benatti, 2011b, 2012a-d, 2013a-c, 2014; Benatti & Marcelli, 2010; Benatti & Elix, 2012; Bungartz *et al.*, 2013; Zhang *et al.*, 2014) from which about 70% are cited from South America, considered by Hale (1976) as its main center of distribution, followed by South Africa. Until recently only six species (*Bulbothrix coronata*, *B. imshaugii*, *B. regnelliana*, *B. subcoronata*, *B. viatica*, and *B. viridescens*) were cited from Argentina (Hale, 1976; Benatti, 2012a; Michlig & Ferraro, 2012; Benatti, 2013a-c), with *B. imshaugii* cited solely for southern Argentina (Calvelo & Adler, 1999).

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The species of *Bulbothrix* are usually characterized by their small, lacinate and often adnate thalli, simple to branched bulbate marginal cilia and rhizines (Benatti, 2011a), cortical atranorin, smooth to coronate (with bulbs) 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, 1993; Benatti, 2011b, 2012a-b, 2013a-c, 2014; Benatti & Elix, 2012). The medullary chemistry is often represented by some chemosyndromes, notably the gyrophoric, lecanoric, lobaric, norstictic, protocetraric and salazinic acids, with several species containing fatty acids (some still undetermined) or no medullary substances at all (Benatti, 2011b, 2012a-d, 2013a-c, 2014; Benatti & Marcelli, 2010; Benatti & Elix, 2012; Bungartz *et al.*, 2013). Crespo *et al.* (2010) recently revised generic concepts of parmelioid lichens based on molecular, morphological and chemical data finding that *Bulbothrix* as currently defined is nested in the *Parmelina* clade, and is a paraphyletic genus as some species (mainly from the salazinic chemical group) are grouped within *Parmelinella*.

Northeastern Argentina is located towards the south of the Neotropical region defined by Morrone (2006), between  $22^{\circ}28'39.28''\text{-}30^{\circ}47'26.15''\text{S}$  and  $53^{\circ}35'46.88''\text{W}$ , with a subtropical climate. Its geographical location confers a high ecoregional diversity, varying its vegetation from subtropical forests to the east (Paranaense Forest) to xerophytic forest to the west (Chaco and Espinal) (Brown *et al.*, 2006). This paper adds six species as new citations from Argentina, one of them being cited for the first time for the Americas. The range within Argentina is expanded of two species which were just recently cited for the country (Benatti, 2012a; Michlig & Ferraro, 2012).

## MATERIAL AND METHODS

Morphological characters were studied using standard stereoscopic (Leica MZ6) and compound light microscopes (Leica CME). Anatomical sections were made using a razor blade by hand. The lichen substances were initially checked by spot tests with 10% potassium hydroxide (K), sodium hypochlorite (C) and paraphenylenediamine (P), and also examined under UV light (360 nm). Chemical constituents were identified by thin-layer chromatography (TLC) using solvent C (Bungartz, 2001), following standard methods described in Elix & Ernst-Russell (1993) and Orange *et al.* (2010). In addition, microcrystalization was performed on some specimens with GAW and GE for detection of gyrophoric acid (Orange *et al.*, 2010).

## RESULTS

### Key for the *Bulbothrix* species in NE Argentina

- |   |                 |
|---|-----------------|
| 1a. Upper surface with isidia.....  | 2               |
| 1b. Upper surface without isidia.....   | 4               |
| 2a. Thalli containing no medullary substances.....  | <i>B. cassa</i> |
| 2b. Thalli containing medullary salazinic acid (K+ yellow <sup>®</sup> dark red, P+ yellow) ..... | 3               |

- 3a. Lower cortex brown overall ..... *B. isidiza*  
 3b. Lower cortex black, with brown to black margins ..... *B. tabacina*  
 4a. Thalli with medullary norstictic acid (K+ yellow<sup>®</sup> reddish orange, P+ orange) ..... 5  
 4b. Thalli without medullary substances (C-, KC-) or with gyrophoric acid (C+ rose, KC+ rose) ..... 6  
 5a. Upper cortex always devoid of laminal ciliary bulbs; cilia often lacking the apical portion or with very short apices; ascospores (5-) 6-10 × 4-6 μm .....  
 ..... *B. regnelliana*  
 5b. Upper cortex often with laminal ciliary bulbs; cilia commonly bearing simple apices; ascospores (10-) 12-15 × 6-10 μm ..... *B. viatica*  
 6a. Medulla without substances; apothecia with ecoronate margin, but bearing tiny bulbs ..... *B. laeviuscula*  
 6b. Medulla with gyrophoric acid; apothecia truly coronate ..... 7  
 7a. Lobes 0.7-1.6 mm wide; conidia weakly bifusiform to bacilliform 5-7 μm long .....  
 ..... *B. coronata*  
 7b. Lobes sublinear to linear, 0.4-0.75 (-1) wide, conidia bacilliform, 7-9 μm long .....  
 ..... *B. affixa*

*Bulbothrix affixa* (Hale & Kurok.) Hale, Phytologia 28: 480 (1974)

**Figs 1-3**

*Thallus* foliose, mineral gray, submembranaceous, corticolous, moderate to tightly adnate to substrate, 1.2-3 cm in diameter; lobes sublinear to linear, anisotomically dichotomously to irregularly branched, 0.4-0.75 (-1) mm wide, contiguous to slightly imbricate, with subtruncate apices; margin entire, with bulbate cilia; cilia abundant, evenly distributed, with simple to bifurcate apices, laminal ciliary bulbs absent. *Upper surface* shiny, smooth, sometimes with some irregular cracks, emaculate. *Isidia*, *soralia*, *pustules* and *dactyls* absent. *Medulla* white. *Lower surface* black, densely rhizinate, with a narrow dark brown marginal zone, shiny, rhizinate; rhizines simple to dichotomously branched, up to 3 times branched, black towards the base turning brownish towards the apices, evenly distributed. *Apothecia* abundant, plane to slightly convex, 0.4-3.2 mm in diameter, sessile, submarginal, margin coronate, entire to crenate; amphithecium with bulbate cilia, with or without apices, disc imperforate, dark brown, epruinose; ascospores ellipsoid to ovoid, (7-) 8-10 × 4-5 μm. *Pycnidia* scarce, submarginal; conidia bacilliform, 7-9 μm long.

