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Morphological and phylogenetic characterisations reveal nine new species of *Chrysosporium* (Onygenaceae, Onygenales) in China

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Abstract

Twenty-three strains of *Chrysosporium* were isolated from soil samples in different provinces of China. Phylogenetic analyses of *ITS* sequence data showed that these strains comprised nine new species and four already known species. The new species could be also distinguished from related species by their morphology. The results of phylogenetic and morphological analyses confirmed the nine new species of *Chrysosporium*. Descriptions and illustrations of these new species are provided.

Keywords: filamentous fungi, phylogeny, taxonomy

Introduction

Chrysosporium was introduced by Corda (1833) with the type species *C. corii* Corda. Saccardo (1901) regarded this genus as a synonym of *Sporotrichum* Link ex Fr. Carmichael (1962) redefined the genus *Chrysosporium* and regarded four genera *Myceliophthora* Costantin, *Geomyces* Traaen, *Blastomyces* Gilchrist & W.R. Stokes and *Emmonsia* Cif. & Montemart. as synonyms of *Chrysosporium*. Oorschot (1980) re-evaluated *Chrysosporium* and confirmed 22 species based on morphological characteristics.

With the development of molecular techniques, molecular systematics has been applied to identification of *Chrysosporium* species. Some new species have been reported based on morphology and *ITS* phylogeny (Han *et al*. 2013, Zhang *et al*. 2017, Li *et al*. 2019, Zhao *et al*. 2018, Zhang *et al*. 2020a). The genus currently comprises 67 species (Wijayawardene *et al*. 2020, Zhang *et al.* 2020a).

Chrysosporium is widely distributed and exists in various habitats such as air, sea, sludge, waste water and animal faeces (Deshmukh 1999, Zhang *et al*. 2016a, Zhang *et al*. 2020), especially in rich keratin substance (Zhang *et al*. 2017). Species of *Chrysosporium* can also be endomycorrhizal fungi of plants and affect the formation of active ingredients (Wang *et al*. 2013). Therefore, it is useful to explore species diversity and habitat distribution of *Chrysosporium* species throughout the world. During the investigation of *Chrysosporium* species from soils in China, 23 strains were discovered and nine new species were identified based on morphological observation and phylogenetic analysis. This paper provides a phylogenetic tree, descriptions, and illustrations of these novel species.

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Materials and methods

Fungal isolation and morphology

Soil samples were collected from different provinces in China, according to the methods described by Zhang *et al*. (2020b). Briefly, sterile chicken feathers and human hairs were combined with the soil samples. Samples were placed in sterile Petri dishes, and moistened with ddH_2O. The baited soil-sample Petri dishes were incubated at 26 °C for 1 month and remoistened as necessary. After that, 2 g portions of sample were added to test tubes containing 9 mL of ddH_2O. The mixture was then diluted to $1:10^3$ and 1 mL of suspension was evenly spread on plates containing Sabouraud's dextrose agar (SDA; 10 g peptone, 40 g dextrose, 20 g agar, 3.3 mL of 1% Bengal red aqueous solution, and 1 L ddH_2O) with anti-bacterial chloramphenicol and cycloheximide. Plates were incubated at 26 °C for 5 days. The axenic strains were then transferred to potato dextrose agar (PDA, Shanghai Bio-way technology Co., China) plates for purification and for preparation of test tube slants for storage at 4 °C. Dried holotype, ex-holotype and ex-isotype strains were deposited in the Institute of Fungus Resources, Guizhou University (formally Herbarium of Guizhou Agricultural College; code GZAC), Guiyang City, Guizhou, China. The pure strains were incubated on PDA at 26 °C in darkness. Macroscopic characterization was performed after 7 days of incubation, and the colony colours (surface and reverse) were observed. Line drawing of diagnostic structures were made following examination by a light microscope.

DNA extraction, *PCR amplification and nucleotide sequencing*

DNA extraction was carried out according to Zhang *et al*. (2016b). The extracted DNA was stored at −20 °C. The internal transcribed spacer (*ITS*) region was amplified using ITS4/ITS5 primers by PCR according to the procedures described by White *et al*. (1990). PCR products were purified using the UNIQ-10 column PCR products purification kit [no. SK1141; Sangon Biotech (Shanghai) Co., Shanghai, China] according to the manufacturer's protocol and sequenced at Sangon Biotech (Shanghai) Co. The resulting sequences were submitted to GenBank (Table 1).

