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The genus *Bulbothrix* (*Parmeliaceae*) in China

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Abstract: The morphology, chemistry and phylogenetic relationships of Chinese populations of *Bulbothrix* are described. Nine species, including two new species *B. mammillaria* Y. Y. Zhang & Li S. Wang sp. nov. and *B. lacinia* Y. Y. Zhang & Li S. Wang sp. nov., and two newly recorded for the flora, *B. scortella* and *B. meizospora*, are reported. *Bulbothrix mammillaria* can be recognized by the sparse cilia that are reduced to a bulbate structure and the broad lobes (3–11 mm). *Bulbothrix lacinia* differs from other species of the genus by dark brown, spherical to short-cylindrical isidia and common lacinulae on the upper surface. Phylogenetic relationships of currently known ITS sequences from *Bulbothrix* were inferred to assess the affinities of the new species. A key to all known species from China is presented.

Key words: bulbate cilia, diversity, lichenized fungi, taxonomy

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Introduction

Hale (1974) erected the genus *Bulbothrix* as a segregate from *Parmelia* Ach. to accommodate species with marginal bulbate cilia; the cilia can be simple or branched. *Bulbothrix* is further characterized by laciniate lobes, cortical atranorin, hyaline unicellular, ellipsoid to bicornute ascospores, and bacilliform to bifusiform conidia (Hale 1976; Elix 1993; Benatti 2014). Bulbate cilia also occur in *Relicina* Hale, which differs, however, in the presence of usnic acid. Although the two genera exhibit similar morphologies, they are quite distinct as *Bulbothrix* belongs to the *Parmelina*-clade, and *Relicina* to the *Parmelia*-clade (Crespo *et al.* 2010). As currently circumscribed, *Bulbothrix* is a polyphyletic genus composed of two clades. The first, the *B. isidiza* group, is sister to *Parmelinella* Elix & Hale (Divakar *et al.* 2006, 2010; Masson *et al.* 2015) which leads to this clade either nesting in the genus *Parmelinella* or being a small genus that contains salazinic acid (Benatti 2013; Kirika *et al.* 2015). The second clade is the *Bulbothrix goebelii* (Zenker) Hale group, and the type species of *Bulbothrix*, *B. semilunata* (Lynge) Hale, may belong in this clade (Kirika *et al.* 2015) on account of its distribution and morphological characteristics.

Twenty-nine species of *Bulbothrix* were accepted by Hale (1976). This number has grown to 61 (Bungartz *et al.* 2013; Benatti 2015; Masson *et al.* 2015), with many species from Brazil, which may be the centre of diversity for *Bulbothrix* (Spielmann & Marcelli 2008; Benatti 2012*a*). The genus is widespread in tropical and subtropical regions and reaches its highest diversity in semi-arid woodlands and secondary forests

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(Hale 1976; Marcano *et al.* 1996). Species concepts within the genus rely primarily on the branching patterns of cilia and rhizines, presence or absence of isidia, maculate or emaculate upper cortex, colour of the lower surface and secondary chemistry in the medulla.

Seven species of *Bulbothrix* have been reported from China: *B. goebelii*, *B. isidiza* (Nyl.) Hale, *B. setschwanensis* (Zahlbr.) Hale, *B. tabacina* (Mont. & Bosch) Hale, *B. asiatica* Y. Y. Zhang & Li S. Wang, *B. subscortea* (Asahina) Marcelli & Benatti and *B. yunnana* (Wang *et al.* 2000; Chen *et al.* 2009; Zhang *et al.* 2014). However, *Bulbothrix yunnana* was transferred to *Parmotrema* as *P. yunnanum* (Sheng L. Wang *et al.*) Marcelli & Benatti because the cilia have an enlarged base instead of being truly bulbate (Benatti & Marcelli 2010).

In this study, two new species are described and two species are newly recorded, resulting in a total of nine species of *Bulbothrix* occurring in China. Morphological and chemical descriptions, phylogenetic analyses together with a key to these species are presented.

Materials and Methods

Morphological and chemical studies

Approximately 400 specimens from the lichen herbarium of the Kunming Institute of Botany (KUN-L) and the Chinese Academy of Sciences were examined. Morphological characters were studied using standard stereoscopy and light microscopy. We sectioned apothecia, thalli, pycnidia and cilia with a razor blade under a NIKON SMZ745T dissecting microscope, then examined and measured traits using a micrometer under a NIKON Eclipse Ci-S microscope. Size of the thallus, apothecia and lobes are based on measurements for each specimen, and ascospore dimensions reflect ten measurements from a single apothecium per specimen. Size is represented by the range between the smallest and largest values. The chemical composition of each specimen was assessed using spot reactions and thin-layer chromatography with solvent system C (Orange *et al.* 2001).

DNA extraction

Total genomic DNA was extracted from dry herbarium or fresh specimens using AxyPrep Multisource Genomic DNA Miniprep Kit 50–prep (Qiagen) according to the manufacturer's instructions. The fungal internal transcribed spacer region (ITS) of the rDNA repeat was amplified via polymerase chain reaction (PCR) using the primers ITS1-LM (Myllys *et al.* 1999) and ITS2-KL (Lohtander *et al.* 1998). Amplifications were performed in a 25 µl volume comprising 12·5 µl of 2× MasterMix (TaqDNA Polymerase, 0·1 units/µl^{-1}; 4 mM $MgCl_2$; 0·4 nMdNTPs; produced by Aidlab Biotechnologies Co., Ltd), 0·4 µl of each primer, 11 µl ddH_2O, and 0·7 µl of DNA. PCR amplifications were performed using the following temperature profile: initial denaturation at 94 °C for 3 min, 30 cycles each composed of 94 °C for 40 s, 50 °C for 1 min, 72 °C for 1·5 min, and a final extension at 72 °C for 7 min. PCR products were sequenced using amplification primers by the company Sangon Biotechnology (Shanghai, China).

Sequence alignments

Sequence fragments obtained were assembled with SeqMan 7.0 (DNAStar) and manually adjusted. DNA sequences were aligned with MAFFT version 7 with the L-INS-I alignment algorithm (Katoh *et al.* 2005) using the web server (http://mafft.cbrc.jp/alignment/server/index.html) with all parameters set to default values.

