



The genus *Leptoxyphium* (Capnodiaceae) from China

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Abstract

Leptoxyphium is a relatively poorly known genus of sooty moulds in Capnodiaceae (Dothideomycetes). This paper introduces one new species of *Leptoxyphium*, *L. glochidion* and the first record of *L. kurandae* for China. *L. glochidion* is introduced as a new species based on morphology and molecular data and is compared with related taxa. Descriptions, illustrations and notes are provided for the two species, which are analyzed by ITS, LSU and SSU sequence data. The phylogenetic analysis shows that the two species cluster in *Leptoxyphium* (Capnodiaceae). *L. glochidion* separates from other species of the genus, while *L. kurandae* clustered with the type strain

Key words: morphology, new species, phylogeny, sooty mould

Introduction

The sooty mould genus *Leptoxyphium* was introduced by Spegazzini (1918), and presently includes 19 species (Kirk *et al.* 2008, Index Fungorum 2013) and belongs to the family Capnodiaceae (Hyde *et al.* 2013). Species are saprobic on sugary exudates produced by sap feeding insects growing on the surface of living leaves. The thallus comprises superficial, irregular, networks of mycelium, which are grey brown to brown and constricted at the septa (Chomnunti *et al.* 2011, 2014). Pycnidia are superficial and gregarious, sometimes with helical twisting, with bulbous swollen bases, and an apex that comprises cylindrical hyphae that expands to become funnel-shaped. Conidia are ellipsoidal, hyaline, with some being 1-septate, pigmented and guttulate when mature (Hughes 1976, Chomnunti *et al.* 2011)

There have been few recent studies on the genus *Leptoxyphium*. *L. kurandae* Crous & R.G. Shivas was introduced as a new species from leaves of *Eucalyptus* sp. from Queensland (Crous *et al.* 2011), while *Leptoxyphium cacuminum* Chomnunti & KD Hyde was introduced from leaves of *Gossypium herbaceum* from Thailand (Chomnunti *et al.* 2011). The latter species differed from other known species in the genus because of its hyaline conidia, never becoming septate or pigmented when mature (Chomnunti *et al.* 2011).

The purpose of the paper is to introduce one new species in the genus *Leptoxyphium*, and add further ITS, LSU and SSU rDNA sequence data for the genus to GenBank. *L. kurandae* is also a new record for China.

Materials and methods

Isolates and morphology

Material was collected in the field and returned to the laboratory in paper envelopes where it was examined under a stereomicroscope (Nikon 80i) for morphological characters. Pure cultures were obtained by single spore isolation following the methods of Chomnunti *et al.* (2011). After one month, cultures were used for molecular work.

The specimens are deposited at the International Fungal Research & Development Centre (IFRD) Herbarium, Kunming, Yunnan, and cultures are stored in the culture collection of the same institution (IFRDCC) and in Mae Fah Luang University Culture Collection (MFLUCC).

DNA isolation, amplification and sequencing

Fungal isolates were grown on PDA for 20 days at 26 °C in the dark. DNA was extracted from the mycelium with a Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, China) according to the manufacturer's instructions (Hangzhou, P.R. China). Polymerase chain reaction (PCR) was carried out using known primer pairs. LROR and LR5 were used to amplify a region spanning the large subunit rDNA (28S, LSU) (Vilgalys & Hester 1990) and internal transcribed spacers (5.8S, ITS) was amplified by primer pairs ITS5 and ITS4 (White *et al.* 1990). The partial small subunit nuclear rDNA (18S, SSU) was amplified by using NS1 and NS4 (Vilgalys & Hester 1990). The amplification reaction mixtures were 50 µL which contained 3.0 µL of DNA template, 1.5 µL of each forward and reverse primers, 25 µL of 2× Easy Taq PCR SuperMix (mixture of *EasyTaq*™ DNA Polymerase, dNTPs, and optimized buffer, Beijing TransGen Biotech Co., Chaoyang District, Beijing, PR China) and 19 µL sterilized water. Amplification conditions were set up for initial denaturation of 3 min at 94°C, followed by 38 cycles of 30 s at 94°C, 40 s at 56°C and 60 s at 72°C, and a final extension period of 10 min at 72°C (Phillips *et al.* 2008).

