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Culturable mycobiota from Karst caves in China, with descriptions of 20 new species

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Key words

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Abstract Karst caves are distinctly characterised by darkness, low to moderate temperatures, high humidity, and scarcity of organic matter. During the years of 2014–2015, we explored the mycobiota in two unnamed Karst caves in Guizhou province, China, and obtained 563 fungal strains via the dilution plate method. Preliminary ITS analyses of these strains suggested that they belonged to 246 species in 116 genera, while 23.5 % were not identified to species level. Among these species, 85.8 % (211 species) belonged to *Ascomycota*; 7.3 % (18 species) belonged to *Basidiomycota*; 6.9 % (17 species) belonged to *Mucoromycotina*. The majority of these species have been previously known from other environments, mostly from plants or animals as pathogens, endophytes or via a mycorrhizal association. We also found that 59 % of these species were discovered for the first time from Karst caves, including 20 new species that are described in this paper. The phylogenetic tree based on LSU sequences revealed 20 new species were distributed in six different orders. In addition, ITS or multi-locus sequences were employed to infer the phylogenetic relationships of new taxa with closely related allies. We conclude that Karst caves encompass a high fungal diversity, including a number of previously unknown species. Novel species described include: *Amphichorda guana*, *Auxarthronopsis guizhouensis*, *Biscogniauxia petrensis*, *Cladorrhinum globisporum*, *Collariella quadrum*, *Gymnoascus exasperatus*, *Humicola limonisporum*, *Metapochonia variabilis*, *Microascus anfractus*, *Microascus globulosus*, *Microdochium chrysanthemoides*, *Paracremonium variiforme*, *Pectinotrichum chinense*, *Phaeosphaeria fusispora*, *Ramophialophora globispora*, *Ramophialophora petraea*, *Scopulariopsis crassa*, *Simplicillium calcicola*, *Volutella aeria*, and *Wardomycopsis longicatenata*.

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INTRODUCTION

Caves differ from the land surface habitats in their darkness, low to moderate temperatures, high humidity, and scarcity of organic matter (Gabriel & Northup 2013). Environmental conditions of the caves may be affected by a variety of factors such as the movement of water (streams or water seeps), air currents, visitors and chemolithoautotrophy (Hose et al. 2000, Barton & Jurado 2007, Gabriel & Northup 2013, Ortiz et al. 2014). All these factors may contribute to the microbial flora in caves (Ogórek et al. 2013). Fungi are an important part of cave microbiota, because they play an important role in the feeding strategies of cave fauna (Nováková 2009).

The earliest study of fungi in caves was published by Humboldt in 1794 as described in Dobat (1967), but unfortunately these data currently provide very little information (Vanderwolf et al. 2013). Lagarde (1913) studied several caves in Europe and described a new species, *Ombrophila speluncarum*, which has been believed to be a true troglobitic species. Studies on cave fungi during 1950s–1980s were mostly about animal pathogens, e.g., *Histoplasma capsulatum* (Ajello et al. 1960a, b, Al-Doory & Rhoades 1968, Di Salvo et al. 1969, Zamora 1977), *Trichophyton mentagrophytes* and other dermatophytes (Lurie & Borok 1955, Lurie & Way 1957, Kajihiro 1965).

Recent studies demonstrated that caves encompass a high diversity of fungi. In the research on Lechuguilla Cave, New Mexico, species from nine genera were isolated and *Aspergillus* and *Penicillium* were found to be the most common genera (Cunningham et al. 1995). Nagai et al. (1998) investigated the alkalophilic and alkali-tolerant fungi in two limestone caves in Japan, and obtained 52 species. Koilraj et al. (1999) investigated six different caves in India and obtained 35 sporulating fungi belonging to 18 genera and seven sterile fungi. Nováková (2009) isolated 195 species belonging to 73 genera from caves in Slovakia, including 92 species from bat droppings and guano. In total, more than 1 000 species of fungi in 528 genera have been documented from caves and mines worldwide by 2012 (Vanderwolf et al. 2013).

Fungal diversity in Karst caves has rarely been documented. Yunnan-Guizhou Plateau, located in Southwest China, is the largest and most complex developing Karst topography in the world (Zhou et al. 2007). During the past two years, fungal communities from two caves were investigated based on a culture dependent method. Samples of air, water, rock, soil, and organic litter were collected and used for isolation of fungi. Strains were identified based on morphological characters and phylogenetic affinities. Novel species are described, illustrated, and compared with similar species.

MATERIAL AND METHODS

Sampling sites

Suiyang county is located in Guizhou province, China, with a typical subtropical monsoon climate. The annual mean temperature is 13.5 °C, and the annual rainfall is 1116–1350 mm (Jiang et al. 2012).

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Fig. 1 Visited caves. a. Entrance to Cave 1; b. stalactite; c. pool at the end of Cave 1; d. sampled rocks; e. soil sediment; f. pellucid tadpole; g. bat guano colonised by fungal mycelia; h–i. faeces from unknown animals.

Two unnamed Karst caves in Suiyang, herein named as Cave 1 (N28°12'629" E107°13'639") and Cave 2 (N28°12'599" E107°13'661"), are located at the edge of Kuankuoshui National Natural Reserve. Both caves are zonal and horizontal and have one entrance hiding in the forest on a hillside (Fig. 1). The two caves are 500 m apart from each other, thus might belong to the same cave system with a subterranean river connection (Fig. 1). Two bat roosts were found in Cave 1, the first one was about 50 m deep and the second one was at the end of the cave (about 390 m deep). Only one bat roost was found in Cave 2 (about 50 m deep). Other animals were also found in both caves, such as pellucid tadpoles, small pellucid snails, and spiders (Fig. 1).

The elevation of Cave 1 is 908 m, the length is 400 m, the humidity is 75–80 %, and the temperature is 21–22 °C. The elevation of Cave 2 is 930 m, the length is 750 m, the humidity is 75–85 %, and the temperature is 20–23 °C.

Sample collection

Samples of air, rock, soil, and water were collected along the two caves and preserved at 4 °C before transfer to the laboratory. From the entrance of the caves, each sampling site was c. 100 m distant from the next.

Air samples were collected using the Koch sedimentation method (Borda et al. 2004, Kuzmina et al. 2012). Three Petri dishes that contained 2 % potato-dextrose agar (PDA, Difco) were exposed to the atmosphere in the cave for 15 min at each sampling site, then sealed with Parafilm and placed in zip-locked plastic bags. Seeping, stream, and pool water was collected for 10 mL per sample, respectively, and kept in 15 mL sterile centrifuge tubes. Ten grams of soil were collected at shallow depth (1–5.0 cm) after removing the surface layer (c. 1 cm) from three sites of each location. Rock samples were collected and packed in zip-locked plastic bags according to Ruibal et al. (2005). At each sample site, five pieces of rock in different orientations were collected. Rocks that were apparently being colonised by fungi were also chipped off and collected along the caves. Organic litter, when discovered, were collected, as well as bat droppings, guano, animal dung, carcasses, and plant debris.

Isolation

Fungi were isolated following a modified dilution plate method (Zhang et al. 2015b). One gram of each soil and organic litter sample (1 mL for each water sample) was suspended in 9 mL sterile water in a 15 mL sterile centrifuge tube. The tubes were shaken with Vortex vibration meter thoroughly. The suspension was then diluted to a series of concentrations, i.e., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. Diluted concentration of 10^{-3} and 10^{-4} appeared to be most convenient for colony pickup in the isolating process from organic litters, while that for water samples and soil samples were 10^{-1} and 10^{-2}, respectively. Two hundred microliters suspensions from each concentration were spread onto PDA containing ampicillin (50 μg/mL) and streptomycin (50 μg/mL) with three replicates.

Rock samples were treated following the protocol of Ruibal et al. (2005) with some modifications. Firstly, the rock surface was washed with 95 % ethanol to eliminate the contamination from dust and airborne spores, and washed once with sterile water containing 0.1 % of Tween 20. The small pieces of rocks were then ground into powder using a mortar and pestle. Suspensions were made by adding sterilised water to the concentration of 10^{-1}. Three different volumes of the rock powder suspension, i.e., 300, 500, and 1000 μL, were respectively placed onto three PDA plates supplemented with ampicillin (50 μg/mL) and streptomycin (50 μg/mL) (Ruibal et al. 2005, Selbmann et al. 2005, Collado et al. 2007).

All the plates were incubated at room temperature (23–25 °C) for 3–4 wk, and from which the single colonies were picked up and inoculated onto new PDA plates every 2 d. All fungal strains were stored at 4 °C for further studies.

Molecular analyses

Total genomic DNAs were extracted following a modified protocol of Doyle (1987). The large subunit (LSU) rDNA, the internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS), the translation elongation factor 1-alpha (*EF-1α*), β-tubulin (*TUB*), and RNA polymerase II second largest subunit (RPB2) regions were amplified using primer pairs LR0R/LR5 (Vilgalys & Hester 1990), ITS1/ITS4 (White et al. 1990), 983F/2218R (Rehner & Buckley 2005), Bt2a/Bt2b (Glass & Donaldson 1995), and RPB2-5F2/fRPB2-7cR (Liu et al. 1999, Sung et al. 2007), respectively. Amplification reactions were performed in a 25 μL reaction volume including 2.5 μL 10× PCR Buffer (Dingguo, Beijing, China), 2 mM $MgCl_2$, 50 μM dNTPs, 0.1 μM of each forward and reverse primer, 0.5 U Taq DNA polymerase and 1–10 ng genomic DNA in amplifier (Dongsheng, EDC-810, China). PCR parameters were as follows: 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at a suitable temperature for 30 s, extension at 72 °C for 30 s and a final elongation step at 72 °C for 10 min. Annealing temperature for each gene were 54 °C for ITS, 51 °C for LSU, and 57 °C for *EF-1α* and *TUB*. Sequencing reactions were performed with the same primer pairs used for amplification by OmegaGenetcis Company Limited, Beijing, China.

All obtained strains were BLASTn searched in NCBI and assigned to potential genera and species. The strains whose ITS sequences had closest similarities below 97 % were recognised as potential new species and further identified through morphological characterisation and multi-locus phylogenetic analyses.

To reveal the order placements of new species described in this paper, an LSU tree was constructed. To reveal the phylogenetic relationships and taxonomic distinctions of novel species, analyses were performed based on ITS, LSU, and genetic markers recommended in recent publications, such as *TUB* and *EF1-α*. All the sequences were aligned using MAFFT (http://www.ebi.ac.uk/Tools/msa/mafft/) (Katoh & Toh 2010) and edited manually using MEGA v. 6 (Tamura et al. 2013). Individual alignments were then concatenated and used to construct the phylogenetic tree. Ambiguously aligned regions were excluded from the analysis.

Bayesian inference (BI) and Maximum Likelihood (ML) methods were used to construct the phylogenetic trees. For Bayesian analysis, the best fit model of evolution was estimated by jModelTest v. 2.1.7 (Guindon & Gascuel 2003, Darriba et al. 2012). Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.1 (Huelsenbeck & Ronquist 2001), using the estimated model of evolution. Six simultaneous Markov chains were run for 1 000 000 generations and trees were sampled every 100th generation (resulting 10 000 total trees). The first 2 000 trees, which represented the burn-in phase of the analyses, were discarded and the remaining 8 000 trees were used to calculate posterior probabilities (PP) in the majority rule consensus tree. The ML analyses were implemented using RAxML v. 7.0.3 (Stamatakis 2006) with 1 000 replicates under the GTR-GAMMA model. The robustness of branches was assessed by bootstrap analysis with 1 000 replicates. Trees were visualized in TreeView (Page 1996). All the sequences generated were deposited in GenBank (Table 1), the multi-locus alignment and tree in TreeBASE (submission no: 19811), and typifications in MycoBank (Crous et al. 2004).

Table 1 Strain numbers and sequence accession numbers of new species.

Species name	Strain number[1]	Sequence accession number				
		ITS	LSU	*TUB*	*EF1-α*	*RPB2*
Amphichorda guana	CGMCC3.17908 T	KU746665	KU746711	KU746757	KX855211	–
	CGMCC3.17909	KU746666	KU746712	KU746758	KX855212	–
Auxarthronopsis guizhouensis	CGMCC3.17910 T	KU746668	KU746714	KU746759	KX855213	–
	CGMCC3.17911	KU746667	KU746713	KU746760	KX855214	–
Biscogniauxia petrensis	CGMCC3.17912 T	KU746669	KU746715	KU746761	KX855215	–
	CGMCC3.17913	KU746670	KU746716	KU746762	KX855216	–
	CGMCC3.17949	KU746671	KU746717	KU746763	KX855217	–
Cladorrhinum globisporum	CGMCC3.17921 T	KU746680	KU746726	KU746771	KX855226	–
	CGMCC3.17922	KU746679	KU746725	KU746772	KX855225	–
Collariella quadrum	CGMCC3.17917 T	KU746675	KU746721	KU746767	KX855221	KY575870
	CGMCC3.17918	KU746676	KU746722	KU746768	KX855222	KY575871
	CGMCC3.17919	KU746677	KU746723	KU746769	KX855223	KY575872
	CGMCC3.17920	KU746678	KU746724	KU746770	KX855224	KY575873
Gymnoascus exasperatus	CGMCC3.17923 T	KU746682	KU746728	KU746773	KX855227	–
	CGMCC3.17924	KU746681	KU746727	KU746774	KX855228	–
Humicola limonisporum	CGMCC3.17914 T	KU746672	KU746718	KU746764	KX855218	KY575867
	CGMCC3.17915	KU746673	KU746719	KU746765	KX855219	KY575868
	CGMCC3.17916	KU746674	KU746720	KU746766	KX855220	KY575869
Metapochonia variabilis	CGMCC3.17925 T	KU746684	KU746730	KU746775	KX855229	–
	CGMCC3.17926	KU746683	KU746729	KU746776	KX855230	–
Microascus anfractus	CGMCC3.17950 T	KU746686	KU746732	KU746777	KX855231	–
	CGMCC3.17951	KU746685	KU746731	KU746778	KX855232	–
Microascus globulosus	CGMCC3.17927 T	KU746688	KU746734	KU746779	KX855233	–
	CGMCC3.17928	KU746687	KU746733	KU746780	KX855234	–
Microdochium chrysanthemoides	CGMCC3.17929 T	KU746690	KU746736	KU746781	KX855235	–
	CGMCC3.17930	KU746689	KU746735	KU746782	KX855236	–
Paracremonium variiforme	CGMCC3.17931 T	KU746691	KU746737	KU746783	KX855237	–
	CGMCC3.17932	KU746692	KU746738	KU746784	KX855238	–
	CGMCC3.17933	KU746693	KU746739	KU746785	KX855239	–
	CGMCC3.17934	KU746694	KU746740	KU746786	KX855240	–
Pectinotrichum chinense	CGMCC3.17935 T	KU746695	KU746741	KU746787	KX855241	–
	CGMCC3.17936	KU746696	KU746742	KU746788	KX855242	–
Phaeosphaeria fusispora	CGMCC3.17937 T	KU746698	KU746744	KU746789	KX855243	–
	CGMCC3.17938	KU746697	KU746743	KU746790	KX855244	–
Ramophialophora globispora	CGMCC3.17939 T	KU746700	KU746746	KU746791	KX855245	–
	CGMCC3.17940	KU746699	KU746745	KU746792	KX855246	–
Ramophialophora petraea	CGMCC3.17952 T	KU746702	KU746748	KU746793	KX855247	–
	CGMCC3.17953	KU746701	KU746747	KU746794	KX855248	–
Scopulariopsis crassa	CGMCC3.17941 T	KU746704	KU746750	KU746795	KX855249	–
	CGMCC3.17942	KU746703	KU746749	KU746796	KX855250	–
Simplicillium calcicola	CGMCC3.17943 T	KU746706	KU746752	KU746797	KX855252	–
	CGMCC3.17944	KU746705	KU746751	KU746798	KX855251	–
Volutella aeria	CGMCC3.17945 T	KU746708	KU746754	KU746799	KX855253	–
	CGMCC3.17946	KU746707	KU746753	KU746800	KX855254	–
Wardomycopsis longicatenata	CGMCC3.17947 T	KU746710	KU746756	KU746801	KX855255	–
	CGMCC3.17948	KU746709	KU746755	KU746802	KX855256	–

[1] Ex-type strains are indicated with T.

Morphological studies

Strains of potentially new species were transferred to new plates of PDA and synthetic nutrient-poor agar (SNA; Nirenberg 1976) and were incubated at room temperature (23–25 °C). Colony characters and pigment production on PDA and SNA were examined after 10 d. Growth rates were measured after 7 d, while slow growing strains were measured after 10 d or even 8 wk. Cultures were examined periodically for the development of reproductive structures. Photomicrographs were taken using a Nikon 80i microscope with differential interference contrast. Measurements for each structure were made according to methods described by Liu et al. (2012). The dry cultures were deposited in the Herbarium of Microbiology, Academia Sinica (HMAS), while living cultures were deposited in the China General Microbiological Culture Collection Center (CGMCC) and LC Culture Collection (personal culture collection held in the laboratory of Dr Lei Cai).

RESULTS

In this study, 85 samples from Cave 1, and 115 samples from Cave 2 were collected and 563 fungal strains were isolated. These strains belong to 116 genera, and 246 species by employing a BLASTn search in GenBank using the ITS sequences. Among these species, 85.8 % (i.e., 211 species, 489 strains) belong to 98 genera of *Ascomycota*; 7.3 % (i.e., 18 species, 29 strains) belong to 13 genera of *Basidiomycota*; 6.9 % (i.e., 17 species, 45 strains) belong to four genera of *Mucoromycotina* (Table 2). The most common genera included: *Aspergillus* (7.7 %), *Penicillium* (7.7 %), *Chaetomium* (3.3 %), *Mortierella* (3.7 %), *Trichoderma* (3.7 %), *Phoma* (2.8 %), *Mucor* (2.4 %), *Arthrinium* (2.4 %), *Xylaria* (2.0 %), and *Fusarium* (2.0 %) (Table 3). The most common species include *Cephalotrichum verrucisporum* (3.9 %), *Aspergillus* (*As.*) *thesauricus* (3.4 %), *As. versicolor* (3.2 %), *Mortierella alpina* (2.7 %), *Eutypella scoparia* (2.5 %), *Chaetomium* (*Ch.*) *trigonosporum* (2.3 %), *Ch. nigricolor* (1.6 %), *Clonostachys rosea* (1.6 %), *Mortierella* sp. 3 (1.3 %), *Alternaria tenuissima* (1.2 %), *Amphichorda felina* (1.2 %), *Bionectria ochroleuca* (1.2 %), *As. candidus* (1.1 %), *Isaria fumosorosea* (1.1 %), *Lecanicillium fusisporum* (1.1 %), and *Trichocladium asperum* (1.1 %). Among the obtained strains, 220 strains were isolated from Cave 1, belonging to 133 species in 75 genera, and 343 strains were isolated from Cave 2,

(text continues on p. 8)

Table 2 An overview of fungal species isolated from the cave samples.

Fungal species[1]	Cave 1					Cave 2				
	Air	Water	Soil	Rock	Organic litter	Air	Water	Soil	Rock	Organic litter
Ascomycota										
Acremonium nepalense	–	–	1	–	–	–	–	–	–	–
A. persicinum	–	–	–	–	–	–	–	–	–	1
A. sp. 1	–	–	–	–	2	–	–	–	–	1
A. sp. 2	–	–	–	–	–	1	–	–	–	–
Acrodontium crateriforme	1	–	–	–	–	–	–	–	–	–
Acrostalagmus luteoalbus	–	–	1	–	–	–	–	–	–	–
Alternaria alternata	–	–	–	–	–	–	1	–	1	–
Al. tamaricis	–	–	–	–	–	–	–	–	1	–
Al. tenuissima	–	3	–	–	1	–	–	1	–	2
Amphichorda felina	–	–	–	–	–	–	–	–	–	7
Am. guana	–	–	–	–	–	–	–	–	–	2
Arthrinium arundinis	–	1	1	–	–	–	–	–	–	–
Ar. malaysianum	–	–	–	–	–	–	–	–	–	1
Ar. marii	–	–	–	–	–	1	–	–	–	–
Ar. phaeospermum	–	–	–	–	–	1	–	1	–	–
Ar. sacchari	1	–	–	–	–	–	–	–	–	–
Ar. sp.	1	–	1	–	–	1	–	–	–	–
Arthroderma curreyi	–	–	–	–	–	–	–	1	–	–
Art. quadrifidum	–	–	–	–	–	–	–	–	–	2
Arthropsis hispanica	–	–	–	–	1	–	–	–	–	–
Aspergillus candidus	–	–	1	–	–	1	–	–	–	4
As. cavernicola	–	*1*	2	–	–	–	–	1	–	–
As. creber	–	–	–	–	–	–	–	–	–	1
As. flavus	–	–	–	–	–	1	1	–	–	–
As. fumigatus	–	–	–	–	–	4	–	–	–	–
As. niger	–	–	–	–	1	–	–	–	–	1
As. niveoglaucus	–	–	–	–	–	–	–	–	–	1
As. pragensis	–	–	–	1	–	–	–	–	–	–
As. reptans	–	–	–	–	–	–	–	–	–	1
As. ruber	1	–	–	–	–	–	–	–	–	1
As. sp.	–	–	1	–	–	–	–	–	–	1
As. spelunceus	–	–	1	–	–	1	–	–	–	–
As. tennesseensis	–	–	–	–	–	–	–	–	–	1
As. thesauricus	–	–	2	6	6	1	–	2	2	–
As. tubingensis	–	–	–	–	–	–	–	–	1	–
As. ustus	–	–	–	–	–	–	–	1	–	–
As. versicolor	–	–	–	–	1	1	–	2	–	14
As. wentii	–	–	–	–	–	–	–	–	–	1
Auxarthron thaxteri	–	–	1	–	–	–	–	–	–	–
Auxarthronopsis guizhouensis	2	–	–	–	–	–	–	–	–	–
Bionectria ochroleuca	–	–	–	1	–	–	–	–	1	5
Biscogniauxia petrensis	–	–	3	–	–	–	–	–	–	–
B. sp.	–	–	1	–	–	–	–	–	–	–
Calcarisporium sp.	–	–	–	–	–	–	–	–	–	3
Capnodium sp.	–	–	–	1	–	–	–	–	–	–
Cephalotrichum verrucisporum	2	–	–	–	4	5	–	1	1	9
Ceratobasidium sp.	–	–	–	–	1	–	–	–	–	–
Chaetomidium arxii	–	–	–	–	–	–	–	–	–	1
Chaetomium bostrychodes	–	–	1	–	–	–	–	–	–	3
C. crispatum	–	–	–	–	–	–	–	–	1	–
C. globosum	–	–	–	1	–	–	–	–	–	–
C. murorum	–	–	–	–	–	–	–	1	–	–
C. nigricolor	1	1	3	2	–	–	–	1	–	1
C. sp.	–	–	–	–	–	–	–	–	–	1
C. trigonosporum	–	–	3	–	–	1	–	1	1	7
C. udagawae	–	–	1	–	–	–	–	–	–	–
Chalara holubovae	–	–	–	–	–	1	–	–	–	–
Chloridium sp.	–	–	1	–	–	–	–	3	–	–
Chrysosporium pseudomerdarium	–	–	–	–	1	–	–	–	–	–
Ch. sp. 1	–	–	–	–	–	–	–	–	–	2
Ch. sp. 2	–	–	–	–	–	–	–	–	–	1
Cladorrhinum globisporum	–	2	–	–	–	–	–	–	–	–
Cl. sp.	–	1	–	–	–	–	–	–	–	–
Cladosporium cladosporioides	1	1	–	–	–	2	–	–	–	–
Clad. sphaerospermum	–	–	1	1	–	2	–	–	–	–
Clonostachys rosea	1	–	–	–	1	–	2	1	1	3
Clon. sp.	–	–	–	–	–	–	–	1	–	–
Collariella quadrum	–	–	–	–	2	–	–	1	–	1
Colletotrichum gloeosporioides	1	–	1	–	–	–	–	–	–	–
Coll. karstii	–	–	–	–	–	–	–	–	–	1
Coll. sp.	2	–	–	–	–	–	–	–	–	–
Corynespora sp.	–	–	–	–	1	–	–	–	–	–
Cylindrocarpon olidun	–	–	1	–	–	–	–	1	–	2
Cyl. sp.	–	–	–	–	–	–	–	–	–	1
Diaporthe phoenicicola	–	–	–	–	–	1	–	1	–	–
Doratomyces columnaris	–	–	–	–	–	–	–	–	–	1

Table 2 (cont.)

