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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.

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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*TM 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
Capnodium coartatum	MFLUCC 10-0069		JN832614	JN832599
Capnodium coartatum	MFLUCC 10-0070		JN832615	JN832600
Capnodium coffeae	CBS 147.52		DQ247800	DQ247808
Cladosporium cladosporioides	CBS 170.54	AY213640	NG_027578	DQ678004
Conidioxyphium gardeniorum	CPC 14327	—	GU301807	GU296143
Davidiella tassiana	CBS 723.79	EU167558	GU214410	EU167558
Dissoconium aciculare	CBS 204.89	AY725520	GU214419	GU214523
Dissoconium commune	CPC 12397	GQ852738	GQ852589	NG_016521
Dothidea insculpta	CBS 189.58	AF027764	DQ247802	DQ247810
Graphiopsis chlorocephala	CPC 11969	EU009458	EU009458	GU214534
Leptoxyphium cacuminum	MFLUCC 10-0049		JN832602	JN832587
Leptoxyphium cacuminum	MFLUCC 10-0059		JN832603	JN832588
Leptoxyphium cacuminum	MFLUCC 10-0086		JN832604	JN832589
Leptoxyphium fumago	CBS 123.26	—	GU214430	GU214535
Leptoxyphium kurandae	CPC:17274	JF951150	JF951170	—
Leptoxyphium glochidion	**IFRDCC 2651**	**KF982307**	**KF982308**	**KF982309**

..... *continued on the next page*

TABLE 1 (continued)

