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Standard Paper

A molecular study of the lichen genus *Byssoloma* Trevisan (*Pilocarpaceae*) with descriptions of three new species from China

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Abstract

A molecular phylogeny of the genus *Byssoloma* is inferred from mtSSU sequences using Bayesian and maximum likelihood phylogenetic analyses. *Byssoloma subdiscordans* is resolved as sister to *B. citricola* rather than to the *B. leucoblepharum* clade, the *B. subundulatum* group (species with a compact apothecial margin) is shown to be a monophyletic group, and three species belonging to this group are described as new to science from Hainan Province in China: *B. brunneodiscum* W. C. Wang & J. C. Wei, with dark brown apothecia, crystals in the excipulum and the presence of 2,5,7-trichloro-3-O-methylnorlichexanthone; *B. rubrofuscum* W. C. Wang & J. C. Wei, with red-brown apothecia, 3–6-septate ascospores and the presence of 5,7-dichloro-3-O-methylnorlichexanthone; *B. melanodiscocarpum* W. C. Wang & J. C. Wei, with pure black apothecia, a K+ olive-black hypothecium and the presence of thiophanic acid.

Key words: foliicolous lichens, Hainan, mtSSU, taxonomy

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Introduction

The genus *Byssoloma*, an almost cosmopolitan group of foliicolous lichens, is most diverse in the tropics; it includes *c.* 55 currently recognized species characterized by their byssoid apothecial margin, *Byssoloma*-type of ascus structure, pycnidia conidiomata, pyriform or bacillar conidia and 1–7-septate ascospores (Vězda 1974, 1975, 1987; Sérusiaux 1979, 1998; Kalb & Vězda 1990; Aptroot & Sipman 1991; Sipman & Aptroot 1992; Fárkas & Vězda 1993; Malcolm & Vězda 1995; Kondratyuk 1996; Aptroot *et al.* 1997, 2003; Cáceres 1999; Thor *et al.* 2000; Lücking *et al.* 2001; Sérusiaux *et al.* 2002; Schubert *et al.* 2003; Hermansson & Thor 2004; Lücking 2006, 2008, 2013; Messuti & de la Rosa 2007; Nguyen *et al.* 2010; Lumbsch *et al.* 2011; Breuss 2013, 2016*a*, *b*; Cáceres *et al.* 2013; Aptroot 2014; Gupta & Sinha 2015; van den Boom 2016; Wei 2017; Elix & McCarthy 2018; Wang & Wei 2018). Most species occur on living leaves and a small number can be found on bark, such as *Byssoloma arboricola* Sérus. & Aptroot, *B. australiense* P.M. McCarthy & Elix, *B. catillariosporum* M. Cáceres *et al.*, *B. fuscum* van den Boom, *B. llimonae* Sérus. *et al.*, *B. maderense* Breuss, *B. marginatum* (Arnold) Sérus., *B. permutans* (Nyl.) Lücking, *B. rubromarginatum* Messuti & de la Rosa, *B. vezdanum* Sérus. and *B. xanthonicum* Aptroot (Aptroot *et al.* 1997; Sérusiaux 1998; Sérusiaux *et al.* 2002; Messuti & de la Rosa 2007; Cáceres *et al.* 2013; Lücking 2013; Aptroot 2014; Breuss 2016*a*, *b*; van den Boom 2016; Elix & McCarthy 2018) or rocks, such as *B. adspersum* Malcolm & Vězda and *B. octomerum* Malcolm & Vězda (Malcolm & Vězda 1995). *Byssoloma lichenophila* S.Y. Kondr. *et al.*, is the only lichenicolous species (Kondratyuk 1996).

This study is based mainly on our own collections from South China and Thailand. The phylogenetic reconstruction with molecular data and detailed analysis of morphological and anatomical characters supports the description of three new species.

Material and Methods

Material of the new species and other samples used for DNA analysis are deposited in the herbaria HMAS-L and RAMK, with an additional four specimens from South America and Europe stored in the private herbaria of Pieter van den Boom (Table 1).

Morphology and chemistry

All specimens were examined using a Motic dissecting microscope and an Olympus CX21 compound microscope. Micrographs were taken with a Leica M125 dissecting microscope equipped with a Leica DFC450 camera. Anatomical mounts were photographed with a Zeiss Imager A2 compound microscope equipped with a Zeiss AxioCam MRc5 camera. For identification purposes, colour reactions of apothecium sections were tested with KOH (a 10% aqueous solution of potassium hydroxide), I (a 10% aqueous solution of potassium iodide) and P (saturated solution of p-phenylenediamine in 95% ethyl alcohol). Lichen substances were identified using standardized thin-layer chromatography techniques (TLC) with solvent system C (Orange *et al.*

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Table 1. Specimens used in the mtSSU-based phylogenetic analysis of *Byssoloma* species together with their voucher information and GenBank Accession numbers. New sequences and new species are in bold.