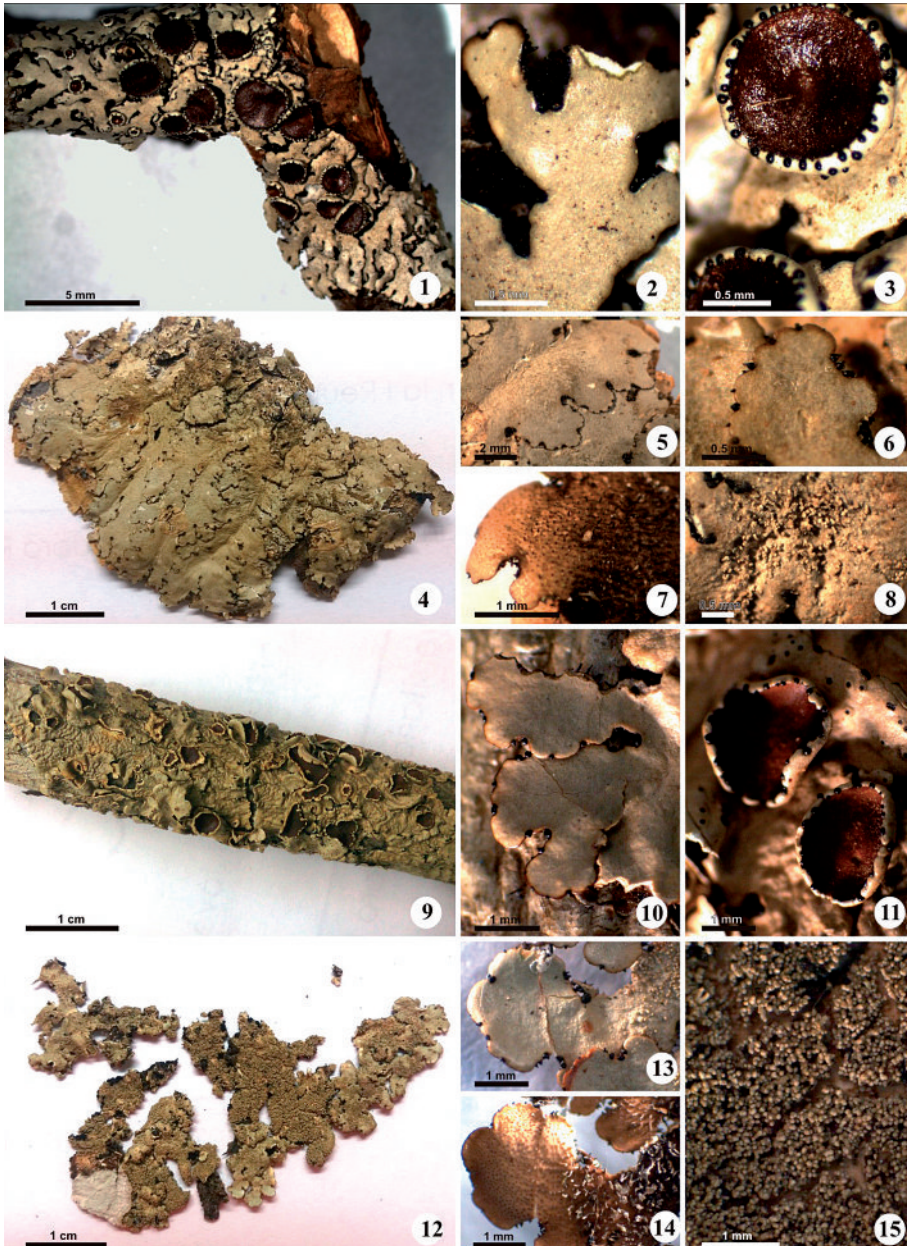
*Spot reactions*: Upper cortex K+ yellow, UV-; medulla K-, C+ rose, KC+ rose, P-, UV-.

*TLC*: Cortical atranorin; medullary gyrophoric acid.

*Specimen examined*: ARGENTINA, Formosa, Bermejo, provincial route N° 9, between Sumayén and El Aibal, 24° 22' 0,5" S 61° 38' 19,7" W, 169 m, in an *Aspidosperma quebracho-blanco* forest, 18 April 2009, A. Michlig, N. Niveiro, L. Ferraro & O. Popoff 1139 (CTES).

*Distribution*: This is a rare species recorded so far only from Africa (Hale & Kurokawa, 1964; Hale, 1976). This is therefore its first report outside this continent, and its first record for the Americas.

*Comments*: *Bulbothrix affixa* is a species with few specimens cited in literature, making it difficult to estimate its morphological variation. The material studied is akin to *B. affixa* in having bulbate cilia with simple to bifurcate apices,



Figs 1-15. 1-3. *B. affixa*: 1. Complete thallus. 2. Lobes with bulbate cilia. 3. Coronate apothecia. 4-8. *B. cassa*: 4. Complete thallus. 5. Maculate upper surface. 6. Lobe with bulbate cilia. 7. Lower surface. 8. Isidia. 9-11. *B. coronata*: 9. Complete thallus. 10. Lobes with bulbate cilia. 11. Coronate apothecia. 12-15. *B. isidiza*: 12. Complete thallus. 13. Lobes with bulbate cilia. 14. Lower surface. 15. Isidia.



and predominantly dichotomous rhizines. In this specimensn the rhizines have a black base which turns brownish and are becoming dichotomous, while in the type material of *B. affixa* they are always black and simple to partially furcate (Benatti, 2013c).