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

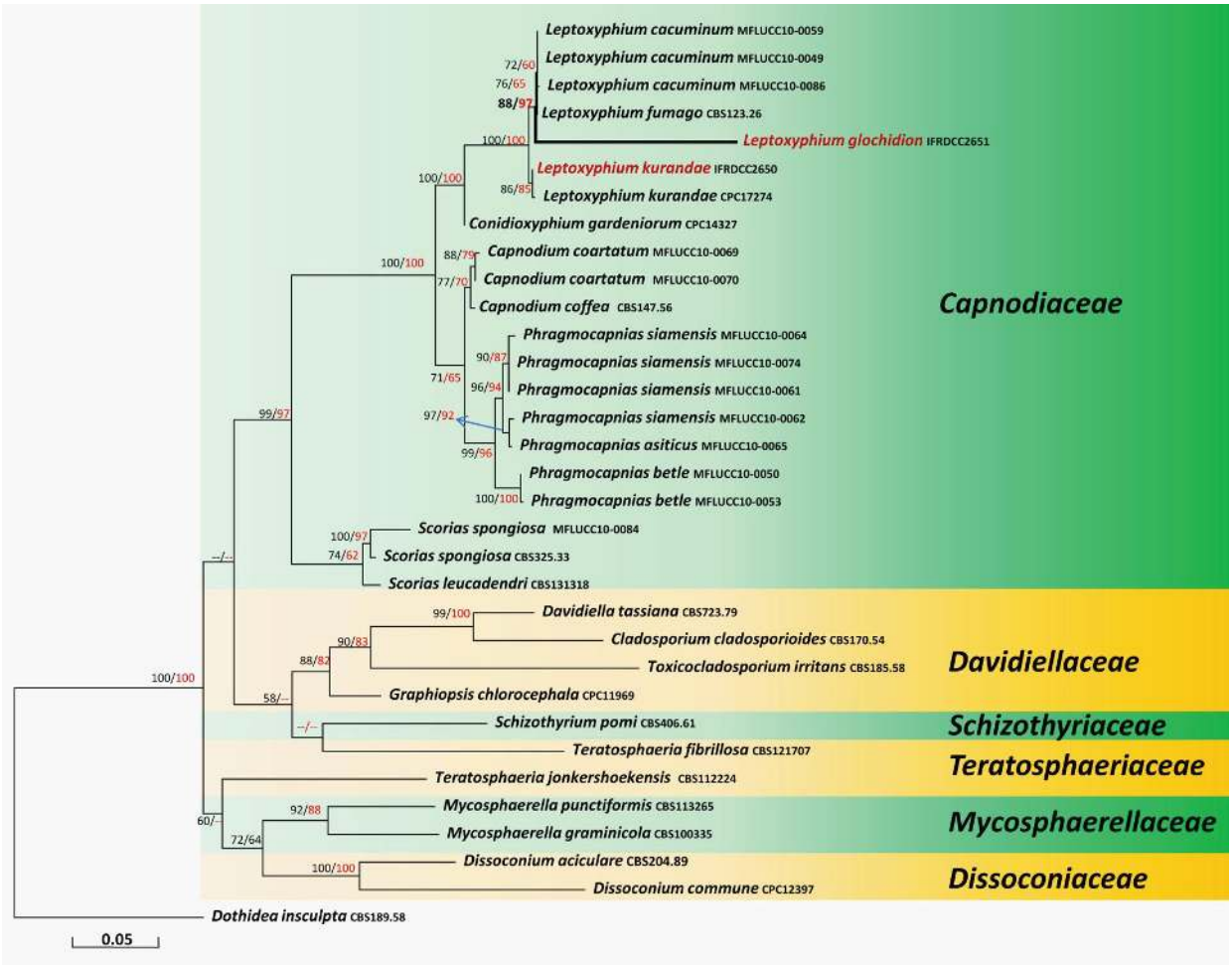

FIGURE 1. RAxML tree generated by the phylogenetic analysis of combined data set of ITS, LSU and SSU rDNA sequence. Bootstrap support values ≥ 50 % are shown below or above the branch. *Dothidea insculpta* is the out-group taxon. The original strain numbers are noted after the species names and names 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 ($\overline{x}$ = 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 ($\overline{x}$ = 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 ($\overline{x}$ = 46 µm, n = 20) wide at the base, the apex 33–46 × 31–47 µm wide ($\overline{x}$ = 39 × 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 × 3.4–4.8 µm ($\overline{x}$ = 7.6 × 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 ($\overline{x}$=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).

