



Two new *Chrysosporium* (Onygenaceae, Onygenales) from China

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Abstract

Fungal isolates GZUIFR-EM14.2002 and GZUIFR-EM66601 were respectively isolated from Chinese soil samples under the snake skin in Guizhou Province and from the soil samples under the feathers in Hubei Province, China. Morphological and molecular evidence support both isolates as new species of *Chrysosporium*. Phylogenetic analysis based on ITS-5.8S rDNA sequences grouped GZUIFR-EM14.2002 together with *C. lucknowense* and *C. mephiticum*. GZUIFR-EM14.2002, which could be distinguished from the latter two species by the presence of abundant intercalary conidia, was named *C. guizhouense* sp. nov. In the phylogenetic tree, GZUIFR-EM66601 was most closely related to *C. submersum* and *C. siglerae*, GZUIFR-EM66601 differed from the other two species in having small obovate to ellipsoidal conidia and no intercalary conidia; this strain was designated as *C. hubeiense* sp. nov. Holotypes and their isolates had been deposited in GZAC, Guiyang, Guizhou Province, China.

Key words Filamentous fungi, taxonomy, identification

Introduction

The genus *Chrysosporium* Corda was introduced by Corda in 1833 (Oorschot 1980) and subsequently practically forgotten. Following Hughes' reintroduction of the name *Chrysosporium* (Hughes 1958), many new species have been reported and their classifications were studied (Carmichael, 1962; Oorschot, 1980). Known telemorphs that have been associated with the described species variously belong to the Gymnoasceae, Onygenaceae, Ascosphaeraceae and Sordariaceae in Ascomycetes, Ascomycota. According the Index Fungorum (<http://www.indexfungorum.org/Names/Names.asp>), 97 species have been reported to date. Excluding synonyms and invalid names, 86 species were currently recognized (Liang *et al.* 2007a).

With the development of molecular technology, phylogenetic analysis based on sequences of internal transcribed spacer regions 1 and 2 and 5.8S rDNA (ITS1-5.8S-ITS2) of 57 *Chrysosporium* species has recently revealed that the genus *Chrysosporium* is a polyphyletic taxon with affiliations to at least two orders of the Ascomycota and should be restricted to anamorphs of Onygenales (Vidal 2000). Pitt *et al.* (2013) studied the genus *Chrysosporium* using nuclear ribosomal large subunit (nrLSU) genes and transferred an extreme xerophilic species, *C. xerophilum* Pitt, to the new genus *Xerochrysium* (Pitt) Pitt. Only six new species have been reported in the past 5 years, namely, *C. guarroi* J. Cabañes & Abarca (Abarca *et al.* 2010), *C. speluncarum* A. Nováková & M. Kolařík (Nováková & Kolařík 2010), *C. longisporum* Stchigel, Deanna A. Sutton, Cano & Guarro (Stchigel *et al.* 2013), *C. magnasporum* Stchigel, Cano, Mac Cormack & Guarro (Crous *et al.* 2013), *C. qinghaiense* Y.F. Han, J.D. Liang & Z.Q. Liang (Han *et al.* 2013), *C. oceanitesii* Stchigel, Cano, Archuby & Guarro (2013) and *C. sanyaense* Y.F. Han & Z.Q. Liang (Zhang *et al.* 2013).

Chrysosporium spp. were mostly saprophytic and keratinolytic. They are widely distributed and can be isolated from various habitats such as air, sea, sludge, waste water (Padhye *et al.* 1967; Ulfig 1991; Ulfig & Korcz 1995; Deshmukh 1999). Vanbreuseghem (1952) and Simpanya & Baxter (1996) have used hair and wool as baits to effectively induce the growth of these fungi. During 2013–2015, in our studies of keratinophilic fungi in Chinese soils, two *Chrysosporium* isolates obtained from the habitats of feather and snakeskin were found to differ morphologically and phylogenetically from other *Chrysosporium* species. In this paper, we introduce them as two new species and provide micrographs and descriptions.