Fungal species[1]	Cave 1					Cave 2				
	Air	Water	Soil	Rock	Organic litter	Air	Water	Soil	Rock	Organic litter
D. nanus	–	–	–	–	–	1	–	–	–	1
D. sp.	–	1	–	–	–	1	–	–	–	–
Epicoccum nigrum	–	–	–	–	–	–	–	–	–	1
Eutypella scoparia	–	–	1	3	2	–	2	5	1	–
Fusarium graminearum	1	–	–	–	–	–	–	–	–	–
F. merismoides	–	–	–	–	–	1	–	–	–	–
F. solani	–	–	–	–	–	–	–	2	–	1
F. sp.	–	–	1	–	–	1	–	–	–	–
F. verticillioides	–	–	–	–	–	–	–	–	1	–
Geotrichum candidum	–	–	–	–	–	–	–	–	–	2
Gibberella moniliformis	–	–	–	–	–	–	–	–	1	–
G. pulicaris	–	–	–	–	–	–	–	–	–	1
G. zeae	2	–	–	–	–	–	–	–	–	–
Gliomastix murorum	–	–	1	–	–	–	–	–	–	1
Gymnoascus exasperatus	–	–	–	–	–	–	–	–	–	2
Gymn. reesii	–	–	2	–	–	–	–	–	–	–
Gyrothrix sp.	–	–	–	–	–	–	–	2	–	–
Humicola limonisporum	–	–	1	–	–	1	–	–	1	–
Hypocrea citrina	–	–	1	–	–	–	–	–	–	–
Hypoxylon perforatum	–	–	–	–	2	–	–	–	–	–
Ilyonectria robusta	–	–	–	–	–	–	–	1	–	–
I. sp.	–	–	1	–	–	–	–	–	–	–
Isaria farinosa	1	–	–	–	–	–	–	–	–	–
Is. fumosorosea	1	–	–	–	–	1	–	2	–	2
Is. tenuipes	–	–	1	–	–	–	–	–	–	–
Kernia sp.	–	–	–	–	–	–	–	–	–	5
Lecanicillium fusisporum	–	–	–	–	–	–	–	–	–	6
Leptosphaeria sp.	1	–	1	–	–	1	–	1	–	–
Lophiostoma corticola	1	–	–	–	–	–	–	–	–	–
L. sp. 1	–	–	1	–	–	–	–	–	–	–
L. sp. 2	–	–	–	–	–	–	–	1	–	–
Massarina sp.	–	–	–	–	1	–	–	–	–	–
Metapochonia bulbilosa	–	–	1	–	–	–	–	–	–	–
M. rubescens	–	–	–	–	1	–	–	–	–	–
M. variabilis	–	–	2	–	–	–	–	–	–	–
Metarhizium anisopliae	–	–	–	–	–	–	–	3	1	1
Met. guizhouense	–	–	–	–	–	–	–	1	–	–
Microascus anfractus	–	–	–	–	–	–	–	–	–	2
Mic. chartarus	–	–	–	–	–	–	–	1	–	–
Mic. globulosus	–	–	–	–	–	–	–	–	–	2
Microdochium chrysanthemoides	–	–	–	–	–	2	–	–	–	–
Microsphaeropsis arundinis	–	–	–	–	–	–	1	–	–	–
Myriodontium keratinophilum	–	–	1	–	1	1	–	–	–	2
Myrothecium sp.	–	–	–	–	–	–	–	–	–	1
Nectria haematococca	–	–	1	–	–	–	–	1	1	–
Nemania bipapillata	–	–	–	1	–	–	–	–	–	–
N. diffusa	–	–	1	1	–	–	–	–	–	–
Neonectria discophora	–	–	–	–	–	–	–	1	–	–
Neurospora intermedia	–	–	–	–	–	–	–	–	1	–
Paecilomyces fumosoroseus	1	–	–	–	–	–	1	2	–	1
P. lilacinus	–	–	–	–	–	1	1	1	1	–
P. sp.	–	–	–	1	–	–	–	–	–	–
Paracremonium variiforme	–	–	–	–	–	–	3	1	–	–
Paraphoma radicina	–	–	–	–	–	–	–	–	–	1
Pectinotrichum chinense	–	–	1	–	–	–	–	1	–	–
Penicillium buchwaldii	–	–	1	–	–	–	–	–	–	–
Pen. camemberti	–	–	–	1	–	–	–	1	–	–
Pen. chrysogenum	–	–	–	–	–	–	–	2	–	–
Pen. coprophilum	–	–	–	–	–	–	1	–	–	–
Pen. expansum	–	–	–	1	–	1	–	–	–	–
Pen. fellutanum	–	–	–	–	–	–	–	1	–	–
Pen. glabrum	–	–	–	–	–	–	–	1	–	–
Pen. herquei	–	1	–	–	–	–	–	–	–	–
Pen. inflatum	–	–	–	–	1	–	–	–	–	–
Pen. janthinellum	–	–	1	–	1	–	–	2	–	–
Pen. lividum	–	1	–	–	–	–	–	–	–	–
Pen. malachiteum	–	–	–	–	–	–	–	1	–	–
Pen. minioluteum	–	–	–	–	–	–	–	2	–	–
Pen. pancosmium	–	–	–	–	–	1	–	–	–	1
Pen. parvulum	–	–	–	–	–	–	–	–	–	1
Pen. pinophilum	–	–	–	–	–	–	–	1	–	–
Pen. sp.	–	–	–	–	–	–	–	–	–	1
Pen. thomii	–	–	–	–	1	–	–	–	–	–
Pen. urticae	–	1	–	–	–	–	–	–	–	–
Pestalotiopsis guepinii	–	–	1	–	–	1	–	–	–	–
Pest. microspora	–	1	–	–	–	–	–	–	–	–
Phaeoacremonium iranianum	–	–	–	–	–	–	1	–	–	–

Table 2 (cont.)

Fungal species[1]	Cave 1					Cave 2				
	Air	Water	Soil	Rock	Organic litter	Air	Water	Soil	Rock	Organic litter
Phaeosphaeria fusispora	–	–	–	–	–	2	–	–	–	–
Phialemonium sp.	–	–	–	–	–	1	–	–	–	–
Phoma herbarum	–	–	1	–	–	–	–	–	–	–
Ph. insulana	–	–	–	–	–	1	–	–	–	–
Ph. macrostoma	–	–	–	–	–	–	1	–	–	–
Ph. radicina	–	–	–	–	1	–	–	–	–	–
Ph. senecionis	–	–	–	–	–	–	1	–	–	–
Ph. sp. 1	–	–	–	–	–	1	–	–	–	–
Ph. sp. 2	–	–	2	–	–	–	–	–	–	1
Phomopsis sp.	–	–	–	–	–	–	–	–	–	1
Plectosphaerella cucumerina	–	–	–	–	–	–	–	1	–	1
Pl. sp.	–	–	–	–	–	1	–	–	–	–
Pleosporales sp.	–	–	2	–	–.	–	–	–	–	–
Preussia aemulans	–	–	1	–	–	–	–	–	–	–
Protocrea farinosa	–	–	1	–	–	–	–	–	–	–
Pseudallescheria fimeti	–	–	–	–	–	1	–	–	–	1
Purpureocillium lilacinum	–	–	–	1	–	1	–	–	–	–
Ramophialophora globispora	–	–	–	–	–	–	–	–	–	2
R. petraea	–	–	–	–	–	–	–	–	2	–
Scopulariopsis crassa	–	–	–	–	–	–	–	–	–	2
S. sp.	–	–	–	–	–	–	–	–	–	1
Scutellinia sp.	–	–	–	–	–	–	–	–	–	1
Simplicillium calcicola	–	–	–	–	–	–	–	–	2	–
Stachybotrys chartarum	–	–	1	–	–	–	–	–	–	–
St. longispora	1	–	–	–	–	–	–	–	–	–
Staphylotrichum boninense	–	–	–	–	–	–	–	–	1	–
Staph. coccosporum	–	–	–	1	–	–	–	–	–	–
Staph. sp.	–	1	–	–	–	–	–	–	–	–
Stephanonectria keithii	–	–	–	–	1	–	–	–	–	3
Talaromyces sp.	–	–	–	–	–	–	–	2	–	–
Thielavia sp.	–	–	–	–	–	1	–	–	1	3
Togninia argentinensis	–	–	–	–	–	1	–	–	–	–
T. viticola	–	–	–	–	–	–	1	–	–	–
Tolypocladium cylindrosporum	–	–	–	–	–	–	2	–	–	–
Torula caligans	–	–	–	–	–	–	3	–	–	–
Tor. herbarum	2	–	–	1	1	–	–	–	1	–
Trichocladium asperum	–	2	–	2	–	–	–	1	1	–
Tr. sp.	–	1	–	–	–	–	–	–	–	–
Trichoderma atroviride	–	–	–	–	–	–	1	–	–	–
Trich. citrinoviride	–	–	–	1	–	–	–	–	–	–
Trich. hamatum	–	–	1	–	1	–	–	2	–	–
Trich. koningiopsis	–	–	–	–	–	–	–	1	–	–
Trich. lixii	–	–	–	–	–	–	–	1	–	–
Trich. longibrachiatum	–	–	–	–	–	–	–	1	–	–
Trich. rossicum	–	–	–	–	–	–	–	–	–	1
Trich. sp. 1	–	–	–	–	1	–	–	–	–	–
Trich. sp. 2	1	–	–	1	–	–	–	–	–	1
Trichosporon akiyoshidainum	–	–	–	–	1	–	–	–	–	–
Trichos. laibachii	–	–	–	–	1	–	–	–	1	–
Trichos. rubrum	–	–	–	1	–	–	–	–	–	–
Verticillium sp.	–	–	–	–	–	1	–	–	–	–
Virgaria nigra	–	–	–	–	1	–	–	–	–	–
Volutella aeria	–	–	–	–	–	2	–	–	–	–
Wardomycopsis longicatenata	–	–	–	–	–	2	–	–	–	–
Xylaria arbuscula	–	–	–	2	–	–	–	–	–	–
X. schweinitzii	–	–	–	–	–	–	–	–	–	1
X. sp. 1	–	–	–	1	–	–	–	–	–	–
X. sp. 2	–	–	–	2	–	1	1	–	–	1
Basidiomycota										
Clitopilus kamaka	–	–	–	2	–	–	–	–	–	–
Coprinellus radians	–	–	1	–	–	–	–	–	–	–
Datronia mollis	–	–	–	–	–	–	–	–	–	2
Ganoderma gibbosum	–	–	–	–	1	–	–	–	–	–
Hyphodermella rosae	–	–	–	–	–	1	–	–	–	–
H. sp.	–	–	–	–	–	–	–	–	–	1
Peniophora cinerea	–	–	–	–	–	–	1	–	–	–
Penioph. limitata	–	–	–	–	–	–	1	–	1	–
Penioph. sp.	–	1	1	1	–	–	–	–	–	–
Periconia sp.	1	–	–	–	–	–	–	–	–	–
Phanerochaete sordida	1	1	–	–	–	–	–	–	–	–
Psathyrella candolleana	–	–	1	–	–	1	–	–	–	–
Ps. cf. *gracilis*	–	–	–	–	1	–	–	–	–	–
Rigidoporus sp.	1	1	–	1	–	–	–	–	–	–
Rig. vinctus	1	1	–	–	–	–	–	–	–	–
Schizophyllum commune	1	–	–	–	–	–	–	–	–	–
Tinctoporellus epimiltinus	–	1	–	–	–	–	–	–	–	–
Trametes versicolor	1	–	–	–	–	1	–	–	–	–

Table 2 (cont.)

Fungal species[1]	Cave 1					Cave 2				
	Air	Water	Soil	Rock	Organic litter	Air	Water	Soil	Rock	Organic litter
Mucoromycotina										
Mortierella alpina	–	–	3	2	1	–	–	4	1	4
Mort. horticola	–	–	1	–	–	–	–	–	–	–
Mort. hyalina	–	–	–	–	1	–	–	–	–	–
Mort. indohii	–	–	–	–	2	–	–	–	–	–
Mort. minutissima	–	–	–	2	–	–	–	–	–	–
Mort. sp. 1	–	–	–	–	–	1	–	–	–	–
Mort. sp. 2	–	–	1	–	–	–	–	–	–	–
Mort. sp. 3	1	1	2	–	–	3	–	3	–	–
Mucor flavus	–	–	–	–	1	–	–	–	–	–
Muc. hiemalis	–	–	–	–	–	–	–	–	–	1
Muc. moelleri	–	–	–	–	–	–	–	–	–	1
Muc. racemosus	–	–	–	–	1	–	–	–	–	1
Muc. sp. 1	–	–	–	–	–	–	–	–	–	1
Muc. sp. 2	–	–	–	–	–	–	–	–	–	1
Muc. sp. 3	–	–	–	1	–	–	–	–	–	–
Rhizomucor variabilis	–	–	1	–	–	–	–	–	–	1
Rhizopus oryzae	1	–	–	–	1	–	–	–	–	–

[1] Names in **bold** signify new species described in this study.

Table 3 Most common genera (≥ 5 species) obtained from karst caves.

Genus	Species	Strains	Genus	Species	Strains
Aspergillus	19	77	*Mucor*	6	7
Arthrinium	6	10	*Penicillium*	19	27
Chaetomium	8	31	*Phoma*	7	9
Fusarium	5	8	*Trichoderma*	9	14
Mortierella	9	34	*Xylaria*	5	9

belonging to 167 species in 83 genera. Forty-four genera were commonly isolated from both caves, and 30 and 39 genera were only obtained from Cave 1 and Cave 2, respectively. For the substrates of isolation, 200 strains from organic litter belong to 98 species in 61 genera; 143 strains from soil belong to 89 species in 52 genera; 96 strains from air belong to 64 species in 47 genera; 73 strains from rock belong to 54 species in 37 genera; and 51 strains from water belong to 39 species in 30 genera. In our study, 28 of the 116 genera and 111 of the 188 identified species (59.0 %) were reported for the first time from caves (Vanderwolf et al. 2013).

The LSU phylogenetic tree (Fig. 2) showed that our 20 new species (marked with **bold** font) scattered in six different orders, i.e., *Hypocreales*, *Microascales*, *Onygenales*, *Pleosporales*, *Sordariales*, and *Xylariales*. Trees presenting the phylogenetic relationships and taxonomic distinction of each species have been deposited in MycoBank. Significant ML bootstrap values (≥ 70 %) and Bayesian posterior probabilities (≥ 75 %) are shown in the phylogenetic tree.

Taxonomy

Amphichorda guana Z.F. Zhang, F. Liu & L. Cai, *sp. nov.* — MycoBank MB818245; Fig. 3

Etymology. Referring to the material (bat guano) from which this fungus was isolated.

Colonies on PDA attaining 14–18 mm diam after 14 d, dense, slightly convex, fimbriate, white to yellowish, with a white margin. Yellowish exudate usually appeared on old colonies. Reverse white at first, slowly becoming pale yellow. Colonies on SNA 13–21 mm diam after 14 d, margin entire, white, mycelia sparse. Reverse white.

Vegetative *hyphae* hyaline, septate, smooth-walled, 1.5–3.5 µm diam, sometimes swollen, up to 7 µm diam. *Synnemata* arising in the centre part of the colony on PDA, up to 15 mm high and 1–3 mm wide, white, cylindrical, tomentose, occasionally branched at the apex. *Conidiophores* arising laterally from hyphae, straight or slightly curved. *Conidiogenous cells* mostly borne on conidiophores, occasionally in simple whorls on lateral branches from the mycelia, fusiform or ellipsoidal, straight or irregularly bent, 7–10 × 2–3 µm. *Conidia* holoblastic, solitary or clumped, hyaline, smooth, broadly ellipsoid to subglobose, unicellular, 4.5–5.5 × 3.5–5 µm (mean = 5.0 ± 0.3 × 3.9 ± 0.3 µm, n = 20). *Chlamydospores* not observed.

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 1, N28°12'629" E107°13'639", on bat guano, 19 July 2014, *X. Zhou* (HMAS 246919 holotype designated here, ex-type living culture CGMCC 3.17908 = LC5815); ibid., CGMCC 3.17909 = LC5819.

Notes — This species should be classified in the genus *Amphichorda* because of its long white synnemata and flask-shaped conidiogenous cells from which 1-celled, terminal holoblastic conidia are produced (De Hoog 1972). *Amphichorda* was established by Fries (1825) and currently comprises only one species, *A. felina* (syn. *Beauveria felina*) (Seifert et al. 2011). *Amphichorda guana* can be distinguished from *A. felina* by its larger conidia (4.5–5.5 × 3.5–4.5 µm vs 3.5–4.0 × 2.5–3 µm). *Amphichorda* is similar to *Beauveria* in morphology, while *Beauveria* is differentiated by its elongate conidiogenous cells with apical denticulate rachis (Rehner et al. 2011, Chen et al. 2013) and the phylogeny based on ITS sequences showed that species of *Amphichorda* clustered in a distinct clade distant from *Beauveria* (phylogenetic tree deposited in MycoBank: MB 818245).

Auxarthronopsis guizhouensis Z.F. Zhang & L. Cai, *sp. nov.* — MycoBank MB818246; Fig. 4

Etymology. Referring to the province where this fungus was collected.

Colonies on PDA 25–31 mm diam after 21 d, felty to cottony, flat, margin entire or dentate, white to yellow-brown. Reverse pale yellow to brown. Colonies on SNA 17–24 mm diam after 21 d, colourless, mycelia extremely scarce. Reverse colourless. Vegetative *hyphae* hyaline, septate, branched, smooth-walled, 1–3 µm wide, sometimes swollen, up to 8.0 µm wide. Fertile hyphae hyaline, occasionally branched. *Conidia* abundant, most arthric, intercalary or few terminal, hyaline, unicellular, solitary, subglobose, ellipsoidal, cylindrical or pyriform, 3.5–9.5 × 2–4.5 µm (mean = 6.1 ± 1.5 × 3.2 ± 0.5 µm, n = 30), frequently separated by 1–3 autolytic connective cells, smooth- and thick-walled; some aleurioconidial, subglobose or ellipsoidal, sessile or extremely short stalked, 3.5–7 × 2–4.5 µm (mean = 4.9 ± 0.8 × 3.1 ± 0.6 µm, n = 20).

