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A new species of *Acrophialophora* from Guizhou Province, China

YAN-WEI ZHANG[1, 2], YAO WANG[1], GUI-PING ZENG[1], WANHAO CHEN[1], ZOU XIAO[1], YAN-FENG HAN[1*], SHU-YI QIU[3*] & ZONG-QI LIANG[1]

[1]*College of Life Science, Guizhou University, Guiyang, Guizhou 550025, China*
**email: swallow1128@126.com*
[2] *School of Chemistry and Life Science, Guizhou Normal University, Guiyang, Guizhou 550018, China*
[3]*College of Liquor and food engineering, Guizhou University, Guiyang, Guizhou 550025, China*
**email: syqiu@gzu.edu.cn*

Abstract

Acrophialophora liboensis, a new fungus from soil samples in Libo County, Guizhou Province, China, is illustrated and described on the basis of morphological and molecular sequences data. Phylogenetic analysis based on internal transcribed spacer (ITS) and β-tubulin sequences demonstrated that *A. liboensis* is a distinct species closely related to *A. cinerea* and *A. furcata*. Morphologically, *A. liboensis* is characterized by solitary and lateral phialides tapering into thin necks and long chains of ellipsoidal or oval conidia. The ex-type living culture has been deposited in CGMCC, Beijing City, China.

Key words: molecular phylogeny, morphology, taxonomy, thermotolerant fungi

Introduction

The genus *Acrophialophora* was established by Edward (1959) with *A. nainiana* Edward as the type. Samson & Mahmood (1970) reintroduced *Acrophialophora* as a thermotolerant genus comprising three species: *A. fusispora* (S.B. Saksena) Samson, *A. levis* Samson & T. Mahmood and *A. nainiana*. Zhang *et al*. (2015) assessed the relationship between the genera *Acrophialophora* and *Taifanglania* through phylogenetic analyses of nuclear ribosomal internal transcribed spacer (ITS) sequences and combined β-tubulin, nuclear small subunit (nuc18S) and ITS sequences. They considered *Taifanglania* to be synonymous with *Acrophialophora* and accordingly transferred all *Taifanglania* species to *Acrophialophora*. In addition, they emended the generic concept of *Acrophialophora* and proposed three new species: *A. acuticonidiata* Yu Zhang & L. Cai, *A. angustiphialis* Yu Zhang & L. Cai and *A. ellipsoidea* Yu Zhang & L. Cai. According to Index Fungorum (http://www.indexfungorum.org/Names/Names.asp March, 2017), 17 species names have been published thus far. Because *A. nainiana* has been treated as a synonym of *A. fusispora* (Samson & Mahmood, 1970), the genus *Acrophialophora* currently includes 16 species.

All *Acrophialophora* species are saprophytic and thermotolerant, and characteristics that may play an important role in cellulose degradation (Liang *et al*. 2007, Han *et al*. 2010b, Wang 2015). Some species can produce highly active laccase and cellulase (Zhang *et al*. 2014; Han *et al*. 2012) and useful thermostable enzymes (Yang *et al*. 2006). Further research is necessary to identify new resources and to explore the potential of these fungi in applied research. During the course of our survey of thermotolerant fungi in China over the past ten years, eight species have been reported (Chu *et al*. 2004; Liang *et al*. 2006, 2007, 2009; Han *et al*. 2007, 2010a; Wang *et al*. 2015). In this paper, we describe and illustrate a new *Acrophialophora* species, *A. liboensis*, which was identified by means of morphological characters and phylogenetic analyses.

Materials and Methods

Sample collection and strain isolation

Strain F0044H was isolated from soil samples collected from Libo County, Guizhou Province, China. According

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to the method of Wang *et al*. (2015), soil samples were added to sterilized water in an Erlenmeyer flask, with 1-mL suspensions then evenly spread on Martin's medium and incubated at 40 °C. The pure cultures were subsequently transferred to potato dextrose agar (PDA) slants and stored at −70 °C at the Institute of Fungus Resources, Guizhou University (GZAC), China; the ex-type living culture is deposited in China General Microbiological Culture Collection Center (CGMCC 3.18309).

