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Standard Paper

Two new species of the lichenized genus *Lasioloma* (*Byssolomataceae*) from Asia

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Abstract

Two new species of the lichenized genus *Lasioloma* are described from Asia: *Lasioloma longiramosum* W. C. Wang & A. Abas (collected from Malaysia), is characterized by a distinct woolly prothallus between dispersed thallus patches, comparatively small, muriform ascospores, long filiform conidia (main branch 22–28 μm in length, the other three branches 65–80 μm) and a foliicolous habitat; *L. verrucosum* W. C. Wang & X. L. Wei (collected from China), is characterized by a warted thallus, filiform conidia (main branch 22–32 μm in length, the other three branches 50–65 μm) and a corticolous habitat. The placement of both new species was confirmed by a molecular phylogenetic approach based on combined ITS, mtSSU and mtLSU sequences, and both are compared in detail to other similar species of the genus. Our study also revealed that the length of the conidial branches, which has not been explored in previous studies, should be regarded as an important feature for species delimitation in *Lasioloma*.

Key words: China, conidia, foliicolous lichens, Malaysia, mtLSU

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Introduction

The genus *Lasioloma* was established by Santesson (1952), based mainly on its woolly prothallus, pilose apothecial margin and muriform ascospores. Santesson recognized three species of the genus from Asia, but at the time did not capture the nature of the campylidioid anamorphs in this genus and considered them to represent a lichenicolous fungus, which he named *Pyrenotrichum staurosporum* comb. ined., based on *Chlorocyphella aeruginascens* var. *staurospora* Keissl.

Vězda (1986) recognized the true nature of these structures for the first time and described in detail the conidial morphology of the type species of the genus, *L. arachnoideum* (Kremp.) R. Sant., as another important feature to delimit the genus *Lasioloma*.

Species of *Lasioloma* are known from foliicolous and corticolous substrata, mainly distributed in tropical rainforests. Foliicolous species are characterized by a woolly prothallus, a dispersed to continuous thallus, and a pilose apothecial margin; corticolous species usually lack a woolly prothallus and their thalli are usually continuous. All known species produce campylidia and branched conidia (Santesson & Lücking 1999; Lücking & Sérusiaux 2001; Breuss 2002; Lücking 2008; van den Boom *et al.* 2018; McCarthy 2020; Lücking *et al.* 2021).

A total of eleven species of *Lasioloma* have been reported and a key has also been produced for these species, except *L. corticola* (McCarthy 2020; Lücking *et al.* 2021), but there has been little recent progress in research on this genus in Asia and so the present study aims to reduce this knowledge gap.

Materials and Methods

The material collected from Malaysia is deposited in the Minnan Normal University (MNNU), and that collected from China (Guangxi Province) is deposited in the Institute of Microbiology of the Chinese Academy of Sciences (HMAS-L).

Micrographs of morphological and anatomical features of specimens studied were taken with a Motic SMZ171 dissecting microscope and a Leica TM500 compound microscope, respectively. Secondary chemistry was assessed following Orange *et al.* (2010).

DNA extraction, PCR amplification and sequencing

PCR amplification of mtSSU rDNA was performed with the primers mrSSU1 and mrSSU3R (Zoller *et al.* 1999), of ITS with the primers ITS1F and ITS4 (White *et al.* 1990), and of mtLSU rDNA with the primers ML3A and ML4 (Printzen 2002). Total DNA extraction, PCR cycling parameters, PCR product purification and sequencing were performed as described in Wang *et al.* (2020).

Sequence alignment and phylogenetic analysis

Geneious v. 6.1.2 (Biomatters Ltd, Auckland, New Zealand) was used to assemble and edit the original sequence reads. A total of 13 newly generated sequences were aligned together with 31

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Table 1. Specimens of *Lasioloma* and outgroup species used in the phylogenetic analyses (Fig. 1) with voucher information and GenBank Accession numbers. New sequences and new species are in bold.