TABLE 1. The strains isolated in this study together with specimen and *ITS* GenBank numbers.

Species name	Dried specimen no.	GenBank no.
Chrysosporium alvearium	EB4301M	MW485632
C. fusiforme	GZAC.I8^T	MW485676
C. fusiforme	GZAC.I9	MW485678
C. fusiforme	GAZC.L17.2	MW485688
C. gansuense	GZAC.C4.1^T	MW485666
C. gansuense	GZAC.C4.2	MW485669
C. guangxiense	EB9001M^T	MW488044
C. hubeiense	GZAC.D14.3	MW485670
C. hubeiense	GZAC.D13.3	MW485668
C. hubeiense	GZAC.E5.2	MW485672
C. irregularum	GZAC.J1.1^T	MW488042
C. irregularum	GZAC.J102	MW485682
C. jiangsuense	GZAC.I10^T	MW485677
C. kaiyangense	EB0402M^T	KY318071
C. keratinophilum	GZAC.E20.2	MW485631
C. multiforme	GZAC.U3^T	MW485687
C. multiforme	GZAC.U302	MW485686
C. ovalisporum	EB0702M	KY318072
C. sichuanense	GZAC.FX8^T	MW485634
C. sichuanense	GZAC.I18	MW485680
C. sichuanense	GZAC.I17	MW485679
C. villiforme	GZAC.L19.4^T	MW485678
C. villiforme	GZAC.L19.5	MW485688

^{T} Ex-type culture

Sequence alignment and phylogenetic analyses

The DNA sequences generated in this study were assembled and edited using Lasergene software (version 6.0, DNASTAR). Sequences of *ITS* were selected based on Han *et al*. (2013), Zhang *et al*. (2017), Li *et al*. (2019), Zhao *et al*. (2018), Zhang *et al*. (2020a), Wijayawardene *et al*. (2020) and the result of a Blast search in GenBank. Multiple sequence alignments for *ITS* were carried out using MAFFT v7.037b (Katoh & Standley 2013). Sequence editing was performed with MEGA6 (Tamura *et al*. 2013) and the resulting output was in Fasta file format. The combined data was analyzed phylogenetically using Bayesian MCMC and maximum likelihood (ML). For the Bayesian analysis, two runs were executed simultaneously for 10,000,000 generations, saving trees every 500 generations, with the SYM+I+G4 nucleotide substitution model across all partitions, in MrBayes 3.2 (Ronquist *et al*. 2012). After the analysis was finished, each run was examined with the program Tracer v1.5 (Drummond & Rambaut 2007) to determine burn-in and confirm that both runs had converged. The ML analysis were implemented using RAxML (Stamatakis 2014), the robustness of branches was assessed by bootstrap analysis with 1000 replicates. The final alignment is available from TreeBASE under submission ID 27786.

Results

The study found 13 species of *Chrysosporium* of which nine were shown to be new species. The other four species, *C. keratinophilum*, *C. alvearium*, *C. hubeiense*, and *C. ovalisporum* have been already described from China.

Sequencing and phylogenetic analysis

The *ITS* sequences from strains were deposited in GenBank (Table 1). The alignment of *ITS* sequences was 589 bp long. The topological structures were similar in both ML and Bayesian analyses (Fig. 1).

The phylogenetic analyses showed that there were several clades in the genus *Chrysosporium.* The 23 *Chrysosporium* strains isolated in this study clustered in different subclades. Sequences MW485687 and MW485686 of *C. multiforme sp. nov.* clustered to a separate subclade and related to *C. keratinophilum.* Both sequences MW485666 and MW485669 of *C. gansuense sp. nov.* were related to *C. shanxiense* and *C. tropicum*. Both sequences MW488042 and MW485682 of *C. irregularum sp. nov.* were in a separate clade with high bootstrap value. *C. jiangsuense sp. nov*. MW485677 clustered in a subclade with *C. indicum* and *C. linfenense*; Three sequences MW485676, MW485678 and MW485688 of *C. fusiforme sp. nov*. clustered in a separate subclade with *C. jiangsuense*, *C. linfenense* and *C. indicum* with high bootstrap value. Sequences MW485684 and MW485683 of *C. villiforme sp. nov*. were closely related to *C. qinghaiense* in a separate subclade. *C. guangxiense sp. nov*. MW488044 was a separate subclade and related to *C. alvearium. C. sichuanense sp. nov.* including three sequences MW485634, MW485680 and MW485679 obviously separated from other *Chrysosporium* spp. with high bootstrap value. *C. kaiyangense sp. nov*. KY318071 was closely related to *C. ovalisporum*. The sequence MW485631 clustered into *C. keratinophilum* clade, sequence MW485632 clustered into *C. alvearium* clade, sequences MW485670, MW485668 and MW485672 clustered into *C. hubeiense* clade, and sequence KY318072 clustered into *C. ovalisporum* clade.