There are few *Bulbothrix* species with similar characters. The most morphologically similar to *B. affixa* is *Bulbothrix silicisrea* Benatti, a saxicolous species recently described from Brazil (Benatti, 2012a). Both species share the coronate apothecia, simple to partially furcate cilia and rhizines, medullary gyrophoric acid (C+ rose, KC+ rose) and the absence of vegetative propagules. *Bulbothrix silicisrea* is characterized by the presence of narrow lobes (0.2-0.6 mm), small rounded to ellipsoid ascospores (5-7 × 4-5 µm), conidia 5-6 µm long, and a saxicolous habit, characters which distinguished it from *B. affixa* (Benatti, 2012d). The material studied of *B. affixa* is corticolous, and has simple to dichotomously branched rhizines, and conidia 7-9 µm long, thus differing from *B. silicisrea*.

Another related species is *Bulbothrix coronata* (Fée) Hale which, just as *B. affixa* and *B. silicisrea*, lacks vegetative propagules and has medullary gyrophoric acid and narrow lobes (0.5-0.9 mm) (Benatti, 2013c). The specimen studied was at first identified as this species, as there are some characters shared. *Bulbothrix coronata* was reported as having black rhizines with brownish apices, which are dichotomously to irregularly branched (Benatti, 2013c), partially coinciding with the material studied are similar in the specimen studied, where the rhizines are simple to dichotomously branched. The cilia apices are also branched being initially simple to furcate, then turning very dichotomous and irregularly branched in *B. coronata* (Benatti, 2013c), and bifurcate in this sample.

***Bulbothrix cassa* Jungbluth, Marcelli & Elix, Mycotaxon 104: 52 (2008) Figs 4-8**

*Thallus* foliose, submembranaceous, corticolous, tightly attached to substrate, 6 cm in diameter; lobes sublinear, irregularly branched, 0.75-1.23 mm wide, contiguous to slightly imbricate, with subrounded apices; margin crenate, with bulbate cilia; cilia distributed mainly in lobe axils or in margin incisions, mainly with apices, simple to rarely bifurcate, completely black or occasionally with brownish to whitish apices. *Upper surface* shiny, smooth to rugose at the center of the thallus, continuous, mostly irregularly cracked, weakly to distinctly maculate; maculae punctiform to irregular, laminal. *Isidia* simple, granular to cylindrical, eciliate, rarely with brownish apices, generally caduceous, partially pycnidiate. *Soralia*, *pustules* and *dactyls* absent. *Medulla* white. *Lower surface* black towards the center, turning dark brown to pale brown, smooth to slightly rugose, shiny, moderately rhizinate, with a pale brown to dark brown, rarely ivory, marginal zone, smooth to slightly rugose, with a narrow zone to the margin not rhizinate, soon papillate; rhizines simple, brown to rarely black, evenly distributed, mostly without basal bulbs. *Apothecia* absent. *Pycnidia* scarce; ostiole brown; conidia bifusiform, 5-6 µm long.

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

*TLC*: Cortical atranorin; medullary substances absent.

*Specimen examined*: ARGENTINA, Misiones, Iguazú, Iguazú falls, in the way to Garganta del Diablo, 2 March 1982, L. Ferraro 2570 (CTES).

*Distribution*: This is an exclusively South American species, known so far only from several localities from São Paulo State in Brazil (Jungbluth *et al.*, 2008; Benatti, 2013b). This is its first report for Argentina.

*Comments:* The isidia in *B. cassa* were initially described as having bulbs (Jungbluth *et al.*, 2008), although when studying the holotype, Benatti (2013b) determined that they are actually pycnidia. This uncommon characteristic is also present in *B. papyrina* (Fée) Hale, which is distinguished by an emaculate upper surface, isidia tortuous when mature, and medullary gyrophoric acid (Benatti & Elix, 2012). Pycnidia were not present over cortex in the material studied.

The material studied has cilia predominantly with apices, which are simple to rarely bifurcate, completely black or occasionally with brownish to whitish apices, partially differing from those described by Benatti (2013b), where the apices are simple when present. Jungbluth *et al.* (2008) did not describe the cilia apices.

The lower surface *B. cassa* was described as being black in older parts, but for most part various shades of brown, and prominently veined except in the distal portion of some lobules, with bulbate rhizines located in veins, black when mature, with whitish apices when younger (Jungbluth *et al.*, 2008). These characters were not observed, as the lower surface in material studied is smooth to slightly rugose, with evenly distributed rhizines, mostly without basal bulbs.

Among morphologically related species are *B. ventricosa* (Hale & Kurok.) Hale, *B. isidiza* and *B. tabacina*, which differ in having non-pycnidiate isidia and by their medullary chemistry. *Bulbothrix ventricosa* has medullary norstictic acid and also differs in the presence of laminal ciliar bulbs (Benatti, 2012a), while *B. isidiza* and *B. tabacina*, both present in NE Argentina, produce medullary salazinic acid, the former differing also by its brown overall lower surface.