Taxon	Locality	Voucher specimens	ITS	mtSSU	mtLSU
Byssoloma leucoblepharum	China	*W. C. Wang* 20180145 (HMAS-L 140613)	MK946971	MK957166	--
B. leucoblepharum	China	*W. C. Wang* HN20170357 (HMAS-L 139782)	MK946977	MK957174	**OL439824**
Calopadia foliicola	Thailand	*W. C. Wang* KYW0068 (RAMK-31790)	MK946951	MK957146	--
C. foliicola	Thailand	*W. C. Wang* KYW0035 (RAMK-31537)	MK946953	MK957148	--
C. puiggarii	China	*W. C. Wang* HN20170381 (HMAS-L 139789)	MK946975	MK957172	**OL439821**
C. puiggarii	China	*W. C. Wang* 20180158 (HMAS-L 140626)	MK946972	MK957167	--
Fellhanera fuscatula	Thailand	*W. C. Wang* KYW0392 (RAMK-31669)	MK946956	MK957151	--
F. fuscatula	Thailand	*W. C. Wang* KYW0336 (RAMK-31862)	MK946959	MK957154	--
F. microdiscus	China	*W. C. Wang* HN20170313 (HMAS-L 139758)	MK946978	MK957175	**OL439829**
Lasioloma antillarum	Netherlands	*Sipman* 54818 (AFTOL-ID 4887) (B)	--	KJ766365	--
L. arachnoideum	Thailand	*W. C. Wang* KYW0595 (RAMK-31616)	MK946960	MK957155	--
L. arachnoideum	Thailand	*W. C. Wang* KYW0646 (RAMK-31913)	MK946979	MK957158	--
L. arachnoideum	Thailand	*W. C. Wang* KYW0282-1 (RAMK-31852)	**OL396968**	**OL412922**	**OL439841**
L. longiramosum	Malaysia	*W. C. Wang & A. Abas* WWC356 (MNNU)	**OL396890**	--	--
L. phycophorum	China	*W. C. Wang* 20192918 (HMAS-L)	**OL396905**	**OL412901**	**OL439805**
L. verrucosum	China	*X. L. Wei & J. H. Wang* 134593 (HMAS-L)	--	--	**OL439784**
Lasioloma sp.	Costa Rica	*Lücking* 16005	AY756467	AY567783	--
Sporopodium antoninianum	Costa Rica	*Lücking* 16002d (BG)	AY756498	AY567785	--
S. asiaticum	China	*W. C. Wang* 20192917 (HMAS-L)	OL396904	OL412900	**OL439804**
S. asiaticum	Thailand	*W. C. Wang* KYW0620 (RAMK-31676)	OL396977	OL412935	**OL439849**

sequences retrieved from GenBank (Table 1). *Byssoloma leucoblepharum* (Nyl.) Vain. was chosen as outgroup based on previous phylogenetic analyses (Wang *et al.* 2020). The assembled sequences were aligned using the online version of MAFFT v. 7 (Katoh *et al.* 2009). Gblocks v. 0.91b (Castresana 2000) was used to delimit ambiguous regions, implementing all the options for a less stringent selection which yielded final alignments of 618 bp (ITS), 837 bp (mtSSU) and 809 bp (mtLSU). Alignments were concatenated in Geneious v. 6.1.2 for multilocus phylogenetic analysis. The final alignment consisted of three ITS, two mtSSU and eight mtLSU sequences newly generated from nine specimens, and 15 ITS and 16 mtSSU sequences downloaded from NCBI (Table 1).

A maximum likelihood (ML) analysis was used to infer phylogenetic trees based on the combined ITS, mtSSU and mtLSU data set, using RaxML-HPC v. 8.2.6 (Stamatakis 2014) on the Cipres Science Gateway (http://www.phylo.org). Bootstrap support values (BS) were based on 1000 non-parametric pseudoreplicates. The phylogenetic tree was visualized with the program FigTree v. 1.4.3. and edited in Adobe Illustrator CC 2019.

Results and Discussion

Based on *a priori* taxonomic assessment, five species of *Lasioloma* were included in our phylogenetic tree (Fig. 1) and they form a well-supported (BS = 87) clade. Most species were represented by singletons, whereas *L. arachnoideum* included two terminals. The Thai sequences identified as *L. arachnoideum* (31616, 31852, 31913; Fig. 2) did not form a clade with a Costa Rican sequence (*Lücking* 16005), which was named as *L. arachnoideum* in GenBank, suggesting two separate species.