Taxonomy

Chrysosporium fusiforme Y.F. Han, W.H. Chen, J.D. Liang & Z.Q. Liang, *sp. nov*. (Fig. 2) Mycobank No.: MB 838863

Type:—CHINA. Shanxi Province: Jinzhong City, Qixian (N37.37°, E112.28°), from soil, August 2017, Y.F. Han, holotype GZAC.I9; ex-type culture GZU.I9.

Colonies on PDA attaining about 35 mm diam. at 26 °C after 7 days, flat, felty, margin villiform, creamy to white from center to margin; reverse creamy to white from center to margin. *Hyphae* septate, smooth, hyaline, 1.0–2.5 µm. *Racquet hyphae* absent. *Conidia* abundant, hyaline, smooth, lateral or terminal, arising from aerial hyphae directly or on short protrusions, unicellular, fusiform, 5.5–10.5 × 2.0–4.0 µm, or ovoid, 3.5–5.0 × 1.0–3.0 µm, with truncate base, basal scars 1.0–1.5 µm; intercalary conidia fusiform, 6.5–9.0 × 2.5–3.0 µm. Chlamydospores absent.

Etymology:—Referring to the shape of conidia.

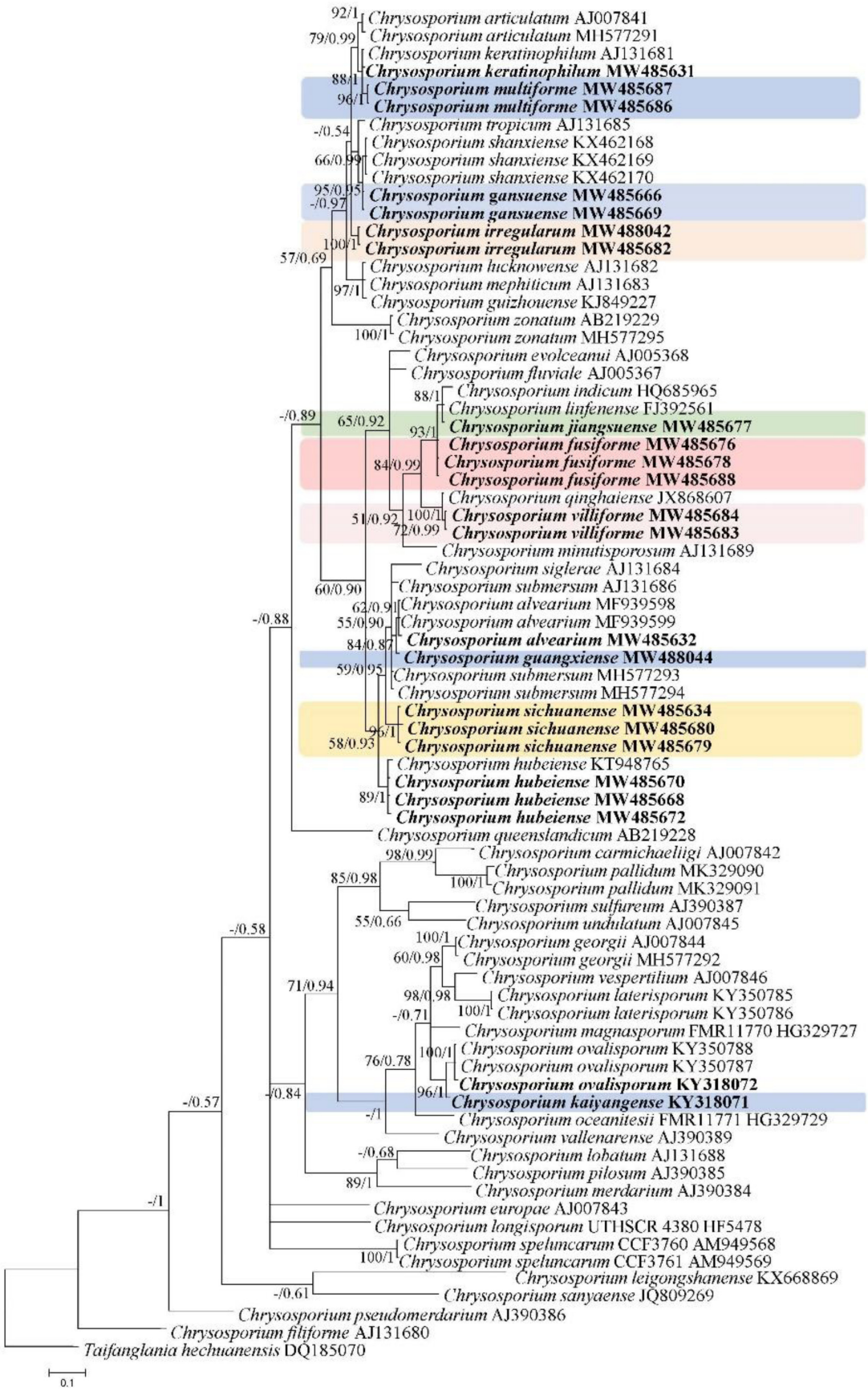

FIGURE 1. Phylogenetic analysis of *Chrysosporium* spp. based on *ITS* sequences. Statistical support values (≥50 %) are shown at nodes, and presented as ML bootstrap support/Bayesian posterior probabilities. Names in black bold are the strains isolated in this study, the coloured names are the new species.

Additional strains examined:—CHINA. Shanxi: Linfen, soil, N36.09°, E110.68°, August 2017, Y.F. Han, GZAC.I8 and GZAC.L17.2, their living cultures GZU.I8 and GZU.L17.2.

Known distribution:—Jinzhong and Linfen city, Shanxi Province, China.

