Phytotaxa 270 (3): 210–216
http://www.mapress.com/j/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.270.3.5

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

YAN-WEI ZHANG[1, 2], WAN-HAO CHEN[1], GUI-PING ZENG[1], YU-RONG WANG[1], XIAO ZOU, YAN-FENG HAN[1]*, SHU-YI QIU[1] & ZONG-QI LIANG[1]

[1]*College of Life Science, Guizhou University, Guiyang, Guizhou 550025, China*
**email: swallow1128@126.com*
[2] *School of Chemistry and Life Science, Guizhou Normal University, Guiyang, Guizhou 550018, China*

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.

 Accepted by Wen-Ying Zhuang: 5 Aug. 2016; published: 19 Aug. 2016

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'- GGTGAGAGATTTCTGTGC -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 Carto 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.
Chrysosporium articulatum	UAMH 4320	AJ007841	*C. pilosum*	IMI 356294	AJ390385
C. carmichaeliigi	CBS 643.79	AJ007842	*C. pseudomerdarium*	CBS 631.79	AJ390386
C. europae	UAMH 4587	AJ007843	*C. qinghaiense*	GZUIFR-11	JX868607
C. evolceanui	RV26475	AJ005368	*C. queenslandicum*	IFM 51121	AB219228
C. filiforme	CBS 187.82	AJ131680	*C. sanyaense*	GZUIFR-A10222M	JQ809269
C. fluviale	FMR 6005	AJ005367	*C. siglerae*	UAMH 6541	AJ131684
C. georgii	CBS 272.66	AJ007844	*C. speluncarum*	CCF3761	AM949569
C. indicum	GZUIFR-3-4	HQ685965	*C. speluncarum*	CCF3760	AM949568
C. keratinophilum	IFO 7584	AJ131681	*C. submersum*	IMI 379911	AJ131686
C. linfenense	GZUIFR-H31	FJ392561	*C. sulfureum*	CBS 634.79	AJ390387
C. luchnowense	IMI 112798	AJ131682	*C. tropicum*	UAMH 691	AJ131685
C. longisporum	UTHSCR4380	HF547873	*C. undulatum*	IMI 375884	AJ007845
C. lobatum	CBS 666.78	AJ131688	*C. vallenarense*	CBS 627.83	AJ390389
C. magnasporum	FMR11770	HG329727	*C. vespertilium*	RV 27093	AJ007846
C. mephiticum	CBS 320.86	AJ131683	*C. zonatum*	IFM 51122	AB219229
C. merdarium	CBS 408.72	AJ390384	***C. guizhouense***	**EM14.2002**	KT948765
C. minutisporosum	IMI 379912	AJ131689	***C. hubeiense***	**EM66601**	KJ849227
C. oceanitesii	MR11771	HG329729	*Myceliophthora thermophila*	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%).