The conidia of the Thai samples have four branches (main branch of *c.* 48–49 µm in length, other three branches 40–42 µm), and are similar to the conidial description of the type species (four branches, main branch 38–56 µm, other three branches *c.* 36 µm) by Vězda (1986), whereas those of neotropical samples have much shorter branches (each branch only 20–30 µm in length) (Lücking 2008). The holotype of *L. arachnoideum* originates from South-East Asia (Santesson 1952) so the Thai specimens should represent that taxon in its strict sense. There is currently no alternative name available for neotropical material thus far identified as *L. arachnoideum*.

The target samples, WWC356 and 134593, were each resolved on a separate singleton branch; they were also revealed as morphologically different from all known species of the genus (see notes below) and therefore we describe these two species here as new.

While *Lasioloma* can be easily recognized at genus level due to its pilose apothecial margin, woolly prothallus and filiform conidia with 3–5 branches originating from a single point, the study of the Asian specimens revealed that there are diagnostic differences in conidial morphology and dimensions between species of this genus, especially the length of the conidial branches, a feature that has not been explored in previous studies but should be regarded as an important additional line of evidence for species delimitation in *Lasioloma*.

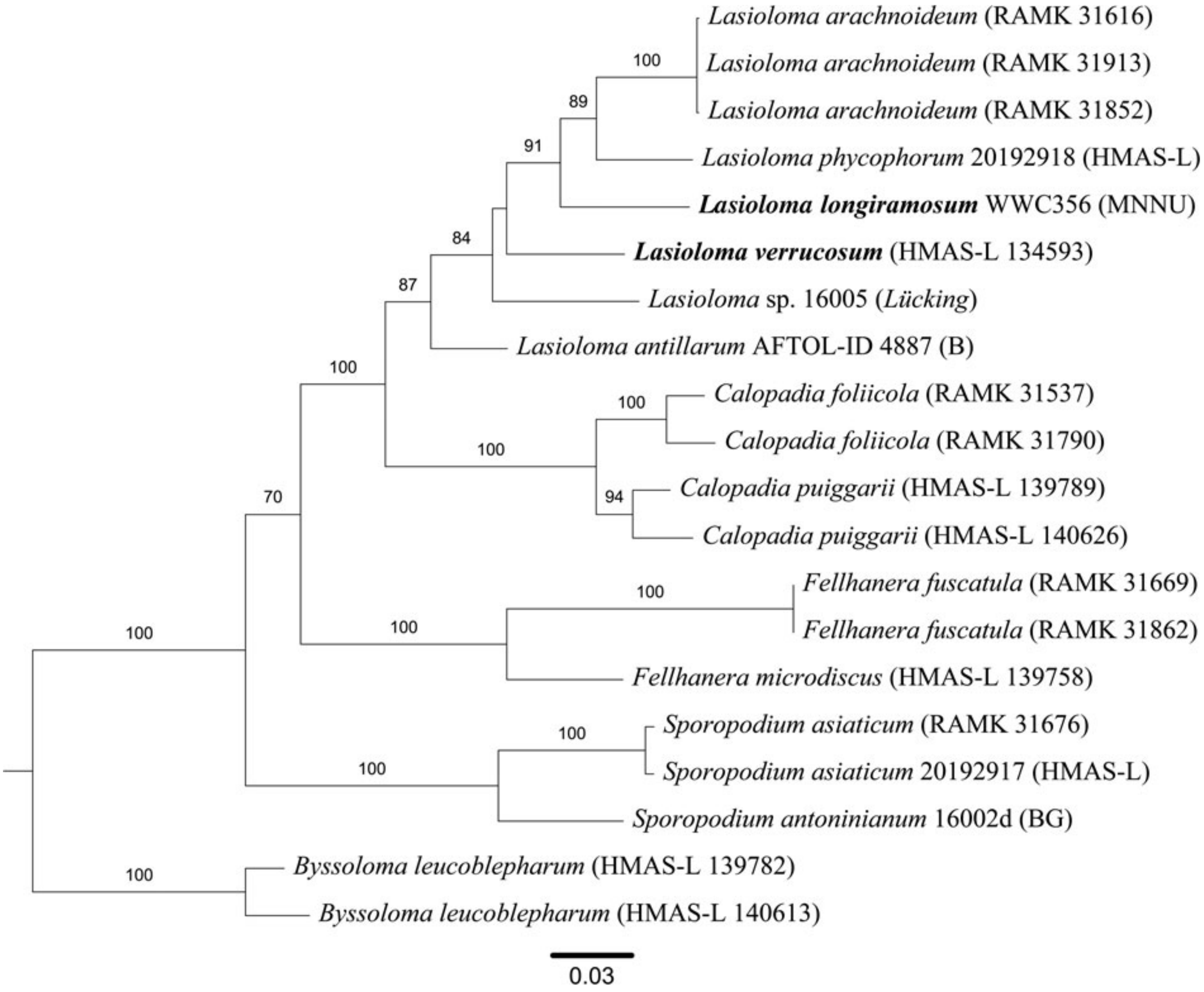

Fig. 1. Phylogram of *Lasioloma* species using maximum likelihood (ML) inferred from a concatenated data set of ITS + mtSSU + mtLSU. Bootstrap values ≥ 75 are indicated at the branches. The tree was rooted using *Byssoloma leucoblepharum*. Scale = 0.03 substitutions per site.

Taxonomic Treatment

Lasioloma longiramosum W. C. Wang & A. Abas sp. nov.

Fungal Names No.: FN 571259

Differs from other foliicolous *Lasioloma* species based on its single, small ascospores (53–61 × 19–25 µm, 2.5–2.8 times as long as wide) and long filiform conidia (three long branches 65–80 µm and one shorter main branch of 22–28 µm).