Morphological identification

Isolates were transferred to PDA and Czapek agar, incubated at 40 °C for 7 days, and subjected to macroscopic examination. Fungal microcharacteristics were examined with a microscope (Motic, Guangzhou, China) and photographed. Diagnostic features were then illustrated on the basis of these observations. Finally, the fungi were morphologically identified according to colony characteristics and conidiogenous structures (Liang *et al*. 2009; Zhang *et al*. 2015; Wang *et al*. 2015).

DNA extraction, PCR amplification and nucleotide sequencing

Total genomic DNA was extracted from fresh sporulating cultures at 25 °C for 7 days using a Fungal DNA mini kit (Omega Biotech, Doraville, GA, USA) according to the manufacturer's protocols and stored at –20 °C. ITS-5.8S rDNA and β-tubulin regions were amplified using the primer sets ITS5/ITS4 (Zhang *et al.* 2016, Wen *et al*. 2015) and Bt2a/Bt2b (Glass & Donaldson 1995), respectively. Amplifications were carried out in 50-μL volumes as outlined by Cai *et al*. (2006). DNA sequencing was performed with the primers mentioned above by Sangon Biotech, Shanghai, China. The generated ITS-5.8S rDNA and β-tubulin sequences were submitted to GenBank (accession numbers KP192127 and KP999978).

Phylogenetic analysis

Phylogenetic analyses were conducted based on ITS sequences and a combination of β-tubulin and ITS sequences data. Isolates information on analyzed species is given in Table 1. Sequences of our isolate along with reference sequences obtained from GenBank were aligned with Clustal X (Thompson *et al*. 1997). Alignments were then manually optimized in BioEdit (Hall 1999). Ambiguously aligned regions were excluded from analyses.

Phylogenetic trees were constructed using maximum parsimony (MP) and Bayesian analysis according to the methods of Zhang *et al*. (2015). MP analysis was performed in PAUP* 4.0b10 (Swofford 2003). Trees were inferred using the heuristic search option with tree-bisection-reconnection branch swapping and 1,000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed and all multiple parsimonious trees were saved. Branch robustness was estimated by conducting a bootstrap analysis of 1,000 replicates, each with 10 replicates of random addition sequences. A Shimodaira-Hasegawa test (Shimodaira & Hasegawa 1999) was performed to evaluate whether trees were significantly different from one another. Trees were visualized in TreeView (Page 1996). For Bayesian analysis, the best-fit model of evolution was estimated in MrModeltest 2.2 (Nylander 2004). Posterior probabilities (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov chain Monte Carlo sampling in MrBayes 3.2.1 (Huelsenbeck & Ronquist 2001) using the estimated model of evolution. Six simultaneous Markov chains were run for 1,000,000 generations, with trees sampled every 100th generation (resulting in 10,000 total trees). The first 2,000 trees, which represented the burn-in phase of the analysis, were discarded; the remaining 8,000 trees were used to calculate posterior probabilities in the majority rule consensus tree. Sequences derived in this study were deposited in GenBank. Sequence alignments were uploaded to TreeBASE (www.treebase.org/treebase/index.html, ID20069) and taxonomic novelties were deposited in MycoBank (www.MycoBank.org).

Results

Phylogenetic analysis

The final aligned dataset of ITS sequences from 30 fungal strains (Table 1) comprised 576 characters, including 76 that were parsimony informative and 406 that were constant. Parsimony analysis generated 100 equally most parsimonious trees, one of which (tree length [TL] = 218, consistency index [CI] = 0.903, retention index [RI] = 0.908, rescaled consistency index [RC] = 0.8208, homoplasy index [HI] = 0.096) is illustrated in Figure 1.