Taxon	Locality	Voucher specimens	mtSSU-GenBank No.
Byssolecania hymenocarpa	Thailand	*Wang* KYW0286 (RAMK-31639)	MK957152
B. hymenocarpa	Thailand	*Wang* KYW0254 (RAMK-31633)	MK957159
Byssolecania sp.	China, Yunnan	*Wang* 20180247 (HMAS-L 144266)	MK957170
Byssoloma annuum	China, Hainan	*Wang* HN20170295 (HMAS-L 139408)	**MN043716**
B. annuum	China, Hainan	*Wang* HN20170297 (HMAS-L 139745)	**MN043726**
B. brunneodiscum	China, Hainan	*Wang* HN20170147 (HMAS-L 139507)	**MN105603**
B. brunneodiscum	China, Hainan	*Wang* HN20170165 (HMAS-L 139422)	**MN105600**
B. citricola	Surinam	*P. v. d. Boom* 50677	**MN043707**
B. leucoblepharum	Thailand	*Wang* KYW0405 (RAMK-31929)	MK957160
B. leucoblepharum	Thailand	*Wang* KYW0188 (RAMK-31585)	MN105612
B. leucoblepharum	China, Yunnan	*Wang* 20180153 (HMAS-L 140621)	MK957165
B. leucoblepharum	China, Yunnan	*Wang* 20180145 (HMAS-L 140613)	MK957166
B. leucoblepharum	China, Hainan	*Wang* HN20170357 (HMAS-L 139782)	MK957174
B. leucoblepharum	Thailand	*Wang* KYW0422 (RAMK-31715)	**MN043694**
B. leucoblepharum	China, Yunnan	*Wang* 20180152 (HMAS-L 140620)	**MN043708**
B. leucoblepharum	China, Fujian	*Wang* 20190428 (HMAS-L 144223)	**MN105604**
B. leucoblepharum	China, Fujian	*Wang* 20190447 (HMAS-L 144222)	**MN105605**
B. leucoblepharum	China, Hainan	*Wang* HN20170091 (HMAS-L 139568)	**MN043720**
B. leucoblepharum	China, Hainan	*Wang* HN20170108 (HMAS-L 139585)	**MN043724**
B. leucoblepharum	Ecuador	*P. v. d. Boom* 54740	**MN105613**
B. cf. *leucoblepharum*	Thailand	*Wang* KYW0440 (RAMK-31945)	**MN043695**
B. cf. *leucoblepharum*	Thailand	*Wang* KYW0451 (RAMK-31730)	**MN043698**
B. cf. *leucoblepharum*	Thailand	*Wang* KYW0418 (RAMK-31711)	**MN105594**
B. cf. *leucoblepharum*	Thailand	*Wang* KYW0419 (RAMK-31933)	**MN043691**
B. cf. *leucoblepharum*	Thailand	*Wang* KYW0184 (RAMK-31582)	**MN043700**
B. cf. *leucoblepharum*	Thailand	*Wang* KYW0187 (RAMK-31584)	**MN105597**
B. cf. *leucoblepharum*	Thailand	*Wang* KYW0415 (RAMK-31708)	**MN043693**
B. cf. *leucoblepharum*	Thailand	*Wang* KYW0375 (RAMK-31881)	**MN043702**
B. cf. *leucoblepharum*	Thailand	*Wang* KYW0444 (RAMK-31725)	**MN105595**
B. cf. *leucoblepharum*	Thailand	*Wang* KYW0445 (RAMK-31726)	**MN043697**
B. cf. *leucoblepharum*	Thailand	*Wang* KYW0441 (RAMK-31946)	**MN043696**
B. melanodiscocarpum	China, Hainan	*Wang* HN20170148 (HMAS-L 139508)	**MN105607**
B. melanodiscocarpum	China, Hainan	*Wang* HN20170153 (HMAS-L 139512)	**MN105608**
B. melanodiscocarpum	China, Hainan	*Wang* HN20170298 (HMAS-L 139744)	**MN105601**
B. rubrofuscum	China, Hainan	*Wang* HN20170295-1 (HMAS-L 144214)	**MN105599**
B. rubrofuscum	China, Hainan	*Wang* HN20170297-1 (HMAS-L 144216)	**MN105602**
B. subdiscordans	Portugal	*P. v. d. Boom* 57130	**MN043703**
B. subdiscordans	Portugal	*P. v. d. Boom* 57021	**MN043704**
B. subdiscordans	China, Hainan	*Wang* HN2014213 (HMAS-L 132508)	**MN105606**
B. subdiscordans	China, Hainan	*Wang* HN20170156 (HMAS-L 139514)	**MN105609**
B. vanderystii	China, Hainan	*Wang* HN20170227 (HMAS-L 139541)	**MN043718**
B. vanderystii	China, Hainan	*Wang* HN20170232 (HMAS-L 139546)	**MN043713**
B. vanderystii	China, Hainan	*Wang* HN20170258 (HMAS-L 139478)	**MN043715**

(*Continued*)

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Table 1. (*Continued*)

Taxon	Locality	Voucher specimens	mtSSU-GenBank No.
B. vanderystii	China, Hainan	*Wang* HN20170261 (HMAS-L 139481)	**MN043714**
B. vanderystii	China, Hainan	*Wang* HN20170268 (HMAS-L 139488)	**MN105598**
B. vanderystii	China, Hainan	*Wang* HN20170102 (HMAS-L 139579)	**MN043712**
B. vanderystii	China, Hainan	*Wang* HN20170097 (HMAS-L 139573)	**MN043719**
B. vanderystii	Thailand	*Wang* KYW0375 (RAMK-31659)	**MN043701**
B. vanderystii	Thailand	*Wang* KYW0056 (RAMK-31553)	**MN105596**
B. vanderystii	Thailand	*Wang* KYW0060 (RAMK-31556)	**MN043699**
B. vanderystii	China, Yunnan	*Wang* 20180144 (HMAS-L 140612)	**MN043710**
Byssoloma sp.	China, Guangxi	*Wang* 20190514 (HMAS-L 144227)	**MN105610**
Byssoloma sp.	China, Guangxi	*Wang* 20190551 (HMAS-L 144217)	**MN105611**

2010). Paraphyses were investigated in 10% KOH when they were indistinct due to strong gelatinization.

DNA extraction, PCR amplification and sequencing

DNA was extracted from 8–30 apothecia per sample using the DNAsecure Plant Kit DP320-03 (200preps; Tiangen, Beijing, China). Apothecia were cut from cleaned thallus material with a razor blade, and extractions were carried out following the instructions of the kit. Finally, the DNA was held in 1.5 ml micro-centrifuge tubes and kept at −20 °C.

PCR amplification of mtSSU rDNA was performed using the primers mrSSU1 and mrSSU3R (Zoller *et al.* 1999). The 25 µl of PCR mix contained 12.5 µl 2 × MasterMix (TaqDNA Polymerase (0.1 units/µl), 4 mM $MgCl_2$, 0.4 nM dNTPs; Aidlab Biotechnologies Co., Ltd, Beijing, China), 1 µl of each primer, 8.5 µl ddH_2O, and 2 µl of DNA. The PCR cycling parameters included an initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 53 °C for 30 s and 72 °C for 1 min 30 s, and a final extension at 72 °C for 8 min. PCR products were purified using the magnetic bead method and Sanger sequenced by the Beijing Ruibio BiotechCo., Ltd (China).