Phylogenetic analysis

Blast searches were made to reveal the closest matches in GenBank. All ITS, LSU and SSU sequences obtained from GenBank (Batzer *et al.* 2008, Crous *et al.* 2007, 2009a, b, 2011, Schoch *et al.* 2006a, b, 2009, Verkley *et al.* 2004) are listed in Table 1. The ITS sequences for *Leptoxyphium cacuminum*, *Capnodium coartatum*, *Phragmocapnias siamensis*, *P. betle* and *Scorias spongiosa* were provided by P. Chomnunti. Representative sequences from closest taxa in different families i.e. Davidiellaceae, Dissoconiaceae, Mycosphaerellaceae, Schizothyriaceae, Teratosphaeriaceae were selected. Multiple sequence alignments were generated with MAFFT v. 6.864b (<http://mafft.cbrc.jp/alignment/server/index.html>). The alignments were checked visually and improved manually where necessary.

TABLE 1. Taxa used in the phylogenetic analysis and their corresponding GenBank numbers.

Species	Strain no.	GenBank accession no.		
		ITS	LSU	SSU
<i>Capnodium coartatum</i>	MFLUCC 10-0069		JN832614	JN832599
<i>Capnodium coartatum</i>	MFLUCC 10-0070		JN832615	JN832600
<i>Capnodium coffeae</i>	CBS 147.52		DQ247800	DQ247808
<i>Cladosporium cladosporioides</i>	CBS 170.54	AY213640	NG_027578	DQ678004
<i>Conidioxyphium gardeniorum</i>	CPC 14327	—	GU301807	GU296143
<i>Davidiella tassiana</i>	CBS 723.79	EU167558	GU214410	EU167558
<i>Dissoconium aciculare</i>	CBS 204.89	AY725520	GU214419	GU214523
<i>Dissoconium commune</i>	CPC 12397	GQ852738	GQ852589	NG_016521
<i>Dothidea insculpta</i>	CBS 189.58	AF027764	DQ247802	DQ247810
<i>Graphiopsis chlorocephala</i>	CPC 11969	EU009458	EU009458	GU214534
<i>Leptoxyphium cacuminum</i>	MFLUCC 10-0049		JN832602	JN832587
<i>Leptoxyphium cacuminum</i>	MFLUCC 10-0059		JN832603	JN832588
<i>Leptoxyphium cacuminum</i>	MFLUCC 10-0086		JN832604	JN832589
<i>Leptoxyphium fumago</i>	CBS 123.26	—	GU214430	GU214535
<i>Leptoxyphium kurandae</i>	CPC:17274	JF951150	JF951170	—
<i>Leptoxyphium glochidion</i>	IFRDCC 2651	KF982307	KF982308	KF982309

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TABLE 1 (continued)

Species	Strain no.	GenBank accession no.		
		ITS	LSU	SSU
<i>Leptoxyphium kurandae</i>	IFRDCC 2650	KF982310	KF982311	KF982312
<i>Mycosphaerella graminicola</i>	CBS 100335	GU062240	EU019298	GU214539
<i>Mycosphaerella punctiformis</i>	CBS 113265	AY490763	AY490776	AY490775
<i>Phragmocapnias asiticus</i>	MFLUCC 10-0062		JN832612	JN832597
<i>Phragmocapnias betle</i>	MFLUCC 10-0050		JN832605	JN832590
<i>Phragmocapnias betle</i>	MFLUCC 10-0053		JN832606	JN832591
<i>Phragmocapnias siamensis</i>	MFLUCC 10-0061		JN832607	JN832592
<i>Phragmocapnias siamensis</i>	MFLUCC 10-0063		JN832608	JN832593
<i>Phragmocapnias siamensis</i>	MFLUCC 10-0064		JN832609	JN832594
<i>Phragmocapnias siamensis</i>	MFLUCC 10-0065		JN8326010	JN832595
<i>Phragmocapnias siamensis</i>	MFLUCC 10-0074		JN8326011	JN832596
<i>Schizothyrium pomi</i>	CBS 406.61	EF134949	EF134949	EF134949
<i>Scorias leucadendri</i>	CBS 131318	JQ044437	JQ044456	—
<i>Scorias spongiosa</i>	MFLUCC 10-0084		JN832601	JN832586
<i>Scorias spongiosa</i>	CBS 325.33	GU214696	GU214696	GU214696
<i>Teratosphaeria fibrillosa</i>	CBS 121707	EU707862	GU323213	GU296199
<i>Teratosphaeria jonkershoekensis</i>	CBS 112224	EU707864	GU301874	GU296200
<i>Toxicocladosporium irritans</i>	CBS 185.58	EU040243	EU040243	GU214619