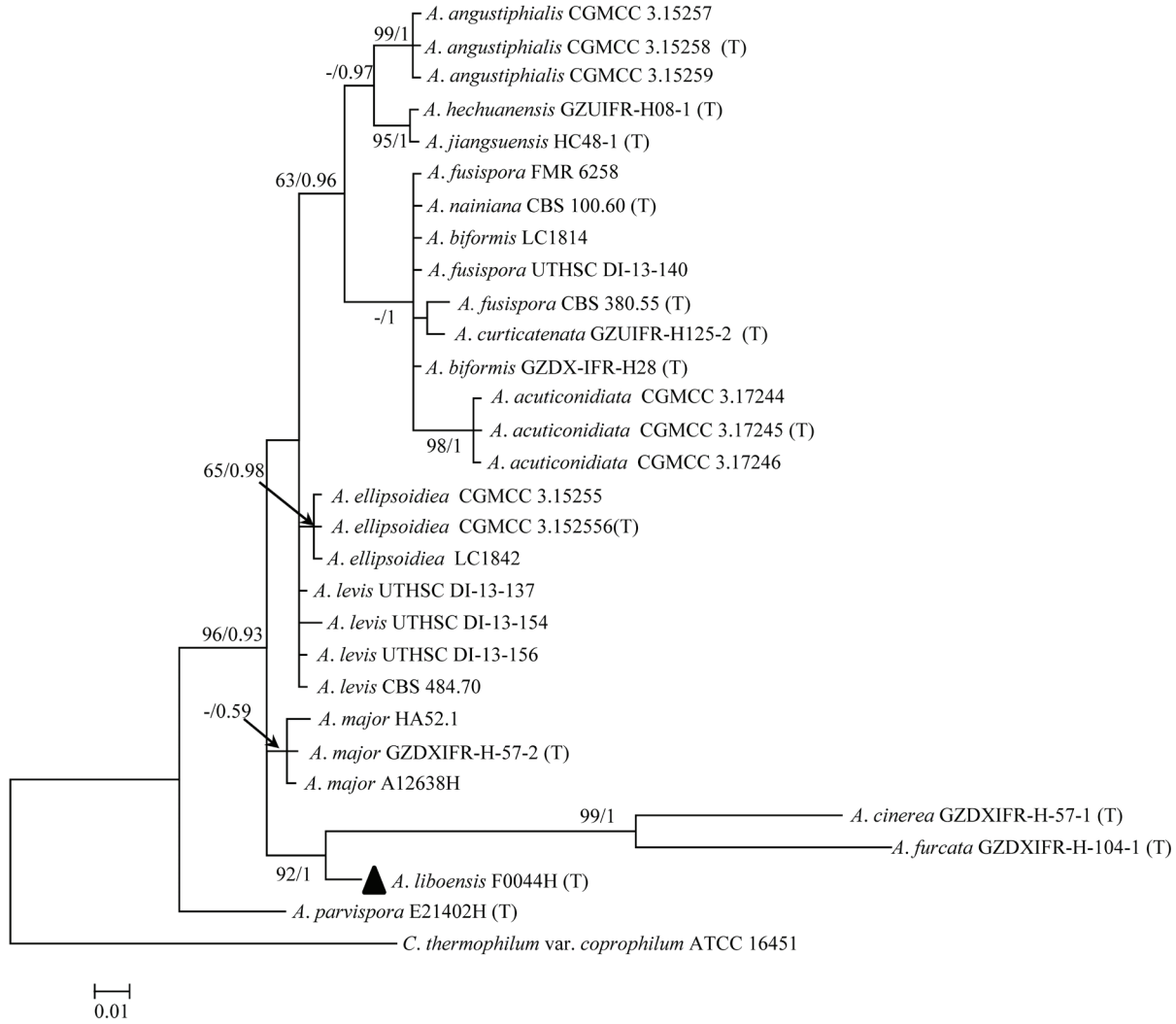

FIGURE 1. Phylogram generated from maximum parsimony analysis of ITS sequences of *Acrophialophora* species. Numbers above branches are parsimony bootstrap values ≥ 75% and significant Bayesian posterior probability values ≥ 0.90. The tree was rooted using *Chaetomium thermophilum* var. *coprophilum* as outgroup.