Phylogenetic analyses

The ITS matrix was analyzed using a maximum likelihood (ML) optimality criterion and a Bayesian (BI) Markov chain Monte Carlo approach (B\MCMC), with *Myelochroa aurulenta* (Tuck.) Elix & Hale selected as outgroup.

ML analyses were performed using RAxML7.0.4 (Stamatakis 2006), implementing default settings. Support values were inferred from the 70% majority-rule tree of all saved trees obtained from 1000 non-parametric bootstrap replicates.

The Bayesian analyses were performed using MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001), with 2 000 000 generations and four incrementally heated chains. MrModeltest 2.3 (Nylander 2005) was used in conjunction with PAUP* (Swofford 2003) to estimate the best-fitting substitution model (i.e., GTR + I + G) based on the AIC by jModelTest 3.7 (Posada 2008). MCMC started from a random tree and was sampled every 1000th generation, with the first 10% of trees discarded as burn-in. The remaining trees were used to generate a majority-rule consensus tree with posterior probability (PP), inferred from consensus values, ≥0·95 considered as strongly supported. We used the program Tracer v1. 6 (Rambaut & Drummond 2003) to ensure that stationarity was achieved by checking whether the log-likelihood values of sample points reached a stable equilibrium. Phylogenetic trees were visualized using the program FigTree 1.4.0 (Rambaut 2012).

Results

The ITS matrix contained a total of 35 sequences, including 12 that were newly generated (Table 1). The ITS topologies

TABLE 1. *Specimen information and GenBank accession numbers for taxa used in this study. Newly obtained sequences are in bold.*

Species	Locality	Voucher specimens	GenBank number
Bulbothrix apophysata	Costa Rica	F 16650b	DQ279481
B. asiatica 1	Cambodia: MondulKiri	KUN 12-37239	KM249891
B. asiatica 2	China: Yunnan	KUN 14-44427	KM285403
B. coronata	South Africa	MAF 13987	DQ279482
B. decurtata	South Africa	MAF 13988	DQ279483
B. goebelii	South Africa	MAF 13985	DQ279484
B. isidiza 1	Madagascar	TinoRuibal: *Ertz* 12878	JN943847
B. isidiza 2	Madagascar	BR 12878	GQ919263
B. isidiza 3	China: Yunnan	KUN 12-33001	**KP776573**
B. isidiza 4	China: Yuanmou	KUN 13-39821	**KP776574**
B. klementii	Costa Rica	F 15170a	DQ279485
B. lacinia 1	China: Yunnan	KUN 13-41296	**KP780410**
B. lacinia 2	China: Yunnan	KUN 13-41301	**KP776570**
B. laevigatula	Costa Rica	F: *Lücking* 15045b	GQ919264
B. lyngei	Cambodia: PreahVihear	KUN 12-37404	KM249892
B. mammillaria 1	China: Yunnan	KUN 14-44233	**KP776571**
B. mammillaria 2	China: Yunnan	KUN 13-41171	**KP776572**
B. mammillaria 3	China: Yunnan	KUN 14-43407	**KT729545**
B. mammillaria 4	China: Yunnan	KUN 13-40814	**KT729546**
B. meizospora 1	India: Uttaranchal	GPGC 02-000786	AY611068
B. meizospora 2	India	TinoRuibal: GPGC 02-000786? BUME351	JN943846
B. scortella 1	China: Yunnan	KUN 14-44441	**KP776565**
B. scortella 2	China: Yunnan	KUN 14-44442	**KP776566**
B. sensibilis 1	Rwanda	*Ertz* 11025	GU994541
B. sensibilis 2	Rwanda	BR 11025	GQ919265
B. setschwanensis 1	China: Yunnan	MAF 10212	AY611069
B. setschwanensis 2	China: Yunnan	KUN 13-41167	**KP776567**
B. setschwanensis 3	China: Yunnan	KUN 13-39866	**KP776568**
B. subscortea 1	Cambodia: Kampot	KUN 12-37479	KM249895
B. subscortea 2	Cambodia: MondulKiri	KUN 12-37270	KM249897
B. suffixa	Madagascar	BR 12889	GQ919266
B. tabacina 1	Kenya	MAF-Lich 16112	GQ919268
B. tabacina 2	Republic of the Congo	MAF-Lich 16111	GQ919267
Myelochroa aurulenta	Canada	*F. Lutzoni & J. Miądlikowska* 07.02.03-3 (DUKE)	JQ301701
Parmelinella wallichiana	China	MAF 10411	DQ279532

obtained by the maximum likelihood and Bayesian approaches were congruent, and ML topology was selected to represent the phylogenetic relationship (Fig. 1).

Two main clades that were identical to Kirika *et al.* (2015) were recovered in the phylogenetic trees. Clade 1 is characterized by narrow lobes usually with a truncate to subtruncate apex, branched rhizines and cilia (dichotomously, trichotomously to irregularly, densely branched), and the absence of medullary substances or containing secondary metabolites such as gyrophoric, conlensoinic, lecanoric, lobaric acids. Clade 2 is characterized by broad lobes (to 11 mm) generally with a round to subrotund to subtruncate apex, simple rhizines and cilia, and the presence of salazinic acid in the medulla. The two new species, *B. mammillaria* and *B. lacinia*, formed highly supported clades (MLBS = 100%, PP = 1·0 and MLBS = 97%, PP = 1·00, respectively). *Bulbothrix lacinia* is sister to *B. subscortea* but differs by granular to short cylindrical isidia that are dark brown or have a dark brown apex, and an upper surface with numerous lacinulae; it is also close to *B. setschwanensis* but differs by the presence of isidia. *Bulbothrix mammillaria* is sister to *B. asiatica* but the cilia of the latter are frequent along