Maximum likelihood analyses including 1000 bootstrap replicates were run using RAxML v. 7.2.6 (Stamatakis & Alachiotis 2010). The online tool Findmodel (<http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html>) was used to find out the best nucleotide substitution model for each partition. The resulting replicates were plotted on to the best scoring tree obtained previously. Maximum Likelihood bootstrap values (MLBP) equal or greater than 50 % are given below or above each node in red (Fig. 1).

MEGA 5.0 (Tamura *et al.* 2011) was used for Minimum Evolution (ME) inference, with default settings except for assuming pairwise deletion, and using the Tamura-Nei nucleotide substitution model. For inferring tree robustness, 1000 bootstrap replicates were carried out and bootstrap values (ME) equal or greater than 50 % are given below or above each node in black (Fig. 1). Maximum Trees are visualized with Tree View (Page 1996).

Results

Phylogeny based on combined ITS, LSU and SSU rDNA data set: the combined ITS, LSU and SSU data set utilized 33 taxa with *Dothidea insculpta* as the outgroup taxon. This resulted in 11.98% missing and gap characters out of a total set of 2350 characters and the Final ML Optimization Likelihood is -9328.036863. Phylogenetic trees obtained from ME and RAxML analyses yielded a best scoring tree with similar overall topology at the genus and family relationship in agreement with previous work based on a RAxML tree (Fig. 1). The phylogenetic hypothesis strongly supports four monophyletic groups, the two new sequences described here cluster within the *Leptoxyphium* (Capnodiaceae) clade, they are unrelated to sequences of species of Davidiellaceae, Dissoconiaceae, Mycosphaerellaceae, Schizothyriaceae and Teratosphaeriaceae. The isolate of the new species *Leptoxyphium glochidion* (IFRDCC 2651) clustered in a subclade with *L. fumago* (CBS 123.26) with high bootstrap support (92% and 86%), while the new strain of *L. kurandae* clustered with the type strain.