***Bulbothrix coronata*** (Fée) Hale, *Phytologia* 28: 480 (1974)

**Figs 9-11**

*Thallus* foliose, grayish to brownish in herbarium, subcoriaceous, corticolous, moderate to tightly adnate to substrate, 2.5-4 cm in diameter; lobes sublinear to subirregular, irregularly to anisotomically dichotomously branched, 0.7-1.6 mm wide, contiguous, with subtruncate to subrotund apices; margin entire to slightly crenate, with bulbate cilia; cilia abundant, evenly distribute but more frequent at crenae and lobe axils, with simple to bifurcate apices, laminal ciliary bulbs absent. *Upper surface* shiny, smooth to slightly rugose, continuous to irregularly cracked at center, emaculate. *Isidia*, *soralia*, *pustules* and *dactyls* absent. *Medulla* white. *Lower surface* black at center, densely rhizinate, shiny, smooth, continuous, brown at margins; rhizines initially simple, then dichotomously branched, up to 3 times branched, with basal bulbs, black, sometimes turning brownish to whitish towards the apices, evenly distributed. *Apothecia* abundant, plane to slightly concave, 0.8-4 mm in diameter, adnate to sessile, submarginal to laminal, margin coronate, crenate; amphithecia with bulbate cilia, disc imperforate, dark to pale brown, epruinose; ascospores ellipsoid to ovoid, 6-10 × 4-5 μm. *Pycnidia* abundant, submarginal to laminal, with black ostioles; conidia weakly bifusiform to bacilliform 5-7 μm long.

*Spot reactions:* Upper cortex K+ yellow, UV-; medulla K-, C+ rose, KC+ rose, P-, UV-.

*TLC:* Cortical atranorin; medullary gyrophoric acid.

*Specimens examined:* ARGENTINA, Corrientes, San General Paz, route 5, Lomas de Vallejos, on fallen tree in "quebrachal", 24 November 1978, A. Schinini *et al.* 16178 (CTES).

*Distribution:* Africa and America (Hale, 1976; Benatti, 2013c), being recorded in South America from Brazil and Paraguay, and erroneously from Argentina (see comments), therefore its presence in this country is here confirmed.

*Comments:* Cilia in material studied have simple to bifurcate apices, partially differing from those describe by Benatti (2013c), who reported cilia which soon become densely dichotomously or irregularly branched.

This species was recorded from Argentina by Osorio (1981), who listed the lichen species until then cited from Misiones province, including *B. coronata* citing as reference the monograph of the genus made by Hale (1976). In this monograph, it is not recorded from Argentina, actually *B. subcoronata* is the species cited on the page mentioned by Osorio (1981).

***Bulbothrix isidiza* (Nyl.) Hale, Phytologia 28: 480 (1974)**

**Figs 12-15**

*Thallus* foliose, mineral gray to greenish gray, brownish in herbarium, subcoriaceous, corticolous, moderate to loosely attached to substrate, 3-5 cm in diameter; lobes sublinear, irregularly branched, 0.9-3 mm wide, contiguous to slightly imbricate, with rounded apices; margin entire to crenate, with bulbate cilia; cilia distributed mainly in lobes axils and in margin incisions, generally with apices, simple to bifurcate. *Upper surface* shiny, smooth, continuous or sometimes with irregular cracks at the center, weakly maculate; maculae punctiform, laminal. *Isidia* abundant, simple to 1-3 branched, sometimes with pale brown apices, eciliate, caducous. *Soralia*, *pustules* and *dactyls* absent. *Medulla* white. *Lower surface* brown, moderately rhizinate, shiny, smooth, continuous, with a narrow naked margin, soon becoming papillate; rhizines simple, black to grayish or brown, occasionally with basal bulbs, evenly distributed, sometimes more abundant in some areas. *Apothecia* and *pycnidia* absent.

*Spot reactions:* upper cortex K+ yellow, UV-; medulla K+ yellow turning dark red, C-, KC-, P+ yellow, UV-.

*TLC:* Cortical atranorin; medullary salazinic and consalazinic acids.

*Specimens examined:* ARGENTINA, Misiones, San Ignacio, Teyú Cuaré Provincial Park, De la Selva trail, near the entry of the path to Parquizado Inferior, 23 May 2009, A. Michlig & N. Niveiro 1747 (CTES); San Pedro, Yaboty Biosphere Reserve, Moconá Provincial Park, surroundings of the Biological Substation Marcio Ayres, 27° 9' 13''S 53° 54' 0,4''W, 318 m, 17 May 2008, A. Michlig, N. Niveiro, A. Cabaña Fader & R. Salas 966a (CTES); idem, surroundings of the Camping area, 27° 9' 13,1''S 54° 54' 5,2''W, 333 m, 12 December 2012, A. Michlig, S. Jimenez, N. Niveiro & Á. Vega 2842 (CTES).

*Distribution:* Known to all continents, except Antarctica and Europe (Hale, 1976; Benatti, 2013a). In South America, it was recorded for Brazil (Brako *et al.*, 1985; Eliasaro & Adler, 1997; Eliasaro, 2001; Fleig & Grüniger, 2000a-b; Fleig & Riquelme, 1991; Hale, 1976, Jungbluth, 2006; Marcelli, 1991, 1993; Pereira & Marcelli, 1989), Chile (Galloway & Quillhot, 1998), Paraguay (Hale, 1976), and Venezuela (López Figueiras, 1986; Marcano *et al.*, 1996). It is here recorded for the first time from Argentina.

*Comments:* Apothecia and pycnidia were not present in the specimens studied, but a detailed description of these structures can be found in Benatti (2013a). Chen *et al.* (2009) mentioned ascospores 15-18 × 7-11 µm for this species, while Elix (1994), Hale (1976), Kurokawa & Lai (2001), and Nash & Elix (2002) cited smaller ascospores of 7-14 × 5-8 µm. Benatti (2013a) found ascospores 10-16 (-17.5) × 5-9 µm, even in the type material, which indicates that there is more variation in ascospores sizes in one specimen than usually reported. Pycnidia are rarely found, cited by Elix (1994) as having bacilliform to slightly bifusiform conidia, 5-6 µm long.