Type: Malaysia, Pahang, Raub, Fraser Hill Forest Park, Jeriau Waterfall Trail, 3°42′50.4″N, 101°44′6″E, 700 m alt., on leaves, 7 September 2019, *W. C. Wang* & *A. Abas* WWC356 (MNNU—holotype!).

(Fig. 3)

Thallus epiphyllous, crustose, dispersed into irregular patches, 8–10 mm across, ecorticate, uneven, pale green, every patch 0.5–0.7 mm wide, composed of colourless and branched hyphae, 2.5–5 µm wide; *prothallus* well developed, between algiferous thallus patches, formed by loosely interwoven hyphae, white to pale grey. *Phycobiont* a species of *Chlorococcaceae*, cells round, green, 5–12.5 µm diam.

Apothecia rounded, 0.25–0.4 mm diam. and 200–240 µm high; *disc* plane, yellowish brown; margin thick, khaki, laterally densely pilose. *Excipulum* paraplectenchymatous, colourless, 16–22 µm wide, laterally with short hairs formed by individual, septate hyphae, up to 110 µm long; *hypothecium* 25–30 µm high, dark brown; apothecial base aeruginous; *epithecium* thin, 5–10 µm high, pale brown; *hymenium* 80–85 µm high, colourless. *Asci* 60–72 × 22–27 µm. *Ascospores* single, oblong, muriform, 53–61 × 19–25 µm, 2.5–2.8 times as long as wide, colourless, halonate.

Campylidia sessile, 0.4–0.5 mm wide; lobe well developed, hood-shaped, dark grey, non-pruinose; socle not apparent. *Conidia* filiform, colourless, with four branches originating from a single point, main branch distinctly shorter than the others, 2–3-septate, 22–28 × 2–2.5 µm; other three branches 5–8-septate, 65–80 × 1.5–2 µm.

Secondary chemistry. No substances detected by TLC.

Etymology. The epithet *longiramosum* refers to the filiform conidia with three long branches.

Habitat and distribution. This species grows on leaf surfaces and was found in the lower stratum of a damp understorey rainforest near a river in a valley in Malaysia.

Notes. So far, four foliicolous species of *Lasioloma* with a single ascospore have been described worldwide: *L. arachnoideum*, *L.*