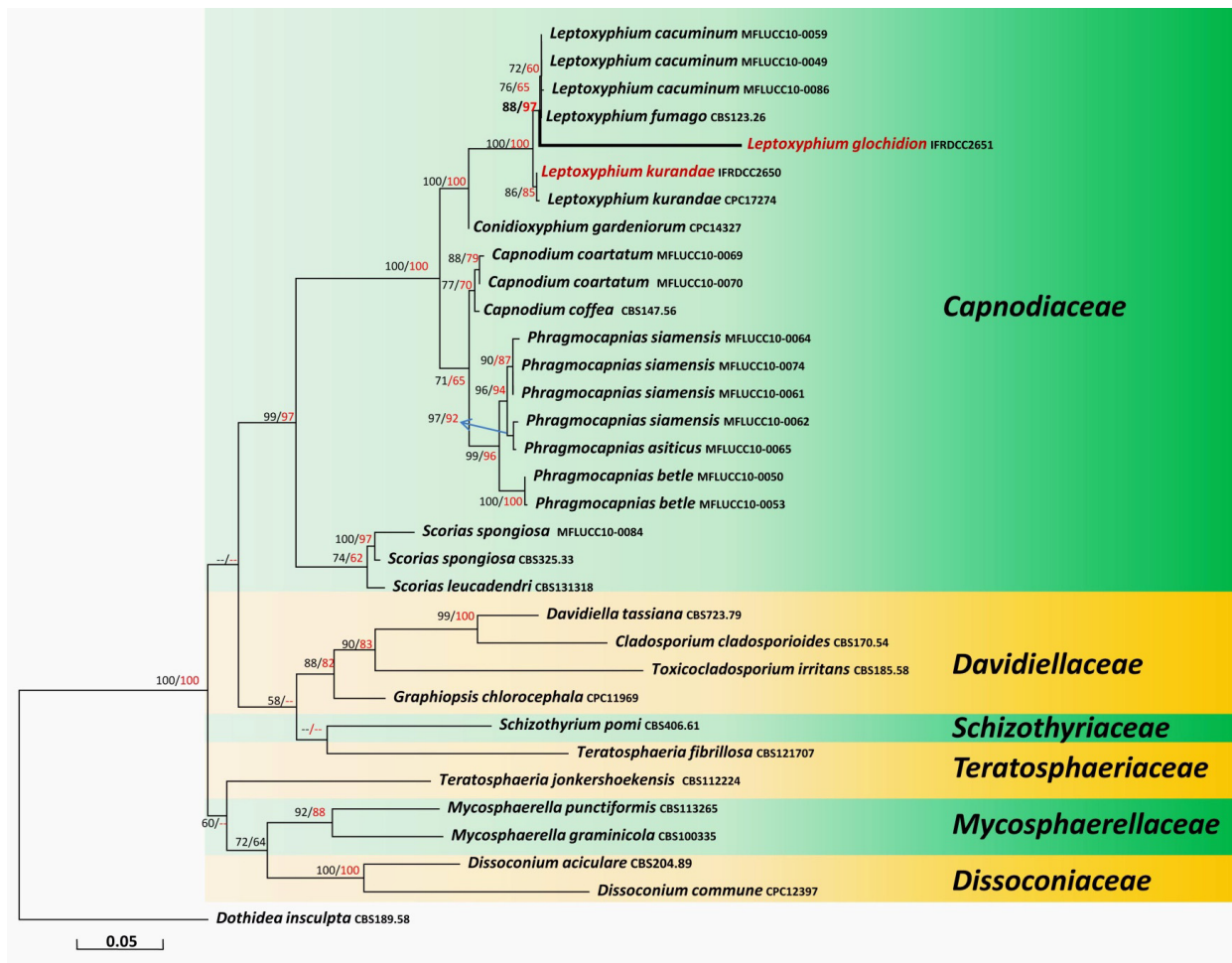


FIGURE 1. RAxML tree generated by the phylogenetic analysis of combined data set of ITS, LSU and SSU rDNA sequence. Bootstrap support values $\geq 50\%$ are shown below or above the branch. *Dothidea insculpta* is the out-group taxon. The original strain numbers are noted after the species names and names of isolates with newly obtained sequences from this study are in bold.

Taxonomy

Leptoxyphium glochidion H. Yang & K.D. Hyde, *sp. nov.* MycoBank: MB807763

Holotype: IFRD 9043 (Figs 2, 3)

Etymology:—in reference to its occurrence on *Glochidion*.

Saprobic sooty moulds, forming on the upper surface of leaves. *Hyphae* 4.8–6.8 μm wide (\bar{x} = 5.8 μm , n = 20), cylindrical or bead-like, guttulate, light brown to dark brown, branched, septate, constricted at the septa, forming an irregular network. Asexual state: *Pycnidia* 306–396 μm high (\bar{x} = 357 μm , n = 10), separate or in groups of two, dark brown to black, straight to slightly flexuous, the basal cell dark brown and slightly swollen, 34–54 μm (\bar{x} = 46 μm , n = 20) wide at the base, the apex 33–46 \times 31–47 μm wide (\bar{x} = 39 \times 37 μm , n = 20), expanding into a funnel-shape, resembling a cupula, light brown. *Conidiogenous cells* arising from the inner cell wall of the cupulate apex, light brown to hyaline, subcylindrical to subulate. *Conidia* 6.5–9.5 \times 3.4–4.8 μm (\bar{x} = 7.6 \times 3.9 μm , n = 20), broadly ellipsoid to oblong or subglobose, round ends, hyaline and lacking septate, with 1–2 obvious guttules, germinating is from both ends of conidia (Fig. 2).