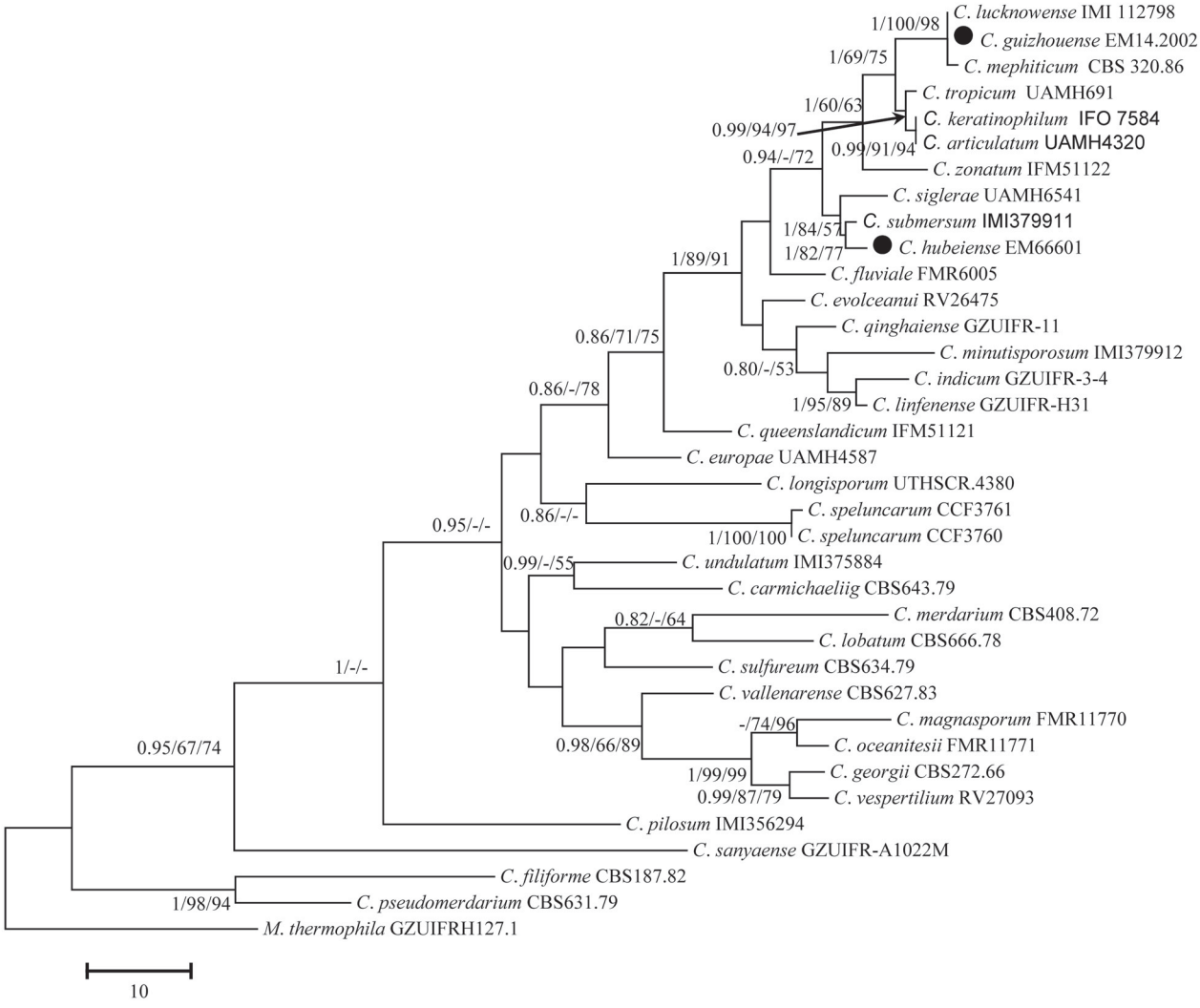

FIGURE 1. Phylogenetic tree of *Chrysosporium* spp. constructed from ITS-5.8S rDNA sequences. Statistical support values (Bayesian posterior probability/maximum likelihood bootstrap percentage/maximum parsimony percentage) are shown at nodes. The tree was rooted using *Myceliophthora thermophila* as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