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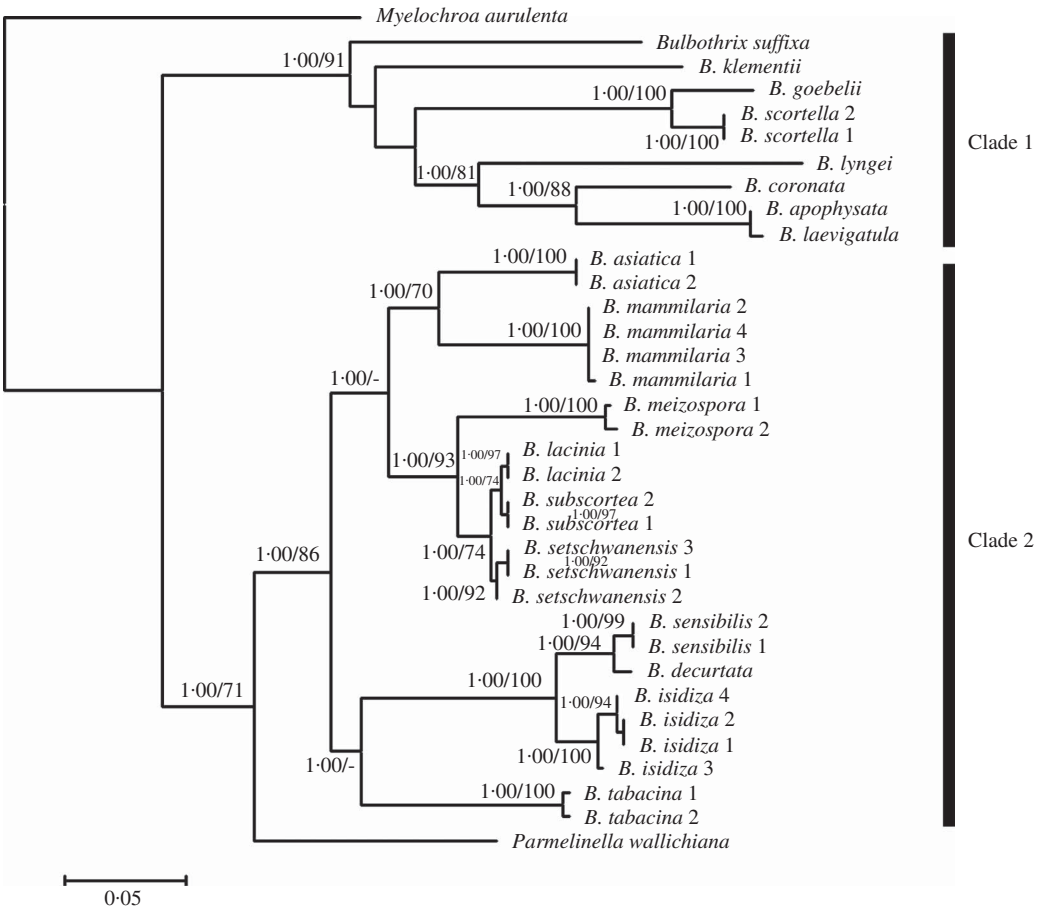

FIG. 1. Phylogenetic relationships of the species used in this study based on Maximum Likelihood and Bayesian analyses of ITS sequences.

the lobe margins with a simple or rarely branched apex and its lower surface is uniformly black with a pale brown margin.

Taxonomy

New species

Bulbothrix mammillaria Y. Y. Zhang & Li S. Wang sp. nov.

MycoBank No.: MB 812997

Similar to *B. asiatica*, but differs by the sparse cilia reduced to a bulbate structure without a tapering apex to rarely with a very short apex, shorter isidia (60–360 μm), broader lobes (3–11 mm), variable colour of lower surface, broader pale brown lower marginal zone (2–5 mm), and rhizines normally with white apices.

Type: China, Yunnan Prov., Yongsheng Co., Dashan Village, on bark, 5 December 2013, *L. S. Wang et al.* 13–41171 (KUN-L22067—holotype).

(Fig. 2)

Thallus greenish grey to tan, to 10 cm diam., submembranaceous to subcoriaceous; upper cortex 12·5–17·0 μm thick, algal layer 12·5–22·0 μm thick, medulla 75–82 μm thick, lower cortex 7·5–25·0 μm thick. *Lobes* irregularly branched, 3–11 mm wide, contiguous and crowded at the centre, very

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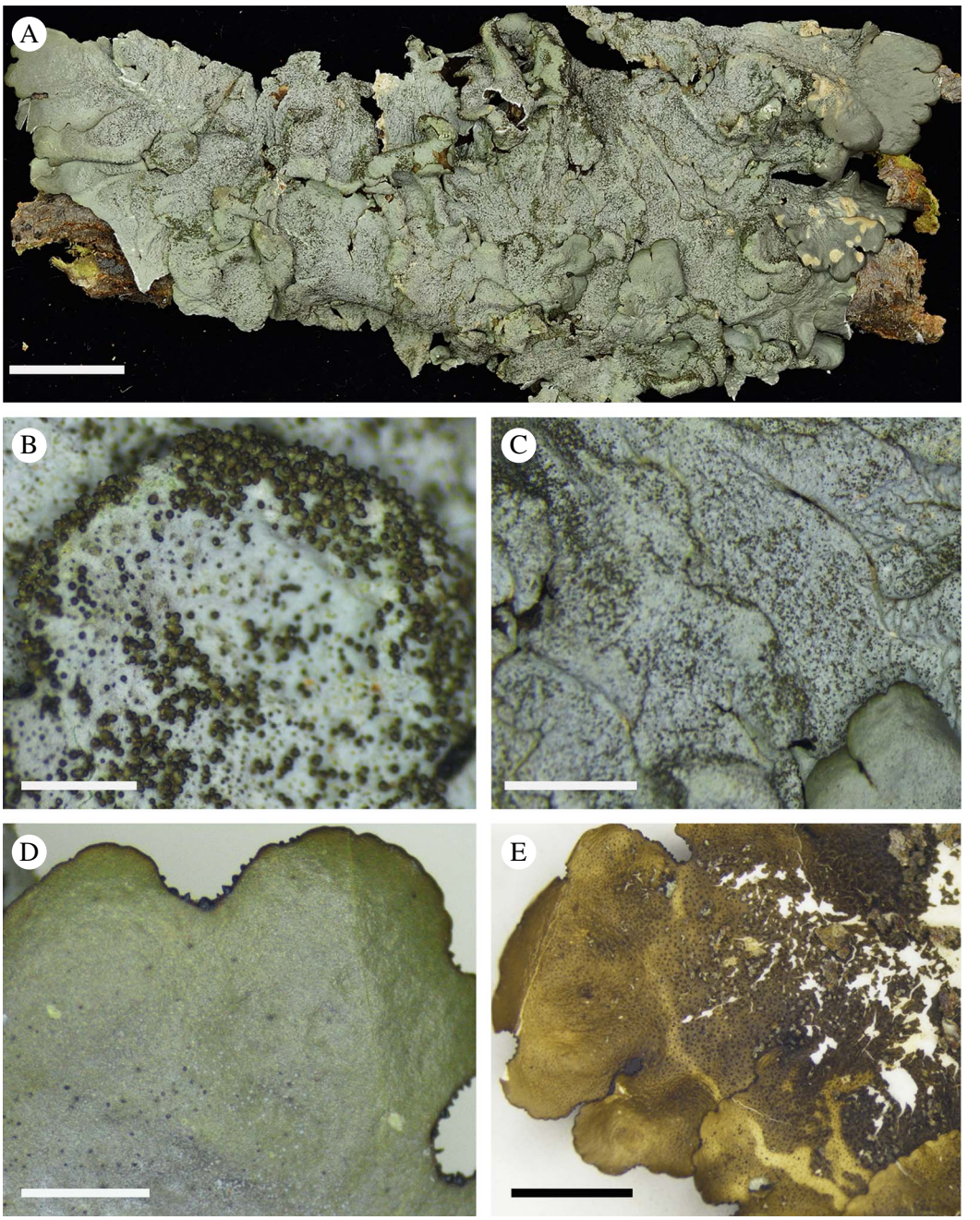