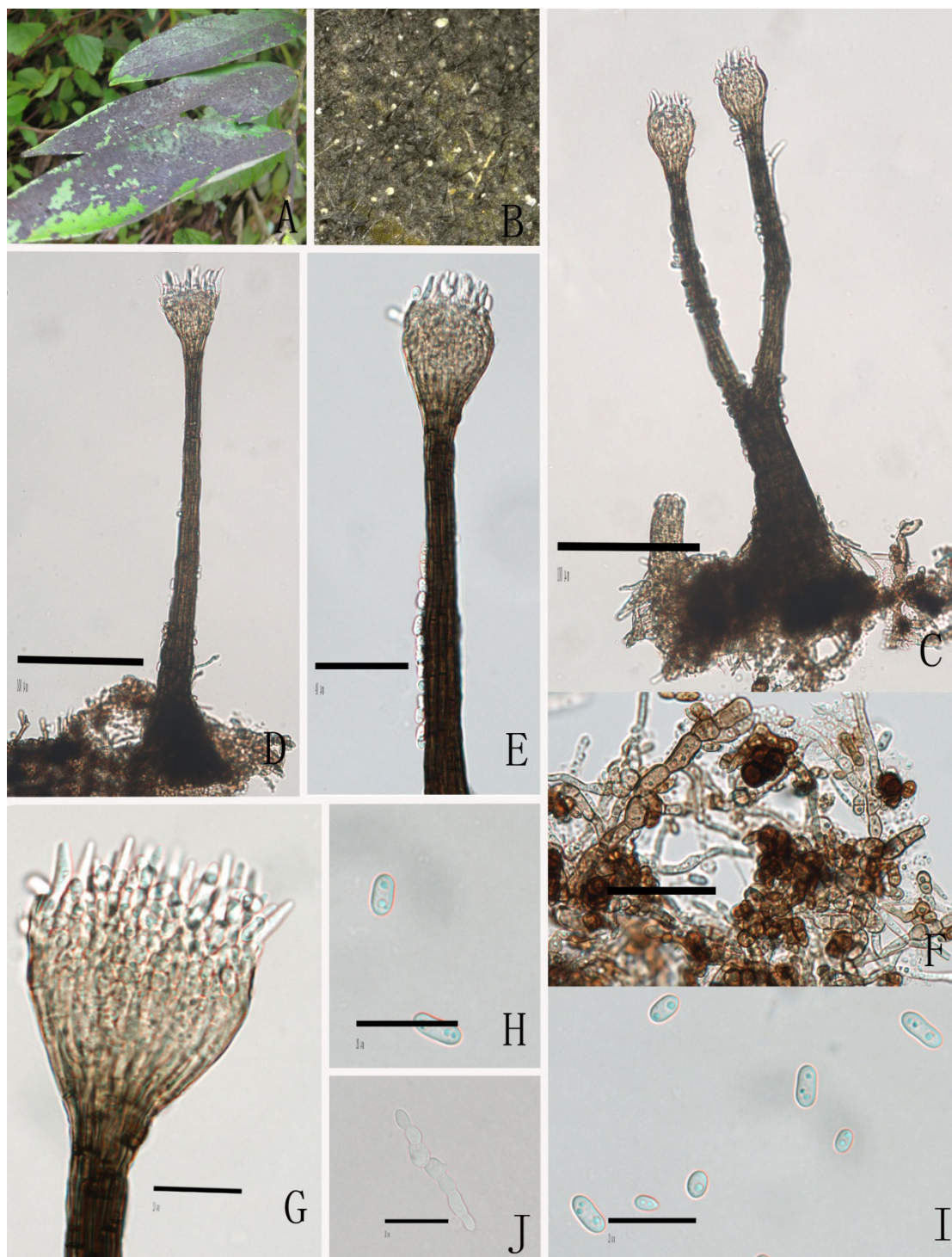


FIGURE 2. *Leptoxyphium glochidion* (holotype). A. Superficial mycelium on host. B. Gregarious pycnidia on host surface. C–E. Stalked pycnidia. F. Reticulate hyphae. G. Black stalked funnel cupulate apex. H–I. Hyaline conidia. J. Germinating conidia. Bars: C = 100 μm , D–G = 40 μm , H–J = 20 μm .

Pycnidium stalked, arising from the basal haphae and swelling like a cupulate at the apex. Stalk brown or dark brown, and brown to light brown at the apex, the cells oblong with septate. Haphae 3.2–5.2 μm wide (\bar{x} =4.5 μm , n =20), brown, cylindrical, branched, septate, constricted at the septa. Conidia broadly subglobose to ellipsoid with round ends, hyaline, no septate, with guttulate (Fig. 3).