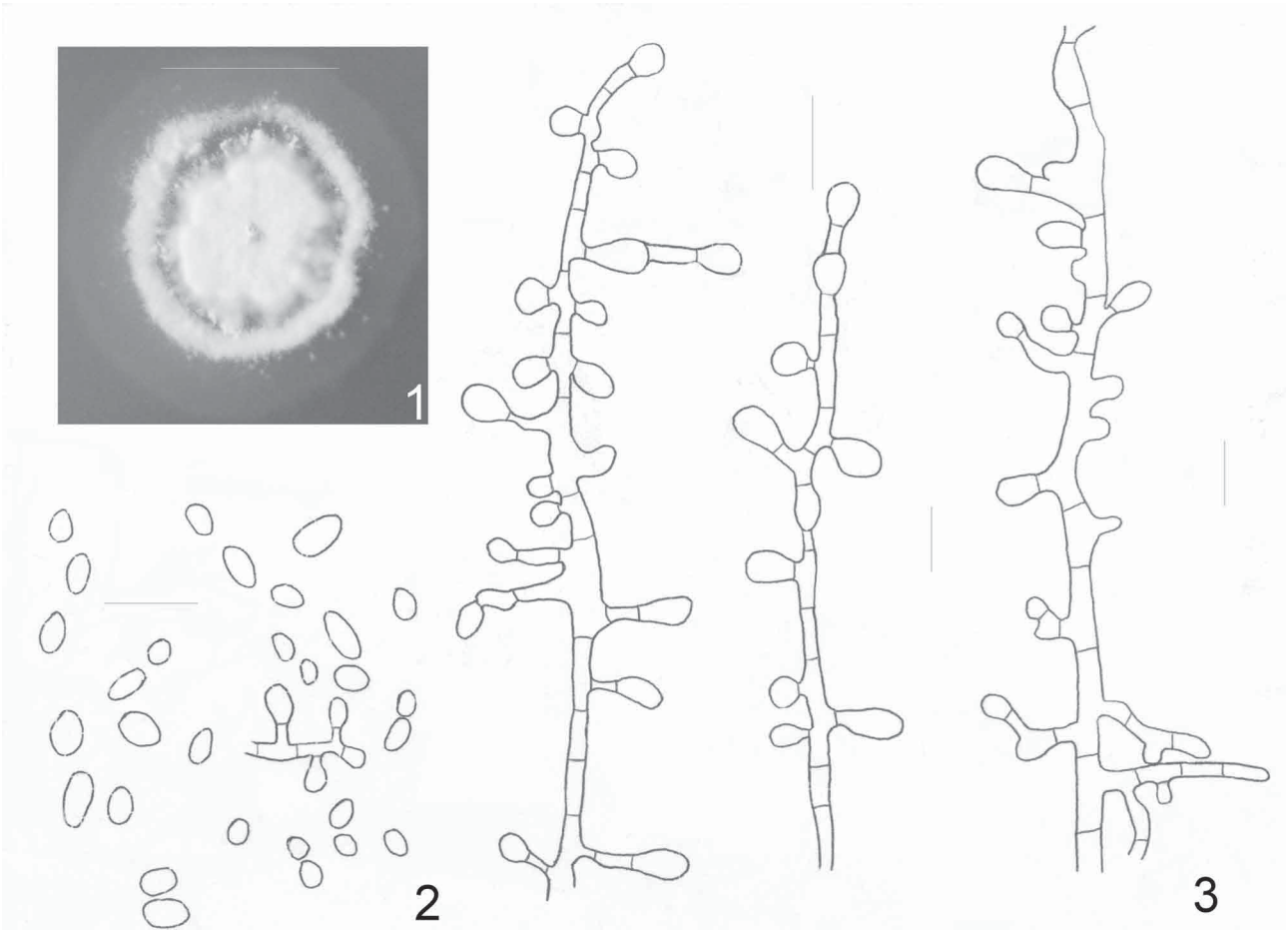

FIGURE 2. *Chrysosporium guizhouense* **(holotype). 1.** Colony; **2.** Conidia **3.** Conidiogenous structures; Bar1=10mm; Bars2-3=10µm.

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 ($\overline{x}$ = 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).