All studied specimens were found on bark, although Elix (1994) cited that this species may also be found over rocks, which could not be confirmed by Benatti (2013a), who located only corticolous specimens. Some authors mentioned the lobes as 2-6 mm wide (Elix 1994; Kurokawa & Lai 2001), whereas in the material studied they are slightly narrower (0.9-3 mm), similar to those described by Eliasaro (2001), Hale (1976), and Jungbluth (2006), who mentioned a lobe width up to 4 mm for this species. Benatti (2013a) found measurements within 1.5-5.5 mm.

Among the isidiate species containing salazinic acid, *B. tabacina* (Mont. & Bosch) Hale, also found in Northeastern Argentina, differs in having a black lower surface, while *B. australiensis* Hale, a rare species apparently endemic to Australia, differs by its narrower (0.8-2 mm), more sublinear lobes with  $\pm$  truncate apices, emaculate upper surface, robust, larger, and often simple cylindrical isidia (Benatti 2013a). As seen by Benatti (2013a) some specimens of *B. isidiza* may eventually develop bulbate bases on some of their rhizines, although not all specimens appear to have them and their mechanism or appearance is yet not clear (Elix, 1994; Benatti, 2013a).

*Bulbothrix cassa* Jungbluth, Marcelli & Elix, recently described from Brazil, can be distinguished by the negative reactions in the medulla (Jungbluth *et al.*, 2008; Benatti, 2012b). *Bulbothrix ventricosa* (Hale & Kurok.) Hale can be differentiated by the variably colored lower cortex (often mottled black brown or with different tinges of brown), firm isidia, coronate apothecia, and the presence of medullary norstictic acid in the medulla (Hale & Kurokawa, 1964; Benatti, 2012b).

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

**Figs 16-18**

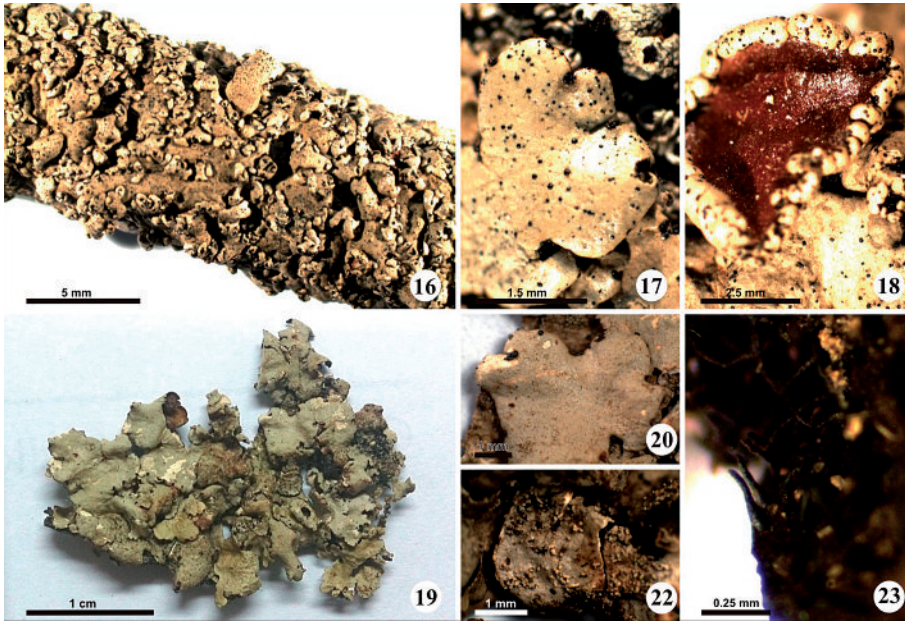
*Thallus* foliose, mineral gray, subcoriaceous, corticolous, moderately attached to substrate, 6 cm in diameter; lobes sublinear, sometimes elongated, irregularly branched, 0.5-1 mm wide, contiguous to slightly imbricate, with subtruncate to truncate apices; margin entire to crenate, with bulbate cilia, developing lobules; cilia distributed mainly in crenae and lobe axils, small (0.05-0.1  $\times$  0.05 mm), generally with simple apices, sometimes brownish. *Upper surface* shiny, smooth to slightly rugose at the center, irregularly cracked, with abundant small laminal bulbs (0.025-0.075 mm), emaculate, with abundant lobules, originated mostly from lobe margins, occasionally adventitious. *Isidia*, *soralia*, and *pustules* absent. *Medulla* white. *Lower surface* black at the center, soon becoming dark brown, shiny, smooth, densely rhizinate, with a narrow pale brown rhizinate margin; rhizines at first simple near the marginal zone, soon becoming dichotomously branched (branched 1-2 times), sometimes trifurcate, completely black or with brownish apices, sometimes at the lobe tips, sometimes completely brown at lobe tips, with basal or displaced bulbs. *Apothecia* slightly concave to plane, 1-5 mm in diameter, sessile, laminal, with a crenate, irregularly coronate margin bearing tiny bulbs, amphithecia also with bulbs, disc imperforate, dark brown, epruinose; ascospores ovoid to ellipsoidal, sometimes subglobose, 7-9  $\times$  5-7  $\mu$ m. *Pycnidia* absent.

*Spot reactions*: Upper\_cortex K+ yellow, UV-; medulla K+ yellow turning dark red, C-, KC-, P-, UV-.

*TLC*: Cortical atranorin; no medullary substances.

*Specimen examined*: ARGENTINA, Corrientes, Monte Caseros, Uruguay River, Itacumbú island, in front of Monte Caseros, eastern shore of the island, 20 August 1977, J. Irigoyen 444 (CTES).





Figs 16-23. **16-18.** *B. laeviuscula*: **16.** Complete thallus. **17.** Lobes showing laminal bulbs. **18.** Apothecia bearing tiny bulbs at margin. **19-23.** *B. tabacina*: **19.** Complete thallus. **20.** Lobes with bulbate cilia. **21.** Isidia. **23.** Rhizines with basal bulbs.