FIGURE 3. *Leptoxyphium glochidion* (holotype) in culture. A–C. Pycnidia. D. Septate hyphae. E–F. Hyaline conidia. Bars: A–D = 40 μm , E–F = 20 μm .

Culture characteristics:—Colonies black, dense, with sparse to moderate aerial mycelium and even margins, reaching 4 cm after 10 days, with slimy water drops on the medium surface. Hyphae black, branched, penetrating the PDA medium, asexual state produced after 40 days.

Material examined:—CHINA. Yunnan Province: Jinghong, on living leaf of *Glochidion wrightii* Benth. (Euphorbiaceae), 20 July 2012, *Hui Yang* MS06 (IFRD 9043, holotype), ex-type living culture = IFRDCC 2651

Sequence data:—ITS = KF982307, LSU = KF982308, SSU = KF982309

Notes:—This species is similar to *Leptoxyphium cacuminum*, *L. fumago* and *L. kurandae*. It is most similar to *L. cacuminum* which was described from *Gossypium herbaceum* in northern Thailand by Chomnunti *et al.* (2011). Our collection differs from *L. cacuminum* in having shorter pycnidia (306–396 μm , \bar{x} = 357 μm , versus 341–446 μm , \bar{x} = 392 μm), with a larger swollen base (34–54 μm , \bar{x} = 46 μm vs 19–30 μm , \bar{x} = 26 μm) and larger conidia (6.5–9.5 \times 3.4–4.8 μm , \bar{x} = 7.6 \times 3.9 μm vs 4.1–6.7 \times 2.1–2.7 μm , \bar{x} = 5.2 \times 2.4 μm) and its *Glochidion wrightii* host. Our collection differs from the other known species in the genus because of its hyaline conidia, that never become septate or pigmented when mature.

Saprobic sooty moulds, forming on the upper surface of leaves. *Hyphae* olivaceous-brown to dark brown, branched, septate, constricted at the septa, bead-like, forming an irregular network. *Pycnidia* 342–422 μm high (\bar{x} = 381 μm , n = 10), dark olivaceous-brown to black, straight to slightly flexuous, the base 37–47 \times 37–43 μm wide (\bar{x} = 44 \times 39 μm , n = 20), the basal cells dark olivaceous-brown, bulbous, apex 29–37 \times 28–32 μm wide (\bar{x} = 34 \times 31 μm , n = 20), funnel-shaped, resembling a cupula. *Conidiogenous cells* arising from the inner cell wall of the cupulate apex, olivaceous-brown to hyaline. *Conidia* 6.2–7.4 \times 2.7–3.4 μm (\bar{x} = 6.7 \times 3 μm , n = 20), broadly ellipsoid with rounded ends, hyaline, aseptate and lacking guttules, germinating both ends of conidia (Fig. 4).