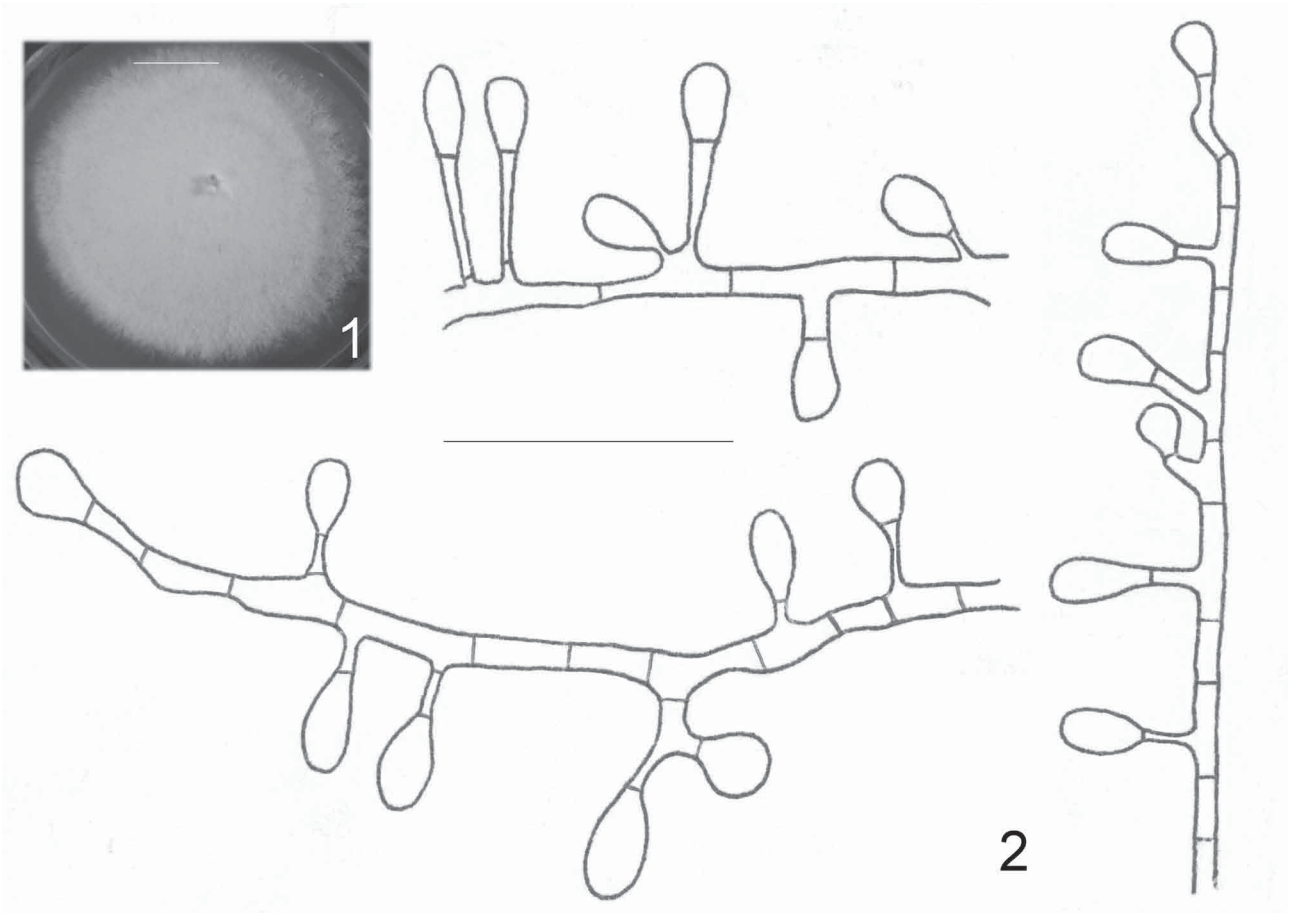

FIGURE 3. *Chrysosporium hubeiense* **(holotype). 1.** Colony; **2.** Conidiogenous structures; Bar1=10mm; Bar2=10µm.

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 chlamydospore 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
C. lucknowense	Present	Subglobose	Smooth	2.5–11×1.5–6	Absent	-	-
C. mephiticum	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
C. siglerae	-	-	Rough	5–30×2–3.5	Present	Cylindrical	Smooth
C. submersum	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	-	-

Acknowledgments

This work was supported by the National Natural Science Foundation of China (nos. 31460010, 31360453, 313600031, 31093440, 31493010, 31493011), Ministry of Science and Technology of China for Fundamental Research (no. 2013FY110400) and Excellent Youth Special Foundation of Guizhou Province (2013-05) and China Scholarship Council. We also thank Dr. Xiong Ting (College of English Languages, Sichuan University) and Dr. Zhang Jian for their careful English revision.

Reference

Abarca, M.L., Castellá, G., Martorell, J. & Cabañes, F.J. (2010) *Chrysosporium guarroi* sp. nov. a new emerging pathogen of pet green iguanas (*Iguana iguana*). *Medical Mycology* 48 (2): 365–372.
http://dx.doi.org/10.3109/13693780903173401

Carmichael, J.W. (1962) *Chrysosporium* and some other aleuriosporic hyphomycetes. *Canadian Journal of Botany* 40 (8): 1137–1173.
http://dx.doi.org/10.1139/b62-104

Crous, P.W., Wingfield, M.J., Guarro, J., Cheewangkoon, R., van der Bank, M., Swart, W.J., Stchigel, A.M., Cano-Lira, J.F., Roux, J., Madrid, H., Damm, U., Wood, A.R., Shuttleworth, L.A., Hodges, C.S., Munster, M., de Jesús Yáñez-Morales, M., Zúñiga-Estrada, L., Cruywagen, E.M., De Hoog, G.S., Silvera, C., Najafzadeh, J., Davison, E.M., Davison, P.J.N., Barrett, M.D., Barrett, R.L., Manamgoda, D.S., Minnis, A.M., Kleczewski, N.M., Flory, S.L., Castlebury, L.A., Clay, K., Hyde, K.D., Maússe-Sitoe, S.N.D., Chen, Shuaifei, Lechat, C., Hairaud, M., Lesage-Meessen, L., Pawłowska, J., Wilk, M., Śliwińska-Wyrzychowska, A., Mętrak, M., Wrzosek, M., Pavlic-Zupanc, D., Maleme, H.M., Slippers, B., Mac Cormack, W.P., Archuby, D. I., Grünwald, N.J., Tellería, M.T., Dueñas, M., Martín, M.P., Marincowitz, S., de Beer, Z.W., Perez, C.A., Gené, J., Marin-Felix, Y. & Groenewald, J.Z. (2013) Fungal Planet description sheets: 154–213. *Persoonia-Molecular Phylogeny and Evolution of Fungi* 31 (1): 188–296.
http://dx.doi.org/10.3767/003158513X675925

Han, Y.F., Wang, Y.R., Liang, J.D. & Liang, Z.Q. (2013) A new species of the genus *Chrysosporium* from the farmland soil of Qinghai province. *Mycosystema* 32 (4): 606–611. [in Chinese]