FIG. 2. *Bulbothrix mammillaria* habitus (holotype). A, thallus; B, isidia; C, upper surface of thallus; D, cilia reduced to a bulbate structure; E, lower surface of thallus. Scales: A = 1 cm; B = 1 mm; C & E = 4 mm; D = 2 mm. In colour online.

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appressed, with subrotund and incised apices. *Upper surface* often rough and rugose, rarely cracked at the centre, but smooth distally. *Maculae* absent. *Cilia* black, sparse, bulbate without a tapering apex or rarely with a very short apex. *Isidia* granular to occasionally short cylindrical, with black to brown apex, occasionally concolorous with upper thallus surface, evenly distributed on upper surface but lacking on distal part of lobes. *Medulla* white. *Lower surface* usually black, dark brown, rarely pale brown, rugose and often with ridge-like folds, with a wide (2–5 mm), papillate or smooth, pale brown, usually shiny marginal zone. *Rhizines* sparse to moderately abundant, pale brown to brown, usually with white apices that are sometimes branched and conglutinated, occasionally with a bulbate base near lobe margin.

Apothecia extremely rare, only one was found among 58 collections, and then small and immature.

Pycnidia not seen.

Chemistry. Upper cortex K+ yellow, medulla K+ yellow to red, C−, KC−; atranorin and salazinic acid.

Ecology and distribution. Growing on bark of *Pinus* spp. and *Quercus* spp., on branches of *Phyllanthus emblica* and *Cotoneaster* spp., and on rocks at an elevation of 1274–3160 m in semi-arid environments; known only from southern China.

Comments. *Bulbothrix mammillaria* is characterized by the sparse and very short cilia which are usually reduced to a bulbate structure, mostly wide lobes (5–11 mm), rugose and tan upper surface if it is exposed to sunlight, rhizines with normally white apices, mostly granular isidia with a black apex, and a black to dark, rarely pale brown lower surface, with a pale brown marginal zone.

Even though *Bulbothrix mammillaria* exhibits a wide range of colour in the upper and lower surfaces and lobe width, it can be recognized by the cilia reduced to a bulbate structure. The phylogenetic tree based on ITS sequence data also supports the evolutionary independence of this species. Specimens collected from the open habitats tend to have a tan-coloured upper surface that may be due to exposure to the sun.

Bulbothrix isidiza resembles this new species in the short cilia and presence of medullary salazinic acid, but differs by the cilia commonly having a simple apex, uniformly pale brown lower surface (Benatti 2013) and a distinctly maculate and pale dusky grey upper surface. *Bulbothrix decurtata* (Kurok.) Hale shares the granular to short cylindrical isidia and black to occasionally dark brown lower surface, but differs by the sparse, reduced cilia and by its broader lobes (3–11 mm). *Bulbothrix subscortea* can be recognized by longer, simple to occasionally branched cilia (200–825 μm), and the consistently pale brown lower surface. *Bulbothrix mammillaria* differs from *B. tabacina* in its rugose and often tan upper surface and the strictly bulbate cilia.

Selected specimens examined (here, and elsewhere, all specimens deposited in KUN-L unless otherwise stated). **China:** *Guangxi Prov.*: Longsheng Co., 760 m, 25°47'N, 110°02'E, decayed wood, 2001, *J. B. Chen et al.* 20178 (HAMS-L). *Sichuan Prov.*: Dukou, Ji Mt., 1200 m, on *Quercus* sp., 1983, *L. S. Wang* 83–65. *Yunnan Prov.*: Fumin Co., Chahe Village, 1540 m, 26°14'N, 101°25'E, on branches, 2013, *L. S. Wang et al.* 13–41275; near Pudu River, 1650 m, 25°31'N, 102°36'E, on rocks, 2014, *L. S. Wang et al.* 14–43075; Lancang Co., Taipingzheng Village, 1537 m, 22°36'N, 99°48'E, on bamboo, 2014, *L. S. Wang et al.* 14–44450; Lijiang Co., from Jinjiang to Heqing, 3160 m, 26°38'N, 100°17'E, on bark, 2013, *L. S. Wang et al.* 13–40923; Luquan Co., from Luquan to Yunlong water reservoir, 1952 m, 25°42'N, 102°29'E, on *Quercus* sp., 2014, *L. S. Wang et al.* 14–43131; Nanjian Co., 1426 m, 24°41'N, 100°04'E, on rocks, 2012, *L. S. Wang et al.* 12–34234; Puer, Tongxin Village, 870 m, 22° 58'N, 101°03'E, on bark, 2014, *L. S. Wang et al.* 14–44579; Yongren Co., 1543 m, 26°13'N, 101°25'E, on rocks, 2013, *L. S. Wang et al.* 13–40814; on bark, *L. S. Wang et al.* 13–40771, 13–40772; Yongsheng Co., Dashan Village, 2200 m, 26°27'N, 101°07'E, on branches, 2013, *L. S. Wang et al.* 13–41208, 13–41169, 13–41187; Yuanmou Co., Langbapu Village, 1521 m, 25° 41'N, 101°41'E, on *Phyllanthus emblica*, 2014, *L. S. Wang et al.* 14–43538; Liangshan Mt., 2148 m, 25°43'N, 101° 57'E, on bark, *L. S. Wang et al.* 13–39736, 13–39823.