*Distribution:* This is an exclusively South American species, supposed to be common, maybe endemic, to the Pampas region, recorded for Brazil and Uruguay (Benatti, 2012c). Here it is recorded for the first time from Argentina.

*Comments:* In the material studied, no pycnidia were found. In the specimens cited by Benatti (2012c), they are common and the conidia are bacilliform to slightly bifusiform, 5-9  $\mu\text{m}$  long, as common for the genus. The material described by Benatti (2012c) has simple to furcate or irregularly branched rhizines, while in the single specimen found by us they are often dichotomously or trichotomously branched, branched up to 2 times.

This species was previously considered as a synonym of *B. viridescens* (Lyngé) Hale (Hale, 1976), sharing characteristics as narrow lobes, small adventitious marginal lacinules over the margins, and the absence of medullary substances, but clearly differs in the absence of laminal and amphithecial bulbs, and the consistent slightly smaller ascospores of  $4.5\text{-}6 \times 4\text{-}5 \mu\text{m}$  (Benatti, 2012c, 2013b). It was recorded for Argentina (Adler, 1988; Ferraro, 1981) The specimen cited by Ferraro (1981) from NE Argentina was studied by us, but it does not belong to *Bulbothrix* because its cilia are not truly bulbate.

The apothecia in *B. laeviuscula* are not regularly coronate; they would be considered as ecoronate, as the laminal and amphithecial bulbs are so numerous that they reach the apothecia rim from the amphithecia development, instead of being regularly formed like the marginal bulbate cilia on the truly coronate species.

*Bulbothrix bulbochaeta* (Hale) Hale is another similar species with commonly laminal ciliary bulbs, differing from *B. laeviuscula* by the wider lobes (1-2.5 mm), a much thicker thallus, and the evident subdichotomous branching

pattern of ramification of the cilia and rhizines. As in *B. viridescens*, it also differs by the smaller and more rounded ascospores  $4\text{-}6 \times 4\text{-}5 \mu\text{m}$  (Benatti, 2013b).

Other similar species with narrow lobes are *B. pseudocoronata* (Gyeln.) Benatti & Marcelli and *B. caribensis* Marcelli & Benatti. Both differ from *B. laeviuscula* by the presence of truly coronate apothecia and development of laminal lacinules/lobules with vegetative propagule functionality. The former also differ in having medullary gyrophoric acid (Benatti, 2012a), while the latter differs by its upper surface without laminal ciliary bulbs, and the frequent to abundant laminal lobules (Benatti, 2011b). *Bulbothrix lopezii* Hale has much larger lobes (1-3 mm wide), ecoronate apothecia and has medullary fatty acids (Hale, 1976; Benatti, 2013b).

***Bulbothrix regnelliana*** Jungbluth, Marcelli & Elix, Mycotaxon 104: 58 (2008)

*Thallus* foliose, mineral gray, subcoriaceous, corticolous, moderately attached to substrate, 4-8 cm in diameter; lobes sublinear, irregularly branched, 1-2 mm wide, partially overlapped, with rounded apices; margin entire to crenate, with bulbate cilia; cilia distributed mainly in lobe axils and margin incisions, without apices or with a very short apex. *Upper surface* shiny, smooth to rugose at the center, predominantly continuous, with some irregular cracks at the center, emaculate, without laminal bulbs. *Isidia*, *soralia* and *pustules* absent. *Medulla* white. *Lower surface* dark to pale brown, moderately rhizinate; rhizines simple, black to brown, rarely with whitish tips, with basal bulbs, evenly distributed. *Apothecia* abundant, cupuliform to plane, 0.5-5.2 mm in diameter, adnate to sessile, laminal, margin coronate, entire to crenate, amphithecia smooth; disc imperforate, dark to pale brown, epruinose; ascospores ellipsoid to subspherical, (5-) 6-10  $\times$  4-6  $\mu\text{m}$ . *Pycnidia* abundant, laminal; conidia bifusiform, 5-8  $\mu\text{m}$  long.

*Spot reactions*: Upper\_cortex K+ yellow, UV-; medulla K+ yellow turning bright orange to reddish orange, C-, KC-, P+ orange, UV-.

*TLC*: Cortical atranorin; medullary norstictic and conorstictic acids.

*Specimens examined*: ARGENTINA, Corrientes, Capital, 500 mts of Route 12, on the way to Santa Ana, in "quebrachal", 5 July 1978, L. Ferraro 1287 (CTES); Concepción, Paso Crucecita, 20 June 1974, L. Ferraro 204 (CTES); Esquina, 10 km of route 126, on the way from Tres Bocas to Paso Yunque, 13 March 1975, A. Krapovickas 28074 (CTES); Ituzaingó, Ituzaingó toll station, 27° 34' 43,2" S 56° 37' 08" W, on *Melia azedarach*, 30 November 2009, J. M. Rodríguez, A. Fazio & B. Moncada s/n (CTES); idem, National Route N° 12, 10 km to the limit with Misiones province, 27° 29' 42,4" S 56° 5' 57,38" W, 163 m, over a fence post, 18 June 2011, A. Michlig & N. Niveiro 2568, 2572 (CTES); San Cosme, Paso de la Patria, San Juan stream and Paraná River, in marginal forest, 4 March 1979, L. Ferraro et al. 1634 (CTES); Formosa, Bermejo, provincial route N° 9, between Sumayén and El Aibal, 24° 22' 0,5" S 61° 38' 19,7" W, 169 m, in an *Aspidosperma quebracho-blanco* forest, 18 April 2009, A. Michlig, N. Niveiro, L. Ferraro & O. Popoff 1125 (CTES); Jujuy, Dpto. Ledesma, 10-20 km from Libertador Gral. San Martín, on the way to Valle Grande, 8 November 1974, L. Ferraro & A. Schinini 574 (CTES).