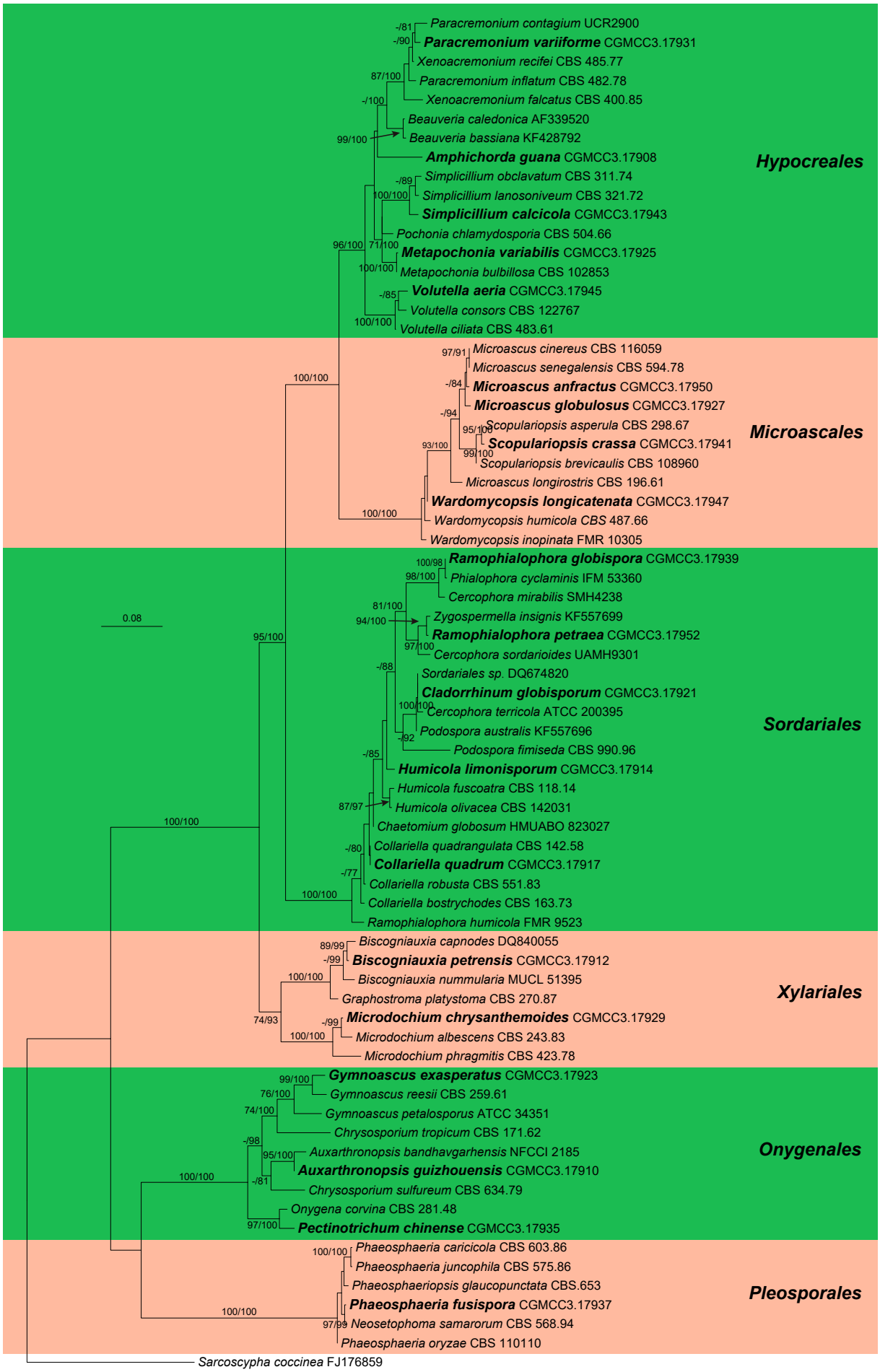

Fig. 2 Maximum likelihood (ML) tree based on LSU sequences showing the order placements of new species described in this study. ML bootstrap values (≥ 70 %) and Bayesian posterior probability (≥ 75 %) are indicated along branches (ML/PP). The tree is rooted with *Sarcoscypha coccinea* (FF176859). Novel species are indicated in **bold** font and the orders are shown on the right side of the figure.

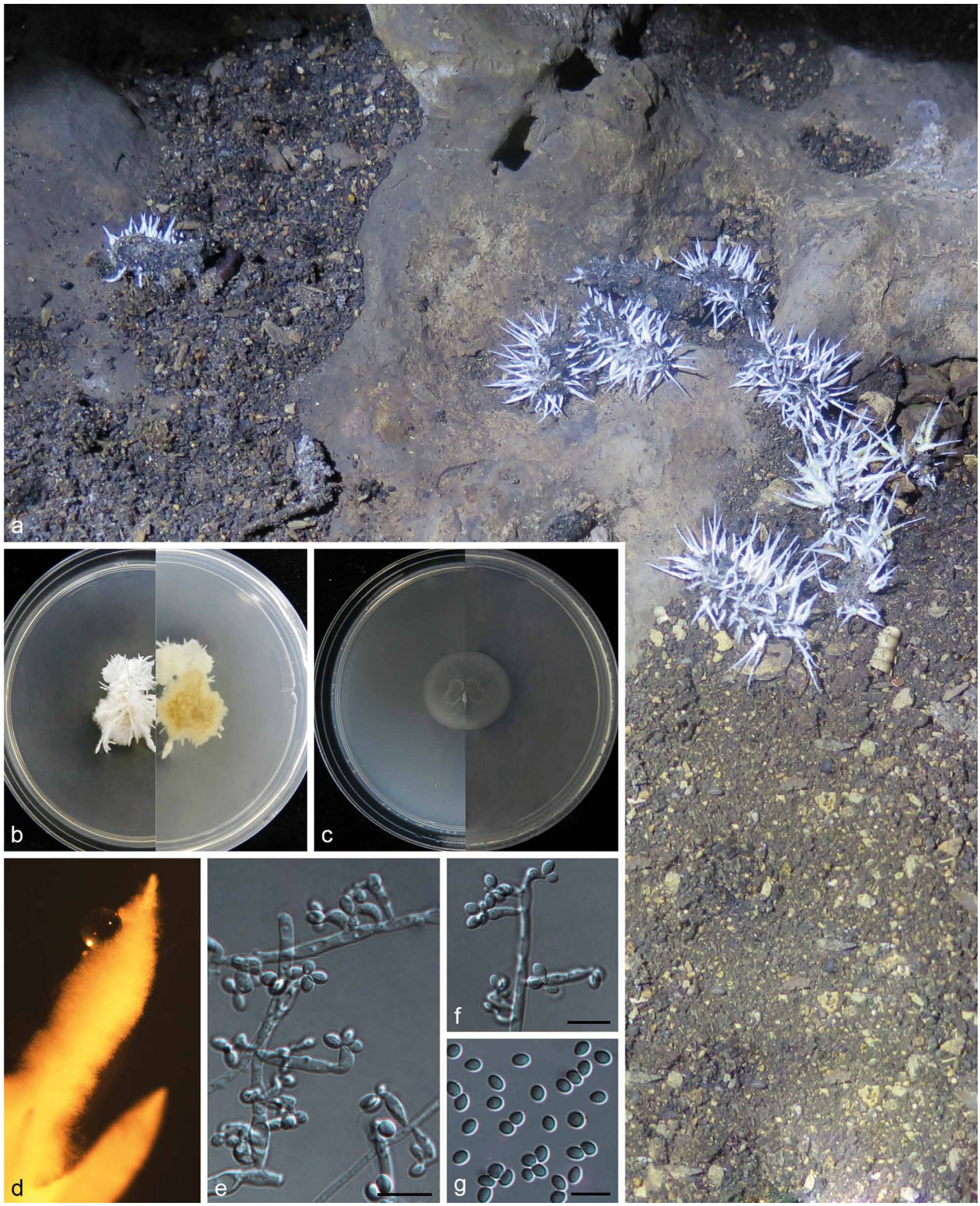

Fig. 3 *Amphichorda guana* (from ex-holotype CGMCC 3.17908). a. *A. guana* on bat guano; b–c. upper and reverse views of cultures on PDA and SNA 10 d after inoculation; d. synnemata; e–f. conidiophores and conidia; g. conidia. — Scale bars: e–g = 10 µm.

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 1, N28°12'629" E107°13'639", from air, 19 July 2014, *Z.F. Zhang* (HMAS 246920 holotype designated here, ex-type living culture CGMCC 3.17910 = LC5705); ibid., CGMCC 3.17911= LC6219.

Notes — The genus *Auxarthronopsis* was established by Sharma et al. (2013) and currently comprises only one species, *A. bandhavgarhensis*. Our isolates are characterised by the intercalary or terminal, hyaline, solitary and aseptate arthric conidia, separated by autolytic connective cells, which is in good agreement with the morphological circumscription of *Auxarthronopsis* (Sharma et al. 2013). Based on the BLASTn research, the closest hit using ITS sequence is the type of *A. bandhavgarhensis*, NFCCI 2185 (HQ164436, identity = 86 %). Although *A. bandhavgarhensis* was not sufficiently described, we roughly measured the conidiogenous cells using f. 3 of Sharma et al. (2013) according to the provided scale bars. We concluded that conidiogenous cells of *A. bandhavgarhensis* were significantly longer (absent or up to 10 µm) than *A. guizhouensis* (absent or shorter than 2 µm). In addition, arthroconidia in *A. guizhouensis* are more abundant than *A. bandhavgarhensis*, while the aleurioconidia are less abundant.

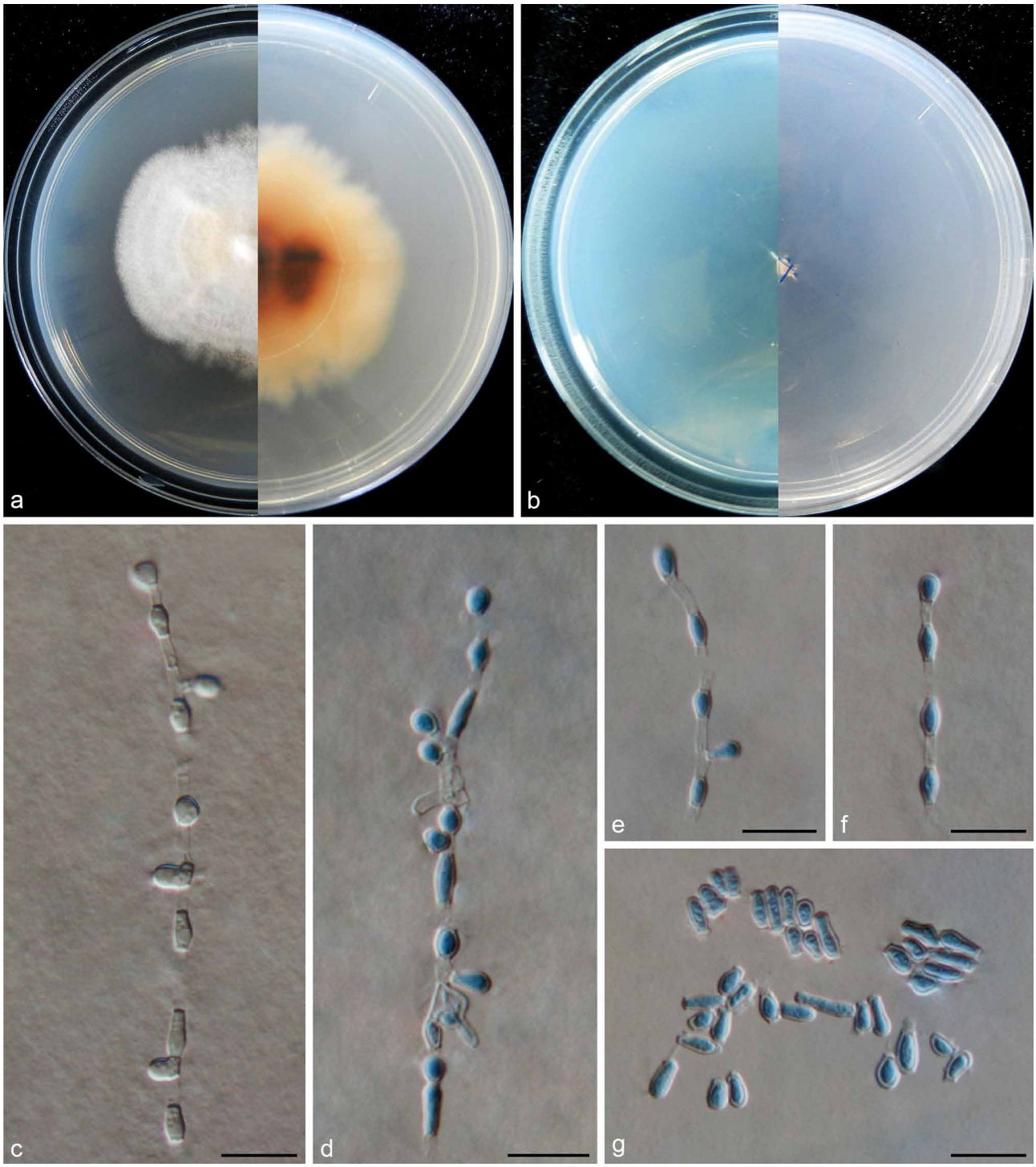

Fig. 4 *Auxarthronopsis guizhouensis* (from ex-holotype CGMCC 3.17910). a–b. Upper and reverse views of cultures on PDA and SNA 21 d after inoculation; c–g. arthroconidia and aleurioconidia (d–g. arthroconidia and aleurioconidia in cotton blue). — Scale bars: c–g = 10 µm.

Biscogniauxia petrensis Z.F. Zhang, F. Liu & L. Cai, *sp. nov.* — MycoBank MB818247; Fig. 5

Etymology. Referring to the material it was isolated from, rock.

Colonies on PDA attaining 80–85 mm diam within 10 d, cottony to woolly, whitish to light pink, aerial mycelia abundant. Red droplets secretions appeared within 2 wk. Reverse yellowish to red-brown. Colonies on SNA attaining 80–85 mm diam within 10 d, fascicular, cottony to woolly, pink-white. Reverse pink-white.

Vegetative *hyphae* hyaline to brown, septate, branched, thin-walled aerial mycelia abundant. *Conidiophores* hyaline to slightly yellowish, rough-walled, composed of main axis, 3–4.5 µm diam, and sometimes one or more major branches, with conidiogenous cells arising terminally or laterally. *Conidiogenous cells* hyaline, swollen at the apex and with conidial secession scars, thin- and rough-walled, cylindrical to oblong, 7–13 × 3–4.5 µm. *Conidia* holoblastic, unicellular, hyaline, smooth, ovoid to clavate, 4.5–7.5 × 2.5–4.5 µm (mean = 5.7 ± 0.6 × 3.3 ± 0.4 µm, n = 35), with obtuse tip and acute truncated base.

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 1, N28°12'629" E107°13'639", rock, 19 July 2014, *Z.F. Zhang* (HMAS 246921 holotype designated here, ex-type living culture CGMCC 3.17912 = LC5697); ibid., CGMCC 3.17913 = LC5698; ibid., CGMCC 3.17949 = LC5751.

Notes — Morphological characteristics of this species fit well with the generic concept of *Biscogniauxia*, i.e., coarse, warty and brown conidiophores, swollen conidiogenous areas with

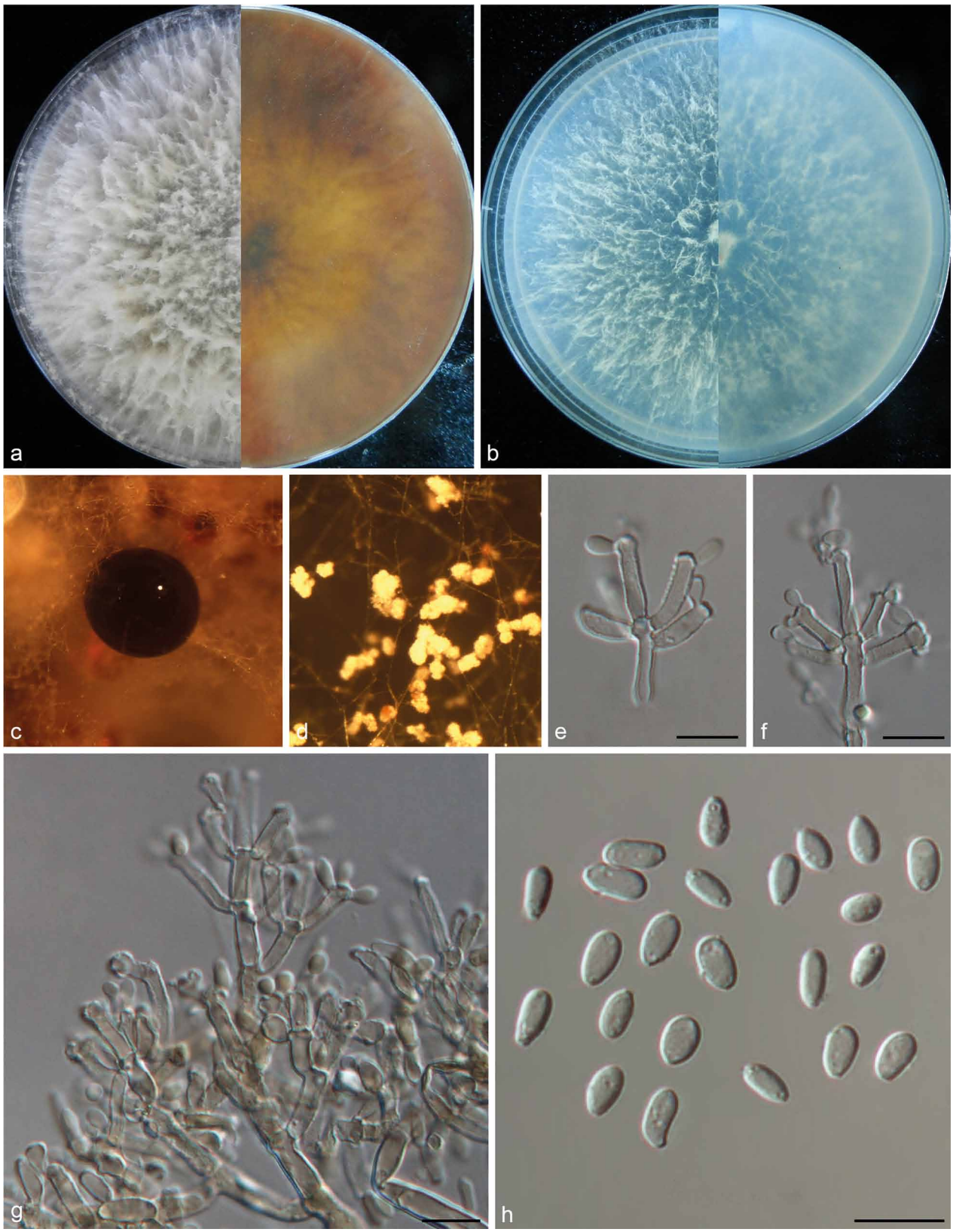

Fig. 5 *Biscogniauxia petrensis* (from ex-holotype CGMCC 3.17912). a–b. Upper and reverse views of cultures on PDA and SNA 14 d after inoculation; c. exudate; d. conidiomata under stereomicroscope; e–g. conidiophores and conidia; h. conidia. — Scale bars: e–h = 10 µm.

conidial secession scars and holoblastically produced conidia (Ju et al. 1998). Three strains formed a distinct clade within the genus *Biscogniauxia*, and are closely related to *B. capnodes* (strain no.: CM-AT-015) (phylogenetic tree deposited in MycoBank: MB818247). While *B. petrensis* differs from *B. capnodes* in the slightly wider conidia and the hyaline to slightly yellowish conidiophores (yellowish to brownish in *B. capnodes*) (Ju et al. 1998).

Cladorrhinum globisporum Z.F. Zhang & L. Cai, *sp. nov*. — MycoBank MB818250; Fig. 6

Etymology. Referring to its globose conidia.

Colonies on PDA 59–71 mm diam after 10 d, cottony to fluffy, flat to slightly plicated, margin entire, pale grey to dark grey. Reverse dark grey to dark olivaceous. Colonies on SNA 60–65 mm diam after 10 d, margin entire, white, aerial mycelia sparse. Reverse white.

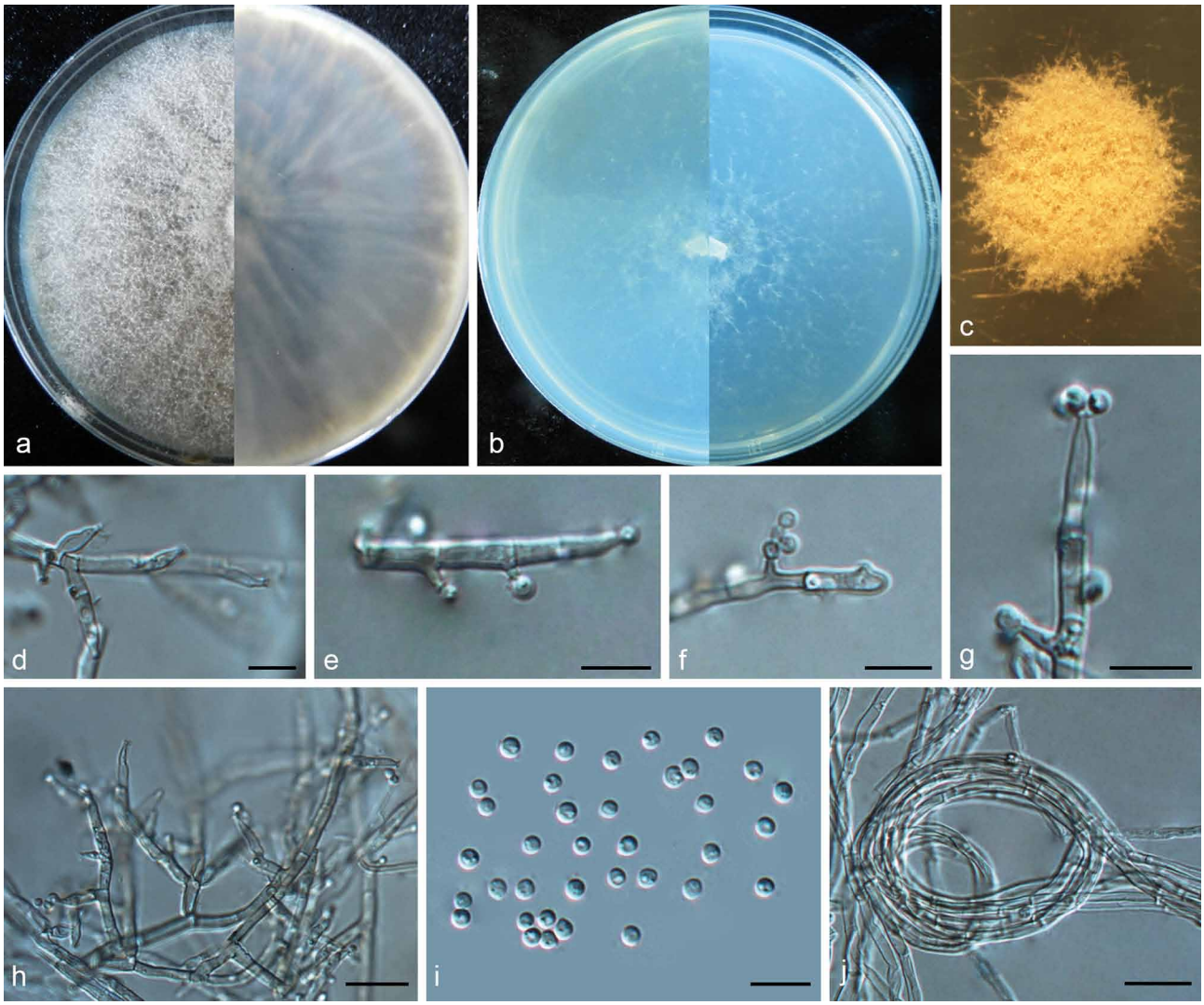

Fig. 6 *Cladorrhinum globisporum* (from ex-holotype CGMCC 3.17921). a–b. Upper and reverse views of cultures on PDA and SNA 21 d after inoculation; c. sporodochium on SNA; d–h. phialides; i. conidia; j. coiled mycelia. — Scale bars: d–j = 10 µm.