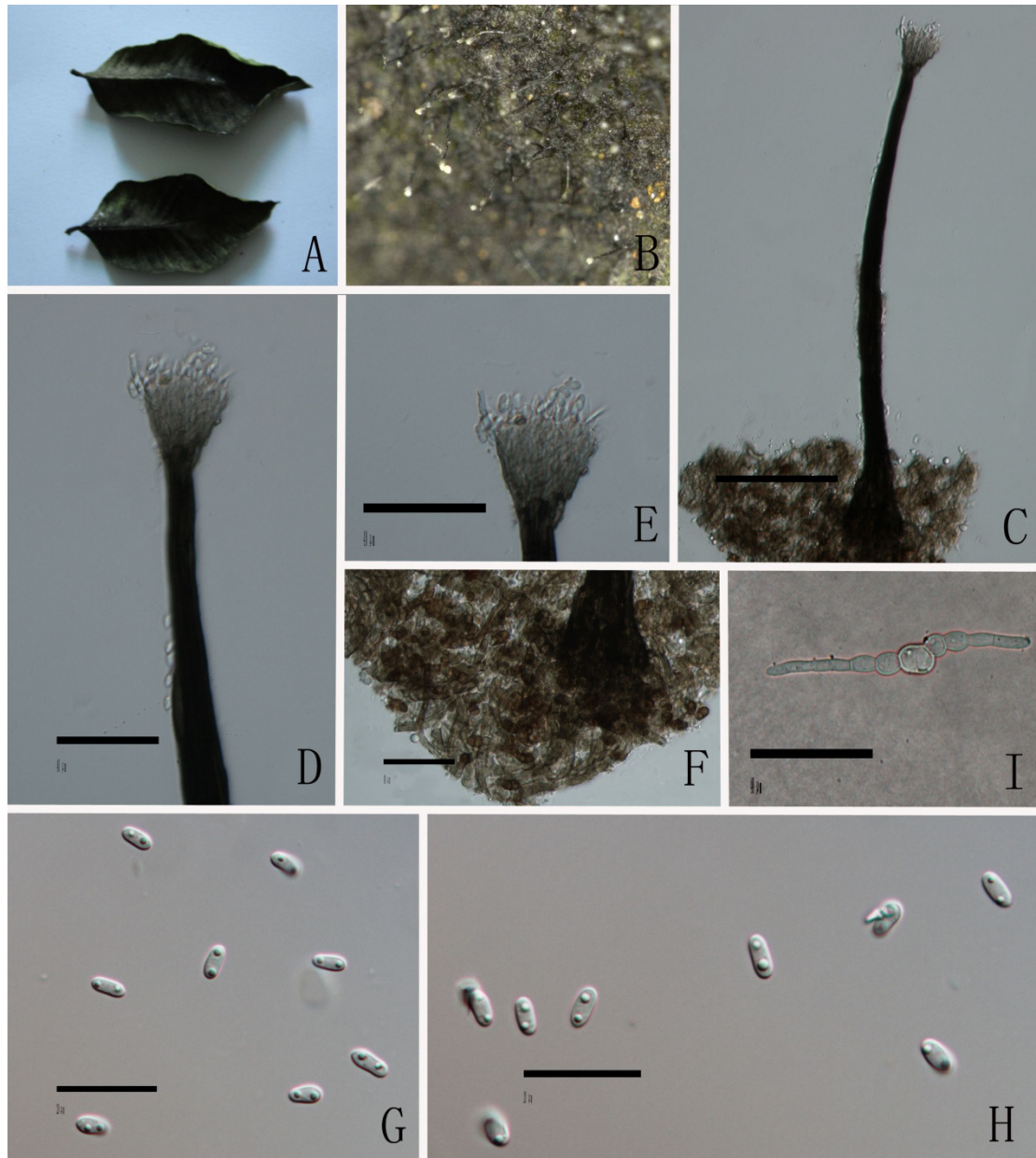


FIGURE 4. *Leptoxyphium kurandae* (IFRD 409-002). A. Superficial mycelium on host. B. Gregarious pycnidia on host surface. C. Stalked pycnidia. D–E. Black stalked and funnel apex. F. Reticulate hyphae. G–H. Hyaline conidia. I. Germinating conidia. Bars: C = 100 μm , D–F, I = 40 μm , G, H = 20 μm .

In culture, pycnidium stalked, verticillate arising from the basal haphae and swelling like a cupulate at the apex. Stalk olivaceous-brown or dark brown, and olivaceous-brown to hyaline at the apex. Hyphae dark brown, branched, septate, constricted at the septa, bead-like. Conidia broadly ellipsoid with round ends, hyaline, no septate, with guttulate (Fig. 5).

Culture characteristics:—Colony surface gray black to olivaceous black, 3 cm diam after 20 days, olivaceous black mycelium dense, gray black mycelium fluffy, with velvet in the center. Asexual state produced on PDA after 40 days.

Material examined:—CHINA. Yunnan Province: Jinghong, on living leaf of *Psidium guajava* L. (Myrtaceae), 20 July 2012, *Hui Yang* MS04-1 (IFRD 409-002) living culture = IFRDCC 2650.

Sequence data:—ITS = KF982310, LSU = KF982311, SSU = KF982312

Notes:—The species was collected from leaves of *Psidium guajava* in Yunnan Province, China. The phylogenetic tree shows that our collection is similar to *Leptoxyphium kurandae*.