Sequence alignment and phylogenetic analysis

A total of 45 new mtSSU sequences were aligned, together with 8 sequences retrieved from GenBank (Table 1). Three sequences of *Byssolecania* Vain. were chosen as outgroup based on previous phylogenetic analyses (Andersen & Ekman 2005) and phenotypic similarity. Primary sequences were assembled and edited with Geneious v.6.1.2 (Biomatters Ltd, Auckland, NZ), and sequence alignments were conducted online using MAFFT v.7 (Katoh *et al.* 2009). Ambiguously aligned sites were excluded with Gblocks v.0.91b (Castresana 2000), implementing all the options for a less stringent selection (http://molevol.cmima.csic.es/castresana/Gblocks_server.html). A matrix consisting of 814 unambiguously aligned mtSSU positions was produced.

Phylogenetic analyses based on mtSSU were carried out using two different approaches, maximum likelihood (ML) and Bayesian inference (BI). Maximum likelihood analysis was performed with RAxML-HPC v.8.2.6 (Stamatakis 2014) on the Cipres Science Gateway (http://www.phylo.org) and support values were inferred from the 70% majority-rule tree of all saved trees obtained from 1000 non-parametric bootstrap pseudoreplicates. Bayesian analysis was performed using MrBayes v.3.2.6 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). For the BI analysis, the GTR + G model was selected, based on jModelTest 2.1.4 (Darriba *et al.* 2012). Markov chain Monte Carlo (MCMC) was initiated from a random tree using 5 million generations and saving every 1000th tree, with the initial 25% of trees discarded as burn-in. Stationarity of analysis was determined by examining the standard deviation of split frequencies (< 0.01). A majority-rule consensus tree was constructed from the remaining trees to obtain posterior probability (PP), with values ≥ 0.95 considered to be significantly supported. The phylogenetic tree was visualised with FigTree v.1.4.3.

Results

The final alignment consisted of 45 mtSSU sequences newly generated from 45 global *Pilocarpaceae* specimens, and 8 mtSSU sequences downloaded from NCBI (Table 1). The two separate analyses, maximum likelihood and Bayesian inference, did not show any conflicts; therefore, only the BI tree is presented, with ML bootstrap support (BS) ≥ 75% and BI posterior probabilities (PP) ≥ 0.95 (Fig. 1).

The mtSSU sequences show that all species with multiple accessions form strongly supported clades in the tree, and the in-group consists of four distinct well-supported clades: sequences of *B. vanderystii* Sérus. are combined in a basal clade, *B. subdiscordans* (Nyl.) P. James and *B. citricola* (Maubl.) Lücking *et al.* form a strongly-supported monophyletic clade, *B. annuum* (Vain.) G. Thor *et al.* is strongly supported as sister to the new species *B. brunneodiscum* and the *B. annuum-brunneodiscum* clade together with *B. melanodiscocarpum*, are sister to *B. rubrofuscum*; consequently these four species form a highly supported clade together with an unnamed species which is basal to this clade. We name this the *B. subundulatum* clade according to Lücking (2008). All sequences of *B. leucoblepharum* (Nyl.) Vain. form a well-supported clade sister to the *B. subundulatum* clade, the sister relationship receiving high support (BS = 99, PP = 1).

Discussion

Our molecular results show that *B. subdiscordans* is sister to *B. citricola*, with which it forms a monophyletic group instead of

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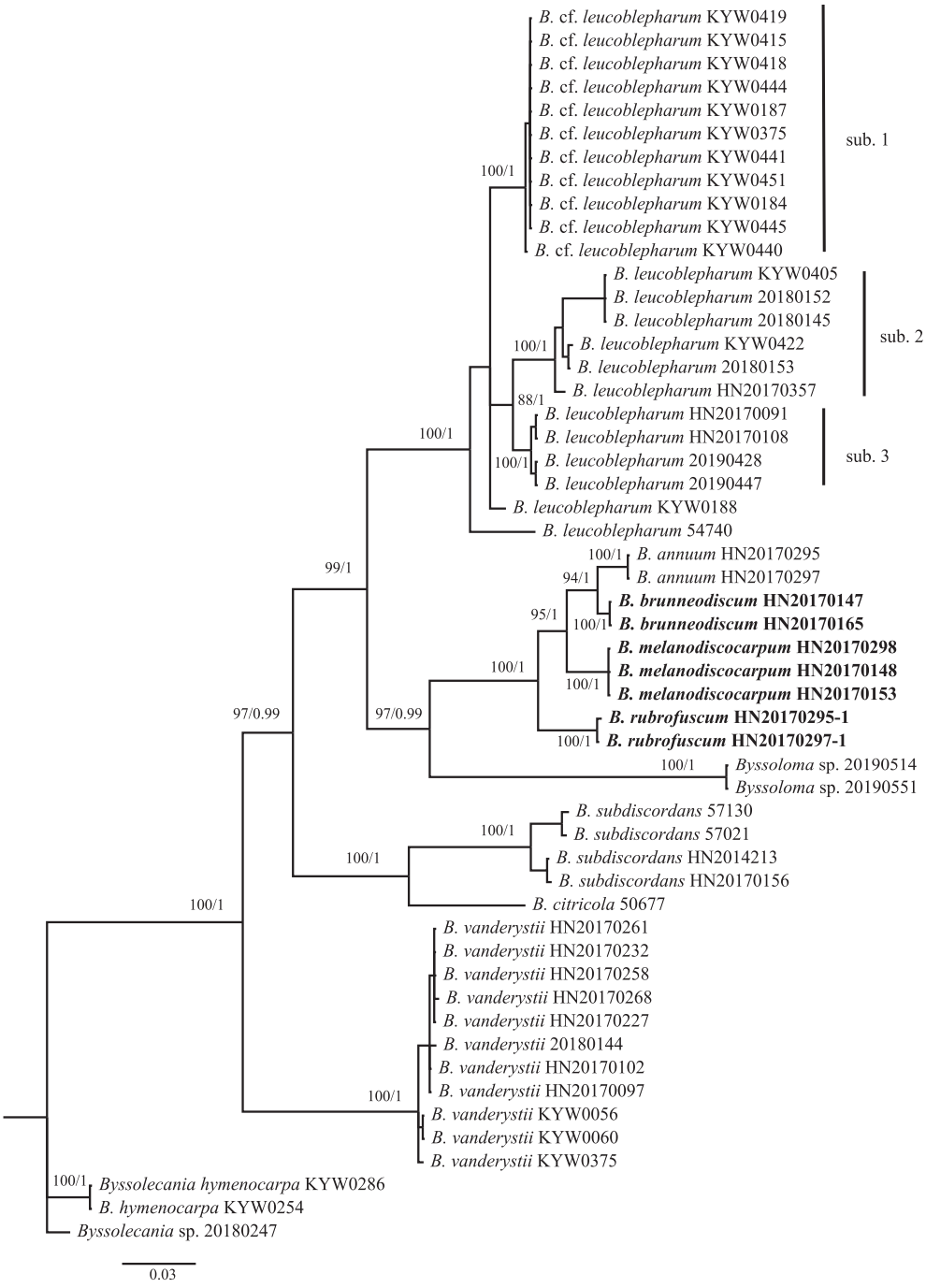