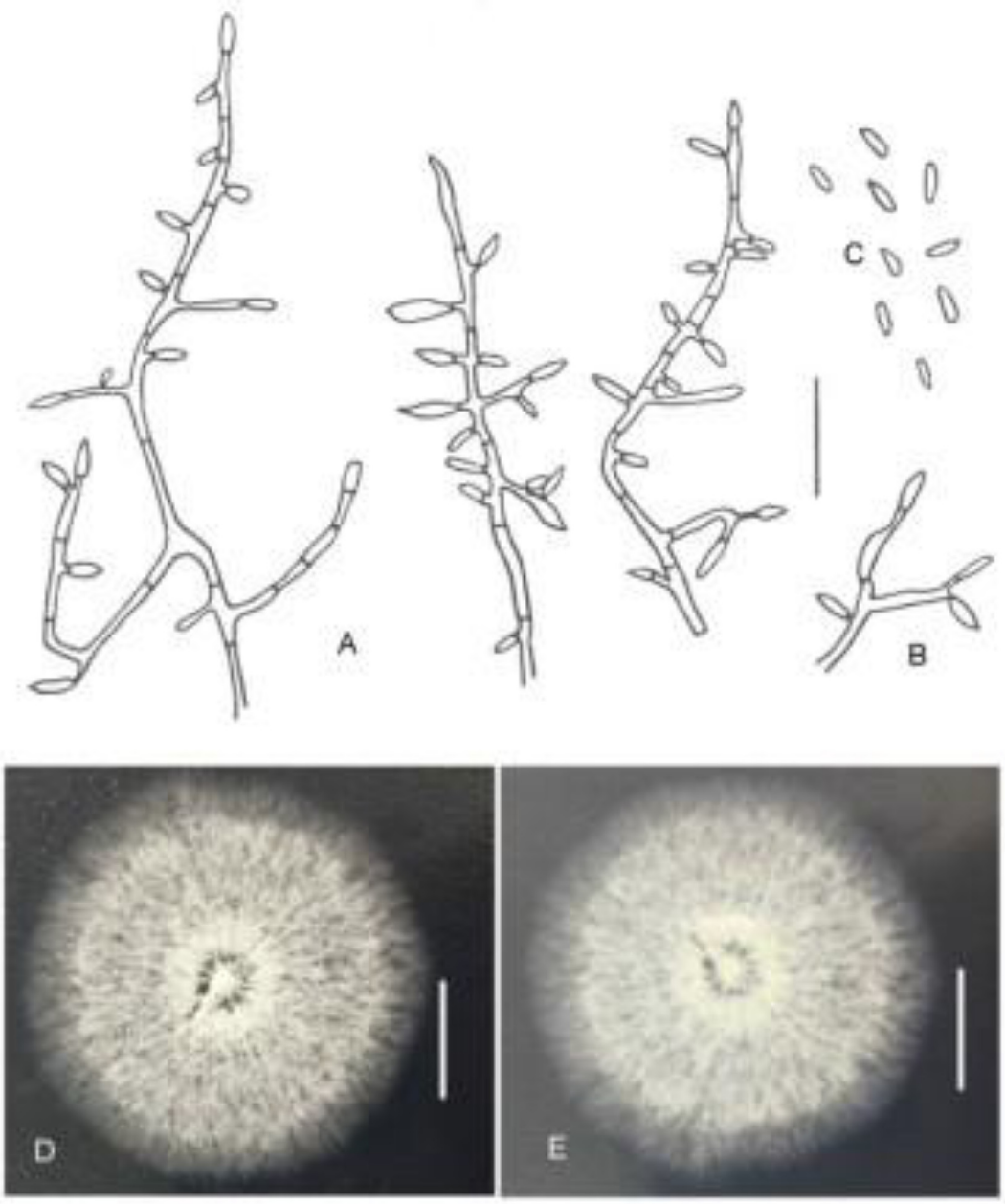

FIGURE 2. *Chrysosporium fusiforme* (holotype). A–B. Conidiogenous structures. C. Conidia. D–E. Colonies (front and reverse) on PDA media. Bars A–C = 20 μm, D–E = 10 mm.

Chrysosporium gansuense Y.F. Han, W.H. Chen, J.D. Liang & Z.Q. Liang, *sp. nov*. (Fig. 3) Mycobank No.: MB 838864

Type:—CHINA. Gansu Province: Jiayuguan City, N39.47°, E98.17°, from soil, August 2017, Y.F. Han, holotype GZAC.C4.1; ex-type culture GZU.C4.1.

Colonies on PDA attaining about 40 mm diam. at 26 °C after 14 days, flat, powdery, margin villiform, creamy to white from center to margin; reverse creamy. *Hyphae* septate, smooth, hyaline, 0.5–2.0 μm. *Racquet hyphae* absent. *Conidia* abundant, hyaline, rough, lateral or terminal, arising from aerial hyphae directly or on short protrusions, unicellular, pyriform or clavate, 3.5–6.0 × 1.5–3.5 μm, or ellipsoidal, 3.5–4.5 × 2.5–4.0 μm, with truncate base, basal scars 0.5–1.0 μm; intercalary conidia ellipsoidal, 3.0–5.0 × 1.5–2.5 μm. Chlamydospores absent.