Materials and Methods

Sample collection and strain isolation

GZUIFR-EM14.2002 was isolated from a soil sample under the snakeskin collected from Kaiyang, Guizhou Province, China, while GZUIFR-EM66601 were isolated from the soil sample under the chicken feather in Songzi, Hubei Province, China. Soil samples were added to sterilized feather powder and kept moist at 25 °C for approximately one month. When fungal growth was observed, the feather powder was mixed with the sterilized water in an Erlenmeyer flask, and 1-mL suspensions were evenly spread on Martin's medium and incubated at 25°C. Then the pure cultures were then transferred to potato dextrose agar (PDA) slants and stored at -70°C at the Institute of Fungus Resources, Guizhou University (GZAC).

Morphological identification

Isolates were transferred to PDA and Czapek agar, incubated at 25 °C for 14 days, and subjected to macroscopic examination. Fungal microcharacteristics were examined with a Motic microscope (Guangzhou, Motic Co., China) and photographed. Diagnosis features were then illustrated on the basis of these observations. Finally, the fungi were morphologically identified according to colony characteristics and conidiogenous structures (Oorschot 1980; Han *et al.* 2013).

DNA extraction, PCR amplification and nucleotide sequencing

Total genomic DNA was extracted from fresh sporulating cultures at 25 °C for 7 days using a Fungal DNA Mini Kit (Omega Biotech, Doraville, GA, USA) according to the manufacturer's protocols and stored at -20 °C. ITS-5.8S rDNA region was amplified with primers ITS5 (5'-GGTGAGAGATTCTGTGC-3') and ITS4 (5'-TCCTCCGCTTAT TGA TATGC-3') (Han *et al.* 2013; Wen *et al.* 2015). The resulting PCR products were sequenced by Sangon Biotech (Shanghai, China) using the same primers. The generated ITS-5.8S rDNA sequences were submitted to GenBank (accession number: KJ849227 and KT948765).

Phylogenetic analysis

Sequences of 33 *Chrysosporium* species identified by Blast searching were downloaded from GenBank. A sequence of *Myceliophthora thermophila* (Apinis) Oorschot was also retrieved for use as an outgroup (Table 1). Alignment of the ITS-5.8S rDNA region of the 34 downloaded sequences and the sequences generated in this study was carried out using MAFFT (Katoh *et al.* 2013), followed by manual adjustment to allow maximum sequence similarity. Editing of sequences was performed in MEGA6 (Tamura *et al.* 2013), which yielded an output file in FASTA format. Phylogenetic analysis of the aligned sequences was performed in MrBayes 3.2 (Ronquist *et al.* 2012). One tree was saved to a file every 1,000 generations for a total of 10,000,000 Markov chain Monte Carlo generations. The GTR+G nucleotide substitution model was used as suggested by Modeltest 3.7 (Posada & Crandall 1998).

TABLE 1. The species list for the phylogeny analysis and the information of ITS1-5.8S-ITS2 rDNA.