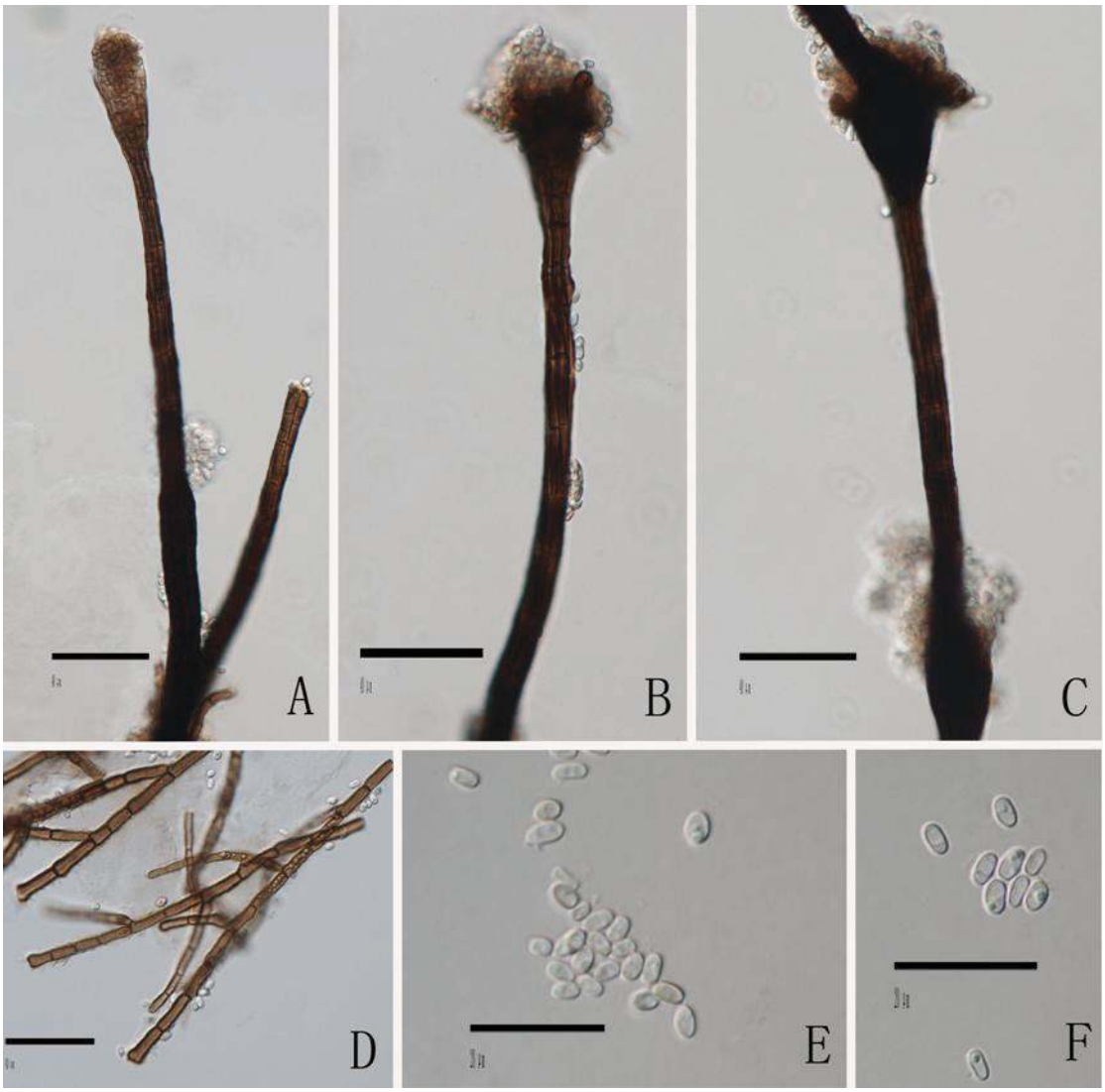

FIGURE 3. *Leptoxyphium glochidion* (holotype) in culture. A–C. Pycnidia. D. Septate hyphae. E–F. Hyaline conidia. Bars: A–D = 40 µm, E–F = 20 µm.

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, $\overline{x}$ = 357 µm, versus 341–446 µm, $\overline{x}$ = 392 µm), with a larger swollen base (34–54 µm, $\overline{x}$ = 46 µm vs 19–30 µm, $\overline{x}$ = 26 µm) and larger conidia (6.5–9.5 × 3.4–4.8 µm, $\overline{x}$ = 7.6 × 3.9 µm vs 4.1–6.7 × 2.1–2.7 µm, $\overline{x}$ = 5.2 × 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.

Leptoxyphium kurandae Crous & R.G. Shivas, in Crous *et al*., Persoonia 26: 145 (2011) MycoBank: 560176 (Figs 4, 5)

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 ($\overline{x}$ = 381 µm, n = 10), dark olivaceous-brown to black, straight to slightly flexuous, the base 37–47 × 37–43 µm wide ($\overline{x}$ = 44 × 39 µm, n = 20), the basal cells dark olivaceous-brown, bulbous, apex 29–37 × 28–32 µm wide ($\overline{x}$ = 34 × 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 × 2.7–3.4 µm ($\overline{x}$ = 6.7 × 3 µm, n = 20), broadly ellipsoid with rounded ends, hyaline, aseptate and lacking guttules, germinating both ends of conidia (Fig. 4).