TABLE 1. Taxa used for the phylogenetic analyses in this study

Species names	Strains No.	GenBank No.	
		ITS	β-tubulin
Acrophialophora acuticonidiata	CGMCC 3.17246	KJ026976	KJ147442
A. acuticonidiata (T)	CGMCC 3.17245	KJ026975	KJ147441
A. acuticonidiata	CGMCC 3.17244	KJ026974	KJ147440
A. angustiphialis	CGMCC 3.15257	KJ026971	KJ147437
A. angustiphialis (T)	CGMCC 3.15258	KJ026972	KJ147438
A. angustiphialis	CGMCC 3.15259	KJ026973	KJ147439
A. biformis	LC1814	KJ026968	KJ147434
A. biformis (T)	GZDX-IFR-H28	DQ191963	---------
A. cinerea (T)	GZDXIFR-H-57-1	DQ243694	KP143110
A. curticatenata (T)	GZUIFR-H125-2	EU004811	---------
A. ellipsoidea	LC1842	KJ026970	KJ147436
A. ellipsoidea (T)	CGMCC 3.15256	KJ026969	KJ147435
A. ellipsoidea	CGMCC 3.15255	KJ026967	KJ147433
A. furcata (T)	GZDXIFR-H-104-1	DQ243695	KP143113

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TABLE 1. (Continued)

Species names	Strains No.	GenBank No.	
		ITS	β-tubulin
A. fusispora	UTHSC DI-13-140	KM995910	LN624451
A. fusispora	FMR 6258	KM995906	LN624447
A. fusispora (T)	CBS 380.55	KP233037	KP233043
A. hechuanensis (T)	GZUIFR-H08-1	DQ185070	KP143115
A. jiangsuensis (T)	HC48-1	KF719171	KP143112
******A. liboensis* (T)	CGMCC 3.18309 (F0044H)	KP192127	KP999978
A. levis	CBS 484.70	KP233038	KP233044
A. levis	UTHSC DI-13-137	KM995882	LN624423
A. levis	UTHSC DI-13-154	KM995895	LN624436
A. levis	UTHSC DI-13-156	KM995897	LN624438
A. major (T)	GZDXIFR-H-57-2	DQ243696	KP143116
A. major	A12638H	KP143099	KP143111
A. major	HA52.1	KF719172	KP143114
A. nainiana (T)	CBS 100.60	KP233036	KP233042
Taifanglania parvispora (T)	E21402H	KF719170	---------
Chaetomium thermophilum var. *coprophilum* (T)	ATCC 16451	JF412013	KP336893