Bulbothrix lacinia Y. Y. Zhang & Li S. Wang sp. nov.

MycoBank No.: MB 812998

Similar to *B. subscortea* (Asahina) Marcelli & Benatti, but differs by common lacinulae on the upper surface,

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spherical to short-cylindrical isidia that are dark brown (longer ones concolorous with thallus but with dark brown apices) and by the narrower lobes (1·0–3·5 mm).

Type: China, Yunnan Prov., Chuxiong, Chahe Village, 2013, *L. S. Wang et al.* 13–41296 (KUN-L22192—holotype).

(Fig. 3)

Thallus pale grey-white, to 8 cm diam., subcoriaceous; upper cortex 10–25 µm thick, algal layer 25–40 µm thick, medulla 50–75 µm thick, lower cortex 7·5–17·5 µm thick. *Lobes* irregularly branched, 1·0–3·5 mm wide, imbricate, very crowded at the centre, appressed, with plane, crenate to subtruncate apices. *Upper surface* smooth and shiny at the apices of lobes, covered with dense isidia in the centre, commonly lacinulate. *Maculae* absent. *Cilia* black, simple, moderately distributed along the margins, with irregularly inflated base, sometimes without bulbs. *Isidia* spherical to short cylindrical (90–500 × 80–160 µm), simple to rarely forked, shiny, dark brown or pale grey with dark brown apex. *Medulla* white. *Lower surface* pale brown, smooth; marginal zone shiny, brown, darker than centre, slightly papillate. *Rhizines* brown, simple, moderately distributed, without bulbate base.

Apothecia not seen.

Pycnidia scarce, with black ostioles, the thallus protruding around them. *Conidia* bacilliform, 5·0–7·5 × 0·75 µm.

Chemistry. Upper cortex K+ yellow, medulla K+ yellow to red, C−, KC−; atranorin and salazinic acid.

Ecology and distribution. Growing on rocks in secondary forest in dry to semi-arid environments; so far known only from Yunnan Province, China.

Comments. *Bulbothrix lacinia* is characterized by the narrow and irregularly branched lobes (1·0–3·5 mm), dense, dark brown, spherical to short-cylindrical isidia, emaculate upper surface, simple cilia and rhizines, irregularly inflated base of cilia, pale brown lower surface and the presence of medullary salazinic acid.

Bulbothrix decurtata resembles *B. lacinia* in terms of lobe size, saxicolous habitat, presence of isidia and medullary salazinic acid, but differs by its very cracked upper surface, black to occasionally dark brown lower surface, rhizines with bulbate bases and the greyish olive-green thallus (Benatti 2013); *Bulbothrix australiensis* Hale differs by having longer isidia (0·5–1·1 mm), rhizines commonly with a bulbate base, pycnidia usually abundant and a corticolous habitat. *Bulbothrix subtabacina* (Elix) Elix can be differentiated by its black lower surface, branched cilia and rhizines, maculate and cracked upper surface, and narrower lobes 0·4–1·1 (–1·5) mm (Benatti 2013), and *B. mammillaria* by its broader lobes (3–11 mm) and cilia reduced to a bulbate structure.

Additional specimens examined. **China:** *Yunnan Prov.*: Chuxiong State, Chahe Village, 1540 m, 26°14'N, 101°25'E, on sandstone, 2013, *L. S. Wang et al.* 13–41301, 13–41265; Fumin Co., beside Pudu River, 1650 m, 25°31'N, 102°36'E, on sandstone, 2014, *L. S. Wang et al.* 14–43065; Yongren Co., from Menghu to Wanma, 1543 m, 26°13'N, 101°25'E, on sandstone, 2013, *L. S. Wang et al.* 13–40794.

New records

Bulbothrix meizospora (Nyl.) Hale

Phytologia **28**(5)**:** 480 (1974); type: India, Nilgiri Mountains (H-NYL 35107—lectotype).

Bulbothrix meizospora is characterized by: a lack of vegetative propagules; a weak to distinctly maculate upper cortex; cilia that are scarce, short, with a bulbate base, without or with simple to double apices; black lower surface with a brown marginal zone (0·5–)2·0–4·0 mm; and rhizines that are black without a bulbate base. Apothecia 0·8–6·2 mm diam., ascospores ellipsoid to oval, pycnidia laminal, and conidia bacilliform. For other details see Benatti (2012*b*).

Chemistry. Upper cortex K+ yellow, medulla K+ yellow to black-red, C−, KC−; atranorin and salazinic acid.

Ecology and distribution. The single specimen from China was growing on *Cinnamomum* sp.