Species names	Strains No.	GenBank No.	Species names	Strains No.	GenBank No.
<i>Chrysosporium articulatum</i>	UAMH 4320	AJ007841	<i>C. pilosum</i>	IMI 356294	AJ390385
<i>C. carmichaeliigi</i>	CBS 643.79	AJ007842	<i>C. pseudomerdarium</i>	CBS 631.79	AJ390386
<i>C. europae</i>	UAMH 4587	AJ007843	<i>C. qinghaiense</i>	GZUIFR-11	JX868607
<i>C. evolceanui</i>	RV26475	AJ005368	<i>C. queenslandicum</i>	IFM 51121	AB219228
<i>C. filiforme</i>	CBS 187.82	AJ131680	<i>C. sanyaense</i>	GZUIFR-A10222M	JQ809269
<i>C. fluviale</i>	FMR 6005	AJ005367	<i>C. siglerae</i>	UAMH 6541	AJ131684
<i>C. georgii</i>	CBS 272.66	AJ007844	<i>C. speluncarum</i>	CCF3761	AM949569
<i>C. indicum</i>	GZUIFR-3-4	HQ685965	<i>C. speluncarum</i>	CCF3760	AM949568
<i>C. keratinophilum</i>	IFO 7584	AJ131681	<i>C. submersum</i>	IMI 379911	AJ131686
<i>C. linfenense</i>	GZUIFR-H31	FJ392561	<i>C. sulfureum</i>	CBS 634.79	AJ390387
<i>C. luchnowense</i>	IMI 112798	AJ131682	<i>C. tropicum</i>	UAMH 691	AJ131685
<i>C. longisporum</i>	UTHSCR4380	HF547873	<i>C. undulatum</i>	IMI 375884	AJ007845
<i>C. lobatum</i>	CBS 666.78	AJ131688	<i>C. vallenarense</i>	CBS 627.83	AJ390389
<i>C. magnasporum</i>	FMR11770	HG329727	<i>C. vespertilium</i>	RV 27093	AJ007846
<i>C. mephiticum</i>	CBS 320.86	AJ131683	<i>C. zonatum</i>	IFM 51122	AB219229
<i>C. merdarium</i>	CBS 408.72	AJ390384	<i>C. guizhouense</i>	EM14.2002	KT948765
<i>C. minutisporosum</i>	IMI 379912	AJ131689	<i>C. hubeiense</i>	EM66601	KJ849227
<i>C. oceanitesii</i>	MR11771	HG329729	<i>Myceliophthora thermophila</i>	H127-1	JX868606

The aligned sequences were also analyzed using maximum parsimony (MP) and maximum likelihood (ML) methods in MEGA 6 (Tamura *et al.* 2013), with gaps treated as missing data and all other parameters following the default condition. Bootstrap support for nodes in the resulting trees was assessed using 1,000 replications per analysis. The final aligned data set is available in TreeBASE under submission ID18631.

Results

Phylogenetic analysis

Three methods (Bayesian inference/ML/MP) were used to phylogenetically analyze the 35 *Chrysosporium* ITS-5.8S rDNA sequences (Figure 1). The resulting three phylogenetic trees were congruent. Consequently, a combined tree is shown in Fig.1 with support values given at nodes for all three methods (Bayesian inference/ML/MP). In this tree, *C. guizhouense* EM14.2002 clusters with strong support (1.0/100%/98%) with *C. lucknowense* and *C. mephiticum*, while *C. hubeiense* EM66601 is grouped with *C. submersum* and *C. siglerae* with credible support (1/84%/57%).