Fig. 1. Phylogenetic relationships among 50 sequences (Table 1) representing 9 species of *Byssoloma* based on a Bayesian analysis of the mtSSU dataset. Bootstrap values ≥ 75 and posterior probabilities ≥ 0.95 are indicated at the branches. Newly described species are in bold. Three subclades are indicated for *B. leucoblepharum*. The tree was rooted using *Byssolecania* spp. Scale = 0.03 substitution per site.

belonging to the *B. leucoblepharum* group as originally suggested by Lücking (2008). *Byssoloma subdiscordans* forms dispersed thalli and pure black apothecia resembling those of *B. citricola*; our molecular data point to the importance of thallus character in taxonomic studies of *Byssoloma*.

In his monograph of foliicolous lichens in the Neotropics, Lücking (2008) combined the species with a compact apothecial margin composed of densely woven hyphae in the *B. subundulatum* group. In our study, five species belonging to this group, *B. annuum*, *B. rubrofuscum*, *B. melanodiscocarpum*, *B. brunneodiscum* and an unnamed species, are sequenced and form a well-supported clade. Our data suggest that the *B. subundulatum* group is probably monophyletic.

The main morphological features separating the *B. vanderystii* clade from other groups in the tree are its bacillar conidia and 7-septate ascospores, while other *Byssoloma* species usually have pyriform conidia and 3–5-septate ascospores.

Byssoloma leucoblepharum has been found to be a highly variable species in terms of pigmentation, shape and size of apothecia, and apothecial anatomy. There are three main subclades (sub. 1, sub. 2, sub. 3) in the *B. leucoblepharum* clade, all of which have

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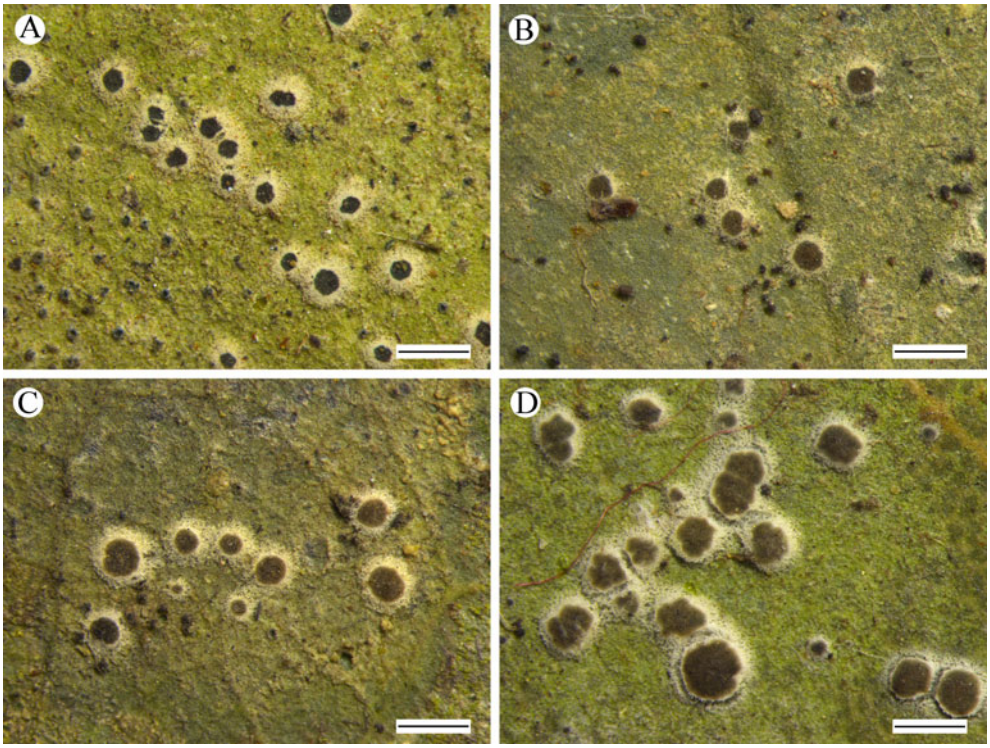

Fig. 2. *Byssoloma leucoblepharum*, thalli with apothecia representing the different subclades in Fig. 1. A, *Wang* KYW0184 (sub. 1). B, *Wang* 20180153 (sub. 2). C, *Wang* 20180152 (sub. 2). D, *Wang* 20190447 (sub.3). Scales: A–D = 0.5 mm.

the same pyriform conidia and are without secondary compounds detected by TLC. However, their morphological distinctions are: sub. 1 has a smooth thallus, small apothecia (0.1–0.2 mm diam.) with a grey-black, flat disc and an indistinct prothallus (accession KYW0188 also has this morphology); sub. 2 has a smooth thallus and flat apothecia, similar to sub. 1 but the apothecia are larger (0.2–0.4 mm diam.) and paler in colour (grey-brown), and the prothallus is grey; sub. 3 is distinguished by even larger apothecia than above (up to 0.6 mm diam.), the disc is pale to dark grey-brown and strongly convex in some specimens (Fig. 2).