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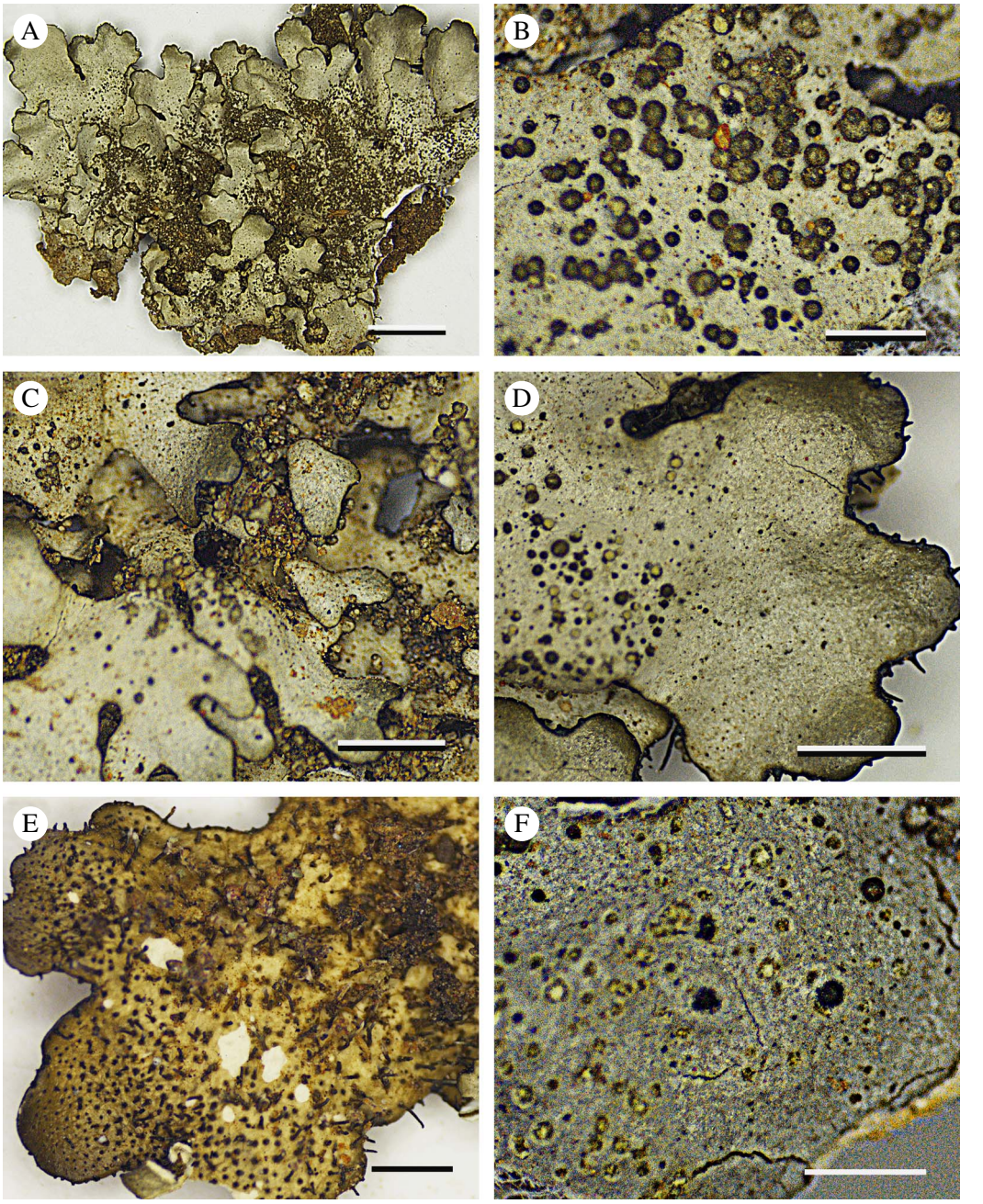

FIG. 3. *Bulbothrix lacina* habitus (holotype). A, thallus; B, spherical and dark black isidia; C, common lacinulae on upper surface of thallus; D, marginal black line and irregular cilia; E, uniformly pale brown lower surface of thallus; F, scarce pycnidia. Scales: A = 4 mm; B, C & F = 0·5 mm; D & E = 1 mm. In colour online.

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World distribution: Asia, Africa and South America (Benatti 2012*b*), and added here to the flora of China.

Comments. This species is similar to *B. setschwanensis*, but can be distinguished by a black lower surface with a pale brown marginal zone, and the maculate upper surface. In Asia, *B. meizospora* has been reported from India, Pakistan, Nepal and Thailand, and only one population is known from Xizang.

Specimen examined. **China:** *Xizang Prov.*: Nyalam Co., 2450 m, 28°N, 86°E, on the bark of *Cinnamomum* sp., 1966, *J. C. Wei & J. B. Chen* 673 (HAMS-L 0021966).

Bulbothrix scortella (Nyl.) Hale

Phytologia **28**(5)**:** 480 (1974); type: USA, Texas, *C. Wright* (FH-Tuck—lectotype (Hale 1976); H-NYL—duplicate).

Bulbothrix scortella is characterized by: a smooth to cracked upper cortex; simple, straight, granular to cylindrical isidia; black to brown, branched cilia; pale brown to brown lower surface with dense rhizines; and rhizines that are pale brown to cream, densely branched with a bulbate base. Apothecia and pycnidia not seen in Chinese specimens, but described by Benatti & Elix (2012).

Chemistry. Upper cortex K+ yellow, medulla K−, C+ rose, KC+ rose; atranorin and gyrophoric acid.

Ecology and distribution. Growing on bark of deciduous trees in China. World distribution: Asia, Africa, Caribbean, USA and South America, as detailed by Benatti & Elix (2012); reported here as new to China.

Comments. Bulbothrix scortella is characterized by simple to occasionally branched isidia, branched rhizines and cilia, and the presence of gyrophoric acid in the medulla.

Specimens of *Bulbothrix scortella* in China were identified as *B. goebelii* by Chen *et al.* (2009) and Lai (2000) who were following the concept of Hale (1976), which is composed of several morphologically and chemically similar species. Benatti & Elix (2012) recently showed that the type specimen, *Parmelia goebelii* Zenker (*Bulbothrix goebelii*), comprises fragments of four different species of *Bulbothrix*, and a lectotype was chosen that conforms to the protologue of Zenker (1827), whereby *B. goebelii* is characterized by the lack of isidia and the presence of lobaric, oxolobaric and colensoic acids but without accessory gyrophoric acid (Benatti & Elix 2012). *Bulbothrix goebelii* previously reported from China has isidia and gyrophoric acid, and is one of the species included in Hale's *B. goebelii* concept. According to the current circumscription of species within that group, these records in fact correspond to *B. scortella*, and *B. goebelii* is therefore excluded from the flora of China.

Bulbothrix scortella is similar to *B. subdissecta* (Nyl.) Hale in sharing the presence of isidia and gyrophoric acid in the medulla, but the latter has a black lower surface and smaller ascospores (Benatti & Elix 2012).