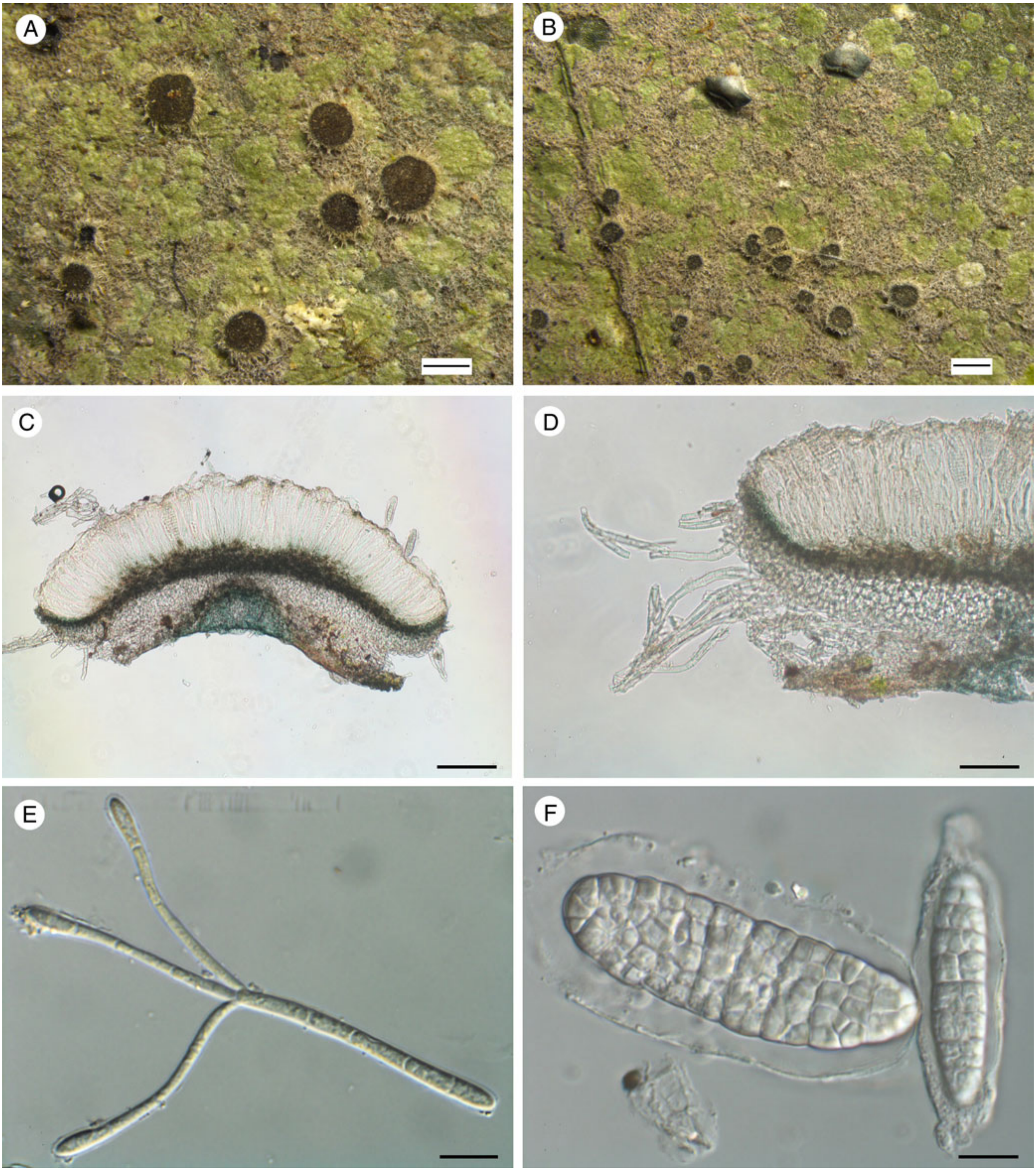

Fig. 2. *Lasioloma arachnoideum* (*W. C. Wang* 31636, RAMK). A, dispersed thallus with apothecia. B, dispersed thallus with apothecia and campylidia. C & D, section of apothecium showing pigmentation. E, filiform conidium with three long branches and a shorter main branch. F, muriform ascospores. Scales: A & B = 500 μm; C = 100 μm; D = 50 μm; E & F = 10 μm. In colour online.

phycophilum (Vain.) R. Sant., *L. phycophorum* (Vain.) R. Sant. and *L. trichophorum* (Vain.) R. Sant. (Lücking *et al.* 2021). All are mainly distributed in South-East Asia.

The new species, *Lasioloma longiramosum*, also has one ascospore per ascus but its small ascospores and long conidia readily distinguish it from the four other known foliicolous species; it is also distinguished phylogenetically from two of the species that have been sequenced (Fig. 1).