The morphological characteristics of *B. leucoblepharum* seem to conform to the geographical distribution of the subclades: the higher the latitude, the larger the apothecia (specimens of sub. 1 are from Thailand, sub. 2 from Xishuangbanna and Hainan in China, and sub. 3 are from Hainan and Fujian in China), and the intermediate transition is obvious. Although specimens from different regions exhibit some minor morphological differences, we tentatively treat these specimens as one species here. A more comprehensive understanding of this species worldwide is necessary before dividing them into different new taxa.

Taxonomic Treatment

Byssoloma brunneodiscum W. C. Wang & J. C. Wei sp. nov.

Fungal Names No.: FN 570659

Similar to *B. annuum* but differs in having brown to dark brown apothecia and a pale brown hypothecium.

Type: China, Hainan Province, Ledong County, Jianfeng Ridge, Mingfeng Valley, 18°44′N, 108°50′E, 960 m alt., on leaves, 6 September 2017, *W. C. Wang* 139422 (HMAS-L—holotype).

(Figs 3A & B, 4A–C)

Thallus epiphyllous, 0.5–1 cm diam., smooth, continuous, green, K−, P−, without hypothallus. *Photobiont* chlorococcoid with globose green cells, 5–7.5 µm diam.

Apothecia rounded, single, sessile, basally constricted, 0.3–0.5 mm diam., 100–120 µm high; *disc* plane, brown to dark brown, dull, epruinose; *margin* white, byssoid but compact, hyphae not forming a rim over the surrounding thallus, thick, prominent and persistent, *c.* 50 µm wide, K−, P−. *Excipulum* and *apothecial base* (the part below hypothecium) composed of abundant colourless loosely interwoven hyphae, encrusted with crystals which dissolve in K, K+ pink or K− (colourless or very pale green in excipulum), P−; excipulum 35–40 µm wide, apothecial base 35–40 µm high; *hypothecium* pale brown, poorly developed, 25–30 µm high, K−, P−; *epithecium* colourless or pale brown, K−, P−; *hymenium* hyaline, mixed with brown pigment, 40–50 µm high; *paraphyses* indistinct, branched and anastomosing. *Asci* clavate, 45–50 × 10–12 µm, 8-spored, ascus apex I+ dark blue, *Byssoloma*-type (Hafellner 1984); *ascospores* 3-septate, without gelatinous perispore, ellipsoid, colourless, 11–14 × 3–4 µm.

Pycnidia sessile, wart-shaped, 10–13 µm wide, with an apical ostiolum when mature, pale grey-brown with black centre. *Conidia* pyriform, colourless, non-septate, 2–2.5 × 1.5 µm.

Chemistry. 2,5,7-trichloro-3-O-methylnorlichexanthone detected by TLC.

Etymology. The epithet of the new species 'brunneodiscum' is a Latin compound consisting of 'brunneo' (= brown) and 'discum' (= disc).

Habitat. Currently known only from Hainan Island, China, where it grows, only sparsely, on leaf surfaces in the damp understory of rainforests at high altitudes on a mountain.

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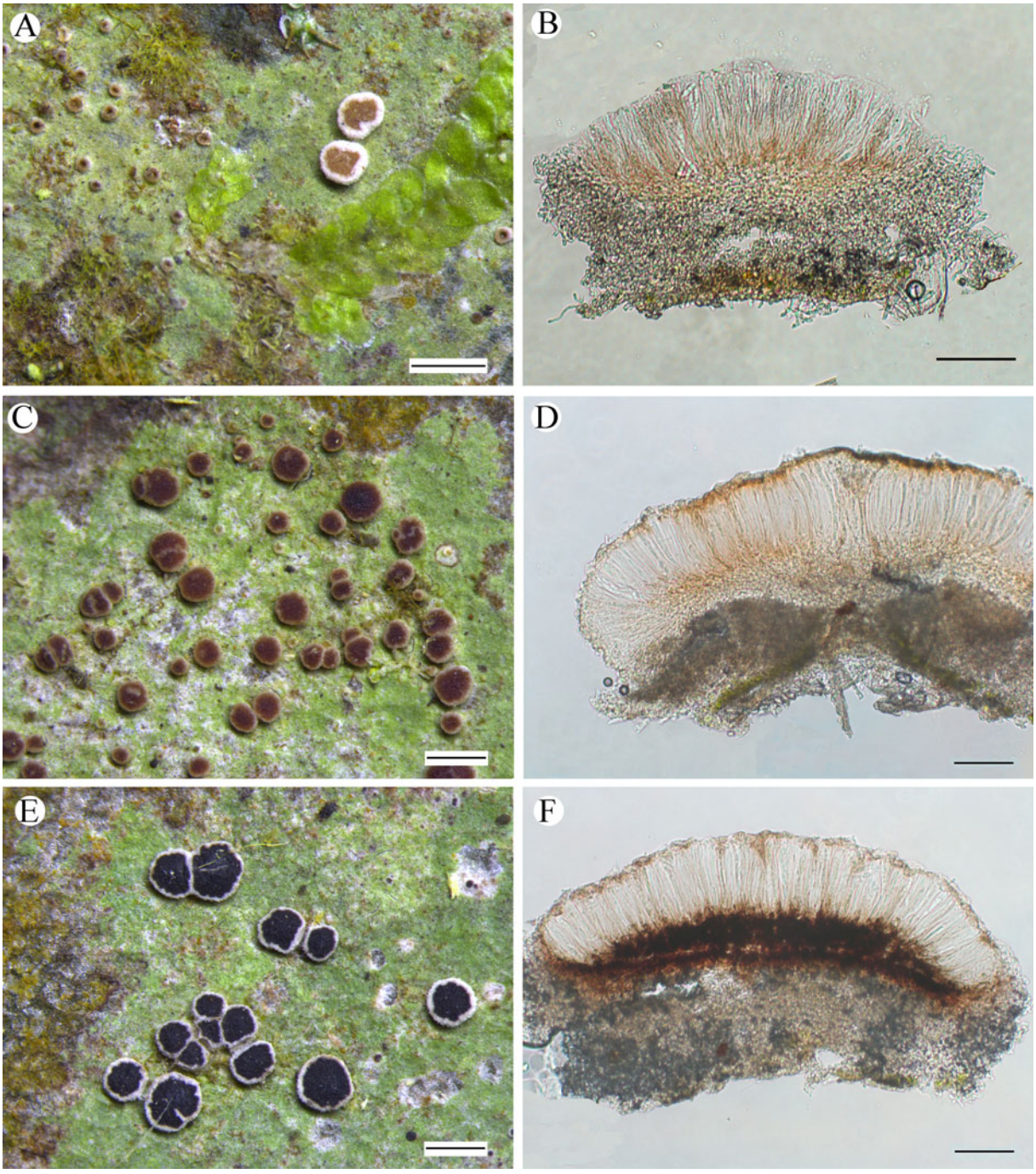