******newly generated isolate used in the study.

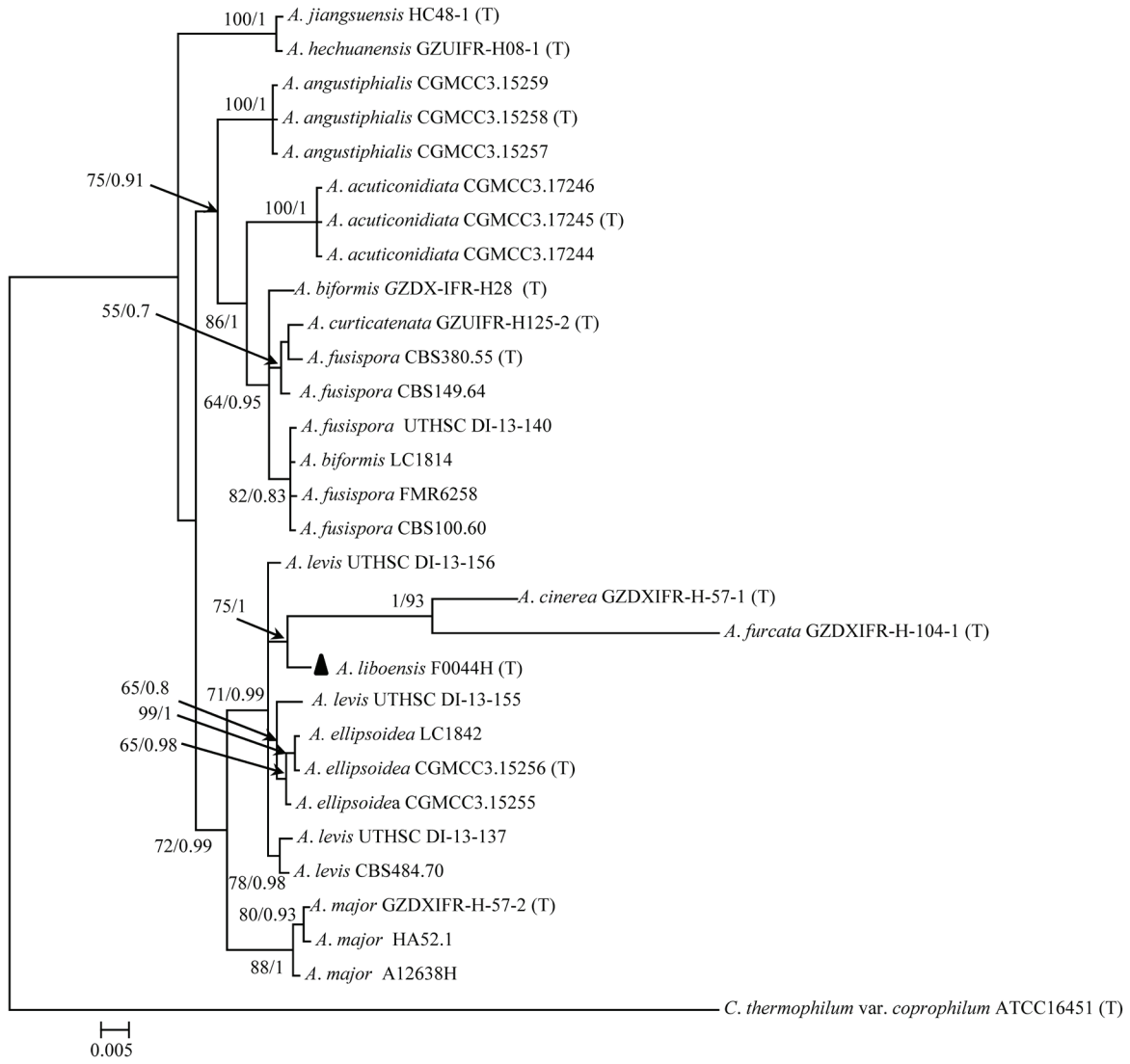

FIGURE 2. Phylogram generated from maximum parsimony analysis of ITS and β-tubulin sequences of *Acrophialophora* species. Numbers above branches are parsimony bootstrap values ≥ 75% and significant Bayesian posterior probability values ≥ 0.90. The tree was rooted using *Chaetomium thermophilum* var. *coprophilum* as an outgroup.

The combined dataset of ITS and β-tubulin sequences from 30 fungal strains (Table 1) comprised 1,017 characters after alignment. Of these, 76 characters were parsimony informative and 831 were constant. Parsimony analysis generated 72 equally most parsimonious trees, one of which (TL = 360, CI = 0.858, RI = 0.878, RC = 0.7536, HI = 0.1417) is illustrated in Figure 2.