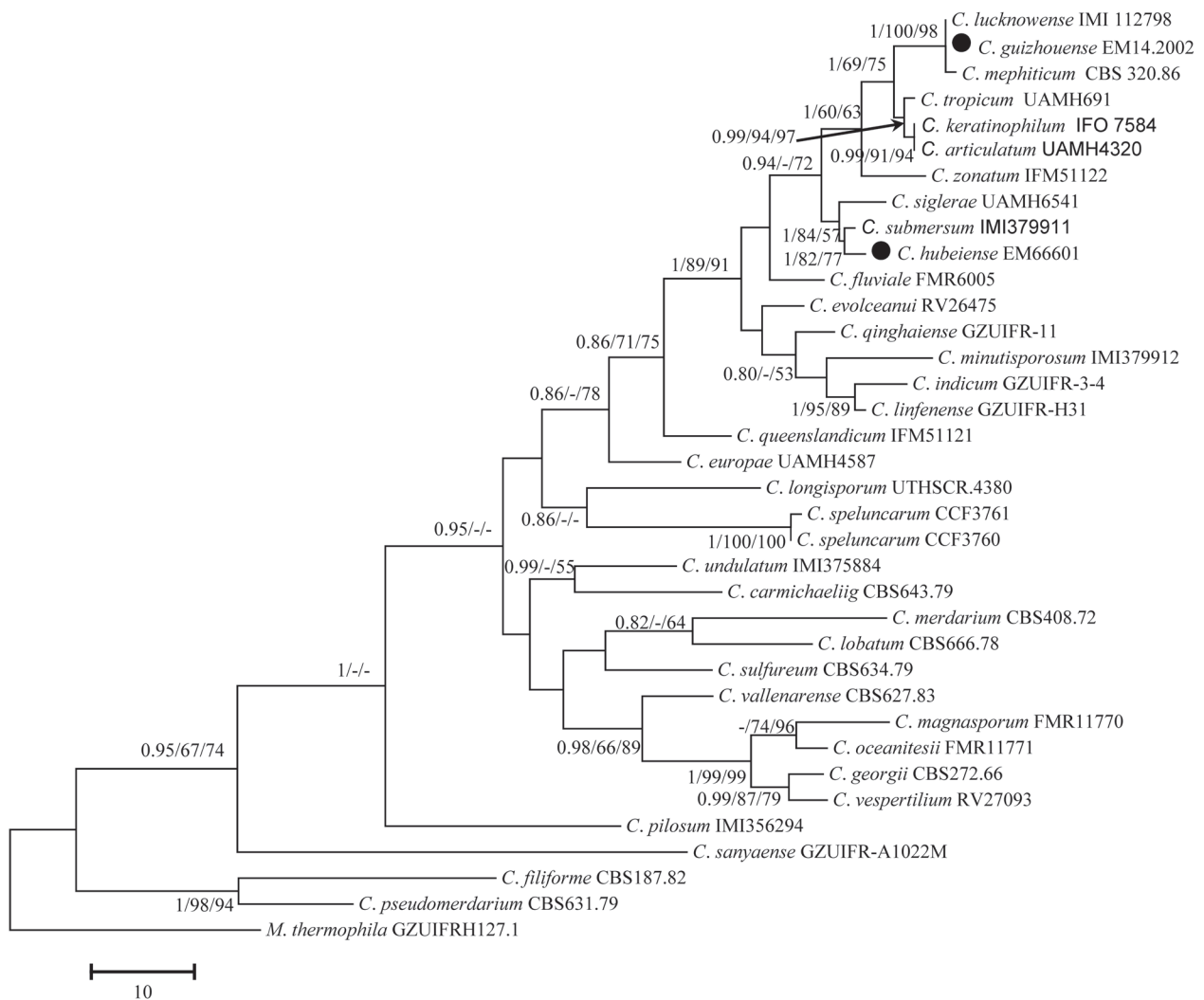


FIGURE 1. Phylogenetic tree of *Chrysosporium* spp. constructed from ITS-5.8S rDNA sequences. Statistical support values (Bayesian posterior probability/maximum likelihood bootstrap percentage/maximum parsimony percentage) are shown at nodes. The tree was rooted using *Myceliophthora thermophila* as an outgroup.

Description and Taxonomy

Chrysosporium guizhouense Y.W. Zhang, Y.F. Han & Z.Q. Liang sp. nov. (Fig.2)

GenBank: KT948765 MycoBank: MB 814991

Type:—CHINA. Guizhou Province: Kaiyang County, N27°19'51.07", E107°09'59.78". Holotype EM14.2002 was isolated from the soil under the dried snakeskin collected in Guizhou Province by Y.R. Wang.

Colonies on Czapek agar, attaining 18–20 mm in 14 d at 25 °C, white, fluffy, round. *Colonies* on PDA attaining 44–54 mm, white, fluffy, dense in the middle, sparse near the margin. Reverse yellowish. *Hyphae* hyaline to subhyaline, septate, smooth, 1.2–4.3 µm wide. *Racquet hyphae* present, 6.5–19.4 × 4.3–7.6 µm. *Terminal and lateral conidia* on short protrusions or side branches, solitary, hyaline, smooth, mostly single-celled, occasionally double-celled, subglobose, 2.2–4.3 µm; obovate to ellipsoidal, 5.4–6.5 × 3.2–4.3 µm (\bar{x} = 5.5 × 3.8, n = 60). *Intercalary conidia* abundant, appearing on the long lateral branches, barrel-shaped, irregularly cylindrical or ellipsoidal, 2.2–24.9 × 1.3–4.3 µm; *basal scars* 0.8–2.5 µm.