Specimens examined. **China:** *Fujian Prov.*: Wuyishan Mt., 850 m, 26°54'N, 116°42'E, on deadwood, 1999, *J. B. Chen & S. L. Wang* 14127 (HAMS-L 021931). *Yunnan Prov.*: Lancang Co., Jinmai Mt., 1135 m, 22°12'N, 100°03'E, on *Alnus nepalensis*, 2014, *L. S. Wang et al.* 14–44441, 14–44442; Mengla Co., Longmen, on bark, 1974, *M. Zang* 528; Manla Village, 1079 m, 22°00'N, 101°26'E, on the branches of *Camellia sinensis*, 2006, *L. S. Wang et al.* 06–27337; Ruili Co., Nongdao, 780 m, on *Quercus*, 1983, *L. S. Wang* 83–2691a; Ninger Co., 1060 m, 23°16'N, 101°09'E, on bark, 2013, *L. S. Wang et al.* 13–40462.

Other species of *Bulbothrix* in China

Bulbothrix asiatica Y. Y. Zhang & Li S. Wang

Bryologist **117:** 379–385 (2014); type: China, Yunnan, *L. S. Wang et al.* (KUN-L 46314—holotype).

Description. See Zhang *et al.* (2014).

Ecology and distribution. Growing on the bark of *Alnus nepalensis*, *Hevea brasiliensis* and other tree species, between 290–1135 m. Cambodia and China.

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Comments. Specimens of this species from China have usually been identified as *Bulbothrix tabacina* (Chen *et al*. 2009). These species are, however, clearly distinct as revealed by the phylogenetic inference (Fig. 1) and morphological characters: *B. tabacina* has a maculate upper surface especially when wet, whereas *B. asiatica* is emaculate.

New collections from Fujian Prov. in China. Wuyishan National Reserve, 980 m, 27°41'N, 117°38'E, on bark, *L. S. Wang et al.* 15–47228, 15–47240.

Bulbothrix isidiza (Nyl.) Hale

Phytologia **28**(5)**:** 480 (1974); type: Angola, Serra Chella, *Newton* (H—lectotype).

Description. See Benatti (2013).

Ecology and distribution. In China this species usually grows on the bark of *Pinus yunnanensis* and some other trees, rarely on rocks, at altitudes of between 1500–2665 m. World distribution: Oceania and North Pacific, Asia, Africa, North America, Central America and South America, as detailed by Benatti (2013). China: Yunnan Province.

Comments. *Bulbothrix isidiza* is characterized by the rough appearance of the upper surface, very delicate and small isidia (Nylander 1884; Hale 1976; Benatti 2013), and commonly distinct maculae on the upper surface. Moreover, specimens from China are usually coriaceous, with a thicker thallus composed of an upper cortex 37·5 – 62·5 µm, algal layer 37·5 – 75·0 µm, medulla 100 – 257 µm and lower cortex 20 – 30 µm thick. The phylogenetic structure in the ITS tree lacks corresponding morphological variation, hence we treat all these specimens as *B. isidiza*.

Selected specimens examined. **China:** *Yunnan Prov.*: Anning, 30 km SW of Kunming, 1750–1800 m, 24°55'N, 102°29'E, on *Pinus yunnanensis*, 1981, *T. Koponen* 37991; Binchuan Co., Mt. Jizushan, 2620 m, 25°57'N, 100°22'E, on bark, 2012, *L. S. Wang & D. Liu* 12–33461; Chuxiong, Mt. Zixishan, 2300 m, 25°04'N, 101°24'E, on rock, 2005, *L. S. Wang et al.* 05–25257; Jianchuan Co., Mt. Shibaoshan, 2665 m, 26°22'N, 100°49'E, on bark, 2011, *L. S. Wang et al.* 11–32529; Jingdong Co., Wenjin, 1492 m, 24°21'N, 100°51'E, on *Ficus*, 2012, *L. S. Wang et al.* 12–34566; Kunming, Mt. Xishan, 1900 m, 1983, *L. S. Wang* 83–37891; Lufeng Co., Qinglongshao, 1700 m, 24°58'N, 102°30'E, on *Pinus yunnanensis*, 1987, *Teuvo Ahti et al.* 46114; Luquan Co., 1952 m, 25°42'N, 102°29'E, on bark, 2014, *L. S. Wang et al.* 14–43117; Nanjian Co., Leqiu Village, 2060 m, 25°00'N, 100°21'E, on *Pinus yunnanensis*, 2012, *L. S. Wang & X. Y. Wang* 12–37816, 12–37812; Mt. Wuliangshan, 2348 m, 24°52'N, 100°35'E, on bark, 2012, *L. S. Wang et al.* 12–32998, 12–33001; Xiaowandong, 1604 m, 24°43'N, 100°08'E, on bark, 2012, *L. S. Wang et al.* 12–34256, 12–34265, 12–34259; Songming Co., Baiyi Village, 2500 m, on *Pinus yunnanensis*, 2008, *L. S. Wang & J. Wang* 08–30314, 08–29619; Tengchong Co., Mt. Kongshan, 1500 m, on *Pinus yunnanensis*, 1996, *L. S. Wang* 96–16791; Xiangyun Co., Mt. Shuimushan, 2440 m, 25°22'N, 100°37'E, on *Pinus yunnanensis*, 2013, *L. S. Wang et al.* 13–38923; Yuanmou Co., Mt. Liangshan, 2148 m, 25°43'N, 101°57'E, 2013, *L. S. Wang et al.* 13–39820, 13–39822, 13–39829 (on bark), 13–39846, 13–39826, 13–39821 (on *Pinus*).

Bulbothrix setschwanensis (Zahlb.) Hale

Phytologia **28**(5)**:** 481 (1974); type: China, Sichuan, *Handel-Mazzetti* 2739 (WU—lectotype).

Description. See Benatti (2012*b*) and Hale (1974).

Ecology and distribution. Usually growing on the bark and branches of *Pinus*, *Quercus* or *Juglans*, at altitudes of 1200–2800 m. World distribution: Asia detailed by Benatti (2012*b*). China: Sichuan and Yunnan Provinces.

Comments. This species is characterized by the uniformly pale brown lower surface, short cilia (≤0·3 mm), delicate and pale brown rhizines, and salazinic acid in the medulla.