Fig. 3. A & B, *Byssoloma brunneodiscum* (holotype, *Wang* 139422). C & D, *B. rubrofuscum* (holotype, *Wang* 144214). E & F, *B. melanodiscocarpum* (holotype, *Wang* 139744). A, C & E, thallus with apothecia. B, D & F, section through apothecium (in water). Scales: A, C & E = 200 μm; B, D & F = 50 μm.

Notes. *Byssoloma annuum* is one of the most closely related species to *B. brunneodiscum* (both contain 2,5,7-trichloro-3-O-methylnorlichexanthone), but it can be distinguished by its orange-yellow apothecia with a colourless hypothecium. A further closely related species is *B. confusum* Farkas & Vězda from Africa, which differs by its excipulum lacking crystals, halonate ascospores and K− apothecial base (Fárkas & Vězda 1993). *Byssoloma brunneodiscum* is also similar to *B. polychromum* (Müll. Arg.) Zahlbr. in its brown apothecia with a compact byssoid white margin and 3-septate ascospores, but the latter differs by its finely verrucose thallus and the presence of a chemosyndrome of 3-O-methylchloro-xanthones with thiophanic acid and 3-O-methylthiophanic acid, as the major components (Elix *et al.* 1995).

Byssoloma brunneodiscum also appears to be closely related to *B. rubrofuscum* and *B. melanodiscocarpum*, the other two new species described below, both of which have a green, smooth continuous thallus, a compact white byssoid apothecial margin and a similar habitat. However, there are a number of differences which set the species apart: *B. rubrofuscum* has red-brown apothecia, 3–6-septate ascospores and produces 5,7-dichloro-3-O-methylnorlichexanthone as a major compound; *B. melanodiscocarpum*

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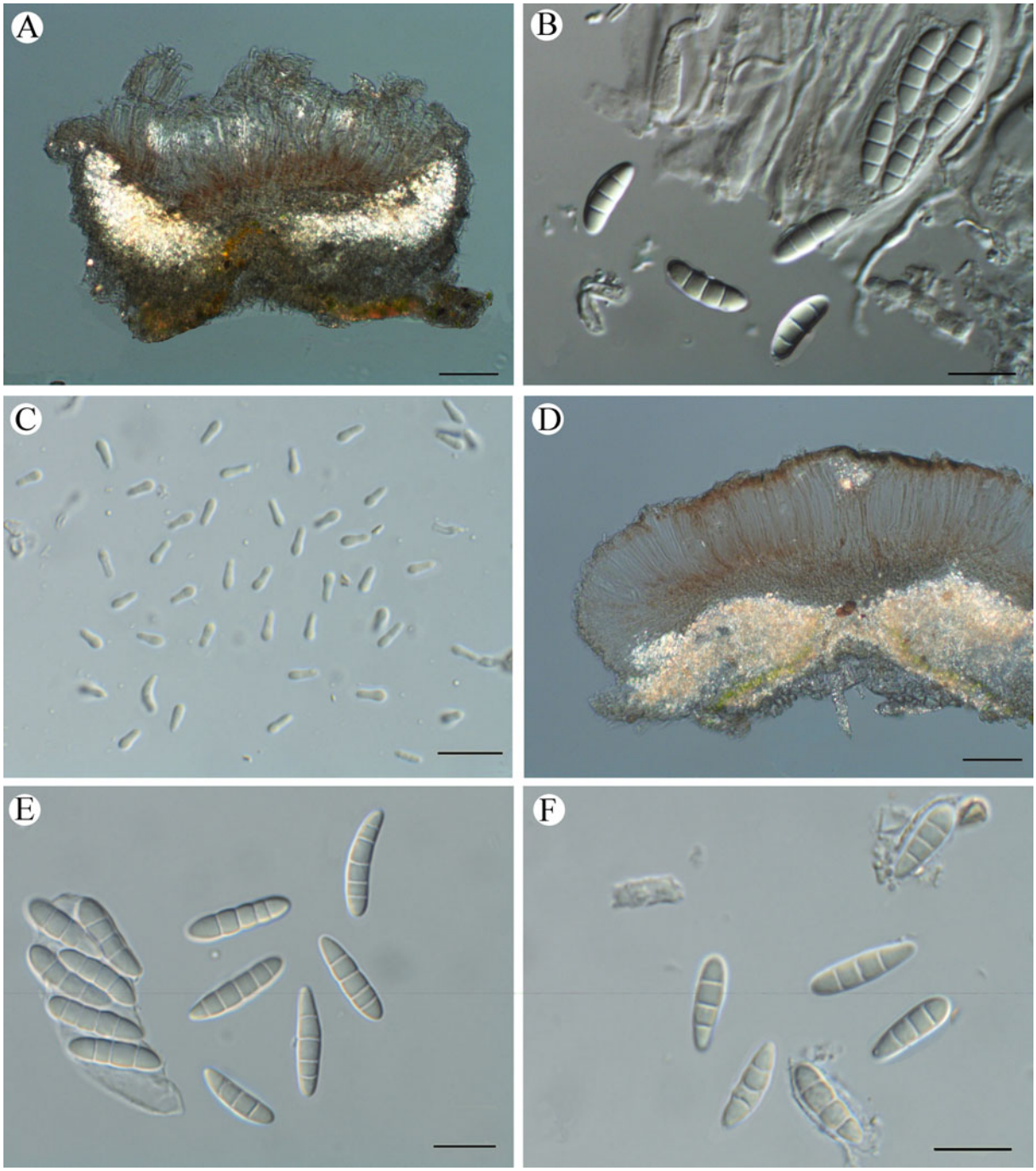