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

Distribution:—Guizhou Province, China

Material examined:—Dried culture EM14.2002 (holotype) and its isolate GZUIFR-EM14.2002 have been deposited at the Institute of Fungal Resource, Guizhou University (GZAC).

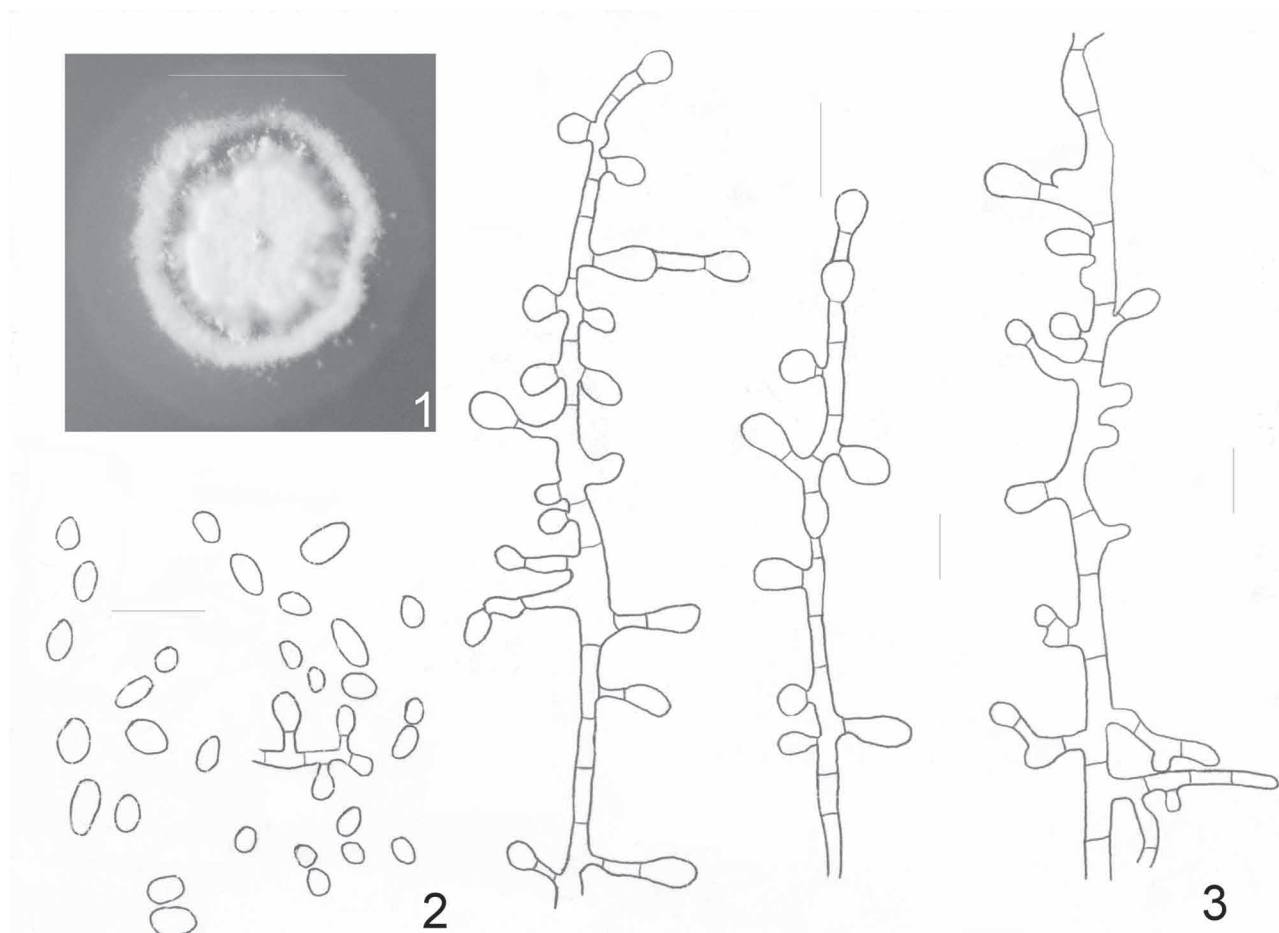


FIGURE 2. *Chrysosporium guizhouense* (holotype). 1. Colony; 2. Conidia 3. Conidiogenous structures; Bar1=10mm; Bars2-3=10µm.