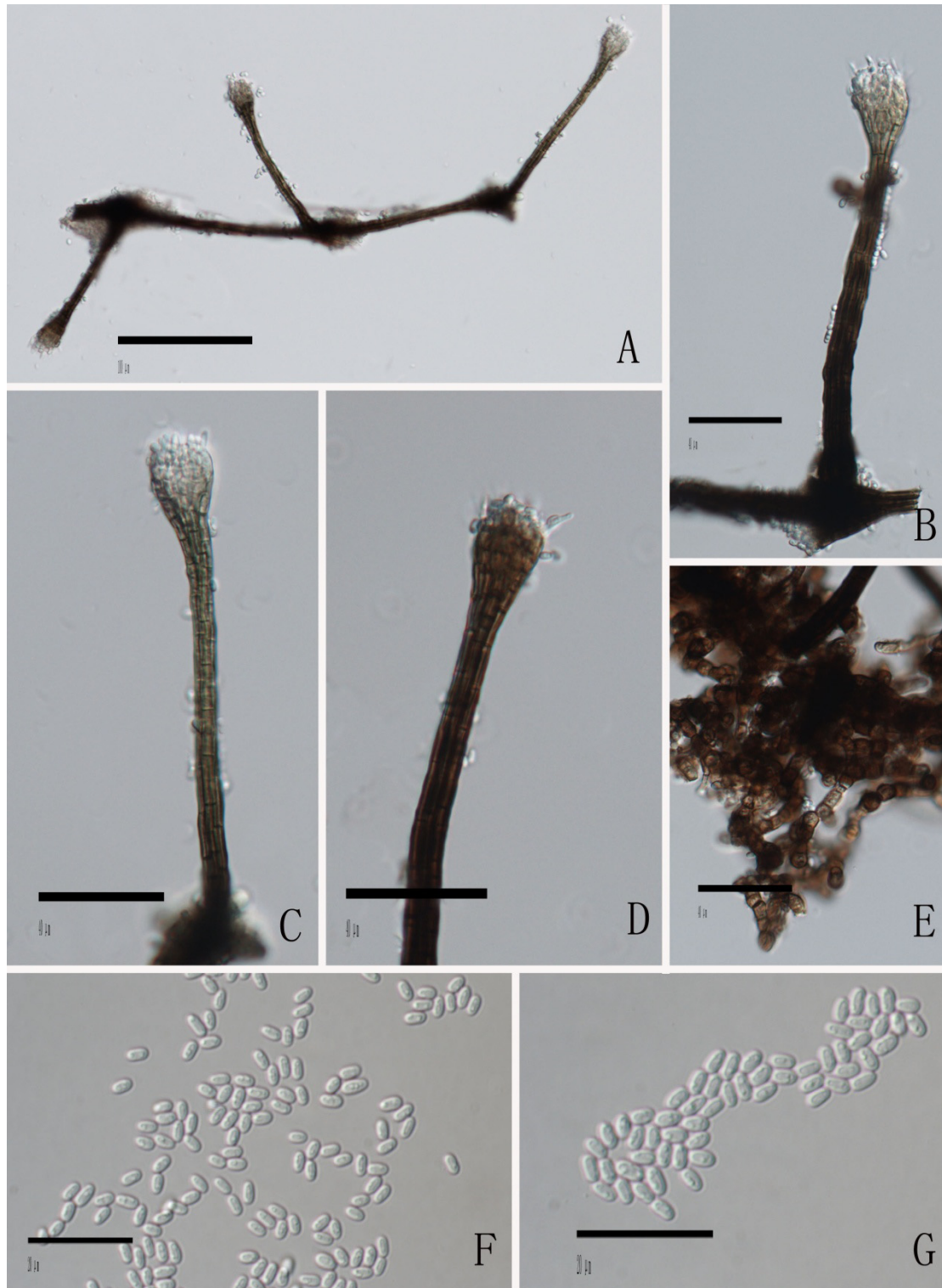


FIGURE 5. *Leptoxyphium kurandae* in culture. A–D. Stalked pycnidia and black stalked funnel cupulate apex. E. Reticulate hyphae. F–G. Hyaline conidia. Bars: A = 100 μ m, B–E = 40 μ m, F–G = 20 μ m.

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