In its gross morphology, the new species resembles *L. arachnoideum*, since both have a dispersed thallus and a distinct woolly prothallus. However, *L. arachnoideum* is distinguished by having shorter conidia (main branch 38–56 μm, the other

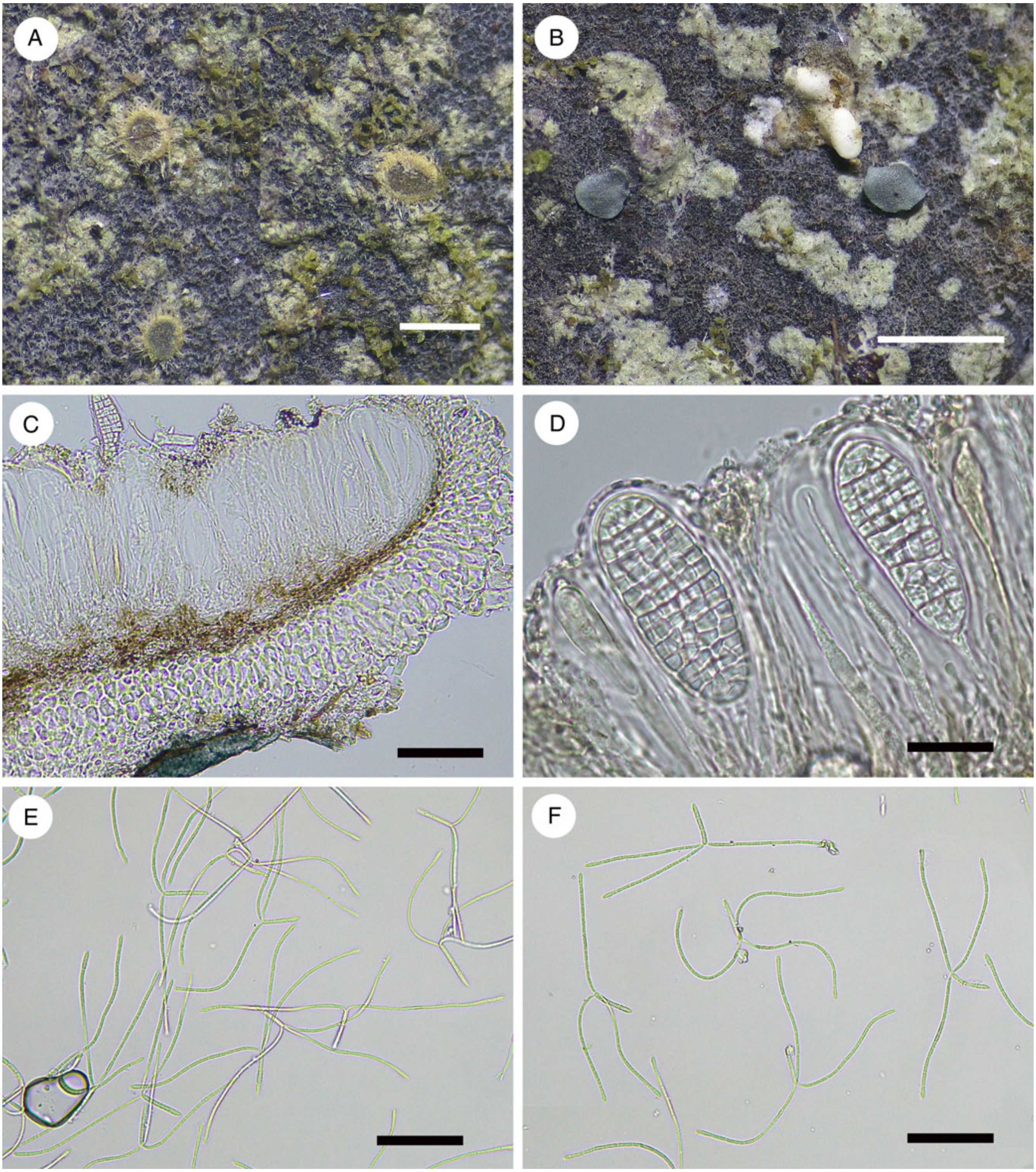

Fig. 3. *Lasioloma longiramosum* (holotype, *W. C. Wang & A. Abas* WWC356 MNNU). A, dispersed thallus with apothecia. B, dispersed thallus with grey campylidia. C, section of apothecium showing pigmentation. D, muriform ascospores (one ascospore per ascus). E & F, filiform conidia with three long branches and a shorter main branch. Scales: A = 500 μm; B = 1 mm; C, E & F = 50 μm; D = 20 μm. In colour online.

three branches *c.* 36 μm), shorter ascospores, and dark (greyish) brown apothecia (Lücking 2008).