*Distribution*: So far known only exclusively from South America, recorded from Brazil (Jungbluth *et al.*, 2008; Benatti, 2012a) and recently from Argentina, where it was cited for Corrientes province (Benatti, 2012a; Michlig & Ferraro, 2012). Its distribution is here extended to Formosa and Jujuy provinces.

*Comments:* The only species closely related to *Bulbothrix regnelliana* are *B. viatica* Spielmann & Marcelli and *B. subcoronata* (Müll. Arg.) Hale, sharing the absence of vegetative propagules, presence of coronate apothecia and medullary norstictic acid. Both can be differentiated by the larger size of their ascospores and the color of the lower surface, which appears to be constantly brown in *B. regnelliana*. According to Benatti (2012a) the absence of laminal ciliary bulbs is another character which distinguished it from the others, being eventually formed only on specimens of *B. viatica*, in variable quantities.

*Bulbothrix regnelliana* and *B. viatica* have many characters in common. According to Benatti (2012a), the more trustworthy difference between these species is the size of the ascospores, which in the former hardly exceed 12 µm, while in the latter rarely they are smaller than 12 µm long. *Bulbothrix viatica* often devolves laminal ciliary bulbs, that Benatti (2012a) mentioned as being scarce to frequent (their appearance in the specimens is probably conditioned to a combined effect triggered by thallus maturity stage and environmental stimuli).

Also as cited by Benatti (2012a) and seen in the material studied, the constantly absence of apices in cilia (or sometimes the presence of very short, not branched apices) in *B. regnelliana* is characteristic, while in *B. viatica* the cilia apices are frequent and longer.

*Bulbothrix subcoronata* differs from *B. regnelliana* in having much narrower lobes (ca. 0.5-1 mm wide), a black lower surface, and even smaller ascospores, rarely surpassing 7 × 5 µm (Benatti 2012a). In Argentina, Ferraro (1981) cited this species from Corrientes province. This material was examined, and actually belongs to *B. regnelliana*. According to the size of the ascospores cited by Hale (1976) the material cited by this author as *B. subcoronata* from Misiones province might also belong to *B. regnelliana*.

***Bulbothrix tabacina* (Mont. & Bosch) Hale, Phytologia 28: 481 (1974) Figs 19-23**

*Thallus* foliose, greenish gray, subcoriaceous, corticolous, moderate to tightly attached to substrate, 2-8 cm in diameter; lobes sublinear, irregularly branched, 1.3-2 mm wide, contiguous to slightly imbricate, with rounded apices; margin entire to slightly crenate, with bulbate cilia; cilia distributed along the margins, mainly in the crenae and lobe axils, where there might be up to 2 contiguous bulbs, with or without apices, simple to rarely bi or trifurcate. *Upper surface* shiny, smooth, continuous to irregularly cracked, weakly maculate; maculae punctiform, laminal. *Isidia* simple, with apices concolorous with the thallus, without cilia, caduceous. *Soralia* and *pustules* absent. *Medulla* white. *Lower surface* black, shiny, smooth, continuous, moderately rhizinate, with a narrow pale brown margin, shiny, smooth to rugose, naked or with short rhizines; rhizines simple, rarely with a thickened basal portion, black, rarely with whitish apices, evenly distributed to rarely irregularly distributed. *Apothecia* and *pycnidia* absent.

*Spot reactions:* upper cortex K+ yellow, UV-; medulla K+ yellow turning dark red, C-, KC-, P+ yellow, UV-.

*TLC:* Cortical atranorin; medullary salazinic and consalazinic acids.

*Specimens examined:* ARGENTINA, Misiones, Guaraní, Yaboty Biosphere Reserve, Caá-Yarí Provincial Park, surroundings of the house of forest rangers, 26° 52' 19,6" S 54° 13' 33,8" W, 526 m, 27 March 2010, A. Michlig, N. Niveiro & O. Popoff 2479 (CTES); San Pedro, Yaboty Biosphere Reserve, Moconá Provincial Park, Piedra Bugre pier, 27° 9' 13" S 53° 54' 4" W, 16 May 2008, A. Michlig,

N. Niveiro, A. Cabaña Fader & R. Salas 884, 984, 990, 997 (CTES); idem, 100 m before Centro de Visitantes, 25 May 2009, A. Michlig & N. Niveiro 1839, 1851 (CTES).

*Distribution:* Known from all continents, except Antarctica and Europe (Hale, 1976; Benatti, 2013a). In South America, it has been recorded from Brazil (Benatti, 2013a; Canêz, 2005; Hale, 1976; Jungbluth, 2006; Marcelli, 1990, 1991, 1993; Osorio, 1989a), Chile (Galloway & Quilhot, 1998), Guyana (Feuerer, 2012), Uruguay (Osorio, 1989b, 1992), and Venezuela (Hale, 1976; López-Figueiras, 1986). It is here recorded for the first time from Argentina.

*Comments:* As in *B. isidiza*, some authors mentioned an emaculate upper surface (Jungbluth, 2006), while others mentioned it as weakly maculate, with punctiform and laminal maculae (Canêz, 2005; Chen *et al.*, 2009). Benatti (2013a) confirmed it as always maculate, ranging from weakly (the commonest) to sometimes more distinctly maculate. The species is described as moderately rhizinate (Hale, 1976; Benatti, 2013a), as is the material studied, although Chen *et al.* (2009) described their material with dense black rhizines.

All studied specimens were found on bark. According to Kurokawa & Lai (2001), Elix (1994) and Benatti (2013a), it may be rarely found on rocks. Some authors mentioned different lobe widths, ranging from 4-7 mm wide (Hale, 1976; Kurokawa & Lai, 2001) to 0.8-3 mm wide (Canêz, 2005; Marcelli, 1993), similar to those in the material studied from Argentina. Benatti (2013a) cited a lobe width (0.6-)-1.6-4.1(-5.4) mm wide.