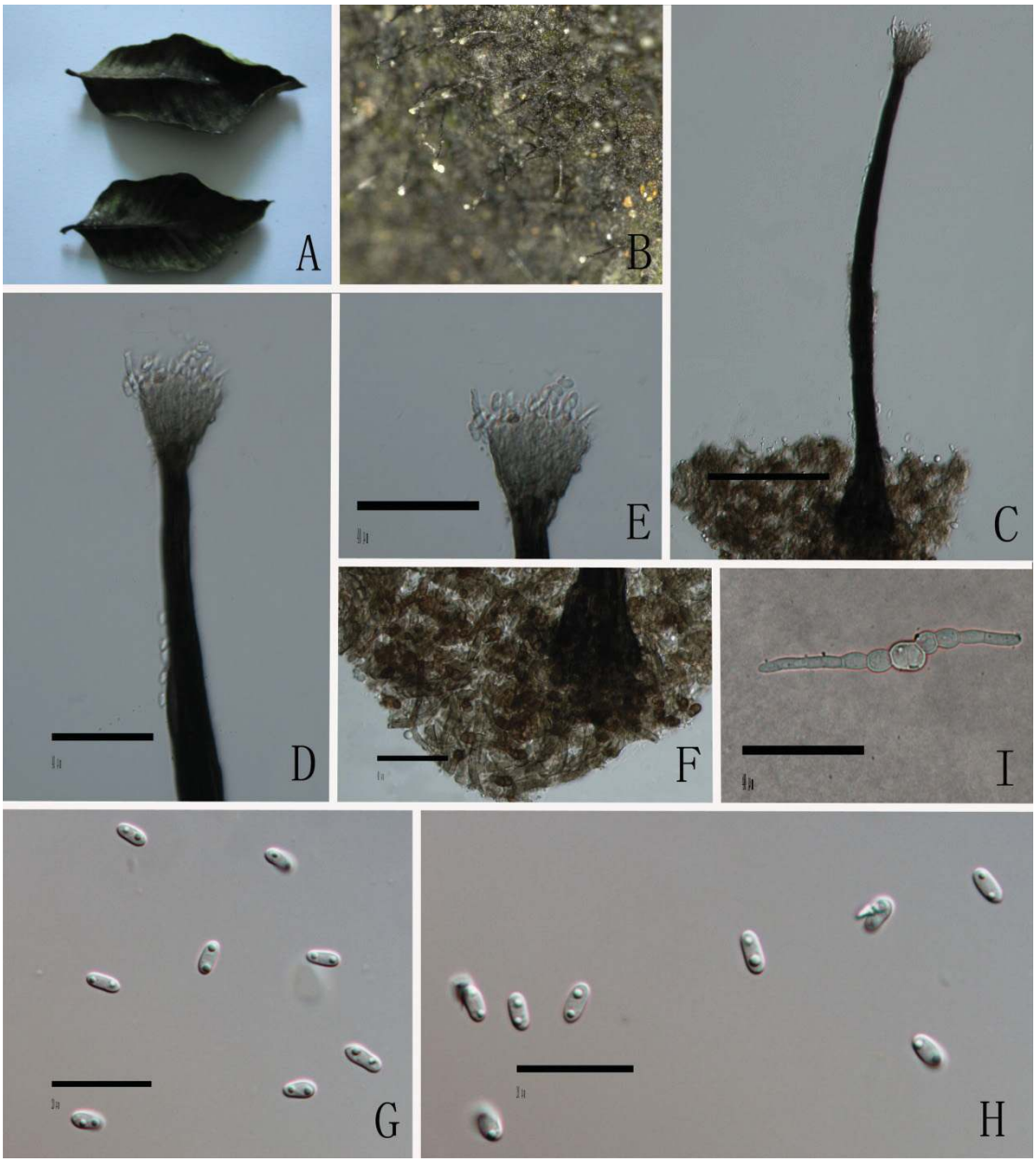

FIGURE 4. *Leptoxyphium kurandae* (IFRD 409-002). A. Superfical mycelium on host. B. Gregarious pycnidia on host surface. C. Stalked pycnidia. D–E. Black stalked and funnel apex. F. Reticulate hyphae. G–H. Hyaline conidia. I. Germinating conidia. Bars: C = 100 µm, D–F, I = 40 µm, G, H = 20 µm.

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*.