Although conidia of the three other foliicolous species (*L. phycophilum*, *L. phycophorum* and *L. trichophorum*) have not been described in the literature, the species usually have vermicular cephalodia, a continuous to marginally dispersed thallus, an indistinct prothallus and longer ascospores (Santesson 1952; Lücking & Sérusiaux 2001; Lücking *et al.* 2021).

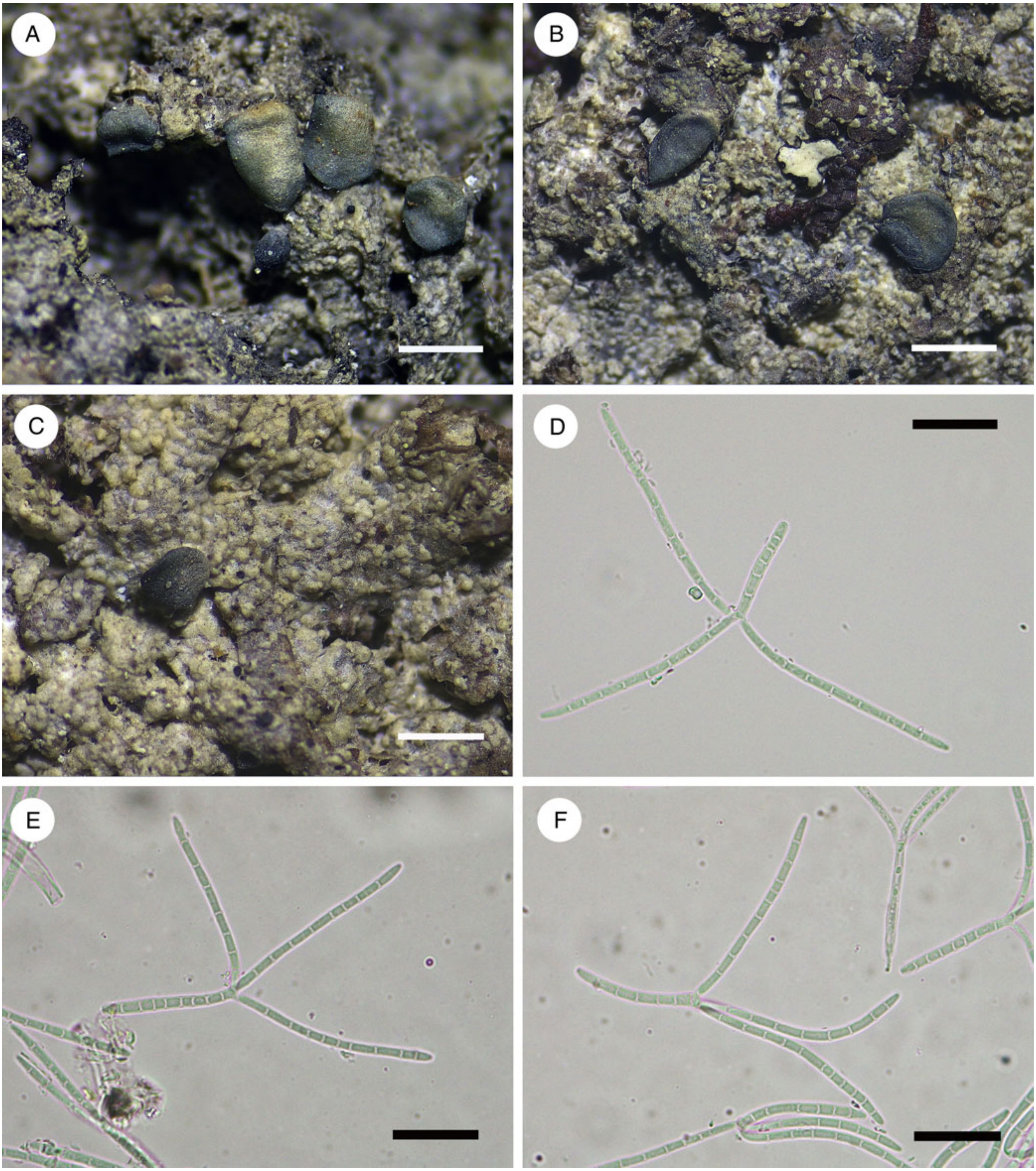

Fig. 4. *Lasioloma verrucosum* (holotype, *X. L. Wei* & *J. H. Wang* 134593 HMAS-L). A–C, warted thallus with campylidia. D–F, filiform conidia with three long branches and a shorter main branch. Scales: A–C = 1 mm; D–F = 20 µm. In colour online.

Lasioloma verrucosum W. C Wang & X. L. Wei sp. nov.

Fungal Names No.: FN 571260

Differs from other corticolous *Lasioloma* species based on its warted thallus of verrucae 0.1–0.15 mm diam., and filiform conidia with three long branches of 56–65 µm and one shorter main branch of 22–32 µm.

Type: China, Guangxi Province, Nanning City, Daming Mountain National Natural Reserve, 23°30′N, 108°26′E, 1231 m alt., on bark of *Betula* sp., 22 May 2015, *X. L. Wei* & *J. H. Wang* GX2015083 (134593 HMAS-L—holotype!).

(Fig. 4)

Thallus corticolous, crustose, continuous, 4–6 cm diam., greyish white (the green likely to fade), irregularly extended, warted, entirely made of densely arranged, compact verrucae; verrucae 0.1–0.15 mm diam., medulla white, K−. *Prothallus* not observed. *Photobiont* chlorococcoid, algal cell globose, green, 7.5–12.5 µm diam.

Apothecia not observed.

Campylidia sessile, 0.5–1 mm wide; lobe well developed, large, hood-shaped, inside dark greyish green, outside pale grey to grey, non-pruinose; socle not apparent. Wall paraplectenchymatous, 50–58 µm thick, laterally with thin layer of densely interwoven hyphae, pale aeruginous, and peripherally with free hyphae composed of thick-walled cells, colourless; conidiogenous cells lining inner wall surface, oblong-papilliform, unbranched.