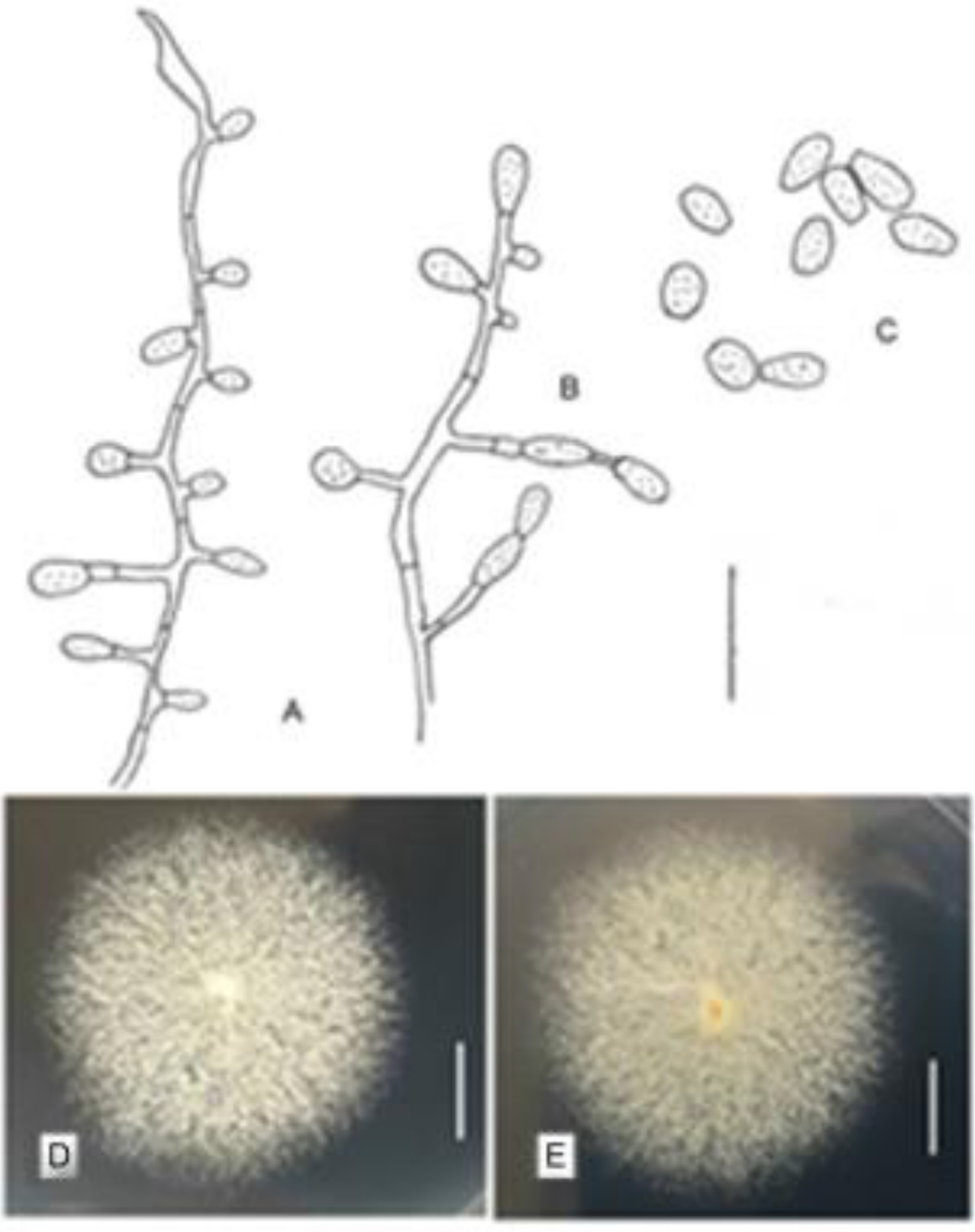

FIGURE 3. *Chrysosporium gansuense* (holotype). A–B. Conidiogenous structures. C. Conidia. D–E. Colonies (front and reverse) on PDA media. Bars A–C = 10 μm, D–E = 10 mm.

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

Additional strains examined:—CHINA. Gansu Province: Jiayuguan, soil, N39.47°, E98.17°, August 2017, J.J. Wang, GZAC.C4.2, living culture GZU.C4.2.

Known distribution:—Jiayuguan city, Gansu Province, China.

Chrysosporium guangxiense Y.F. Han, W.H. Chen, J.D. Liang & Z.Q. Liang, *sp. nov*. (Fig. 4) Mycobank No.: MB 838865

Type:—CHINA. Guangxi Province: Guilin City, N24°18'', E09°45'', from soil, August 2017, Y.F. Han, holotype GZAC.EB9001M; ex-type culture GZU.EB9001M.

Colonies on PDA attaining about 45–50 mm diam. at 26 °C after 14 days, flat, felty, obvious annulation in the center, margin villiform, white; reverse creamy to yellowish. *Hyphae* septate, smooth, hyaline, 1.0–3.0 µm. *Racquet hyphae* present, 17–50 × 2.5–5.5 µm. *Conidia* abundant, hyaline, smooth, lateral or terminal, arising from aerial hyphae directly or on short protrusions, unicellular, solitary or in cluster of 2, long ovoid, 5.0–8.5 × 3.5–7.0 µm, or clavate, 7.0–13 × 2.5–3.0 µm, with truncate base, basal scars 0.5–1.0 µm; intercalary conidia ellipsoidal, 5.5–10.0 × 2.0–2.5 µm. Chlamydospores absent.

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

Known distribution:—Guilin city, Guangxi Province, China.