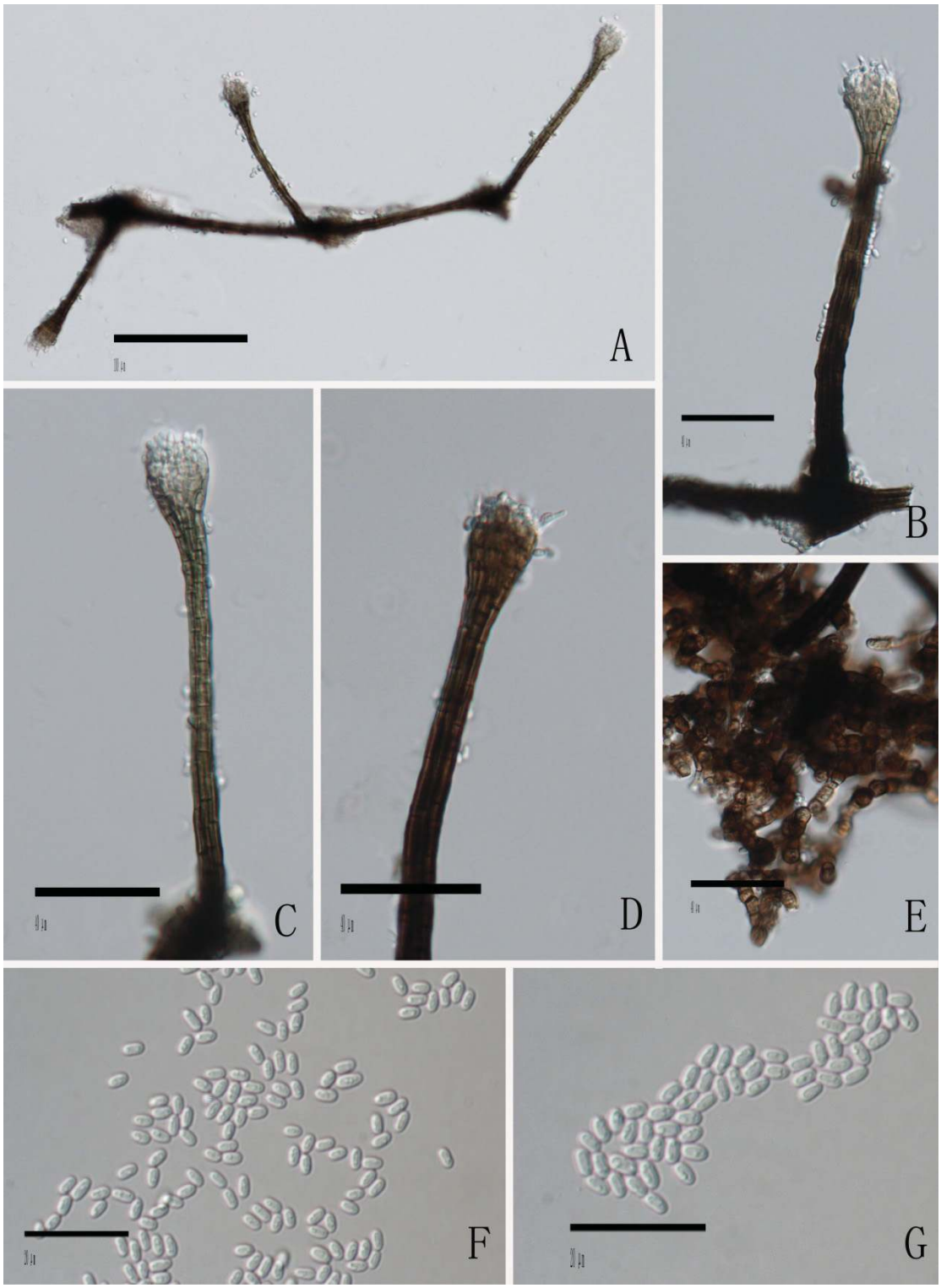

FIGURE 5. *Leptoxyphium kurandae* in culture. A–D. Stalked pycnidia and black stalked funnel cupulate apex. E. Reticulate hyphae. F–G. Hyaline conidia. Bars: A = 100 µm, B–E = 40 µm, F–G = 20 µm.