Conidia filiform, colourless, with four branches originating from a single point, the main branch shorter than the others, 4–8-septate, 22–32 × 2.5 µm, the other three branches 8–13-septate, 50–65 × 2.5 µm, all conidial branches without short terminal appendages.

Secondary chemistry. No substances detected by TLC.

Etymology. The epithet *verrucosum* refers to the warted thallus composed of densely compact verrucae.

Habitat and distribution. *Lasioloma verrucosum* is known only from the bark of *Betula* sp. in montane rainforest in subtropical areas of China.

Notes. Among the 11 species of *Lasioloma* currently known, there are five corticolous species: *L. antillarum* Lücking *et al.*, *L. appendiculatum* Breuss, *L. corticola* P. M. McCarthy, *L. pauciseptatum* van den Boom and *L. stephanellum* (Nyl.) Lücking & Sérus. (McCarthy 2020; Lücking *et al.* 2021).

Lasioloma corticola was described from Queensland in Australia. Its gross morphology resembles that of *L. verrucosum*, since both species have grey campylidia and lack apothecia. However, *L. corticola* is distinguished from *L. verrucosum* by having a smooth thallus and conidia with 4–5 branches (the main branch longer than the other branches), whereas *L. verrucosum* has a warty thallus and conidia with four branches (the main branch shorter than the other branches).

Lasioloma appendiculatum also lacks apothecia but its conidia have five short branches (the main branch 27–35 µm and the other four branches 15–20 µm), and the conidial branches have short terminal appendages.

Lasioloma verrucosum is similar to *L. stephanellum* in having a warted thallus. However, in *L. stephanellum* the medulla is yellow to reddish. There is also a slight resemblance with *L. pauciseptatum* but that species has a smooth thallus and the conidia have 3–5 branches (the main branch longer than the other branches).

Lasioloma antillarum can be distinguished most readily by the smooth thallus and the length of the conidial branches (each branch 3–5-septate, 30–40 µm, the main branch slightly longer and thicker than the others).

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