Hughes, S.J. (1958) Revisiones hyphomycetum aliquot cum appendice de nominibus rejiciendis. *Canadian Journal of Botany* 36 (6):727–836.
http://dx.doi.org/10.1139/b58-067

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30 (4): 772–780.
http://dx.doi.org/10.1093/molbev/mst010

Kiss, L., Schoch, C.L., Seifert, K.A., Caldeira, K., Myhrvold, N.P., Alvarez, R.A. & Mullins, R.D. (2012) Limits of nuclear ribosomal DNA internal transcribed spacer (ITS) sequences as species barcodes for Fungi. *Proceedings of the National Academy of Sciences* 109: 10741–10742.
http://dx.doi.org/: 10.1073/pnas.1207143109

Liang, J.D., Han, Y.F., Liang, Z.Q. & Zhang, J.W. (2007a) Primary screening of keratinase-producing strains of thermotolerant *Chrysosporium*. *Journal of Fungal Research* 5 (4): 210–213. [in Chinese]
http://dx.doi.org/10.13341/j.jfr.2007.04.010

Nováková, A. & Kolařík, M. (2010) *Chrysosporium speluncarum*, a new species resembling Ajellomyces capsulatus, obtained from bat guano in caves of temperate Europe. *Mycological Progress* 9 (2): 253–260.
http://dx.doi.org/10.1007/s11557-009-0634-0

Oorschot, C.A.N. Von (1980) A revision of *Chrysosporium* and allied genera. *Studies in Mycology* 20: 1–89.

Padhye, A.A., Pawar, V.H., Sukapure, R.S. & Thirumalachar, M.J. (1967) Keratinophilic fungi from marine soils of Bombay, India. *Hindustan Antibiotics Bulletin* 10: 138–141.

Pitt, J.I., Lantz, H., Pettersson, O.V. & Leong, S.L.L. (2013) *Xerochrysium* gen. nov. and Bettsia, genera encompassing xerophilic species of *Chrysosporium*. *IMA Fungus* 4 (2): 229–241.
http://dx.doi.org/10.5598/imafungus.2013.04.02.08

Posada, D. & Crandall, K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
http://dx.doi.org/10.1093/bioinformatics/14.9.817

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
http://dx.doi.org/10.1093/sysbio/sys029

Simpanya, M.F. & Baxter, M. (1996) Isolation of fungi from soil using the keratin-baiting technique. *Mycopathologia* 136: 85–49.
http://dx.doi.org/10.1007/BF00437500

Stchigel, A.M., Sutton, D.A., Cano-Lira, J.F., Cabañes, F.J., Abarca, L., Tintelnot, K., Wickes, B.L., García, D. & Guarro, J. (2013) Phylogeny of chrysosporia infecting reptiles: proposal of the new family Nannizziopsiaceae and five new species. *Persoonia-Molecular Phylogeny and Evolution of Fungi* 31 (1): 86–100.
http://dx.doi.org/10.3767/003158513X669698

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30 (12): 2725–2729.
http://dx.doi.org/10.1093/molbev/mst197

Ulfig, K. (1991) Occurrence of keratinophilic fungi in soil of homestead attached gardens polluted by industrial emissions. *Roczniki Panstwowego Zakladu Higieny (Poland)* 62 (4): 445–449.

Ulfig, K. & Korcz, M. (1995) Isolation of keratinolytic fungi from coal mine dump. *Mycopathologia* 129: 83–86.
http://dx.doi.org/10.1007/BF01103466

Vanbreuseghem, R. (1952) Technique biologique pour l'isolement des dermatophytes du sol. *Annales de la Société Belge de Medicine Tropicale* 32: 173–177.

Vidal, P., Vinuesa, M.D.L.A., Sánchez-Puelles, J.M. & Guarro, J. (2000) Phylogeny of the anamorphic genus *Chrysosporium*and related taxa based on rDNA internal transcribed spacer sequences. *Revista Iberoam Micologia* 17: 22–29.

Wen, T.C., Zha, L.S., Xiao, Y.P., Wang, Q., Kang, J.C. & Hyde, K.D. (2015) *Metacordyceps shibinensis* sp.nov. from larvae of Lepidoptera in Guizhou Province, southwest China. *Phytotaxa* 226 (1): 51–62.
http://dx.doi.org/10.11646/phytotaxa.226.1.5

Zhang, Y.W., Han, Y.F., Liang, J.D. & Liang, Z.Q. (2013) A new species of the genus *Chrysosporium* from the rhizosphere soil of China. *Mycosystema* 32 (4): 612–616.