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References

Batzer, J.C., Arias, M.M., Harrington, T.C., Gleason, M.L., Groenewald, J.Z. & Crous, P.W. (2008) Four species of *Zygophiala* (*Schizothyriaceae*, Capnodiales) are associated with the sooty blotch and flyspeck complex on apple. *Mycologia* 100(2): 246–258.
http://dx.doi.10.3852/mycologia.100.2.246

Chomnunti, P., Schoch, C.L., Aguirre-Hudson, B., Ko Ko, T.W., Hongsanan, S., Jones, E.B.G., Kodsueb, R., Phookamsak, R., Chukeatirote, E., Bahkali, A.H. & Hyde, K.D. (2011) *Capnodiaceae*. *Fungal Diversity* 51: 103–134.
http://dx.doi.10.1007/s13225-011-0145-6

Chomnunti, P., Hongsanan, S., Aguirre-Hudson, B., Tian, Q., Peršoh, D., Dhami, M.K., Alias, A.S., Xu, J.C., Liu, X.Z., Stadler, M. & Hyde, K.D. (2014) The sooty moulds. *Fungal Diversity* (on line).
http://dx.doi.10.1007/s13225-014-0278-5

Crous, P.W., Braun, U., Schubert, K. & Groenewald, J.Z. (2007) Delimiting *Cladosporium* from morphologically similar genera *Studies in Mycology* 58: 33–56.
http://dx.doi.10.3114/sim.2007.58.02

Crous, P.W., Schoch, C.L., Hyde, K.D., Wood, A.R., Gueidan, C., de Hoog, G.S. & Groenewald, J.Z. (2009a) Phylogenetic lineages in the Capnodiales. *Studies in Mycology* 64: 17–47.
http://dx.doi.10.3114/sim.2009.64.02

Crous, P.W., Summerell, B.A., Carnegie, A.J., Wingfield, M.J., Hunter, G.C., Burgess, T.I., Andjic, V., Barber, P.A. & Groenewald, J.Z. (2009b) Unravelling *Mycosphaerella*: do you believe in genera? *Persoonia* 23: 99–118.
http://dx.doi.10.3767/003158509X479487

Crous, P.W., Groenewald, J.Z., Shivas, R.G., Edwards, J., Seifert, K.A., Alfenas, A.C., Alfenas, R.F., Burgess, T.I., Carnegie, A.J., Hardy, G.E.St.J., Hiscock, N., Hüberli, D., Jung, T., Louis-Seize, G., Okada, G., Pereira, O.L., Stukely, M.J.C., Wang, W., White, G.P., Young, A.J., McTaggart, A.R., Pascoe, I.G., Porter, I.J. & Quaedvlieg, W. (2011) Fungal Planet description sheets: 69–91. *Persoonia* 26: 108–156.
http://dx.doi.10.3767/003158511X581723

Hughes S.J. (1976) Sooty moulds. *Mycologia* 68: 693–820.
http://dx.doi.org/10.2307/3758799

Hyde, K.D., Jones, E.B.G., Liu, J.K., Ariyawansa, H., Boehm, E., Boonmee, S., Braun, U., Chomnunti, P., Crous, P.W., Dai, D.Q., Diederich, P., Dissanayake, A., Doilom, M., Doveri, F., Hongsanan, S., Jayawardena, R., Lawrey, J.D., Li, Y.M., Liu, Y.X., Lücking, R., Monkai, J., Muggia, L., Nelsen, M.P., Pang, K.L., Phookamsak, R., Senanayake, I., Shearer, C.A., Suetrong, S., Tanaka, K., Thambugala, K.M., Wijayawardene, N.N., Wikee, S., Wu, H.X., Zhang, Y., Aguirre-Hudson, B., Alias, S.A., Aptroot, A., Bahkali, A.H., Bezerra, J.L., Bhat, D.J., Camporesi, E., Chukeatirote, E., Gueidan, C., Hawksworth, D.L., Hirayama, K., Hoog, S.D., Kang, J.C., Knudsen, K., Li, W.J., Li, X.H., Liu Z.Y., Mapook, A., McKenzie, E.H.C., Miller, A.N., Mortimer, P.E., Phillips, A.J.L., Raja, H.A., Scheuer, C., Schumm, F., Taylor, J.E., Tian Q., Tibpromma, S., Wanasinghe D.N., Wang, Y., Xu J.C., Yan J.Y., Yacharoen, S. & Zhang, M. (2013) Families of Dothideomycetes. *Fungal Diversity* 63: 1–313.
http://dx.doi.10.1007/s13225-013-0263-4

Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008) *Dictionary of the fungi*. 10th edn. CABI Bioscience, UK.

Page, R.D.M. (1996) TreeView: an application to display phylogenetic trees on personal computers. *Computer Application in the Biosciences* 12: 357–358.

Phillips, A.J.L., Alves, A., Pennycook, S.R., Johnston, P.R., Ramaley, A., Akulov, A. & Crous, P.W. (2008) Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the *Botryosphaeriaceae*. *Persoonia* 21: 29–55.

Schoch, C.L., Shoemaker, R.A., Seifert, K.A., Hambleton, S., Spatafora, J.W. & Crous, P.W. (2006a) A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia* 98(6): 1041–1052.
http://dx.doi.10.3852/mycologia.98.6.1041

Schoch, C.L., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Tsui, C.K. & Spatafora, J.W. (2006b) The halotolerant fungus *Glomerobolus gelineus* is a member of the Ostropales. *Mycological Research* 110(3): 257–263.
http://dx.doi.org/10.1016/j.mycres.2005.10.001

Schoch, C.L., Crous, P.W., Groenewald, J.Z., Boehm, E.W.A., Burgess, T.I., de Hoog, G.S., Dixon, L.J., Grube, M., Gueidan, C., Harada, Y., Hatakeyama, S., Hirayama, K., Hosoya, T., Huhndorf, S.M., Hyde, K.D., Jones, E.B.G., Kohlmeyer, J.,

Kruys, A., Li, Y.M., Lucking, R., Lumbsch, H.T., Marvanova, L., Mbatchou, J.S., McVay, A.H., Miller, A.N., Mugambi, G.K., Muggia, L., Nelsen, M.P., Nelson, P., Owensby, C.A., Phillips, A.J.L., Phongpaichit, S., Pointing, S.B., Pujade-Renaud, V., Raja, H.A., Plata, E.R., Robbertse, B., Ruibal, C., Sakayaroj, J., Sano, T., Selbmann, L., Shearer, C.A., Shirouzu, T., Slippers, B., Suetrong, S., Tanaka, K., Volkmann-Kohlmeyer, B., Wingfield, M.J., Wood, A.R., Woudenberg, J.H.C., Yonezawa, H., Zhang, Y. & Spatafora, J.W. (2009) A class-wide phylogenetic assessment of Dothideomycetes. *Studies in Mycology* 64: 1–15.

Spegazzini, C. (1918) Notas Micológicas. *Physis (Buenos Aires)* 4: 281–295.

Stamatakis, A. & Alachiotis, N. (2010) Time and memory efficient likelihood-based tree searches on phylogenomic alignments with missing data. *Bioinformatics* 26 (12): 132–139.
http://dx.doi.10.1093/bioinformatics/btq205

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28(10): 2731–2739.
http://dx.doi.10.1093/molbev/msr121.

Verkley, G.J., Crous, P.W., Groenewald, J.Z., Braun, U. & Aptroot, A. (2004) *Mycosphaerella punctiformis* revisited: morphology, phylogeny, and epitypification of the type species of the genus *Mycosphaerella* (Dothideales, Ascomycota). *Mycological Research* 108 (11): 1271–1282.
http://dx.doi.org/10.1017/S0953756204001054

Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246.

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols: a guide to methods and applications*. San Diego, Academic Press. pp. 315–322.