Fig. 4. A–C, *Byssoloma brunneodiscum* (139422). D & E, *B. rubrofuscum* (144214). F, *B. melanodiscocarpum* (139744). A, section through apothecium showing colourless crystals in exciple and apothecial base (using cross-polarized light). B, E & F, ascospores (using differential interference contrast microscopy). C, pyriform conidia. D, section through apothecium showing yellow crystals in apothecial base (using cross-polarized light). Scales: A & D = 50 μm; B, C, E & F = 10 μm. In colour online.

has pure black apothecia without crystals, produces thiophanic acid as a major secondary compound, and its hypothecium is dark reddish brown and K+ olive-black.

Fellhanera albidocincta (Vain.) Lücking is similar in general appearance but has translucent apothecia with a thin chamois-coloured margin and a paraplectenchymatous excipulum (at least in internal parts), while *Bysolloma brunneodiscum* has opaque apothecia with a thick white margin, and its excipulum is entirely composed of free, intricate hyphae with cylindrical cells which extend to the apothecial base.

Additional specimen examined. **China:** *Hainan*: Ledong County, Jianfeng Ridge, Mingfeng Valley, 18°44′N, 108°50′E, 960 m alt., on leaves, 2017, *W. C. Wang* 139507 (HMAS-L).

Byssoloma rubrofuscum W. C. Wang & J .C. Wei sp. nov.

Fungal Names No.: FN 570657

Similar to *B. confusum* but differs in having red-brown apothecia, yellow crystals in the excipulum and apothecial base,

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3–6-septate ascospores and containing 5,7-dichloro-3-O-methylnorlichexanthone.

Type: China, Hainan Province, Wuzhishan City, Wuzhishan National Forest Park, 18°54′N, 109°41′E, 800 m alt., on leaves, 8 September 2017, *W. C. Wang* 144214 (HMAS-L—holotype).

(Figs 3C & D, 4D & E)

Thallus epiphyllous, 1–2.5 cm diam., smooth, continuous, green, K−, P−; *margin* irregular or dispersed into small patches, without hypothallus. *Photobiont* chlorococcoid with globose green cells, 10–15 µm diam.

Apothecia rounded, single or some clustered in groups, sessile, basally constricted, 0.25–0.75 mm diam., 150–170 µm high; *disc* plane, red-brown, or dark red-brown in old specimens, epruinose; *margin* white, byssoid but compact, hyphae not forming a rim over the surrounding thallus, thick, prominent and persistent, *c.* 50 µm wide, K−, P−. *Excipulum* and *apothecial base* composed of abundant colourless, loosely interwoven hyphae, encrusted with yellow crystals which partly dissolve in K, K+ pale yellow, P−, but in some samples inner part extending from hypothecium is well developed, made of radially arranged rows of tight elongated cells instead of loosely interwoven hyphae, without crystals, and the original excipulum (composed of loosely interwoven hyphae) becomes vestigial (Fig. 3D); excipulum 35–40 µm wide, apothecial base 50–60 µm high; *hypothecium* pale brown, 45–55 µm high, K+ brown or orange; *epithecium* well developed, pale brown to brown, K−; *hymenium* with a pale brownish pigment, 60–65 µm high; *paraphyses* indistinct, branched and anastomosing. *Asci* clavate, 40–50 × 10–12.5 µm, 8-spored, ascus apex I+ dark blue, *Byssoloma*-type (Hafellner 1984); *ascospores* 3–5(–6)-septate, without gelatinous perispore, ellipsoid, colourless, 14–18 × 3.5–4 µm.

Pycnidia not seen.

Chemistry. 5,7-dichloro-3-O-methylnorlichexanthone detected by TLC.

Etymology. The epithet of the new species 'rubrofuscum' refers to its reddish brown apothecium.

Habitat. Currently known only from Hainan Province, China, growing on leaf surfaces in damp understory of rainforests at high altitudes of a mountain or near to a river in a forested valley. It is usually associated with *B. melanodiscocarpum*.

Notes. The new species is unique in the genus in having predominantly 3–6-septate ascospores, and absent to poorly developed superficial hyphal strands.

Byssoloma sprucei (C. Bab. ex Müll. Arg.) Lücking & M. Cáceres, a member of the *B. subundulatum* group, has red-brown apothecia very similar to *B. rubrofuscum* but differs in its farinose pale blue thallus, brown apothecial base and 3-septate ascospores. *Byssoloma sprucei* (syn. *B. pernambucense* M. Cáceres) is a neotropical species previously reported only from Brazil.

Additional specimens examined. **China:** *Hainan*: Wuzhishan City, Wuzhishan National Forest Park, 18°54′N, 109°41′E, 800 m alt., on leaves, 2017, *W. C. Wang* 144216 (HMAS-L); Ledong County, Jianfeng Ridge, 18°44′N, 109°10′E, 900 m alt., on leaves, 1993, *Y. M. Jiang* & *S. Y. Guo* 138605, 138623, 138626, 138639 (HMAS-L); *ibid.*, 18°44′N, 109°10′E, 1000 m alt., on leaves, 2000, *M. R. Huang* 112918, 112963 (HMAS-L); *ibid.*, hydrometric station, 18°44′N, 109°10′E, on leaves, 2006, *J. C. Wei* 112786, 112811, 112813, 112815, 112816, 112947 (HMAS-L).