Both trees had similar topologies. Our putative new species F0044H occupied a distinct position in the tree but appeared to be closely related to *Acrophialophora cinerea* (Z.Q. Liang, H.L. Chu & Y.F. Han) Yu Zhang & L. Cai and *A. furcate* (Z.Q. Liang, H.L. Chu & Y.F. Han) Yu Zhang & L. Cai.

Description and Taxonomy

Acrophialophora liboensis Y.W. Zhang, Y. Wang, Y.F. Han & Z.Q. Liang sp. nov. (Fig. 3)
GenBank: KP192127 and KP999978 MycoBank: MB 818669

***Type*:**—CHINA. Guizhou Province: Libo County, N25°24′39.98″, E107°53′13″, soil, Y. Wang, May, 2013, Holotype GZUIFR-F0044 (dried culture), ex-type living culture CGMCC 3.18309 (F0044H).

Colonies on Czapek agar 50–52 mm diam. after 7 d at 40 °C, flat, white, densely fluffy, irregular margined, lightly wavy, reverse brown in the center, gray white in margins. *Aerial mycelia* smooth-walled, hyaline, 1–2 µm ($\bar{x}$ = 1.4 ± 0.2, *n* = 30) wide. *Conidiophores* absent. *Phialides* single, borne laterally on vegetative hyphae, smooth-walled, 5–15 × 1–3 µm ($\bar{x}$ = 11.2 ± 2.2 × 1.6 ± 0.3, *n* = 30), with a cylindrical or ellipsoidal basal portion, tapering into a distinct neck, sometimes proliferating phialides. *Conidia* 2.5–5.4 × 1.5–3 µm ($\bar{x}$ = 3.8 ± 1.1 × 2.6 ± 0.2, *n* = 50), one-celled, ellipsoidal to oval, smooth-walled, forming long chains.

Etymology:—Refers to the region from which the fungus was isolated.

Distribution:—Guizhou Province, China.