Vegetative *hyphae* hyaline to pale olivaceous, branched, septate, thin-walled, slightly rough, 1.5–4 µm diam, sometimes swollen, occasionally coiled. Microsclerotia not observed. *Sporodochium* forming on SNA within 50 d or longer, margin round, yellowish, scattered over entire colony. Fertile hyphae 2–3.5 µm wide, tufted, lateral branched, bearing terminal, lateral *phialides* or lateral phialidic openings of intercalary conidiogenous cells. Terminal and lateral phialides flask-shape, hyaline to pale olivaceous, straight or slightly sinuous, 8–18(–21) × 2–3.5 µm; intercalary conidiogenous openings with short flaring collarette, 2.5–5.0 × 1.5–2.5 µm. *Conidia* enteroblastic, hyaline, smooth- and thin-walled, globose, 2–3 µm diam (mean = 2.4 ± 0.3 µm, n = 40), guttulate, aggregated in slimy head.

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 1, N28°12'629" E107°13'639", water, 19 July 2014, *Q. Chen* (HMAS 246924 holotype designated here, ex-type living culture CGMCC 3.17921 = LC5415); ibid., CGMCC 3.17922 = LC5370.

Notes — Our isolates are morphologically similar to the cladorrhinum-like anamorph of *Cercophora* and *Podospora*. The BLASTn search also showed that the closest hits using ITS sequence of these isolates are the sequences of *Cercophora*, *Cladorrhinum*, and *Podospora* species. *Cladorrhinum* and *Phialophora* are anamorph typified genera related to *Cercophora* and *Podospora* (Miller & Huhndorf 2005, Madrid et al. 2011, Kruys et al. 2015). Previous studies showed that these genera are morphologically similar but polyphyletic (Cai et al. 2005, 2006, Miller & Huhndorf 2005, Madrid et al. 2011, Kruys et al. 2015). *Cladorrhinum* can be distinguished from *Phialophora* by the relative abundance of intercalary phialides and the phylogenetic affinities (Mouchacca & Gams 1993, Madrid et al. 2011).

Phylogenetic analysis based on ITS and LSU showed that *Cl. globisporum* clustered in the clade of *Cladorrhinum* (phylogenetic tree deposited in MycoBank: MB818250) and the morphology fitted well to genus *Cladorrhinum*, which is characterised by the relative abundance of intercalary vs terminal phialides, the pigmentation of mycelia, and the conidial shape (Mouchacca & Gams 1993, Madrid et al. 2011). *Cladorrhinum globisporum* is morphologically similar to several species of *Cladorrhinum* producing globose to dacryoid conidia, e.g., *Cl. bulbillosum*, *Cl. flexuosum*, *Cl. foecundissimum*, *Cl. samala*, and the anamorphs of *Cercophora striata* and *Podospora fimiseda* (Madrid et al. 2011). However, *Cl. globisporum* does not produce blackish microsclerotia in culture, which are present in cultures of *Cl. bulbillosum*, and the anamorph of *Ce. striata*. *Cladorrhinum globisporum* differs from *Cl. samala* in the absence of dark, thick-walled setiform hyphae; from *Cl. flexuosum* in the rather regular conidiophores, which are strongly flexuous in *Cl. flexuosum*; from *Cl. foecundissimum* in the longer terminal and lateral phialides (8–18 µm vs 5.0–11 µm) and the globose conidia, which are dacryoid to almost globose in *Cl. foecundissimum*; from the asexual morph of *P. fimiseda* in its smaller conidia (2–3 µm vs 3–4 µm).

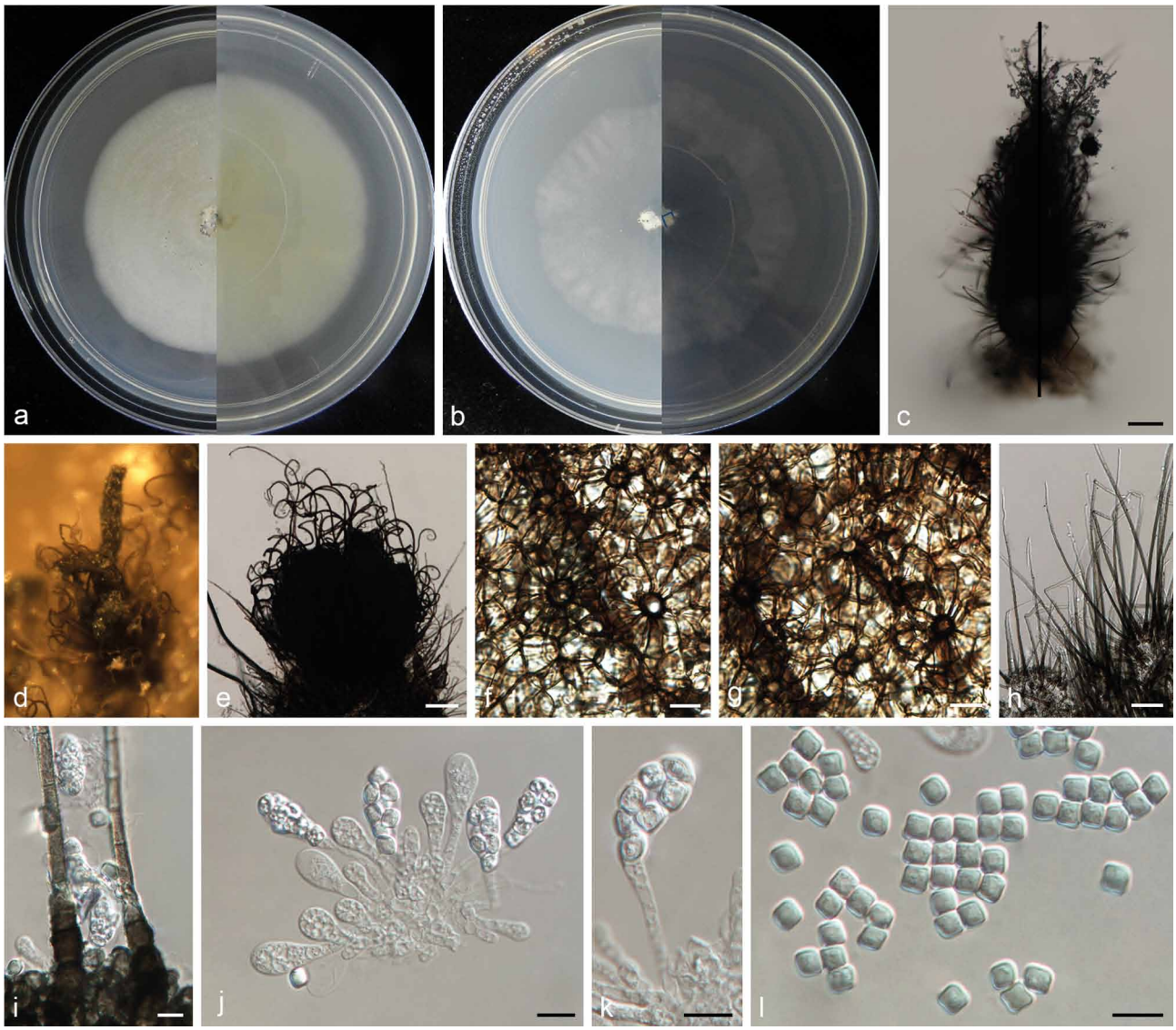

Fig. 7 *Collariella quadrum* (from ex-holotype CGMCC 3.17917). a–b. Upper and reverse views of cultures on PDA and SNA 18 d after inoculation; c, e. ascomata; d. masses of ascospores; f–g. outer surface of peridium; h–i. ascomatal hairs; j–k. asci; l. ascospores. — Scale bars: c, e = 100 µm; h = 50 µm; f–g, i–l = 10 µm.

Collariella quadrum Z.F. Zhang, F. Liu & L. Cai, *sp. nov*. — MycoBank MB818249; Fig. 7

Etymology. Referring to the shape of its ascospores.

Colonies on PDA attaining 34–37 mm diam after 10 d, felty, margin entire, white to pale yellow. Reverse pale cream-yellow. Colonies on SNA attaining 40–42 mm diam after 10 d, flat, aerial mycelia sparse, white. Reverse white.
Vegetative *hyphae* hyaline, septate, branched, smooth-walled, 2–6 µm diam. *Ascomata* black, grey-green, subglobose, oval to fusiform, 250–500 µm high, 200–280 µm diam, with rounded base, ostiolate, neck unconspicuous. *Peridium* brown, comprised of *textura angularis*, arranged in a petaloid pattern. *Ascomatal hair* 260–450 µm long, 4–7 µm diam at base, tapering, unbranched, septate, straight at first, then straight below, spirally and loosely coiled upper, brown, verrucose. *Asci* fasciculate, clavate, eight-spored, long-stalked, 30–60 × 9.5–13 µm. *Ascospores* exuded as elongated cirrhi, biseriately arranged, pale olivaceous, square-pillow-shaped, quadrangular in frontal view, with obtuse angle, 4.5–5.5 × 4.5–5.5 × 4–4.5 µm (mean = 5.0 ± 0.1 × 4.9 ± 0.1 × 4.2 ± 0.2 µm, n = 30).

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 2, N28°12'599" E107°13'661", soil, 19 July 2014, *X. Zhou* (HMAS 246923 holotype designated here, ex-type living culture CGMCC 3.17917 = LC5446); ibid., CGMCC 3.17918 = LC5693; ibid., CGMCC 3.17919 = LC5781; ibid., CGMCC 3.17920 = LC5782.

Notes — The genus *Collariella* was recently established to accommodate several species previously accommodated in *Chaetomium*, based on both phylogenetic and morphological data (Wang et al. 2016). *Collariella quadrum* is morphologically and phylogenetically most closely related to *C. quadrangulata* (phylogenetic tree deposited in MycoBank: MB818248). However, *C. quadrum* differs from *C. quadrangulatum* in producing smaller ascospores (4.5–5.5 × 4.5–5.5 × 4.0–4.5 µm vs 6.5–7.5 × 6–7 × 4–5 µm) exuded as elongated cirrhi (Fig. 7d).

Gymnoascus exasperatus Z.F. Zhang, F. Liu & L. Cai, *sp. nov*. — MycoBank MB818251; Fig. 8

Etymology. Referring to the texture of its conidial wall, rough.

Colonies on PDA attaining 17–21 mm diam after 10 d, felty, flat, plicate, margin entire, white to pale pink. Reverse plicate, white to pale pink. Colonies on SNA attaining 16–18 mm diam after 10 d, felty, annular, margin entire, white to pale yellow, aerial mycelia sparse. Reverse white to pale yellow.
Vegetative *hyphae* pale yellow to yellow, septate, branched, smooth or slightly rough, sometimes fascicular, 1.5–5 µm diam; racquet hyphae present, 'racquet' 10 µm wide. Fertile

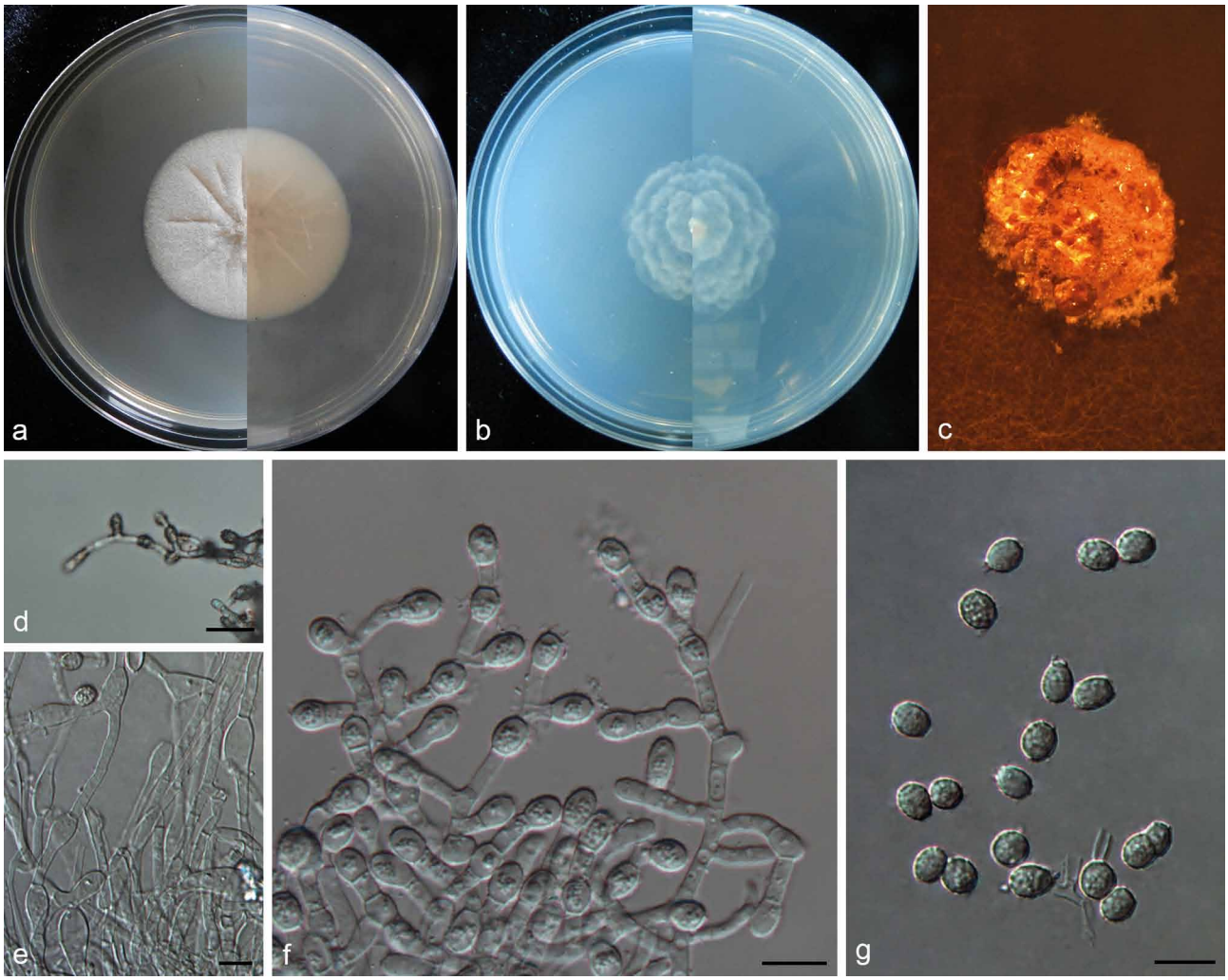

Fig. 8 *Gymnoascus exasperatus* (from ex-holotype CGMCC 3.17923). a–b. Upper and reverse views of cultures on PDA and SNA 21 d after inoculation; c. fertile mycelial structure; d, f. terminal, lateral and intercalary conidia; e. racquet hyphae; g. conidia. — Scale bars: d = 20 µm; e–g = 10 µm.

mycelia usually gathered into a special, superficial structure, where conidia borne mostly. *Conidia* terminal, lateral or intercalary, sessile or borne on short protrusions or side branches, solitary, frequently separated by one hyphal cell, subhyaline to pale brown, rough- and thin-walled; terminal and lateral conidia subglobose, obovoid to ellipsoidal, intercalary conidia cylindrical, 5–8 × 4–6 µm (mean = 6.4 ± 0.7 × 4.8 ± 0.5 µm, n = 30), with one or two sides truncated bases.

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 2, N28°12'599" E107°13'661", bat guano, 19 July 2014, *Z.F. Zhang* (HMAS 246925 holotype designated here, ex-type living culture CGMCC 3.17923 = LC5640); ibid., CGMCC 3.17924 = LC6217.

Notes — *Gymnoascus exasperatus* was isolated from bat guano in the cave. Phylogenetically, it forms a distinct clade sister to *G. reessii* (CBS 410.72), the type species of *Gymnoascus* (Fig. 2, phylogenetic tree deposited in MycoBank: MB818251). *Gymnoascus exasperates* is unique in the genus as it is only known from the asexual morph. The sexual morph of *G. exasperatus* was not observed despite repeated attempts using OA, PDA, and SNA media, as well as human hair and nails as inducer (Orr & Kuehn 1972).

Humicola limonisporum Z.F. Zhang & L. Cai, *sp. nov*. — MycoBank MB818248; Fig. 9

Etymology. Referring to its limoniform ascospores.

Colonies on PDA attaining 53–56 mm diam after 21 d, slightly raised near the centre, floccose, yellowish to yellow-brown. Reverse pale yellow to brown. Colonies on SNA attaining 69–72 mm diam after 21 d, flat, white, aerial mycelia sparse. Reverse white.

Vegetative *hyphae* hyaline, septate, branched, smooth-walled, 2.0–5.5 µm diam. Ascomata appeared after about 40 d, scattered over the colonies, brown to black-brown, oval to subglobose, 180–270 µm high, 120–200 µm diam, with rounded base, ostiolate, neck unconspicuous. Peridium brown, *textura irregularis*. *Rhizoids* well developed, septate, pale brown, 2.5–5.5 µm wide. *Ascomatal hair* 220–720 µm long, 2.0–5.0 µm diam at base, tapering, unbranched, septate, straight at base, spirally and loosely coiled upper, yellow to brown, verrucose. *Asci* fasciculate, clavate, eight-spored, long-stalked, 45–87 × 14.5–21 µm. *Ascospores* dark brown, thick-walled, limoniform to subglobose, 7.5–10.5 × 5.5–9.5 µm (mean = 9.1 ± 0.6 × 7.3 ± 1.1 µm, n = 50), with an apical germ pore. *Aleurioconidia* subhyaline to pale brown, subglobose to globose, solitary, unicellular, 8.5–13.5 µm diam (mean = 10.9 ± 1.1 µm, n = 45).

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 1, N28°12'599" E107°13'661", soil, 19 July 2014, *X. Zhou* (HMAS 246922 holotype designated here, ex-type living culture CGMCC 3.17914 = LC5610); ibid., CGMCC 3.17915= LC5707; ibid., CGMCC 3.17916 = LC5708.

Notes — Traditionally morphologically defined *Chaetomium* was heterogeneous and thus has been recently revised (Wang et al. 2016). Most previously reported *Chaetomium* species do not produce an asexual morph, but few species have a humicola-like anamorph. A distinct clade including the type of

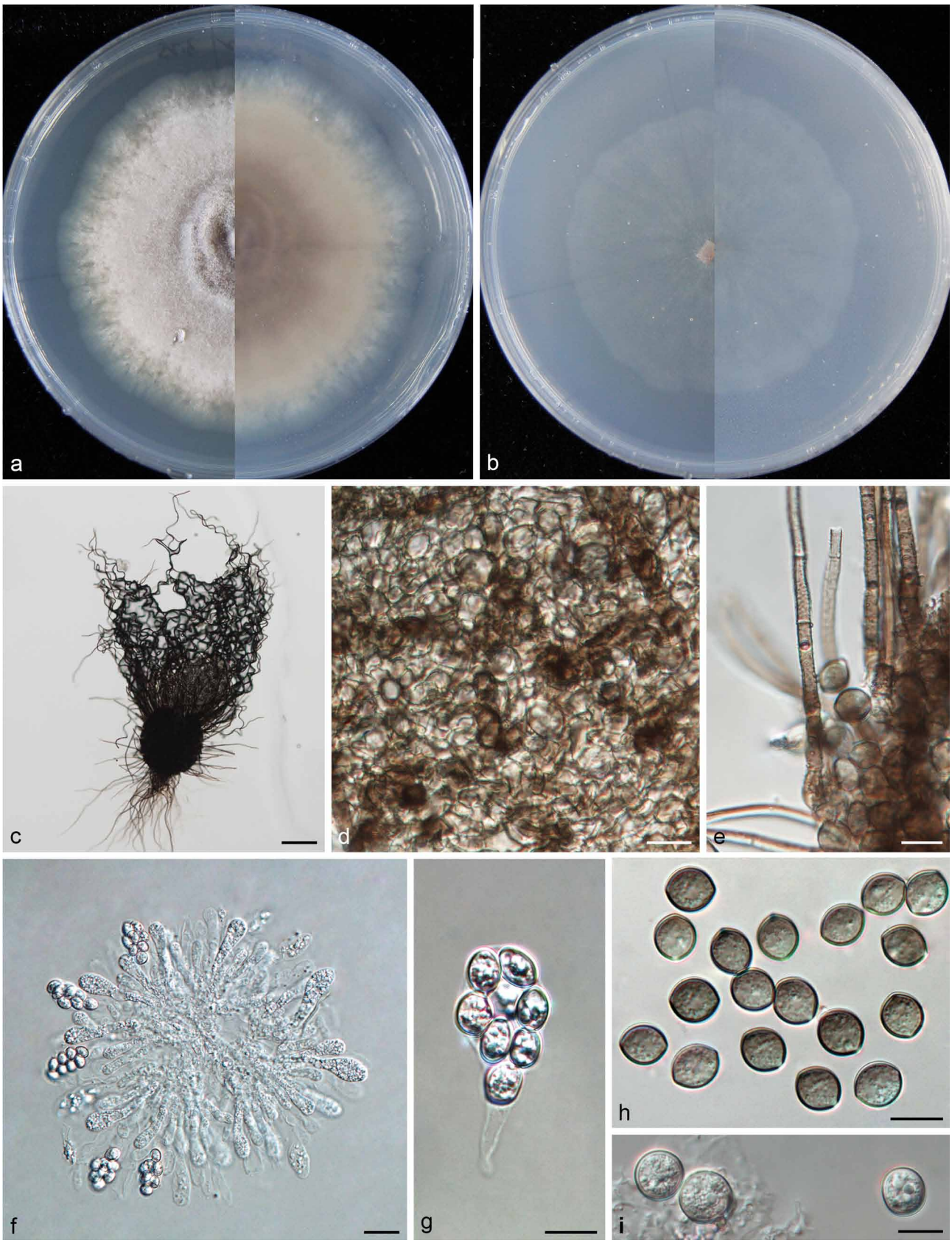

Fig. 9 *Humicola limonisporum* (from ex-holotype CGMCC 3.17914). a–b. Upper and reverse views of cultures on PDA and SNA 20 d after inoculation; c. ascomata; d. outer surface of peridium; e. ascomatal hairs; f–g. asci; h. ascospores; i. aleurioconidia. — Scale bars: c = 100 µm; f = 50 µm; d–e, g–i = 10 µm.