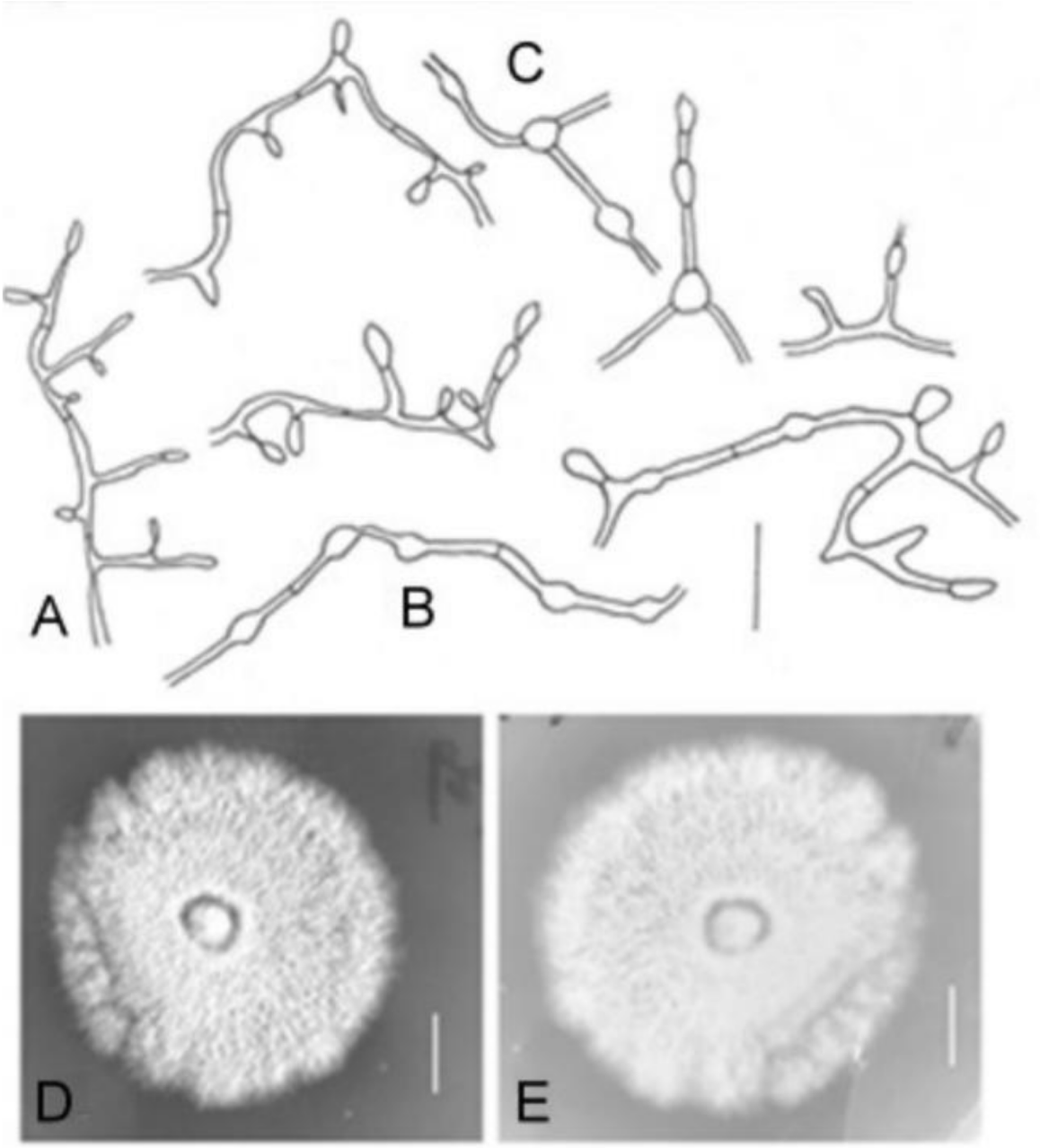

FIGURE 4. *Chrysosporium guangxiense* (holotype). A. Conidiogenous structures. B. Racquet hyphae. C. Intercalary conidia. D–E. Colonies (front and reverse) on PDA. Bars: A–C = 20 µm, D–E = 10 mm.