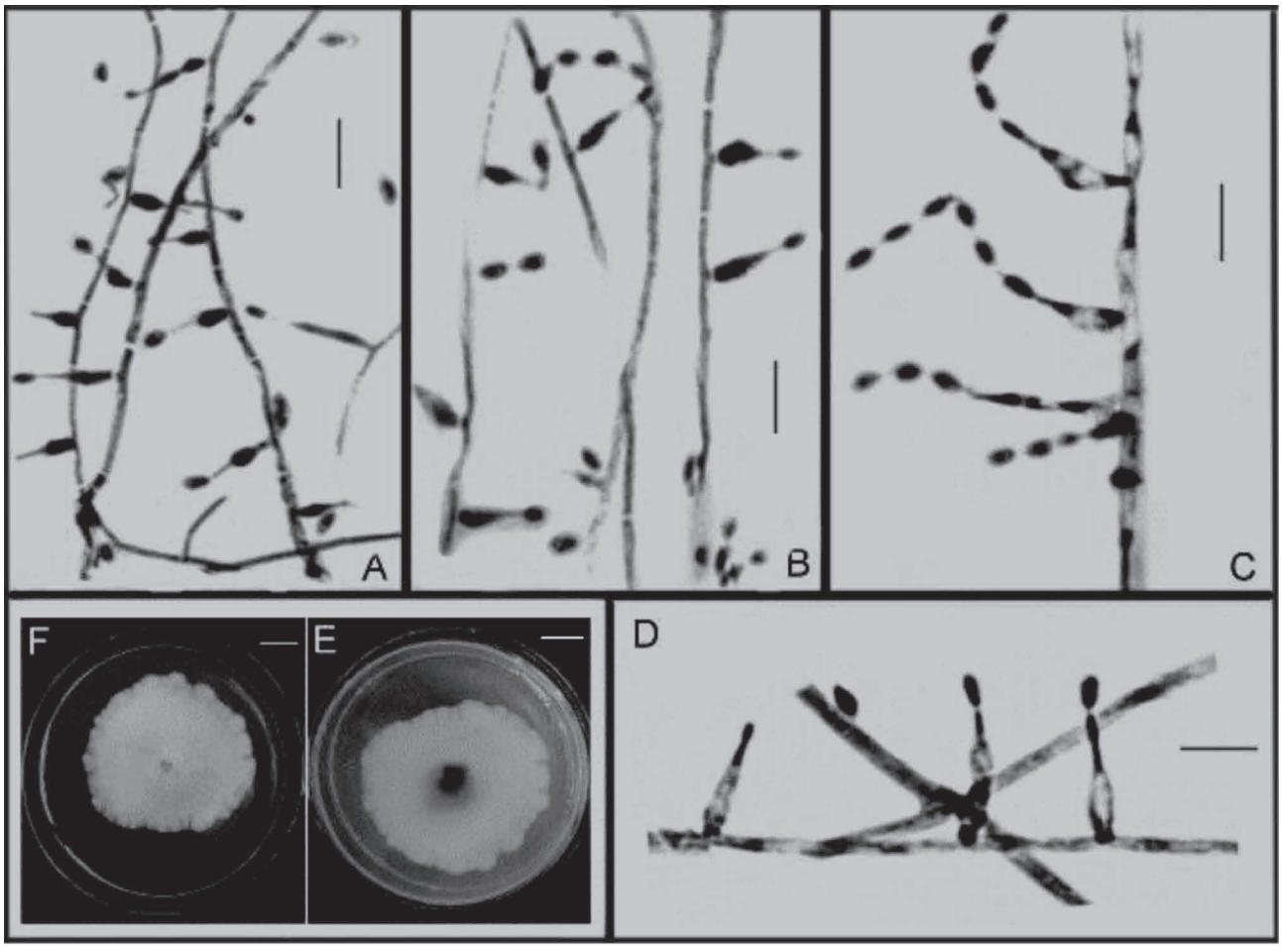

FIGURE 3. *Taifanglania liboensis* **(holotype)**. **A–D:** Conidiogenous structures; **E:** Colony in Czapek agar; **F:** Colony in potato dextrose agar. Bars: A–D = 10 µm; E–F = 10 mm.

Discussion

Acrophialophora is a monophyletic genus belonging to the family Chaetomiaceae (Zhang *et al.* 2015). Our phylogenetic analyses of ITS and combined ITS and β-tubulin sequence datasets support *A. liboensis* as a distinct species from other existing species *Acrophialophora* (Figs. 1 & 2). *Acrophialophora liboensis* is phylogenetically close to *A. cinerea* and *A. furcata*; however, *A. furcata* often has polyphialidic conidiogenous cells with two necks, while *A. cinerea* differs in conidial size (3–13 × 1.5–5.5 μm vs. 2.5–5.4 × 1.5–3 μm) (Liang *et al*. 2009, Liang *et al*. 2006). Morphologically, *A. liboensis* is most similar to *A. angustiphialis* and *A. ellipsoidea* in having monophialidic conidiogenous cells (Zhang *et al*. 2015), but the latter two species are phylogenetically distinct from *A. liboensis*. In addition, *A. liboensis* is also similar to *A. curticatenata* (Z.Q. Liang & Y.F. Han) Yu Zhang & L. Cai and *A. jiangsuensis* (Z.Q. Liang & Y.F. Han) Yu Zhang & L. Cai in conidial shape; however, *A. curticatenata* has slightly rough conidia (Han *et al*. 2007) and *A. jiangsuensis* often has conidial chains with slimy heads (Han *et al*. 2010). The new species *A. liboensis* is characterized by smooth conidia that formed long chains.

Zhang *et al*. (2015) transferred the *Taifanglania* species to the genus *Acrophialophora*. *T. parvispora*, which was isolated from the river mud and was reported as a new species in the genus *Taifanglania* (Wang et al. 2015), was distinct as a separated clade in the ITS tree, they need more strains and more other genes data to confirm its taxonomic status.

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