Humicola, i.e., *H. fuscoatra* (Traaen 1914), was recognised by Wang et al. (2016). In this study, *H. limonisporum* clustered in this clade, closely related to *H. fuscoatra* (the type species of *Humicola*) and *H. olivacea* (phylogenetic tree deposited in MycoBank: MB818248). *Humicola limonisporum* differs from *H. olivacea* in producing white colonies on SNA (grey olivaceous for *H. olivacea*); from *H. fuscoatra* in producing subglobose to globose aleurioconidia (subglobose to obpyriform for *H. fuscoatra*). The genus *Humicola* remains polyphyletic however, as most of the currently known species do not cluster with the type of the genus (Wang et al. 2016). Morphologically, the asexual morph of *H. limonisporum* is comparable to *H. globosa*. However, it differs in having yellow-brown colonies and hyaline somatic hyphae on PDA. In contrast, colonies of *H. globosa*

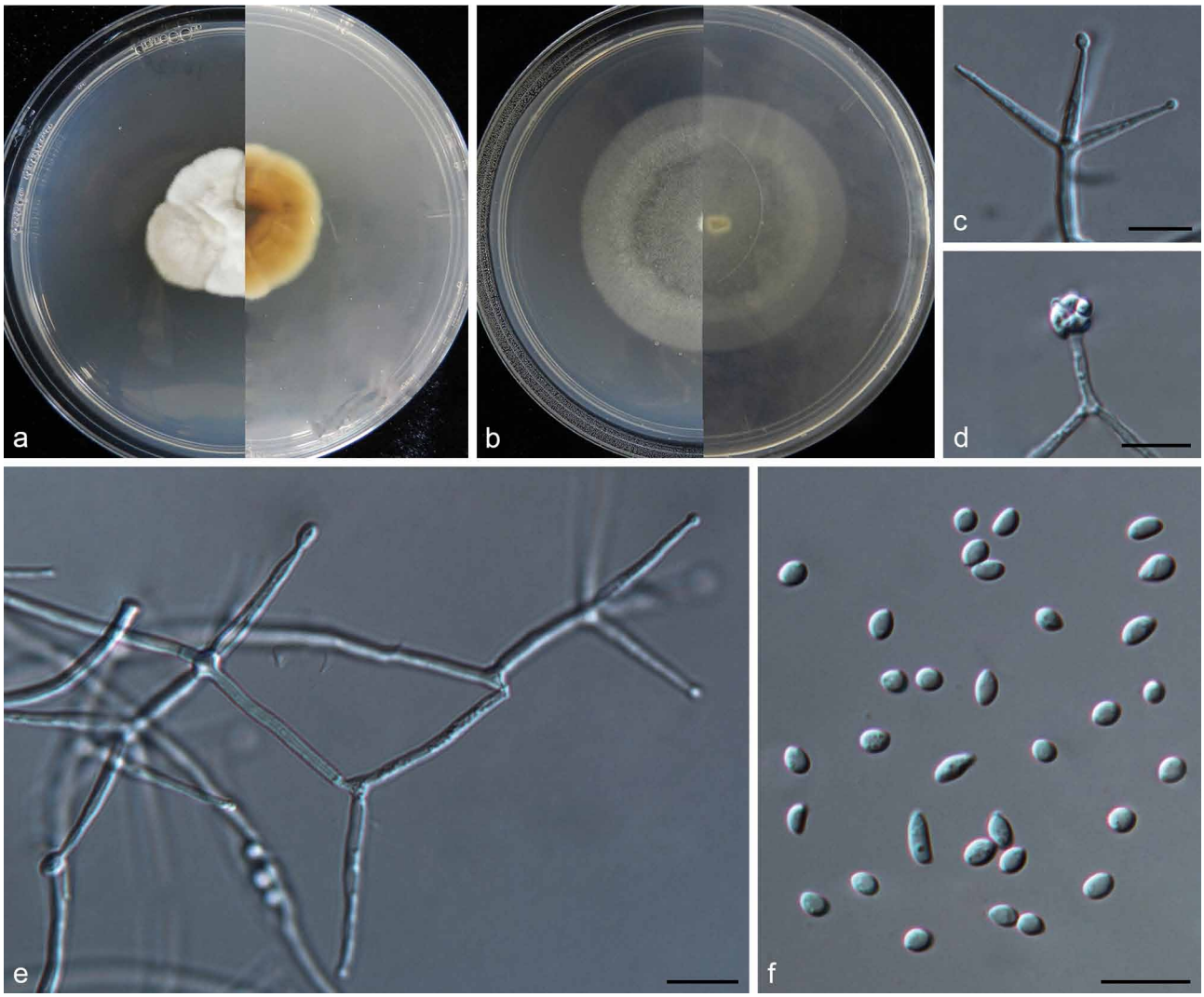

Fig. 10 *Metapochonia variabilis* (from ex-holotype CGMCC 3.17925). a–b. Upper and reverse views of cultures on PDA (10 d) and SNA (21 d); c, e. phialides; d. conidia aggregated in slimy head; f. conidia. — Scale bars: c–f = 10 µm.

on PDA are dark green to black and the somatic hyphae are predominantly brown.

Metapochonia variabilis Z.F. Zhang, F. Liu & L. Cai, *sp. nov*. — MycoBank MB818252; Fig. 10

Etymology. Referring to its various conidial shapes.

Colonies on PDA attaining 22–26 mm diam after 10 d, pulvinate, compact, sometimes plicated, white to pale brown. Reverse yellow-brown to brown. Colonies on SNA attaining 17–28 mm diam after 10 d, flat, margin entire, white to yellowish, aerial mycelia sparse. Reverse white to yellowish.
Vegetative *hyphae* hyaline, smooth-walled, branched, septate. *Conidiophores* arising from prostrate aerial hyphae, straight, hyaline, with 1–4 phialides per node lateral or in whorls of 3–6 phialides terminal, c. 2 µm diam. *Phialides* arising from aerial hyphae or conidiophore, slender, awl-shaped phialides, hyaline, 16–26.5 µm long, 1.5–2.5 µm diam at base, tapering toward the tip. *Conidia* single or aggregated in slimy heads, 1-celled, hyaline, falcate, fusiform, pyriform, ellipse to subglobose, or some other irregularly shapes, 3–6(–8) × 2–3.5 µm (mean = 4.9 ± 1.1 × 2.4 ± 0.3 µm, n = 30). *Dictyochlamydospores* not observed. *Crystals* absent.

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 1, N28°12'629" E107°13'639", soil, 19 July 2014, *X. Zhou* (HMAS 246926 holotype designated here, ex-type living culture CGMCC 3.17925 = LC5717); ibid., CGMCC 3.17926 = LC6221.

Notes — *Metapochonia* was established by Kepler et al. (2014) and currently comprises five species. *Metapochonia* species are verticillium-like, producing conidia on slender, awl-shaped phialides that may be whorled or singular, and most species are also known to produce stalked, thick-walled, and multicellular dictyochlamydospores (Gams & Zare 2001, Zare & Gams 2007, Kepler et al. 2014). Based on the phylogenetic analysis of ITS sequences, our isolates clustered together with other *Metapochonia* species but formed a distinct clade with high support value (phylogenetic tree deposited in MycoBank: MB818252). Morphologically, *M. variabilis* differs from the closely related species *M. bulbillosa* in producing wider conidia (2–3.5 µm vs 1.2–2 µm); from *M. gonioides* in producing different shaped, larger conidia (3–6(–8) × 2–3.5 µm vs 1.8–2.5 µm diam).

Microascus anfractus Z.F. Zhang, F. Liu & L. Cai, *sp. nov*. — MycoBank MB818253; Fig. 11

Etymology. Referring to its coiled mycelia.

Colonies on PDA 10–13 mm diam after 10 d, felty, compact, margin entire to undulate, plicated, low convex, pink to salmon. Reverse salmon. Colonies on SNA 9–12 mm diam after 10 d, compact, margin entire to undulate, white to yellowish, aerial mycelia sparse. Reverse white to yellowish.
Vegetative *hyphae* hyaline to pale brown, septate, branched, smooth-walled, 1–2.5 µm diam, sometimes coiled. The tip of

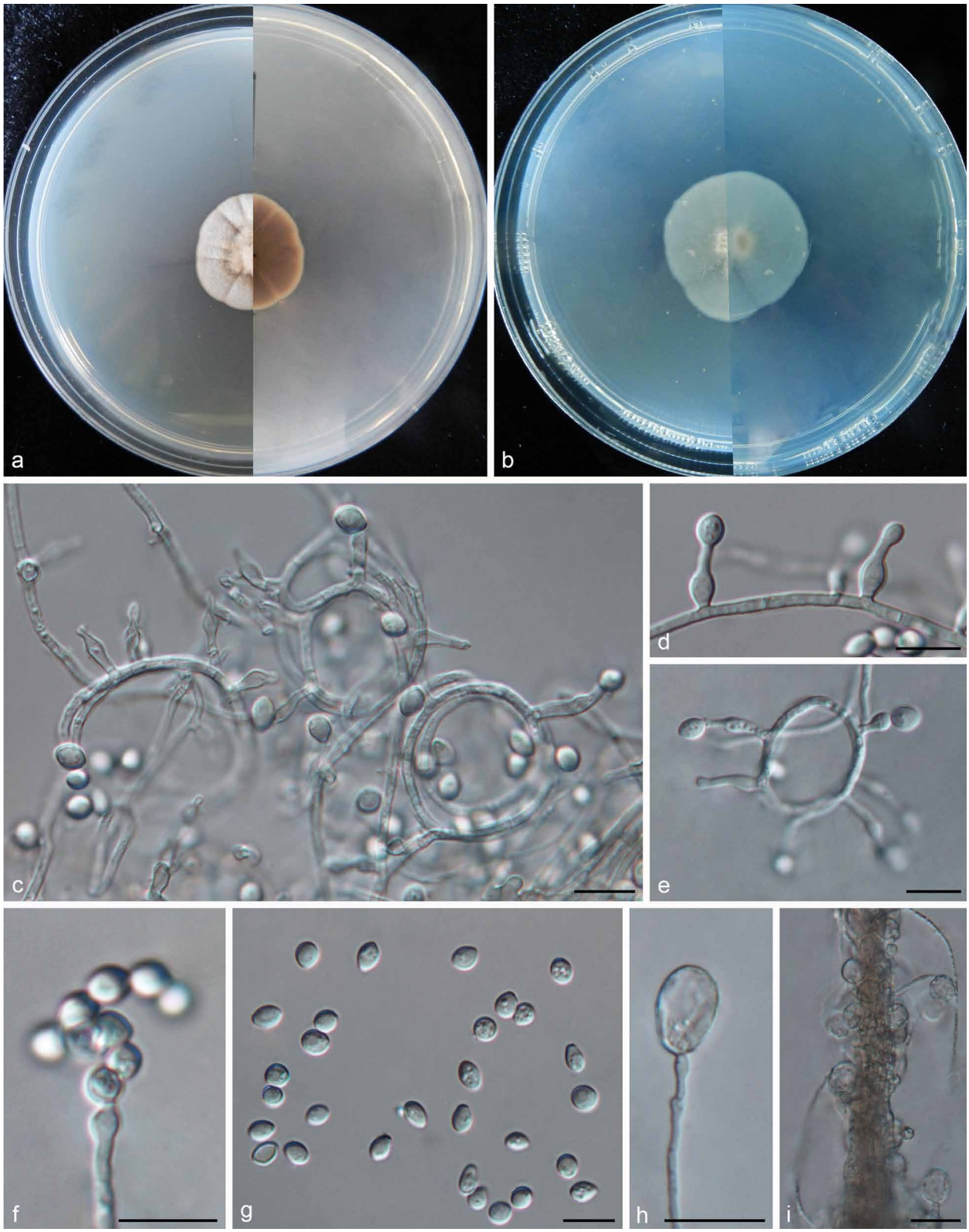

Fig. 11 *Microascus anfractus* (from ex-holotype CGMCC 3.17950). a–b. Upper and reverse views of cultures on PDA and SNA 21 d after inoculation; c, e. coiled mycelia; d–f. conidiogenous cells and conidia; g. conidia; h–i. swollen mycelia. — Scale bars: c–h = 10 µm; i = 20 µm.

semi-immerged mycelia sometimes swollen, globose, up to 20 µm diam. *Conidiogenous cells* borne laterally on aerial hyphae, solitary, hyaline, smooth-walled, lageniform, ampulliform or pyriform, straight or slightly curved, 7–13.5(–28.5) × 2–3.5 µm. *Conidia* formed in chains, hyaline, smooth- and thin-walled, ellipsoidal, fusiform to globose, 3.5–6 × 3.5–5 µm (mean = 5.0 ± 0.6 × 4.1 ± 0.5 µm, n = 30).

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 2, N28°12'599" E107°13'661", plant debris, 19 July 2014, *Z.F. Zhang* (HMAS 246927 holotype designated here, ex-type living culture CGMCC 3.17950 = LC5843); ibid., CGMCC 3.17951 = LC6224.

Notes — Phylogenetically, *M. anfractus* nested within the *Microascus* clade based on ITS, LSU, *TUB*, and *EF1-α* sequences (phylogenetic tree deposited in MycoBank: MB818253) and its morphological characteristics fit well to this genus, i.e., ampulliform or lageniform conidiogenous cells and smooth- and thin-walled or finely rough- and thick-walled conidia (Sandoval-Denis et al. 2016).

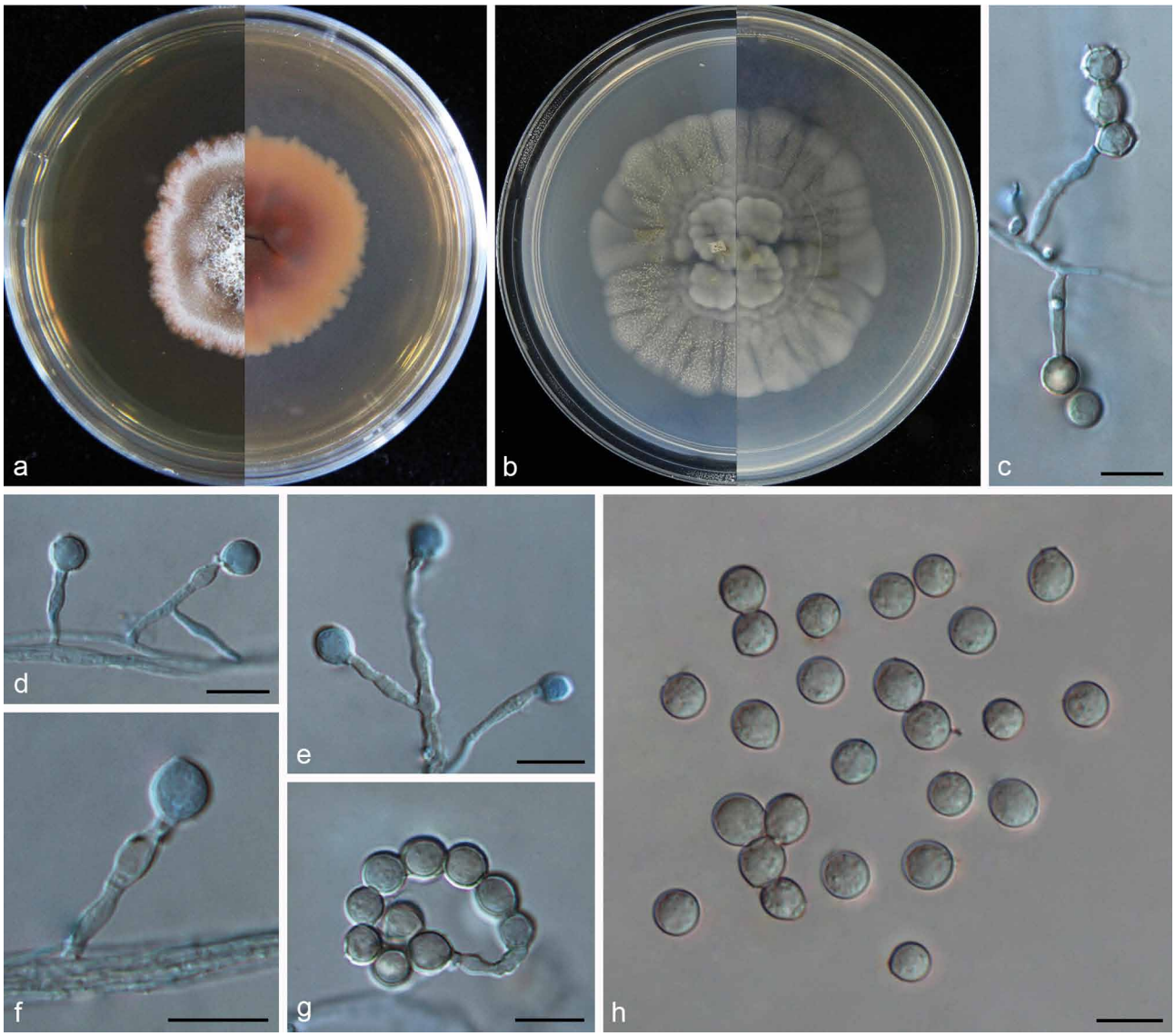

Fig. 12 *Microascus globulosus* (from ex-holotype CGMCC 3.17927). a–b. Upper and reverse views of cultures on PDA (21 d) and SNA (42 d); c–f. conidiogenous cells and conidia in cotton blue; g–h. conidia. — Scale bars: c–h = 10 µm.

Microascus anfractus is morphologically and phylogenetically comparable to three species in *Microascus*, i.e., *M. campaniformis*, *M. cirrosus*, and *M. croci*. The conidiogenous cells of *M. anfractus* arise from hyphae singly, while those of *M. cirrosus* arise from conidiophores and are arranged in whorls of three to five. *Microascus anfractus* can be distinguished from *M. campaniformis* and *M. croci* in its wider conidia (3.5–5 µm vs 2.5–3.5 µm for *M. campaniformis* and 2.5–3.5 µm for *M. croci*).

Microascus globulosus Z.F. Zhang, F. Liu & L. Cai, *sp. nov*. — MycoBank MB818254; Fig. 12

Etymology. Referring to its globose conidia.

Colonies on PDA attaining 29–35 mm diam after 15 d, felty, compact, plicated, umbonate, pink, salmon to rusty red, with white margin, aerial mycelia sparse. Reverse tawny to brown. Colonies on SNA attaining 17–19 mm after 15 d, flocculent, margin radially striate with lobate edge, conspicuously radial gaps, pale grey, aerial mycelia sparse. Reverse pale grey. Vegetative *hyphae* hyaline, septate, branched, thin- and smooth-walled, 1.2–2.8 µm diam. *Conidiogenous cells* lateral or terminal on aerial hyphae, solitary, hyaline, smooth-walled, cylindrical, ampulliform or irregular shaped, erect or curved, constricted at base, occasionally branched, 5.5–24 × 1–3 µm. *Conidia* formed in chains, globose to subglobose, thick- and smooth-walled, hyaline to pale brown, 6.5–9 µm diam (mean = 7.5 ± 0.9 µm, n = 40).

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 2, N28°12'599" E107°13'661", bat guano, 19 July 2014, *Z.F. Zhang* (HMAS 246928 holotype designated here, ex-type living culture CGMCC 3.17927 = LC5820); ibid., CGMCC 3.17928 = LC6223.

Notes — *Microascus globulosus* is phylogenetically closely related to *M. chartarus* based on ITS, LSU, *TUB*, and *EF1-α* sequences (phylogenetic tree deposited in MycoBank: MB818254). However, the conidia of *M. chartarus* are ovate, green-brown, and often with a pointed end, as compared to the globose to subglobose, hyaline to pale brown conidia of *M. globulosus*.

Microdochium chrysanthemoides Z.F. Zhang, F. Liu & L. Cai, *sp. nov*. — MycoBank MB818255; Fig. 13

Etymology. Referring to the shape of its sporodochium, chrysanthemum-like (Fig. 13d).

