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

YAN-WEI ZHANG¹,², WAN-HAO CHEN¹, GUI-PING ZENG¹, YU-RONG WANG¹, XIAO ZOU, YAN-FENG HAN¹*, SHU-YI QIU¹ & ZONG-QI LIANG¹

¹College of Life Science, Guizhou University, Guiyang, Guizhou 550025, China
*email: swallow1128@126.com
²School 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.
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’- GTGAGAGATTCTGTCG -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: 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.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrysosporium articulatum</td>
<td>UAMH 4320</td>
<td>AJ007841</td>
<td>C. pilosum</td>
<td>IMI 356294</td>
<td>AJ03588</td>
</tr>
<tr>
<td>C. carmichaeliigi</td>
<td>CBS 643.79</td>
<td>AJ007842</td>
<td>C. pseudomeridarium</td>
<td>CBS 631.79</td>
<td>AJ03588</td>
</tr>
<tr>
<td>C. europae</td>
<td>UAMH 4587</td>
<td>AJ007843</td>
<td>C. qinghaiense</td>
<td>GZUIFR-11</td>
<td>JX868607</td>
</tr>
<tr>
<td>C. evolceanui</td>
<td>RV26475</td>
<td>AJ005368</td>
<td>C. queenslandicum</td>
<td>IFM 51121</td>
<td>AB219228</td>
</tr>
<tr>
<td>C. filiforme</td>
<td>CBS 187.82</td>
<td>AJ131680</td>
<td>C. sanyaense</td>
<td>GZUIFR-A10222M</td>
<td>JQ809269</td>
</tr>
<tr>
<td>C. fluviale</td>
<td>FMR 6005</td>
<td>AJ005367</td>
<td>C. siglerae</td>
<td>UAMH 6541</td>
<td>AJ131684</td>
</tr>
<tr>
<td>C. georgii</td>
<td>CBS 272.66</td>
<td>AJ007844</td>
<td>C. speluncarum</td>
<td>CCF3761</td>
<td>AM949568</td>
</tr>
<tr>
<td>C. indicum</td>
<td>GZUIFR-3-4</td>
<td>HQ685965</td>
<td>C. speluncarum</td>
<td>CCF3760</td>
<td>AM949568</td>
</tr>
<tr>
<td>C. keratinophilum</td>
<td>IFO 7584</td>
<td>AJ131681</td>
<td>C. submersum</td>
<td>IMI 379911</td>
<td>AJ131686</td>
</tr>
<tr>
<td>C. linfenense</td>
<td>GZUIFR-H31</td>
<td>FJ392561</td>
<td>C. sulfurifer</td>
<td>CBS 634.79</td>
<td>AJ039037</td>
</tr>
<tr>
<td>C. luchnovense</td>
<td>IMI 112798</td>
<td>AJ131682</td>
<td>C. tropicum</td>
<td>UAMH 691</td>
<td>AJ131685</td>
</tr>
<tr>
<td>C. longisporum</td>
<td>UTHSRC4380</td>
<td>HF547873</td>
<td>C. undulatum</td>
<td>IMI 375884</td>
<td>AJ007845</td>
</tr>
<tr>
<td>C. lobatum</td>
<td>CBS 666.78</td>
<td>AJ131688</td>
<td>C. vallennarese</td>
<td>CBS 627.83</td>
<td>AJ390389</td>
</tr>
<tr>
<td>C. magnasporum</td>
<td>FMR11770</td>
<td>HG329727</td>
<td>C. vespertilium</td>
<td>RV 27093</td>
<td>AJ007846</td>
</tr>
<tr>
<td>C. mephiticum</td>
<td>CBS 320.86</td>
<td>AJ131683</td>
<td>C. zonatum</td>
<td>IFM 51122</td>
<td>AB219229</td>
</tr>
<tr>
<td>C. merdarium</td>
<td>CBS 408.72</td>
<td>AJ390384</td>
<td>C. guizhouense</td>
<td>EM14.2002</td>
<td>KT948765</td>
</tr>
<tr>
<td>C. minutisporosum</td>
<td>IMI 379912</td>
<td>AJ131689</td>
<td>C. hubeiensc</td>
<td>EM66601</td>
<td>KJ849227</td>
</tr>
<tr>
<td>C. oceaniitessii</td>
<td>MR11771</td>
<td>HG329729</td>
<td>Myceliophthora thermophila</td>
<td>H127-1</td>
<td>JX868606</td>
</tr>
</tbody>
</table>

TWO NEW CHrysosporium (ONYGENACEAE) Phytophaga 270 (3) © 2016 Magnolia Press • 211
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 (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 (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, subglobe 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 elliptoidal; and intercalary conidia and chlamydomspore 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>
<thead>
<tr>
<th>Species</th>
<th>Racquet hyphae</th>
<th>Conidia shape</th>
<th>Conidia surface</th>
<th>Conidia size (µm)</th>
<th>Intercalary conidia</th>
<th>Intercalary conidia shape</th>
<th>Intercalary conidia surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. lucknowense</td>
<td>Present</td>
<td>Subglobose</td>
<td>Smooth</td>
<td>2.5–11×1.5–6</td>
<td>Absent</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. mephiticum</td>
<td>Present</td>
<td>-</td>
<td>Smooth</td>
<td>2.5–3.5×2.5–3</td>
<td>Absent</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EM14.2002</td>
<td>Present</td>
<td>Subglobose to obovate</td>
<td>Smooth</td>
<td>2.2–4.3×5.4–6.5</td>
<td>Present</td>
<td>Barrel-shaped or cylindrical</td>
<td>Smooth</td>
</tr>
<tr>
<td>C. siglerae</td>
<td>-</td>
<td>-</td>
<td>Rough</td>
<td>5–30×2–3.5</td>
<td>Present</td>
<td>Cylindrical</td>
<td>Smooth</td>
</tr>
<tr>
<td>C. submersum</td>
<td>Present</td>
<td>Clavate to subglobose</td>
<td>Rough</td>
<td>4–35×2.5–5</td>
<td>Present</td>
<td>Barrel-shaped, inflated at one end</td>
<td>Smooth</td>
</tr>
<tr>
<td>EM66601</td>
<td>Present</td>
<td>Obovate to ellipsoidal</td>
<td>Smooth</td>
<td>2.2–4.3×1.6–3.2</td>
<td>Absent</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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


http://dx.doi.org/10.13341/j.jfr.2007.04.010

http://dx.doi.org/10.1007/s11557-009-0634-0


http://dx.doi.org/10.5598/imafungus.2013.04.02.08

http://dx.doi.org/10.1093/bioinformatics/14.9.817

http://dx.doi.org/10.1093/sysbio/sys029

http://dx.doi.org/10.1007/BF00437500

http://dx.doi.org/10.3767/003158513X669698

http://dx.doi.org/10.1093/molbev/mst197


http://dx.doi.org/10.1007/BF01103466


http://dx.doi.org/10.11646/phytotaxa.226.1.5