Selected specimens examined. **China:** *Sichuan Prov.*: Yanbian Co., Yankou, 1960 m, 1983, *L. S. Wang* 83–535a; from Dukou to Yanbian, 2300 m, 1983, *L. S. Wang* 83–225, 83–232; Miyi Co., Baiposhan Mt., 1983, *L. S. Wang* 83–735. *Yunnan Prov.*: Kunming, Xishan Mt., Xiaoshilin, 2345 m, 24°55'N, 102°37'E, on *Pinus*, 2013, *D. Liu* 13–39868b; Lijiang Co., Xiangshan, 2500 m, on branches, 1985, *L. S. Wang* 85–0017, 85–0210a; Yufengsi, 2780 m, on *Juglans* sp., 1985, *L. S. Wang* 85–0104; 2500 m, on *Pinus armandii*, 1982, *L. S. Wang* 82–1087; Yongsheng Co., Dashan Village, 2200 m, 26°27'N, 101°07'E, on branches, 2013, *L. S. Wang et al.* 13–41164, 13–41167; Yuanmou Co., Liangshan Village, Mt. Liangshan, 2148 m, 25°43'N, 101°57'E, 2013, *L. S. Wang et al.* 13–39853 (on *Pinus yunnanensis*), 13–39743, 13–39789 (on *Quercus*), 13–39692, 13–39747 (on bark).

Bulbothrix subscortea (Asahina) Marcelli & Benatti

Mycosphere **3:** 46–55 (2012); type: China, Taiwan, *Asahina* 3324 (TNS—lectotype).

Description. See Benatti (2012*c*) and Zhang *et al*. (2014).

Ecology and distribution. Growing on rocks, shrubs, and on *Quercus* spp., *Pinus* spp., in open secondary forest, at elevations between 430–2850 m. World distribution: Asia. China: Fujian, Guangxi, Hainan, Hong Kong, Sichuan, Taiwan and Yunnan.

Comments. Bulbothrix subscortea is morphologically very variable: cilia vary in length (between 200–825 μm), lobes range from linear elongate to irregularly elongate regardless of habitat, and rhizines are with or without basal or displaced bulbs. However, it can be identified by the following characteristics: emaculate upper surface, pale brown lower surface and salazinic acid in the medulla.

New collections from Fujian Prov. in China. Wuyishan National Reserve, 980 m, 27°41'N, 117°38'E, on bark, *L. S. Wang et al.* 15–47233; 580 m, 27°43'N, 117°42'E, on bark, *L. S. Wang et al.* 15–47053; 430 m, 27°41'N, 117°44'E, on *Quercus* sp., *L. S. Wang et al.* 15–46797.

Bulbothrix tabacina (Mont. & Bosch) Hale

Phytologia **28**(5)**:** 481 (1974); type: Java, *Junghuhn* 335 (L—lectotype; P—isolectotype).

Description. See Benatti (2013) and Hale (1974).

Ecology and distribution. The single specimen cited here grew on branches at an elevation of 860 m in Hainan. World distribution: Oceania, Asia, Africa, North America, Caribbean and South America. This species was also reported from Taiwan by Hale (1976) and Lai (2000).

Comments. This species can be recognized by the maculate upper surface, black lower surface with a pale brown marginal zone, and the presence of salazinic acid in the medulla.

Specimen examined. **China:** *Hainan Prov.*: Ledong Co., Jianfengling, 860 m, 18°44'N, 109°10'E, 2001, *J. B. Chen et al.* 20770 (HAMS-L).

Discussion

Bulbothrix and *Relicina* can be easily recognized by their bulbate cilia, and can be clearly distinguished by secondary metabolites, with the former containing atranorin versus usnic acid in the latter. Although similar overall, these genera belong to distinct clades within the *Parmeliaceae*: *Bulbothrix* shares a common ancestor with *Parmelina*, whereas *Relicina* belongs to a group centred on *Parmelia* (Crespo *et al*. 2010). Furthermore, our phylogenetic trees agree with previous studies (Divakar *et al*. 2006, 2010; Kirika *et al*. 2015), whereby *Bulbothrix* species with bulbate base cilia and atranorin do not compose a monophyletic group. The *Bulbothrix isidiza* group (Clade 2) is sister to *Parmelinella* (Divakar *et al*. 2006, 2010; Masson *et al*. 2015) leading to this clade either nesting in the genus *Parmelinella* or being a small genus that contains salazinic acid. The type species of *Bulbothrix* (i.e., *B. semilunata*) may belong to Clade 1 (Kirika *et al*. 2015) on account of its distribution and morphological characteristics. The genus concept for *Bulbothrix* thus requires further revision and a more extensive sampling of species to resolve the relationships and circumscription of *Bulbothrix* and *Parmelinella*.

Our work builds on studies on Cambodian *Bulbothrix* (Zhang *et al*. 2014), clarifies the species of *Bulbothrix* from China, and thereby contributes to our knowledge of the lichen flora of Asia. Our phylogenetic inference shows that all *Bulbothrix* species from China belong to Clade 2 and have broader lobes, simple to rarely branched cilia and rhizines, and salazinic acid except for *B. scortella*, and confirms that Clade 2 is predominantly paleotropical as reported by Kirika *et al*. (2015). We discovered two new species, *B. mammillaria* and *B. lacinia*, that are distinguished by morphology and ITS sequences. Even so, more new species in this genus may eventually be found in southern China, given the great biodiversity of this region.

Key to species of *Bulbothrix* from China

1 Gyrophoric acid present (medulla C+ red), rhizines branched . . . . . **B. scortella**
Salazinic acid present (medulla C−), rhizines simple 2

2(1) Isidia absent 3
Isidia present 4

3(2) Lower surface pale brown . . . . . **B. setschwanensis**
Lower surface black, with a pale brown marginal zone . . . . . **B. meizospora**

4(2) Cilia reduced to a bulbate structure, without a hairy apex . . . . . **B. mammillaria**
Cilia with a bulbate base and a simple or branched tapering apex 5

5(4) Lower surface pale brown 6
Lower surface black, with a pale brown marginal zone 7

6(5) Numerous lacinulae, isidia spherical to short cylindrical, dark black . . . . . **B. lacinia**
Scarce lacinulae, isidia granular to slender cylindrical, concolorous with thalli or with a brown apex 8

7(5) Upper surface maculate, with a rough appearance . . . . . **B. tabacina**
Upper surface emaculate, with a smooth appearance . . . . . **B. asiatica**

8(6) Upper surface maculate, with a rough appearance . . . . . **B. isidiza**
Upper surface emaculate, with a smooth appearance . . . . . **B. subscortea**

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