Colonies on PDA attaining 40–46 mm diam after 10 d, felty, compact, erose or dentate, white initially, then becoming yellowish with age. Exudate occasionally appeared on old sporodochia. Reverse yellowish to orange, due to the soluble pigment secreted. Colonies on SNA 55–57 mm diam after 10 d, entire, white, aerial mycelia sparse. Exudate absent. Reverse white. Vegetative *hyphae* hyaline, abundant, branched, septate, thin-

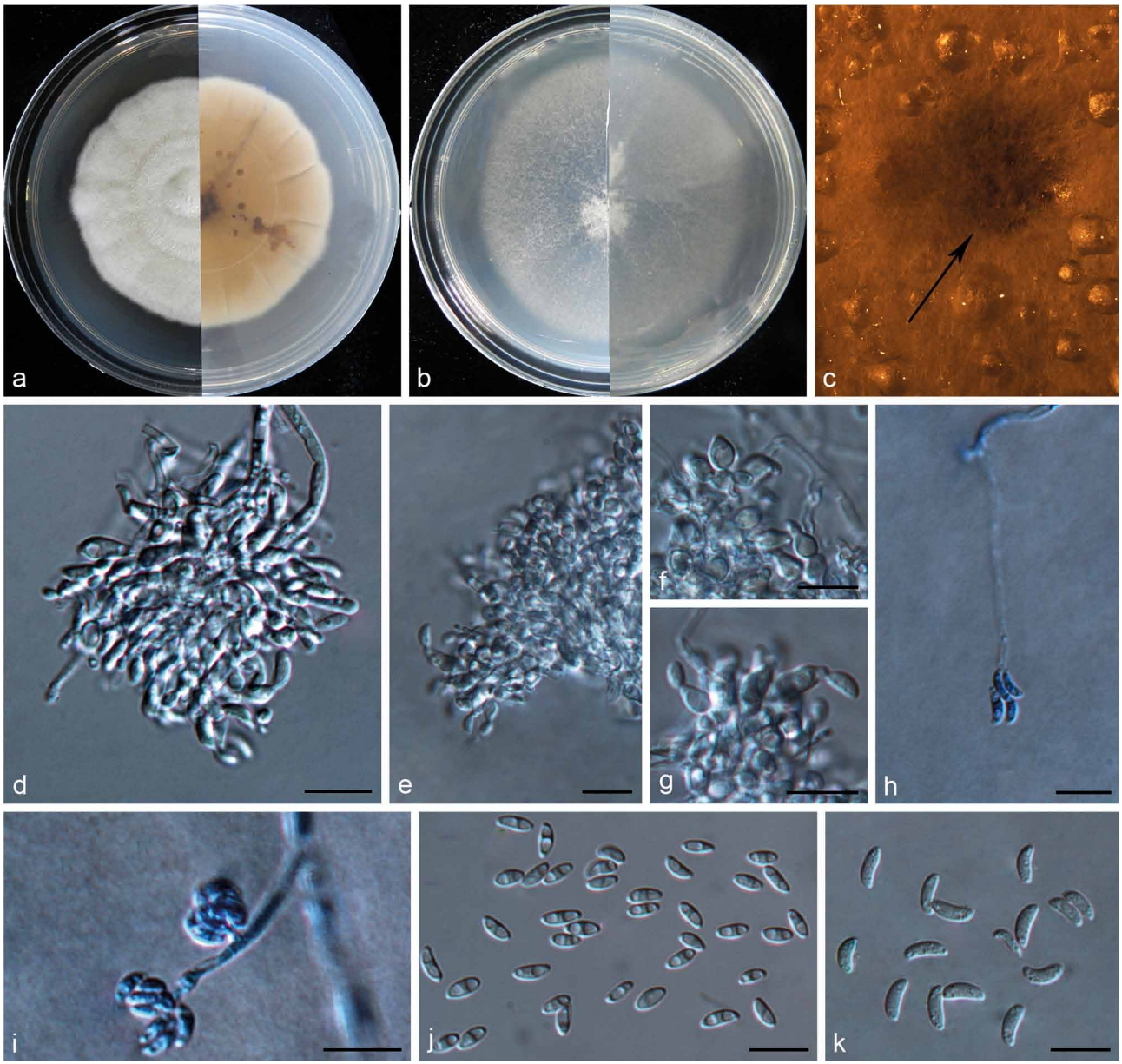

Fig. 13 *Microdochium chrysanthemoides* (from ex-holotype CGMCC 3.17929). a–b. Upper and reverse views of cultures on PDA (10 d) and SNA (14 d); c. sporodochia semi-submerged in agar (black arrow); d–e. sporodochia; f–g. conidiogenous cells from sporodochia; h–i. conidia forming on conidiophore directly (stained with cotton blue); j–k. conidia. — Scale bars: d–k = 10 µm.

walled. *Conidiophores* aggregated in a sporodochium, or borne directly from the hyphae, hyaline, unbranched. Sporodochia appeared within 7 d or longer, yellowish to salmon, semi-submerged. *Conidiophores* borne from hyphae straight or slightly curved, aseptate, 21–58 × 1–1.5 µm, with conidia formed terminal or lateral, solitary or aggregated in a mass. *Conidiogenous cells* holoblastic, solitary, hyaline, apical, simple, ampulliform, lageniform, cylindrical to ellipsoidal, straight or bent, 5–12 × 3.0–4.5 µm; denticles not observed. *Conidia* aseptate, ellipsoid or allantoid, straight or curved, obtuse, guttulate in mature conidia, 4.5–7 × 2–3 µm (mean = 5.5 ± 0.7 × 2.7 ± 0.3 µm, n = 35).

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 2, N28°12'599" E107°13'661", air, 19 July 2014, *Z.F. Zhang* (HMAS 246929 holotype designated here, ex-type living culture CGMCC 3.17929 = LC5363); ibid., CGMCC 3.17930 = LC5466.

Notes — *Microdochium* has been regarded as the asexual morph of *Monographella* (Zhang et al. 2015a, Hernández-Restrepo et al. 2016). Although these two genera were described in the same journal in 1924 (Petrak 1924, Sydow 1924), *Microdochium* has more species and has been more frequently used in literature (Hernández-Restrepo et al. 2016), and thus the name should be protected with the implementation of 'one fungus one name' approach (Hawksworth et al. 2011). *Microdochium* is characterised by its verticillate conidiophores, holoblastic, discrete, small papillate conoid conidiogenous cells and solitary, fusiform to subfalcate, hyaline conidia (Hernández-Restrepo et al. 2016). *Microdochium chrysanthemoides* is phylogenetically closely allied to *M. neoqueenslandicum* (CBS 445.95 and CBS 108926) and formed a distinct clade (phylogenetic tree deposited in MycoBank: MB818255). Morphologically, conidia of *M. chrysanthemoides* are ellipsoid or falcate, straight or curved, and guttulate, while that in *M. neoqueenslandicum* are consistently lunate, allantoid, curved and non-guttullate (Hernández-Restrepo et al. 2016).

Paracremonium variiforme Z.F. Zhang, F. Liu & L. Cai, *sp. nov*. — MycoBank MB818264; Fig. 14

Etymology. Refers to the various shapes of the conidia.

Colonies on PDA attaining 43–49 mm diam after 21 d, flat, margin entire, milk-white to yellow-white, aerial mycelia sparse.

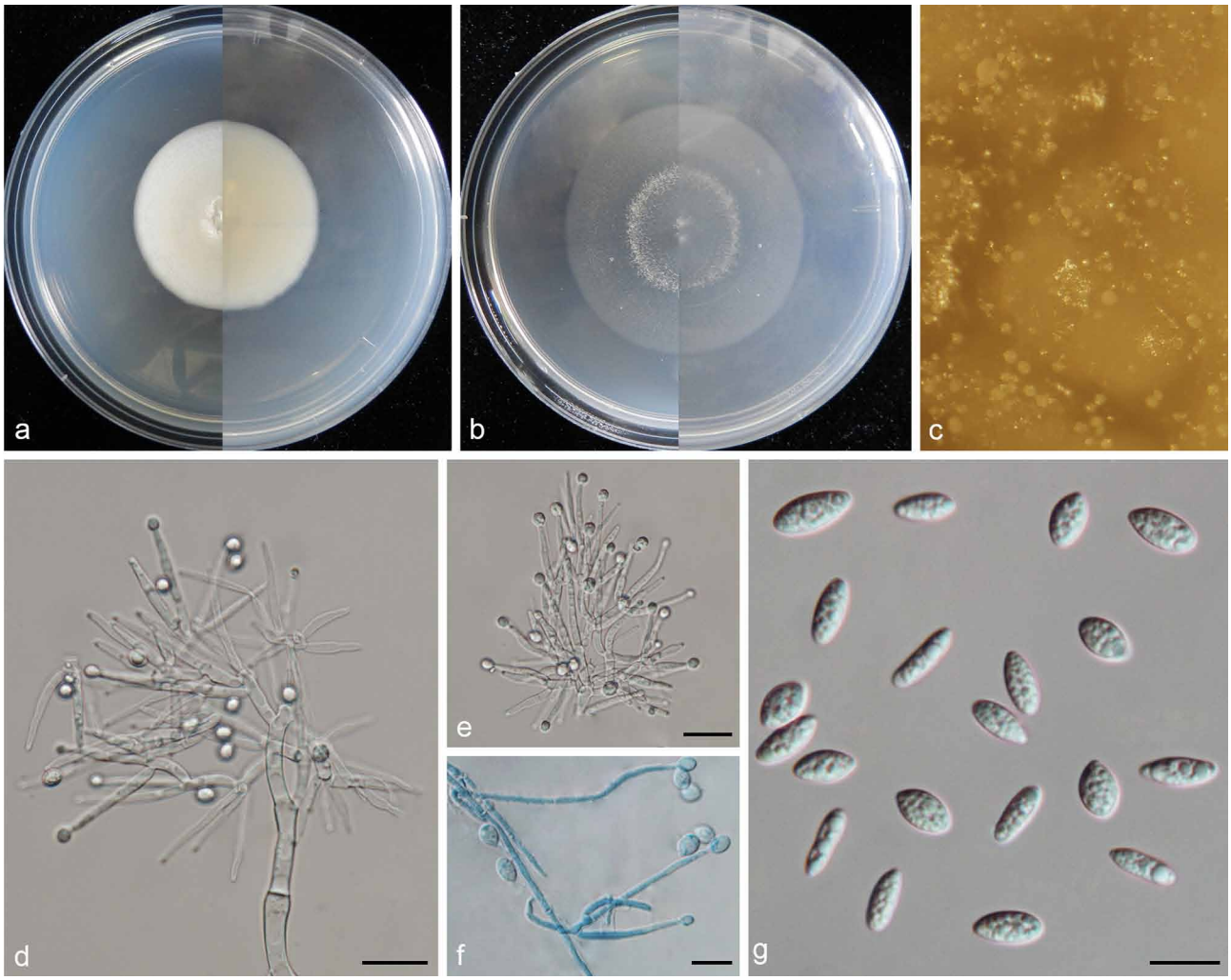

Fig. 14 *Paracremonium variiforme* (from ex-holotype CGMCC 3.17931). a–b. Upper and reverse views of cultures on PDA and SNA 21 d after inoculation; c. sporulation on PDA under stereomicroscope; d–e. conidiophores, phialides and conidia; f. phialides and conidia in cotton blue; g. conidia. — Scale bars: d–e = 20 µm; f–g = 10 µm.

Reverse milk-white to yellow-white. Colonies on SNA attaining 43–46 mm diam after 21 d, margin entire, white. Reverse white. Vegetative *hyphae* hyaline, smooth- and thin-walled, septate, branched, 1.5–9.5 µm diam, inconspicuously swollen at the hyphal septa. Sporulation abundant, mostly phalacrogenous, varying to nematogenous. *Conidiophores* erect, simple or mostly branched, septate, bearing whorls of 2–4 conidiogenous cells. *Conidiogenous cells* terminal or lateral, straight, acicular or elongate-ampulliform, tapering towards apex, hyaline, 18–41 × 2–3.5 µm, with prominent periclinal thickening and inconspicuous collarette, 1–1.5 µm diam. *Conidia* unicellular, hyaline, clavate, ovoid or elliptical, thick- and smooth-walled, with slightly apiculate base, 9–14.5 × 4–6 µm (mean = 11.1 ± 1.3 × 4.9 ± 0.6 µm, n = 40). *Chlamydospores* not observed.

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 2, N28°12'599" E107°13'661", water, 19 July 2014, *Z.F. Zhang*, *L. Cai*, *Q. Chen* & *X. Zhou* (HMAS 246930 holotype designated here, ex-type living culture CGMCC 3.17931 = LC5806); ibid., CGMCC 3.17932 = LC5809; ibid., CGMCC 3.17933 = LC5832; ibid., CGMCC 3.17934 = LC5837.

Notes — Phylogenetic analysis based on ITS, LSU and *TUB* sequences showed that our isolates clustered within the genus *Paracremonium* and formed a distinct clade (MB818264). According to the description of Lombard et al. (2015), *Paracremonium* is distinguished from other acremonium-like genera by the formation of sterile coils from which conidiophores radiate with inconspicuously swollen septa in the hyphae. However, sterile coils were not observed in our isolates. *Paracremonium variiforme* is phylogenetically most closely related to *P. inflatum* and *P. contagium* (phylogenetic tree deposited in MycoBank: MB818264), but could be easily differentiated from them by its branched conidiophores.

Pectinotrichum chinense Z.F. Zhang & L. Cai, *sp. nov*. — MycoBank MB818256; Fig. 15

Etymology. Referring to the country where the fungus was firstly discovered.

Colonies on PDA 27–29 mm diam after 10 d, fluffy, flat, margin entire, white to yellow, powdery. Yellow pigment secreted. Reverse yellow to white from centre to margin. Colonies on SNA 21–22 mm diam after 10 d, yellowish, aerial mycelia extremely sparse. Yellow pigment secreted. Reverse pale yellow.
Vegetative *hyphae* hyaline, 2–3.2 µm diam. *Conidiophores* absent or reduced to conidiogenous cells, formed on aerial hyphae, hyaline, straight or slightly curved, 20–45 × 1.5–3 µm or longer, irregularly branched, sometimes two or three times branched, with conidia formed on branches terminal or lateral. *Conidia* borne on conidiophores or seldom on aerial hyphae directly or with a short stem, 1-celled, hyaline, smooth-walled, cylindrical, oval or pyriform, base rounded or with inconspicuous scars, 3.5–7.5 × 1–2.5 µm (mean = 4.4 ± 1.1 × 1.7 ± 0.3 µm, n = 25).

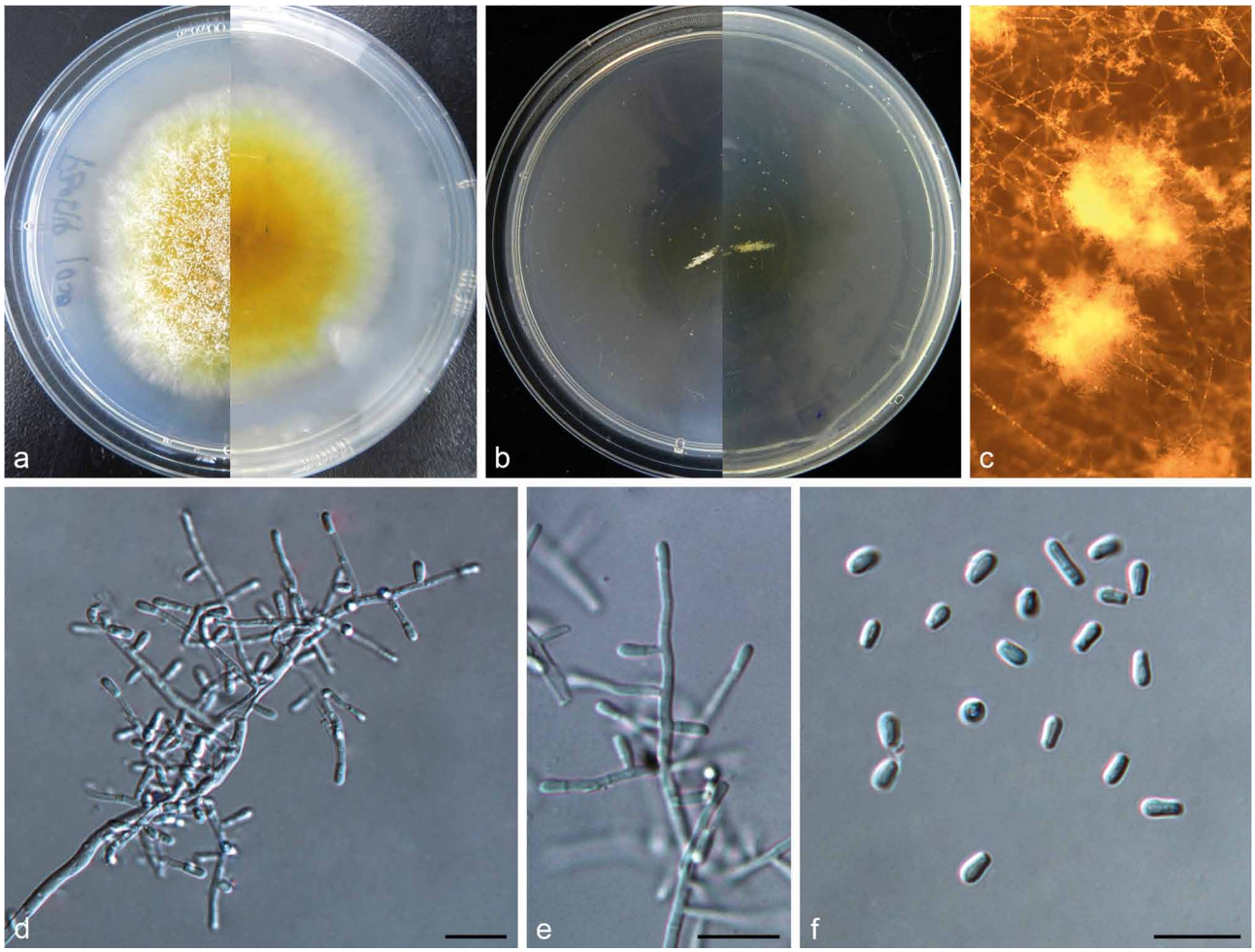

Fig. 15 *Pectinotrichum chinense* (from ex-holotype CGMCC 3.17935). a–b. Upper and reverse views of cultures on PDA and SNA 20 d after inoculation; c. aerial mycelia; d–e. conidiophores and conidiogenous cells; f. conidia. — Scale bars: d–f = 10 µm.

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 1, N28°12'629" E107°13'639", soil, 19 July 2014, *X. Zhou* (HMAS 246931 holotype designated here, ex-type living culture CGMCC 3.17935 = LC5811); ibid., CGMCC 3.17936 = LC5824.

Notes — *Pectinotrichum* was established by Varsavsky & Orr (1971) and currently contains only one species, *P. llanense*, which was reported as a keratinophilic fungus and usually isolated via the hair-bait technique. Based on a BLASTn search the closest hit using ITS sequence of *P. chinense* is that from the ex-type strain of *P. llanense* CBS 882.71 (NR119467, identity = 96 %), and the other BLAST results are all below 90 % identity. *Pectinotrichum chinense* differs from *P. llanense* in its narrower, sometimes cylindrical conidia (3.5–7.5 × 1–2.5 µm, mean = 4.4 ± 1.1 × 1.7 ± 0.3 µm, vs 4–6.5 × 2–3 µm) (Van Oorschot 1980).

Phaeosphaeria fusispora Z.F. Zhang, F. Liu & L. Cai, *sp. nov.* — MycoBank MB818257; Fig. 16

Etymology. Referring to its fusiform ascospores.

Colonies on PDA attaining 36–48 mm diam after 3 wk, felty, flat, margin entire, white to olivaceous from edge to centre. Yellow-brown to black brown secretions exuded. Reverse grey-green, with pale yellow margin. Colonies on SNA attaining 27–29 mm diam after 3 wk, felty, margin entire, white to light brown, aerial mycelia sparse. Reverse white to olivaceous, with an olivaceous circle.

Vegetative *hyphae* hyaline to brown, septate, branched, smooth-walled. *Ascomata* uniloculate, scattered, immersed, globose, glabrous, 120–225 µm high, 150–250 µm diam, with an unconspicuous beak at the apex. *Peridia* 15–30 µm, composed of 3–6 layers of polygonal pseudoparenchymatic cells. *Asci* hyaline to pale brown, cylindrical, clavate to long fusiform, 8-spored, bitunicate, 60–110 × 8–15 µm, with short stipes. *Ascospores* hyaline to pale brown, smooth-walled, fusiform, slightly curved, guttulate, 3-septate, occasionally 4-septate, slightly constricted at septum, L/W = 6.0, 25–40 × 4–6 µm (mean = 30.3 ± 3.5 × 5.0 ± 0.4 µm, n = 30).

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 2, N28°12'599" E107°13'661", air, 19 July 2014, *Z.F. Zhang* (HMAS 246932 holotype designated here, ex-type living culture CGMCC 3.17937 = LC5367); ibid., CGMCC 3.17938 = LC6215.

Notes — This species should be classified in *Phaeosphaeria* because of its typical characters: uniloculate, scattered, immersed, globose, and glabrous ascomata with a beak; 8-spored, bitunicate asci; fusiform, 3-septate ascospores with weak constriction (Shoemaker & Babcock 1989, Quaedvlieg et al. 2013). Although *Phaeosphaeriopsis* (*Ps.*) was similar to *Phaeosphaeria* (*Pa.*) and had a high identity of LSU sequence with our isolates, the ascospores of *Phaeosphaeriopsis* are cylindrical rather than narrowly fusiform (Câmara et al. 2003, Quaedvlieg et al. 2013) and our isolates are phylogenetically allied to *Phaeosphaeria* (phylogenetic tree deposited in MycoBank: MB818257). *Phaeosphaeria fusispora* is morphologically similar to *Pa. franklinensis*, *Pa. juncicola*, and *Pa. juncinella*. While *Pa. fusispora* differs from *Pa. franklinensis* and *Pa. juncicola* in the longer asci (60–110 µm vs 45–70 µm for *Pa. franklinensis* and 45–60 µm for *Pa. juncicola*); it differs from *Pa. juncinella* in having shorter asci (60–110 µm vs 100–140 µm) and constricted septa. Phylogenetically, the new species clustered apart from morphologically similar species.

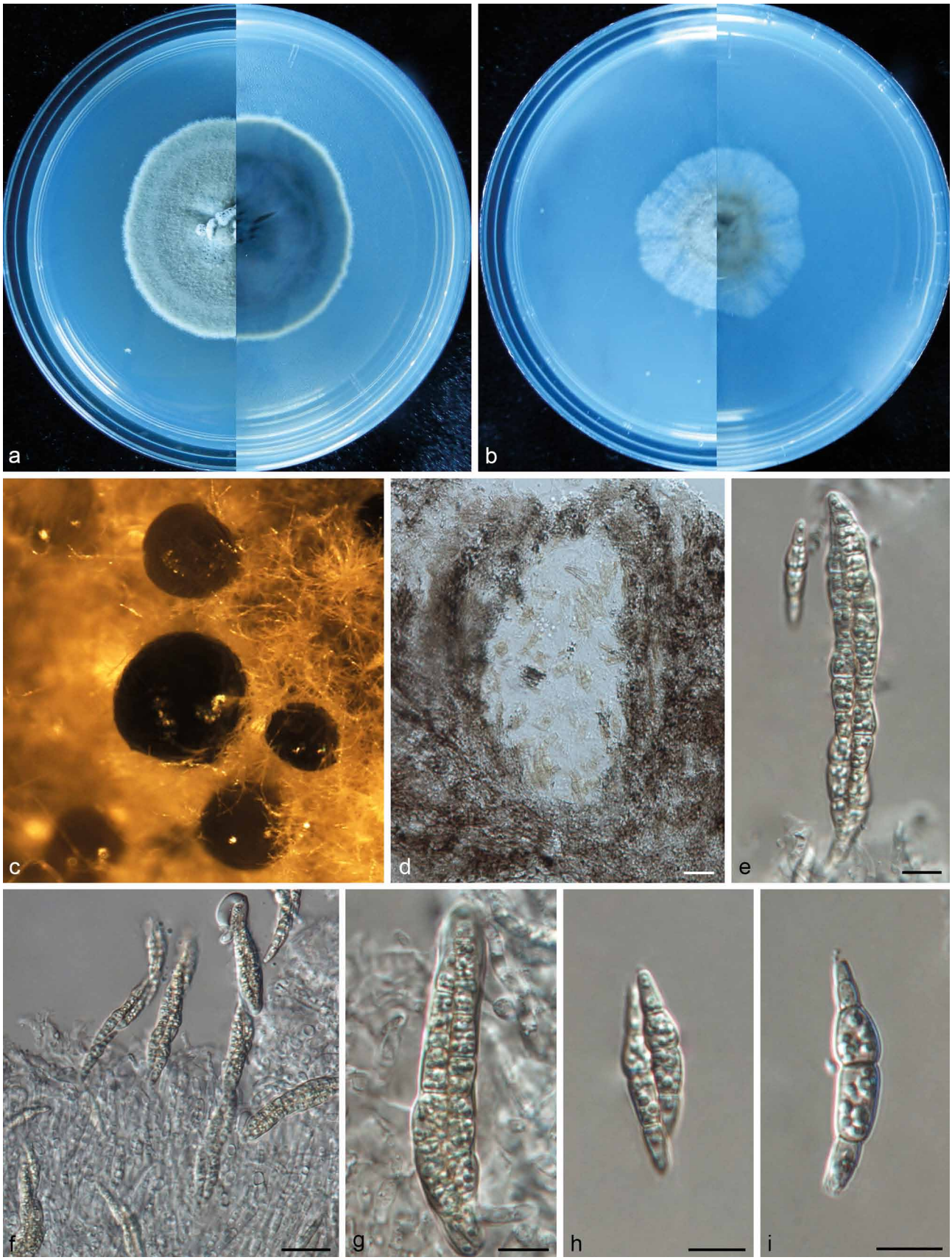

Fig. 16 *Phaeosphaeria fusispora* (from ex-holotype CGMCC 3.17937). a–b. Upper and reverse views of cultures on PDA and SNA 28 d after inoculation; c. exudates; d. section of ascomata; e–i. asci and ascospores. — Scale bars: d, f = 50 µm; e, g = 20 µm; h–i = 10 µm.