Chrysosporium hubeiense Y.W. Zhang, Y.F. Han & Z.Q. Liang sp. nov. (Fig.3)

GenBank: KJ849227 MycoBank: MB 814992

Type:—CHINA. Hubei Province: Songzi, N30°10'22.11", E111°46'13.49". Holotype EM66601 was isolated from the soil under the feather collected in Guizhou Province by Y.R. Wang.

Colonies on Czapek agar, attaining 35–39 mm in 14 d at 25 °C, white, powdery, irregular at the margin. *Colonies* on PDA attaining 65–67 mm, gray white to white, flat, powdery, dense in the middle, sparse villiform near the margin. Reverse yellowish. *Hyphae* hyaline, septate, smooth, 1.1–2.2 µm wide. *Racquet hyphae* present, 5.4–7.6 × 2.2–3.2 µm. *Terminal and lateral conidia* on long or short protrusions perpendicular to hyphae, solitary, hyaline, smooth, obovate to ellipsoidal, 2.2–4.3 × 1.6–3.2 µm (\bar{x} = 3.1 × 2.0, n = 60); *basal scars* 2.2–3.2 µm wide. *Intercalary conidia* and *chlamydospores* absent.

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

Distribution:—Hubei Province, China.

Material examined:—Dried culture EM66601 (holotype) and its isolate GZUIFR–EM66601 have been deposited at the Institute of Fungal Resource, Guizhou University (GZAC).