Apothecia and pycnidia were not found in specimens from Argentina. According to Elix (1994) and Benatti (2013a) the apothecia are rare, concave to cup-shaped, sessile to substipitate, 0.5-5 mm wide, ecoronate, the thalline excipule eventually isidiate, and ascospores ellipsoid, 9-15 (-16.5) × 5-8 (-10) µm in size. Pycnidia are described as laminal, with a black ostioles, and conidia bacilliform to weakly bifusiform, 4-6 µm long (Jungbluth, 2006; Benatti, 2013a).

*Bulbothrix isidiza*, also present in northeastern Argentina, is morphologically similar to *B. tabacina*, and differentiated by its overall brown lower surface. *Bulbothrix decurtata* (Kurok.) Hale is a saxicolous species endemic to Africa, similar to *B. tabacina*, both sharing a black lower cortex, medullary salazinic acid and formation of isidia (Hale, 1976; Benatti, 2013a). According to Benatti (2013a), *B. decurtata* has an emaculate, commonly fissured upper surface, narrower, sublinear lobes (ca. 0.5-3.0 mm wide) with more truncate apices, granular (short, with a ± papillary aspect) to rarely cylindrical, blackened isidia, and large bulbs of the marginal cilia, which often lack the apical portion.

*Bulbothrix ventricosa* (Hale & Kurok.) Hale differs from *B. tabacina* by the variable color of the lower surface often tending to mixed black and brown or different tinges of brown, denser isidia, coronate apothecia, and the medullary norstictic acid (Hale, 1976; Benatti, 2012a). As seen by Benatti (2012a), thalli of *B. ventricosa* constantly form laminal ciliary bulbs, structures also not present in *B. tabacina*. *Bulbothrix subtabacina* (Elix) Elix is differentiated by its sublinear, elongate narrower lobes (0.5-1 mm wide), thinner cilia, dichotomously branched rhizines, and ciliate isidia (Elix, 1994; Benatti, 2012a).

***Bulbothrix viatica*** Spielmann & Marcelli, Mycotaxon 103: 201 (2008)

*Thallus* foliose, mineral gray to greenish gray, submembranaceous, corticolous, moderately attached to substrate, 1.5-4 cm in diameter; sublinear lobes, irregularly branched, 0.9-1.5 wide, contiguous to slightly imbricate, with rounded



apices; margin entire to crenate, with bulbate cilia; cilia restricted to crenae or lobe axils, where there might be up to 2 contiguous bulbs, with or without apices, which are mainly developed in cilia in lobe axils, simple to sometimes bifurcate. *Upper surface* shiny, smooth to slightly rugose at center, continuous or with some irregular cracks, emaculate to slightly maculate; maculae punctiform, laminal. *Isidia*, *soralia*, and *pustules* absent. *Medulla* white. *Lower surface* dark to pale brown, shiny, smooth to subrugose, continuous, moderate to abundantly rhizinate, with a narrow to moderately broad pale brown margin, lustrose, smooth to slightly rugose, rhizinate or papillate; rhizines simple, black to dark brown, occasionally with brownish to whitish apices, with or without basal bulb, evenly distributed. *Apothecia* abundant, cupuliform to plane, 0.4-5 mm in diameter, sessile, laminal, margin coronate, entire to crenate, amphitecium smooth, disc imperforate, pale to dark brown, epruinose; ascospores ellipsoidal (10-) 12-16 × (5-) 6-10 μm. *Pycnidia* laminal, scarce to abundant; conidia bifusiform, 6-7 μm long.

*Spot reactions*: Upper cortex K+ yellow, UV-; medulla K+ yellow turning orange, C-, KC-, P+ orange, UV-.

*TLC*: Cortical atranorin; medullary norstictic and conorstictic acids.

*Specimens examined*: ARGENTINA, Corrientes, San Roque, route 33, 3 kms W from Santa Lucía river, 1978, A. Schinini 16186 (CTES); Misiones, Guaraní, Yaboty Biosphere Reserve, Caá-Yarí Provincial Park, surroundings of the house of forest rangers, 26° 52' 19,6" S 54° 13' 33,8" W, 526 m, 27 February 2010, A. Michlig, N. Niveiro & O. Popoff 2471 (CTES); idem, on a fallen "paraíso" trunk, 10 November 2011, A. Michlig, S. Jimenez, N. Niveiro & Á. Vega 2781 (CTES).

*Distribution*: This is an exclusively South American species, recorded from Brazil (Spielmann & Marcelli, 2008; Benatti, 2012a) and recently from Argentina (Michlig & Ferraro, 2012). Its distribution is now extended to Misiones province.

*Comments*: Laminal ciliary bulbs were not described in the original description (Spielmann & Marcelli, 2008), although Benatti (2012a) when studying the material cited by these authors reported they are common in this species, usually frequent, mainly on young distal parts. In the material studied by us, there are no specimens with ciliary bulbs.

The upper cortex in *B. viatica* has been described as being emaculate (Spielmann & Marcelli, 2008; Benatti, 2012a), but in the material studied it was found to be emaculate to slightly maculate, with punctiform laminal maculae. The lower surface in material studied is dark to pale brown, but in this species it could vary from black mottled with brown in variable intermediary levels to completely brown (Benatti, 2012a).

The species which are morphologically most similar to *B. viatica* are *B. regnelliana* and *B. subcoronata*, which also are characterized by the medullary norstictic acid and the absence of vegetative propagules. *Bulbothrix regnelliana*, frequent in northern Argentina, also has a brown lower surface, but differs in having smaller ascospores (7-9 × 5-7 μm). *Bulbothrix subcoronata* differs in having narrower lobes (0.5-1 mm wide), no laminal bulbs, a black lower surface with distinct brown margins, and retrorse rhizines on apothecia (Benatti, 2012a).

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