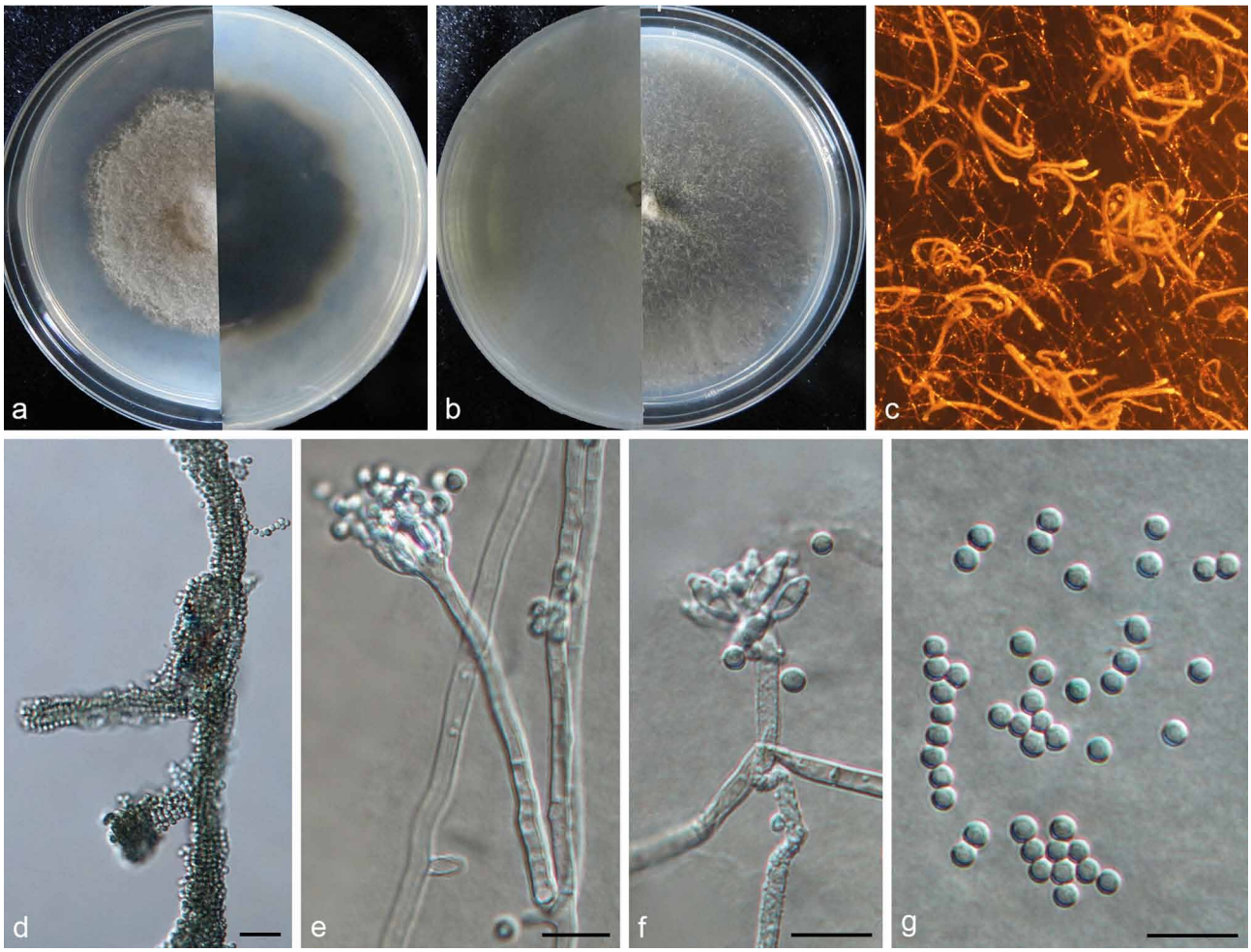

Fig. 17 *Ramophialophora globispora* (from ex-holotype CGMCC 3.17939). a–b. Upper and reverse views of cultures on PDA and SNA 21 d after inoculation; c. conidial beam on SNA under stereomicroscope; d. conidial beam; e–f. rough-walled conidiophores; g. conidia. — Scale bars: d = 20 µm; e–g = 10 µm.

Ramophialophora globispora Z.F. Zhang, F. Liu & L. Cai, *sp. nov.* — MycoBank MB818258; Fig. 17

Etymology. Referring to its globose conidia.

Colonies on PDA 43–52 mm diam after 3 wk, felty to cottony, flat, margin entire, grey-white to brown-grey. Reverse yellow-green to black. Colonies on SNA 51–58 mm diam after 3 wk, cottony, margin entire, taupe, aerial mycelia sparse. Reverse taupe.

Vegetative *hyphae* hyaline to pale yellow, branched, septate, smooth-walled. *Conidiophores* arise from prostrate aerial hyphae solitary, erect, straight, septate, unbranched, hyaline to pale yellow, thin- and rough-walled, 42–200 × 2–3 µm, globose at the apex, bearing tufty and terminally conidiogenous cells. *Phialides* often penicillate, hyaline, smooth-walled, elliptical, with inconspicuous apical collarette, 5–11 × 2.5–3.5 µm. *Conidia* enteroblastic, hyaline, globose, thin- and smooth-walled, 2–3 µm diam (mean = 2.3 ± 0.2 µm, n = 30), in chains, long macroscopic conidial beam formed with the aggregation of conidial chains.

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 1, N28°12'629" E107°13'639", plant debris, 19 July 2014, *Z.F. Zhang* (HMAS 246933 holotype designated here, ex-type living culture CGMCC 3.17939 = LC5696); ibid., CGMCC 3.17940 = LC6218.

Notes — *Ramophialophora* was established by Calduch et al. (2004) to accommodate several species traditionally classified in *Phialophora* but bear phylogenetic affinity to *Sordariales*. Currently the genus includes only two species. Multilocus phylogenetic analysis based on ITS, LSU, and *TUB* sequences showed that *R. globispora* and *R. petraea* clustered with *Ramophialophora*, and several *Cercophora* and *Podospora* species (phylogenetic tree deposited in MycoBank: MB818258). Morphologically, *R. globispora* and *R. petraea* are well allied to *Ramophialophora*. *Ramophialophora globispora* can easily be distinguished from *R. humicola* and *R. vesiculosa* by the unbranched conidiophores, discrete conidiogenous cells, and spherical conidia without protuberant basal hila. The ex-type strain of *Phialophora cyclaminis*, CBS 166.42, also clustered in *Sordariales*, thus might need to be transferred to *Ramophialophora*. *Ramophialophora globispora* can easily be distinguished from *P. cyclaminis* by its unbranched and rough-walled conidiophores vs the sparsely branched and smooth-walled conidiophores in *P. cyclaminis*.

Ramophialophora petraea Z.F. Zhang, F. Liu & L. Cai, *sp. nov.* — MycoBank MB818259; Fig. 18

Etymology. Referring to the sample where this species was first isolated from.

Colonies on PDA attaining 39–41 mm diam after 21 d, flat, margin entire, grey-green at the centre and white to the margin. Reverse pale brown to white. Colonies on SNA attaining 30–34 mm diam after 21 d, margin entire, white, aerial mycelia sparse. Reverse white.

Vegetative *hyphae* hyaline to pale green, septate, branched, thin- and smooth-walled, 2–3 µm diam, sometimes swollen. *Conidiophores* reduced to the conidiogenous cells, or one supporting cell. *Phialides* not abundant, arising laterally or terminally from aerial mycelia, or from the supporting cells of the conidiophores, solitary, ampulliform or sometimes irregular,

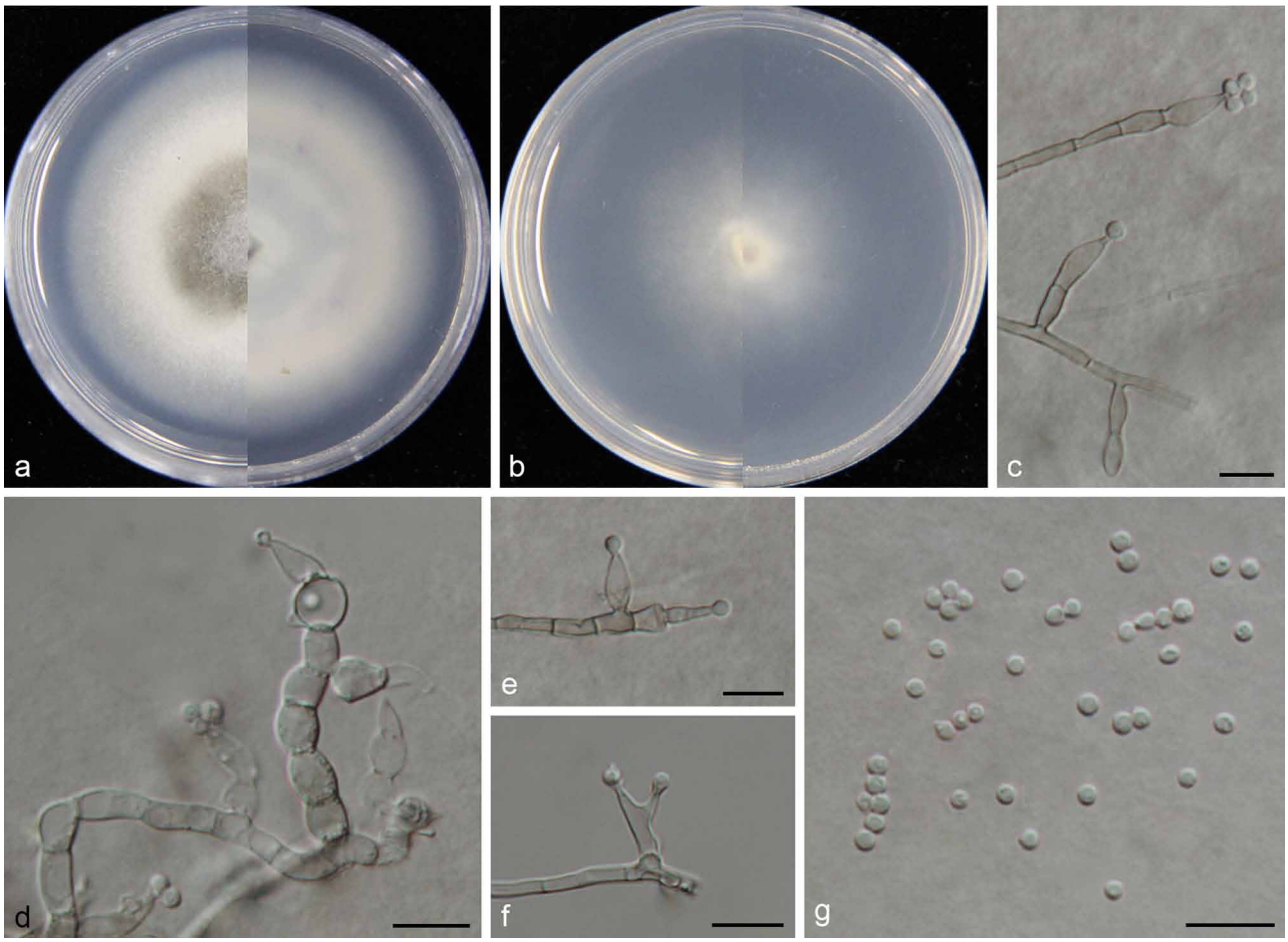

Fig. 18 *Ramophialophora petraea* (from ex-holotype CGMCC 3.17952). a–b. Upper and reverse views of cultures on PDA and SNA 21 d after inoculation; c–f. phialides and conidia; g. conidia. — Scale bars: c–g = 10 µm.

straight or curved, slightly constricted at the base and gradually tapering toward the apex, 7–15 × 2–4 µm, with one or occasionally two conspicuous collarettes. *Conidia* aggregated in small slimy heads, enteroblastic, globose, smooth, hyaline, 1.5–3 µm diam (mean = 2.2 ± 0.3 µm, n = 30), sometimes with a basal hilum.

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 2, N28°12'599" E107°13'661", rock, 19 July 2014, *Z.F. Zhang* (HMAS 246934 holotype designated here, ex-type living culture CGMCC 3.17952 = LC5789); ibid., CGMCC 3.17953 = LC6222.

Notes — This fungus is phylogenetically allied to *Ramophialophora* (MB818259), which was established by Calduch et al. (2004), and the morphological features were also similar. *Ramophialophora petraea* differs from the known species in the genus in its phialides which arise laterally or terminally from aerial mycelia or the supporting cells, and with 1 or 2 conspicuous collarettes.

Scopulariopsis crassa Z.F. Zhang, F. Liu & L. Cai, *sp. nov*. — MycoBank MB818260; Fig. 19

Etymology. Referring to its thick-walled conidia.

Colonies on PDA 23–35 mm diam after 10 d, felty, flat, margin fimbriate, pale brown, aerial mycelia sparse. Reverse pale brown. Colonies on SNA 28–32 mm diam after 10 d, flat, margin fimbriate, pale yellow. Reverse pale yellow.
Vegetative *hyphae* hyaline to pale brown, septate, branched, smooth- and thick-walled. *Conidiophores* arise from prostrate hyphae or aggregated in conidiomata, erect, straight or slightly curved, branched, septate, smooth-walled, hyaline to pale brown, 2–3.5 µm diam. *Conidiogenous cells* borne on aerial hyphae or conidiophores in whorls of 1–3, annellidic, hyaline, cylindrical, or some irregular shapes, slightly curved, occasionally septate, 15–43 × 3–6 µm. *Conidia* in chains, hyaline to pale brown, thick-walled, smooth or finely verrucose, globose or subglobose, 5–10.5 × 5–8.5 µm (mean = 7.8 ± 1.2 × 6.6 ± 1.1 µm, n = 40), with truncated bases.

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 2, N28°12'599" E107°13'661", soil, 19 July 2014, *X. Zhou* (HMAS 246935 holotype designated here, ex-type living culture CGMCC 3.17941 = LC5847); ibid., CGMCC 3.17942 = LC6225.

Notes — *Scopulariopsis crassa* is phylogenetically closely related to *S. asperula* and *S. candida* based on the analysis of ITS, LSU, *TUB*, and *EF1-α* sequences (phylogenetic tree deposited in MycoBank: MB818260). However, *S. crassa* can be differentiated from these two species in producing longer conidiogenous cells (15–43 µm for *S. crassa* vs 5–27 µm for *S. asperula*, 5–16 µm for *S. candida*).

Simplicillium calcicola Z.F. Zhang, F. Liu & L. Cai, *sp. nov*. — MycoBank MB818261; Fig. 20

Etymology. Referring to the substrate it was isolated from, calcaire.

Colonies on PDA attaining 34–38 mm diam after 10 d, cottony, compact, margin entire, white. Yellow pigment produced with aging. Reverse pale yellow to yellow. Colonies on SNA attaining 33–38 mm diam after 10 d, fluffy, margin entire, white. Reverse white.
Vegetative *hyphae* hyaline, aseptate, unbranched, smooth-walled. *Phialides* arise from prostrate hyphae or synnemata, solitary or up to 2–3 in whorls, straight or a little curved, tapering towards the apex, without basal septum, 14–38 × 1–2 µm.

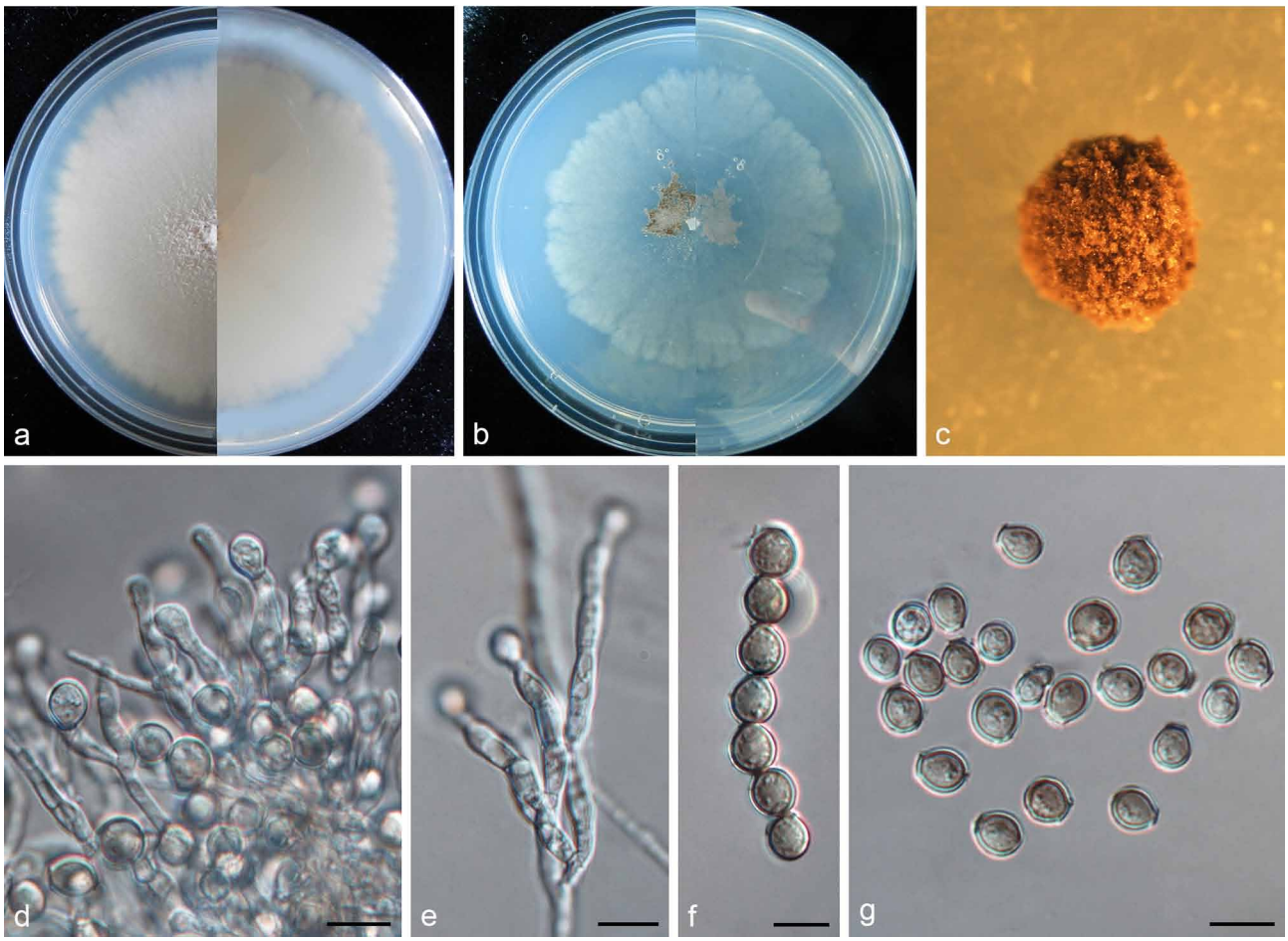

Fig. 19 *Scopulariopsis crassa* (from ex-holotype CGMCC 3.17941). a–b. Upper and reverse views of cultures on PDA and SNA 21 d after inoculation; c. conidiomata; d–e. conidiophores; f–g. conidia. — Scale bars: d–g = 10 µm.

Conidia variable in size and shape, 1-celled, smooth-walled; microconidia globose, oval or ellipsoidal, 2–3.5 × 1–1.5 µm (mean = 2.5 ± 0.3 × 1.4 ± 0.1 µm, n = 20), macroconidia fusiform, 4.5–8 × 1–2 µm (mean = 5.8 ± 0.9 × 1.4 ± 0.3 µm, n = 20). Octahedral crystals absent.

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 2, N28°12'599" E107°13'661", rock, 19 July 2014, *X. Zhou* (HMAS 246936 holotype designated here, ex-type living culture CGMCC 3.17943 = LC5586); ibid., CGMCC 3.17944 = LC5371.

Notes — *Simplicillium* is characterised by predominantly solitary phialides, conidial masses either in globose slimy heads, short chains, or formed in sympodial succession (Zare & Gams 2001, Nonaka et al. 2013). *Simplicillium lamellicola* is similar to *S. calcicola* in producing both microconidia and macroconidia. However, the octahedral crystals of *S. calcicola* are absent and its marcoconidia are wider than those of *S. lamellicola* (1–2 µm vs 0.8–1.2 µm).

Volutella aeria Z.F. Zhang & L. Cai, *sp. nov*. — MycoBank MB818262; Fig. 21

Etymology. Referring to the sample where this species was first isolated from.

Colonies on PDA attaining 37–43 mm diam after 14 d, ulotrichy, margin slightly undulate, white to pale brown. Reverse plicated, yellowish to brown. Colonies on SNA attaining 46–54 mm diam after 14 d, margin erose, white, aerial mycelia sparse. Reverse white.