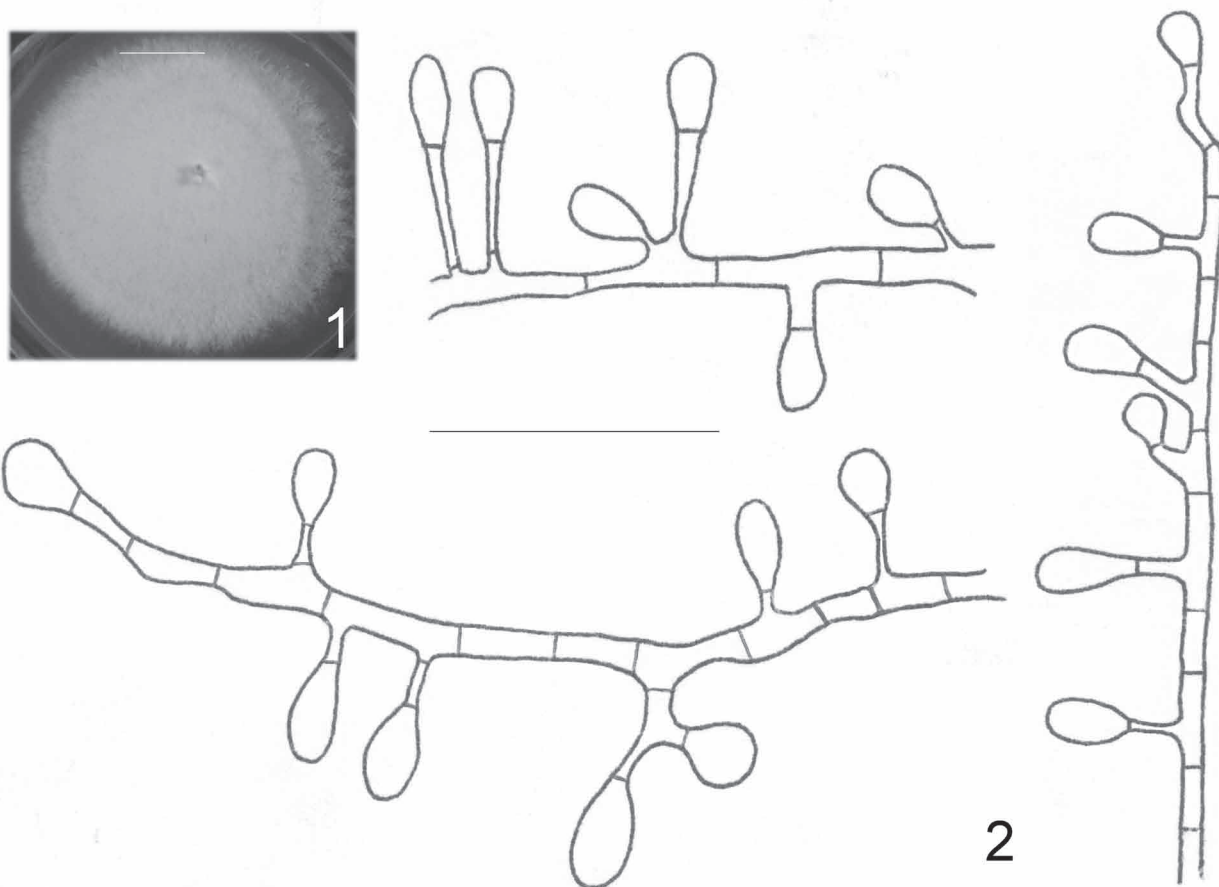


FIGURE 3. *Chrysosporium hubeiense* (holotype). 1. Colony; 2. Conidiogenous structures; Bar1=10mm; Bar2=10 μ m.

Discussion

Although *C. guizhouense* is closely related to *C. lucknowense* and *C. mephiticum* according to the phylogenetic tree, the latter two species do not produce intercalary conidia (Table 2) (Oorschot 1980). As a newly described species, the diagnostic characters of *C. guizhouense* are as follows: racquet hyphae present, terminal and lateral conidia mostly single-celled, occasionally double-celled, subglobose to obovate. Intercalary conidia abundantly present.

Chrysosporium hubeiense is clustered with *C. submersum* and *C. siglerae* in the phylogenetic tree. Because it lacks intercalary conidia (Table 2) (Oorschot 1980), however, *C. hubeiense* is morphologically different from the other two species and can be described as a new species on the basis of the following diagnostic characteristics: racquet hyphae present; terminal and lateral conidia obovate to ellipsoidal; and intercalary conidia and chlamyospore absent.

DNA sequences, especially those of ITS-5.8S rDNA, have become an important tool in evolutionary biology. Numerous studies worldwide have demonstrated that ITS-5.8S rDNA has the highest probability of successfully distinguishing most fungal species, an important characteristic for fungal identification and recognition (Kiss *et al.* 2012). Although both ITS-5.8S rDNA and nrLSU rDNA have been applied for phylogenetic reconstruction of the genus *Chrysosporium*, ITS-5.8S rDNA sequences were able to successfully distinguish the *Chrysosporium* species in this study.

In conclusion, our combined morphological and molecular analysis has confirmed EM14.2002 and EM66601 as two new taxa in the genus *Chrysosporium*.

TABLE 2. Morphological comparison among EM14.2002, EM 66601 and their allied species.

Species	Racquet hyphae	Conidia shape	Conidia surface	Conidia size (μm)	Intercalary conidia	Intercalary conidia shape	Intercalary conidia surface
<i>C. lucknowense</i>	Present	Subglobose	Smooth	2.5–11×1.5–6	Absent	-	-
<i>C. mephiticum</i>	Present	-	Smooth	2.5–3.5×2.5–3	Absent	-	-
EM14.2002	Present	Subglobose to obovate	Smooth	2.2–4.3×5.4–6.5	Present	Barrel-shaped or cylindrical	Smooth
<i>C. siglerae</i>	-	-	Rough	5–30×2–3.5	Present	Cylindrical	Smooth
<i>C. submersum</i>	Present	Clavate to subglobose	Rough	4–35×2.5–5	Present	Barrel-shaped, inflated at one end	Smooth
EM66601	Present	Obovate to ellipsoidal	Smooth	2.2–4.3×1.6–3.2	Absent	-	-

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