Byssoloma melanodiscocarpum W. C. Wang & J. C. Wei sp. nov.

Fungal Names No.: FN 570658

Similar to *B. discordans* var. *flavescens* G. Thor *et al.* but differs in having a continuous, smooth green thallus, pure black apothecia and a K+ olive-black hypothecium.

Type: China, Hainan Province, Wuzhishan City, Wuzhishan National Forest Park, 18°54′N, 109°41′E, 800 m alt., on leaves, 8 September 2017, *W. C. Wang* 139744 (HMAS-L—holotype).

(Figs 3E & F, 4F)

Thallus epiphyllous, 1–2.5 cm in diam., smooth, continuous, green, K−, P−; *margin* irregular or sometimes dispersed into small patches, without hypothallus. *Photobiont* chlorococcoid with globose green cells, 7.5–15 µm diam.

Apothecia rounded, single or some clustered in groups, sessile, basally constricted, 0.25–0.75 mm diam., 180–200 µm high; *disc* plane, pure black, epruinose; *margin* white, byssoid but compact, hyphae not forming a rim over the surrounding thallus, prominent and persistent, thick, *c.* 50 µm wide, K−, P−. *Excipulum* and *apothecial base* well developed, without crystals, composed of abundant, colourless, loosely interwoven hyphae, mixed with granular substances which dissolve in K, K+ pale yellowish green, P−, excipulum 35–40 µm wide, apothecial base 70–80 µm high; *hypothecium* dark brown to dark reddish brown, 45–55 µm high, K+ olive-black (the red tinge disappears), P−; *epithecium* well developed, pale brown, turning colourless in K, P−; *hymenium* hyaline, mixed with pale brown pigment, K+ olive, P−, 60–65 µm high; *paraphyses* indistinct, branched and anastomosing. *Asci* clavate, 45–50 × 7.5–10 µm, 8-spored, ascus apex I+ dark blue, *Byssoloma*-type (Hafellner 1984); *ascospores* 3-septate, slightly constricted at the septa, some with a gelatinous perispore, ellipsoid, colourless, 12–15 × 3–4 µm.

Pycnidia not seen.

Chemistry. Thiophanic acid detected by TLC.

Etymology. The epithet of the new species 'melanodiscocarpum' is a Greek compound consisting of 'melano' (= black), 'disco' (= disc) and 'carpus' (= fruit).

Habitat. This species is abundant in Jianfeng Ridge and Mt Wuzhishan (Hainan Island, China) and is also present in Thailand, growing on living leaves, such as monocotyledons, in the damp understory of rainforests at high altitudes of a mountain or near the river in a valley.

Notes. *Byssoloma melanodiscocarpum* is closely related to *B. discordans* var. *flavescens* described from Japan (Thor *et al.* 2000), which also has almost black apothecia with a white compact margin and 3-septate ascospores. However, in *B. discordans* var. *flavescens*, the thallus is pale green to blue-white, and the apothecia are dark brown to black-brown with a K− hypothecium and crystals in the excipulum. *Byssoloma melanodiscocarpum* is also close to *B. confusum*, which has similar apothecia with a

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white compact margin, but which differ in colour (pale brown) and have a K− brown hypothecium.

Byssoloma melanodiscocarpum should not be confused with a member of the genus *Eugeniella* Lücking *et al.* They might be similar at first glance, but *Eugeniella* is characterized by a smooth and slightly prominent apothecial margin, distinct, unbranched or slightly branched paraphyses, fusiform to oblong conidia, and an excipulum composed of strongly compacted, moniliform hyphae with globose cells and constricted septa; in contrast, *B. melanodiscocarpum* has a byssoid apothecial margin, indistinct, sparsely to densely branched and anastomosing paraphyses, and an excipulum typically composed of free, loosely interwoven, branched and septate hyphae with cylindrical cells and lacking constrictions.

Additional specimens examined. **China:** *Hainan*: Ledong County, Jianfeng Ridge, 18°44′N, 109°10′E, 900 m alt., on leaves, 1993, *Y. M. Jiang* & *S. Y. Guo* 138617, 138618, 138627, 138629, 138636, 138637, 138638 (HMAS-L); *ibid.*, 2006, *J. C. Wei* 112790, 112791, 112812, 112824, 112828, 112830, 112831, 112942, 112943, 112949, 112950, 112954, 112955, 112958, 113055 (HMAS-L); *ibid.*, 18°44′N, 109°1′E, 1000 m alt., on leaves, 2000, *M. R. Huang* 108866, 108865, 108868, 112792, 112795, 112834, 112920, 112922 (HMAS-L); *ibid.*, Mingfeng Valley, 18° 44′N, 108°50′E, 960 m alt., on leaves, 2017, *W. C. Wang* 139435, 139499, 139502, 139505, 139506, 139507, 139508, 139510, 139512, 139513, 139515 (HMAS-L); Wuzhishan City, Wuzhishan National Forest Park, 18°54′N, 109°41′E, 800 m alt., on leaves, 2017, *W. C. Wang* 144215 (HMAS-L); Qiongzhong County, Mt Limu, 19°10′N, 109°44′E, 800 m alt., on leaves, 2017, *W. C. Wang* 139786 (HMAS-L); Wuzhishan City, Mt Wuzhishan, 18.53°N, 109.41°E, 900 m alt., on leaves, 2000, *J. C. Wei* 112810, 113047 (HMAS-L); Changjiang County, Bawang Ridge, 19°15′N, 109°02′E, 670 m alt., on leaves, 2007, *T. Zhang* 126591, 126593, 126602, 126605, 126606, 126607, 126609, 126767 (HMAS-L); Lingshui County, Mt Diaoluo, 19.02° N, 109.5°E, 1000 m alt., on leaves, 2000, *M. R. Huang* 112796, 112800, 112802, 112837, 112898, 112904, 112906 (HMAS-L).— **Thailand:** *Nakon Ratchasima*: Khao Yai National Park, Pakchong District, forest surrounding the cliff 'Diao Dai', 14°22′N, 101° 23′E, 1281 m alt., on leaves, 1999, *K. Papong* 4605 (RAMK).

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