Vegetative *hyphae* hyaline to brown, septate, branched, thin- and smooth-walled. Setae hyaline, aseptate, thick-walled, tapering to end, 300–600 µm long, 2.5–4 µm wide at base, swollen terminally or intermediately. *Sporodochia* sessile, globose, cream yellow, 100–220 µm diam, 150–280 µm high, with several marginal setae. *Conidiophores* hyaline, cylindrical, branched 1–3 times, 1.5–2.5 µm wide. *Conidiogenous cells* hyaline, cylindrical, 9–15 × 1.8–2.5 µm, gathered into a dense parallel layer. *Conidia* forming slimy heads on sporodochium, 1-celled, hyaline, bacillary, 5.5–8 × 1.5–2 µm (mean = 6.6 ± 0.6 × 1.7 ± 0.1 µm, n = 35). Verticillium-like synasexual morph present. *Conidiophores* on aerial hyphae hyaline, branched, septate, the axis 2–3 µm, producing 1–5 phialides per node laterally or in whorls of 3–6 phialides terminally. *Phialides* hyaline, aseptate, slender, tapering to end, 16–34 µm long, 1.5–2.5 µm wide at base. *Conidia* hyaline, smooth, cylindrical, with obtuse ends, solitary, 5.5–11.5 × 2–3.5 µm (mean = 7.5 ± 1.5 × 2.7 ± 0.4 µm, n = 40).

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 2, N28°12'599" E107°13'661", air, 19 July 2014, *Z.F. Zhang* (HMAS 246937 holotype deposited here, ex-type living culture CGMCC 3.17945 = LC5434); ibid., CGMCC 3.17946 = LC6216.

Notes — *Volutella* is characterised by discoid sporodochia with marginal setae, simple to verticillate conidiophores, compact and phialidic conidiogenous cells, and 1-celled, ovoid to oblong conidia; synasexual morph present in some species and with two or more whorls of conidiogenous cells (Gräfenhan et al. 2011, Luo & Zhuang 2012, Lombard et al. 2015). Only four species in the genus were known to produce sporodochia and verticillium-like synasexual morphs, i.e., *V. asiana*, *V. ciliata*, *V. consors*, and the new species *V. aeria* described in this study. *Volutella aeria* differs from *V. consors* in producing longer setae (300–600 µm vs 250–260 µm) and the absence of flaring collarette on sporodochia; from *V. ciliata* in its longer conidia (5.5–

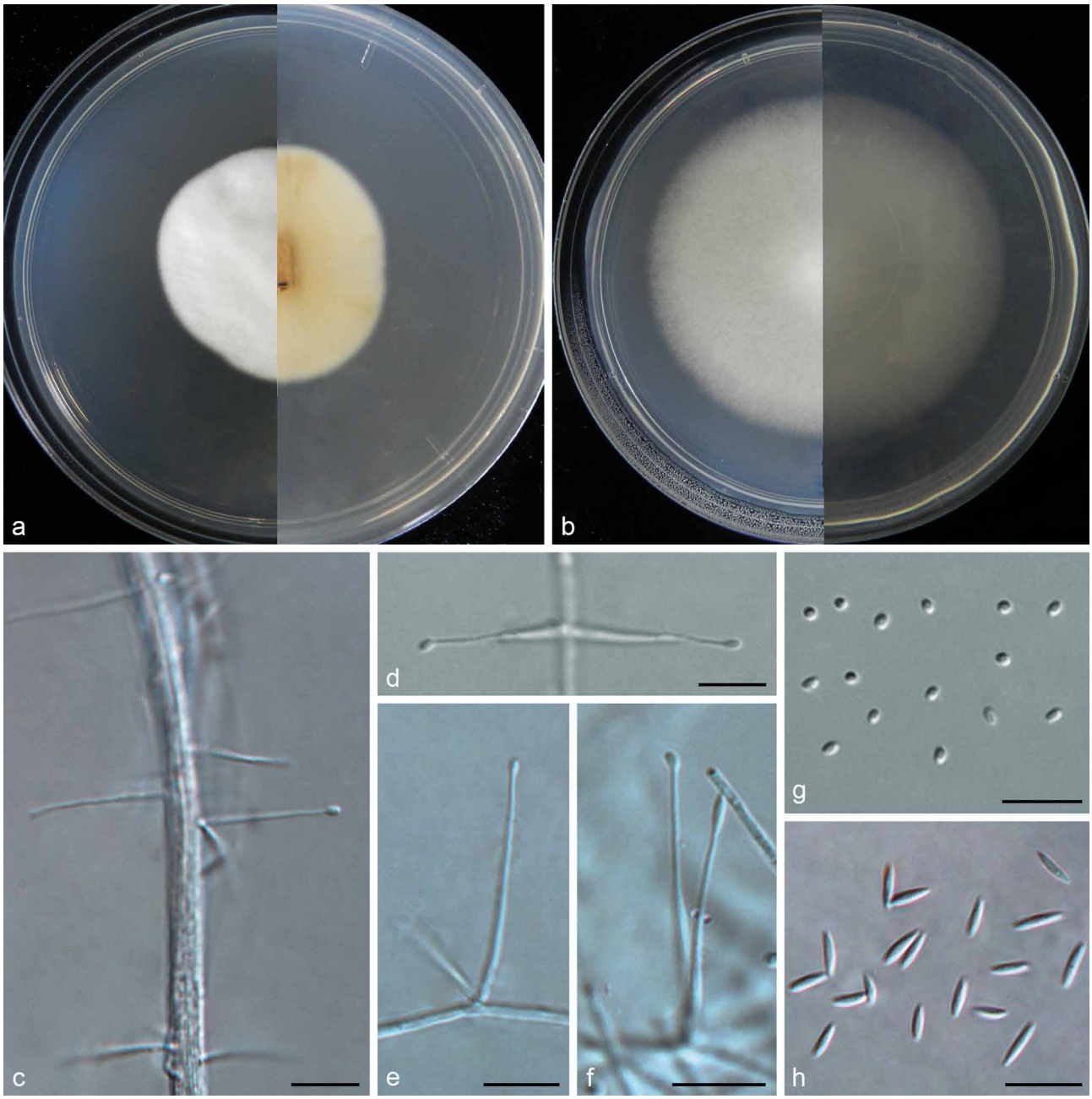

Fig. 20 *Simplicillium calcicola* (from ex-holotype CGMCC 3.17943). a–b. Upper and reverse views of cultures on PDA (10 d) and SNA (21 d); c. phialides on synnemata; d–f. phialides; g–h. microconidia and macroconidia. — Scale bars: c–h = 10 µm.

11.5 µm vs 3–5.5 µm); from *V. asiana* in producing conidiophores with whorls of phialides on aerial mycelia, while conidiophores in *V. asiana* are simple with a single phialide.

Wardomycopsis longicatenata Z.F. Zhang, F. Liu & L. Cai, *sp. nov.* — MycoBank MB818263; Fig. 22

Etymology. Referring to its long conidial chains.

Colonies on PDA 40–45 mm diam after 8 wk, felty, slightly raised, margin entire, yellow-green to dark grey. Reverse yellow-green to dark green. Colonies on SNA 45–51 mm diam after 8 wk, compact, aerial mycelia sparse, margin fimbriate, white to dark green. Reverse dark green.

Vegetative *hyphae* hyaline to pale brown, septate, branched, thin- and smooth-walled. *Ascomata* dark brown to black, immersed, globose or subglobose, 200–330 µm diam, 210–310 µm high, with unconspicuous ostiole. *Peridia* of *textura angularis*, olive green, appendages lacking. *Asci* ovate, globose or subglobose, 8-spored, 7–10.5 × 6–7.5 µm. *Ascospores* triangular to lunate, hyaline to pale red-brown, 3–4.5 × 2–3 µm (mean = 3.8 ± 0.3 × 2.7 ± 0.2 µm, n = 25). *Conidiophores* arising from hyphae, straight or flexuous, septate, occasionally branched one or two times, smooth, hyaline to pale brown. *Conidiogenous cells* solitary on aerial hyphae, or in whorls of 2–3 on apex of conidiophores, hyaline to pale brown, ampulliform, cylindrical, slightly curved, 3–6(–8.5) × 1.5–2.5 µm. *Conidia* in a long chain, brown, thick-walled, ellipsoidal, 4–6 × 2–2.5 µm (mean = 5.4 ± 0.4 × 2.1 ± 0.1 µm, n = 30), with truncated base and median longitudinal germ slit.

Specimens examined. China, Guizhou, Kuankuoshui National Natural Reserve, unnamed Karst Cave 2, N28°12'599" E107°13'661", air, 19 July 2014, *Z.F. Zhang* (HMAS 246938 holotype designated here, ex-type living culture CGMCC 3.17947 = LC5709); ibid., CGMCC 3.17948 = LC6226.

Notes — *Wardomycopsis* was established to accommodate asexual morphs of *Microascus* (Udagawa & Furuya 1978), and characterised by dark, globose, thick-walled conidia with germ slits that form short chains on annellidic conidiogenous cells (Silvera-Simón et al. 2008). However, recent phylogenetic study

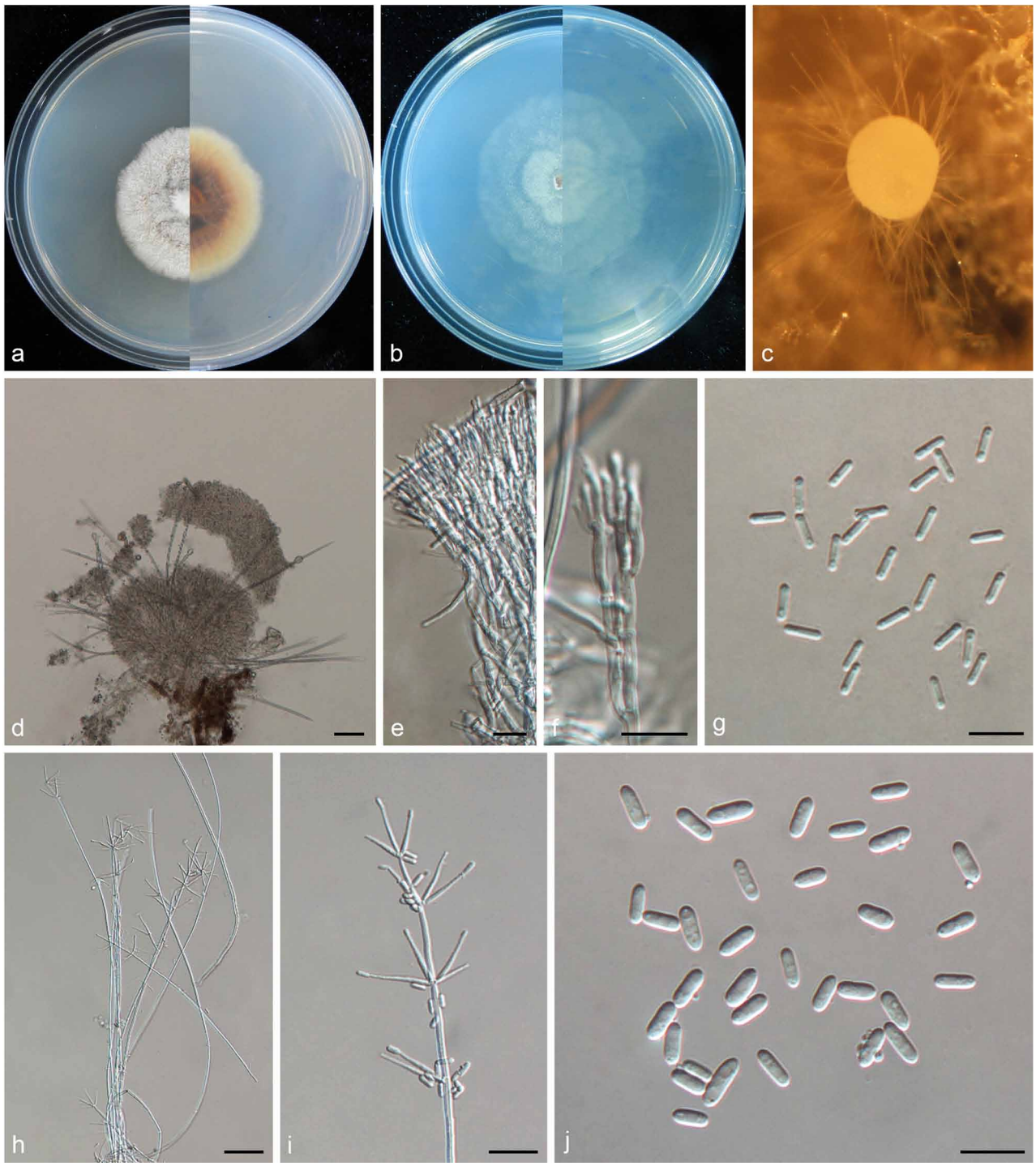

Fig. 21 *Volutella aeria* (from ex-holotype CGMCC 3.17945). a–b. Upper and reverse views of cultures on PDA and SNA 14 d after inoculation; c–d. sporodochia under stereomicroscope and microscope; e–f. conidiophores and phialides from sporodochia; g. conidia from sporodochia; h–i. conidiophores and phialides from aerial mycelia; j. conidia from aerial mycelia. — Scale bars: d = 100 µm; e–g, j = 10 µm; h = 50 µm; i = 20 µm.

based on the ITS and LSU sequences suggested that *Wardomycopsis* and *Microascus* are both monophyletic but distinct from each other (Sandoval-Denis et al. 2016). *Wardomycopsis longicatenata* clustered within *Wardomycopsis* and formed a distinct clade with high support value based on the ITS, LSU, *TUB*, and *EF1-α* sequence analysis (phylogenetic tree deposited in MycoBank: MB818263). Currently, this genus has four species. Morphologically *W. longicatenata* should be compared to *W. humicola* which produces similar ellipsoidal conidia. While they can easily be distinguished from one other by the different shapes of conidiogenous cells, which is ampulliform in *W. longicatenata* but ovoid to subglobose in *W. humicola*.

DISCUSSION

This study significantly improved our understanding of the mycobiota in caves and our data further suggested that fungal communities among different caves are largely different from each other (59 % of identified species in this study were reported for the first time from caves). The total number of species of *Ascomycota* was much higher than that of *Basidiomycota*, which may be explained by the lack of large, nutrient rich substrates such as plant debris or dung in the caves.

Our data also suggested that the majority of fungi documented from caves originated from the outside environment, in agreement to that of Vanderwolf et al. (2013). The most common species revealed in this study are very similar to that listed

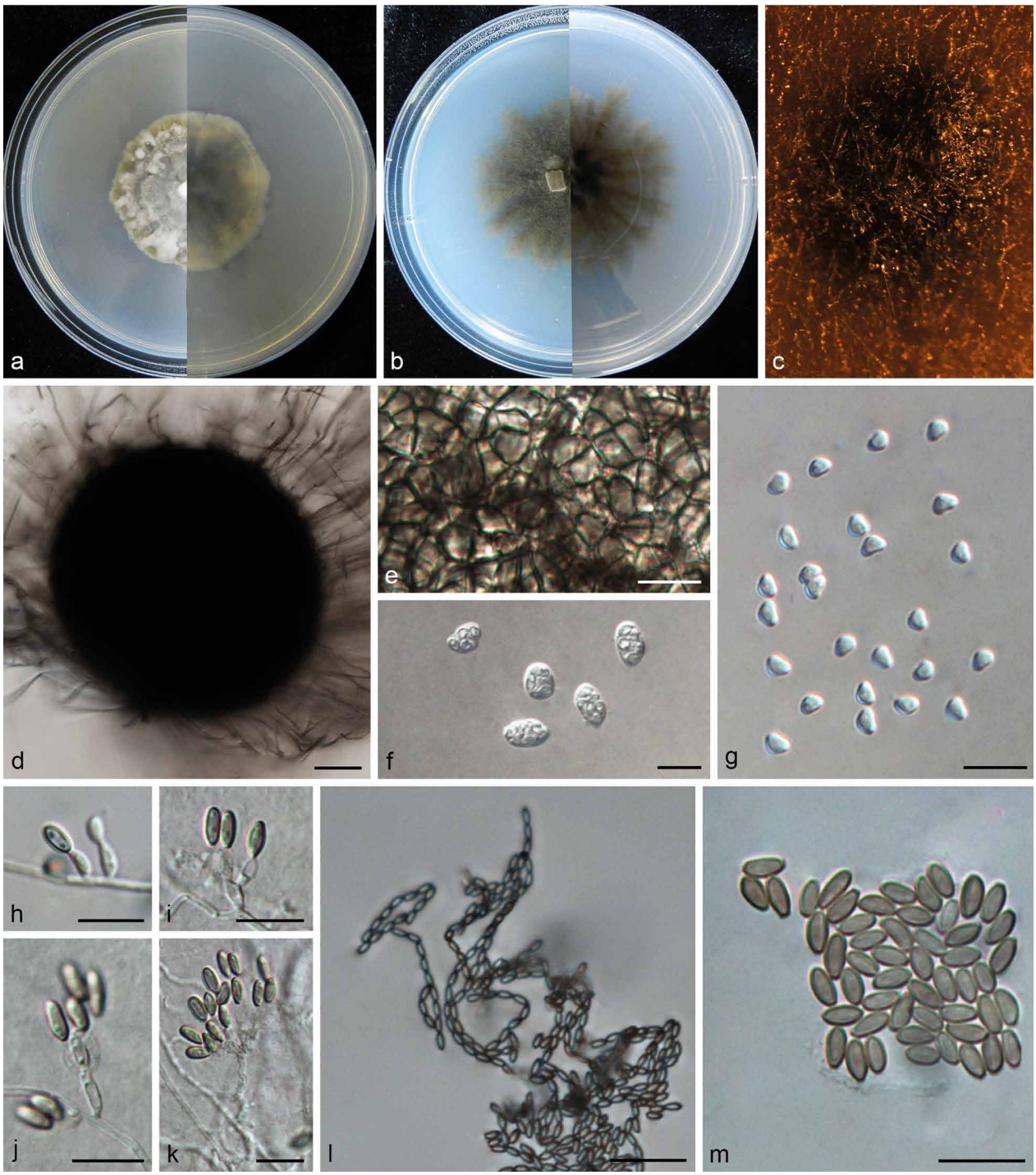

Fig. 22 *Wardomycopsis longicatenata* (from ex-holotype CGMCC 3.17947). a–b. Upper and reverse views of cultures on PDA and SNA 8 wk after inoculation; c. immersed ascoma; d. ascoma; e. peridium; f. asci; g. ascospore; h–k. conidiophores and conidiogenous cells; l–m. conidia. — Scale bars: d = 100 µm; e, l = 20 µm; f–k, m = 10 µm.

by Vanderwolf et al. (2013), which are commonly found in the above-ground environments. All genera recorded in this study are known from the other environments, and most species (83 %) have also been reported from other environments.

Kuzmina et al. (2012) suggested that cave systems might be a good harbour for the development and preservation of allochthonous microorganisms, including pathogenic species. Many species we obtained in this study were known as plant endophytic or pathogenic species. They may originate from outside environments; dispersed through water or air flow; and remain alive in caves. For example, *Diaporthe phoenicicola* isolated from soil in Cave 2, is a species known to cause fungal scleral keratitis in humans (Gajjar et al. 2011). *Fusarium graminearum* isolated from the air in Cave 1, is a plant pathogen which causes head blight of wheat (Bai & Shaner 2004). *Pestalotiopsis guepinii*, isolated from water on Cave 1, was reported to cause azalea petal blight in Argentina (Rivera & Wright 2000). Many species of *Colletotrichum*, *Cylindrocarpon*, and *Phoma* complexes investigated in this study are also well-known plant pathogenic fungi.

The distribution of microbial colonies in caves appeared to be largely determined by the bio-receptivity and susceptibility of the host materials, and the internal micro-environmental conditions, especially water availability and the mobilisation of nutrients in favour of the predominant mass and energy fluxes (Cuezva et al. 2009, Jurado et al. 2009). In our study, most fungi (99 species belonging to 59 genera) were isolated from organic litter compared to other samples, followed by soil, air,

rock, and water samples. Therefore, oligotrophy might be a major limitation of fungal colonisation in caves and higher fungal diversity would be discovered on samples with higher organic carbon concentration in caves (Bastian et al. 2010, Jurado et al. 2010, Kuzmina et al. 2012).

Whether obligate troglobitic fungi exist in caves is a very interesting question, but it needs further investigation. There are many species that have been exclusively isolated from caves (e.g., *Aspergillus baeticus*, *As. spelunceus*, *As. thesauricus*, *Chrysosporium* (*Ch.*) *chiropterorum*, *Ch. speluncarum*, *Microascus caviariformis*, *Mucor troglophilus*, *Ochroconis anomala*, *Ochroconis lascauxensis*, *Ombrophila speluncarum*, *Trichosporon* (*Tr.*) *akiyoshidainum*, *Tr. cavernicola*, *Tr. chiropterorum*) (Vanderwolf et al. 2013). Several of these species were also obtained in this study, such as *As. thesauricus* and *Tr. akiyoshidainum*. Currently we could not conclude if the 20 new species described in this study are obligate troglobitic fungi, or opportunistic colonisers that have dispersed from outside environments.

Several species obtained from this study may be potentially highly valuable. We obtained seven strains of *Amphichorda felina* (syn. *Beauveria felina*, *Isaria felina*), which is a widely known species in producing insecticidal cyclodepsipeptide (Baute et al. 1981, Langenfeld et al. 2011, Seifert et al. 2011). It would be interesting to investigate whether our new species *Amphichorda guana* generates insecticidal activity. Another example is *Trichoderma hamatum* isolated from soils from Cave 1 and Cave 2, a species that has been used as biocontrol agents against fungal diseases of plants (Harman 2006). *Trichoderma longibrachiatum*, a xylanase producing species, was isolated from soils in Cave 2 (Felse & Panda 1999).

In summary, our investigation of the culturable mycobiota in caves revealed a high fungal diversity, including a number of new species scattered in different families and orders. Fungal communities in different caves are largely different from each other but most of the identified species have been reported from other environments, and the outside environments appear to be the major source of fungal flora in caves. Although a number of new species were discovered in this study, this and previous studies on cave fungi did not find any new genera or families, indicating a lack of independently evolved fungal lineage in caves. This is possibly a reflection of the fact that the geographic history of caves (mostly shorter than two million years, Zhang et al. 2000) on earth is relatively short for fungal evolution and speciation. Future study should incorporate culture-independent methods to better reveal the overall picture of fungal diversity and community compositions in caves.

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