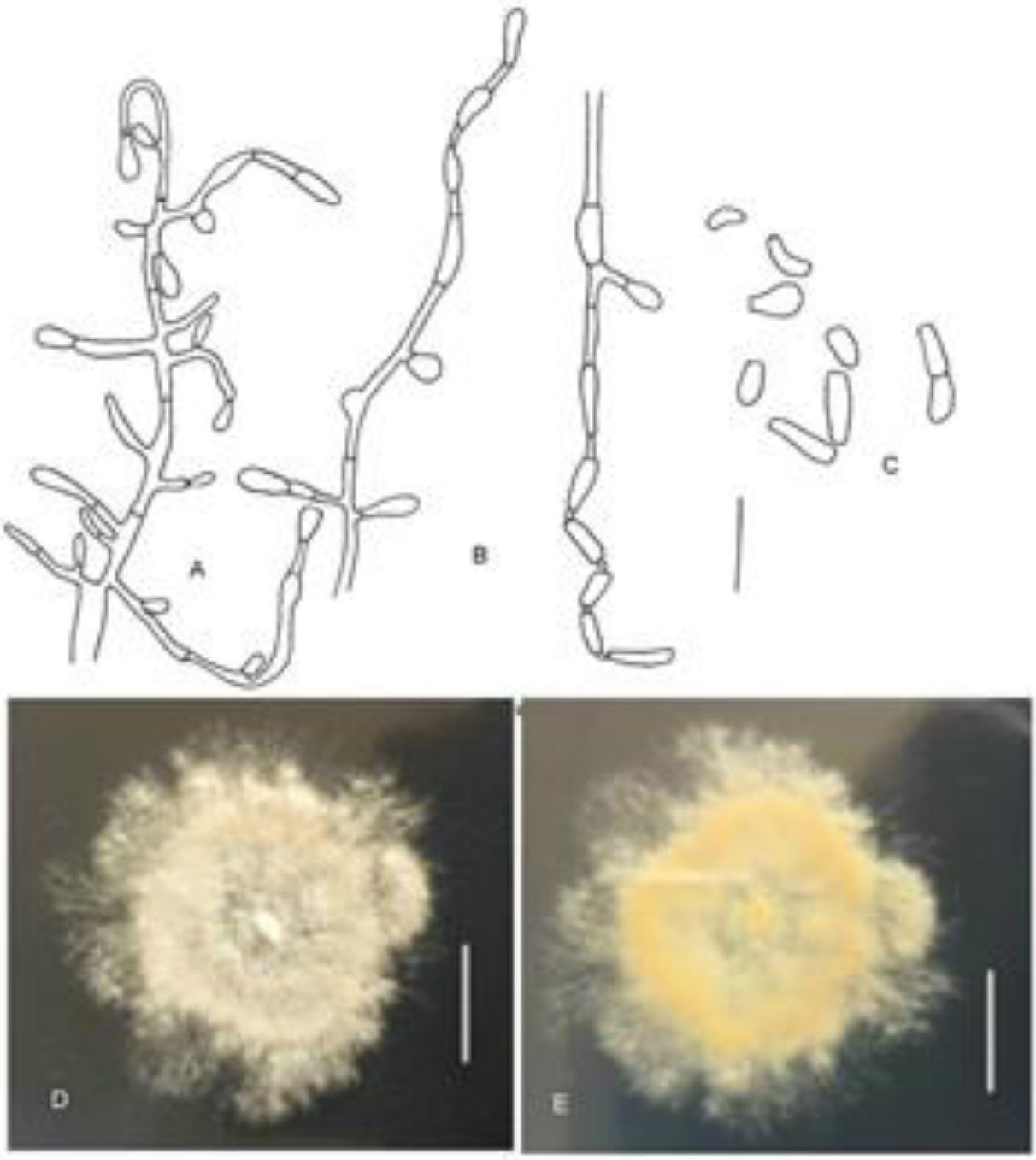

FIGURE 5. *Chrysosporium irregularum* (holotype). A. Conidiogenous structures. B. Intercalary conidia. C. Conidia. D–E. Colonies (front and reverse) on PDA. Bars: A–C = 20 μm, D–E= 10 mm.

Chrysosporium irregularum Y.F. Han, W.H. Chen, J.D. Liang & Z.Q. Liang, *sp. nov*. (Fig. 5)
Mycobank No.: MB 838866

Type:—CHINA. Gansu Province: Dunhuang City, Yumenguan, N40°21', E93°51', from soil, August 2017, J.J. Wang, holotype GZAC.J1.1; ex-type culture GZU.J1.1.

Colonies on PDA attaining about 35 mm diam. at 26 °C after 14 days, lightly raised in the center, densely villiform, margin sparsely villiform, irregular, yellow; reverse creamy to yellowish. *Hyphae* septate, smooth, hyaline, 0.5–3.0 μm. *Racquet hyphae* absent. *Conidia* abundant, hyaline, smooth, lateral or terminal, arising from aerial hyphae directly or short protrusions, unicellular or bicellular, solitary or in pairs, cylindrical, 3.5–9.5 × 1.0–2.5 μm, or pyriform, 3.5–5.0 × 1.5–3.0 μm, or irregularly reniform, 3.0–5.0 × 1.5–3.5 μm, with truncate base, basal scars 0.5–1.0 μm; intercalary conidia ellipsoidal, 2.0–15.0 × 1.0–4.0 μm. *Chlamydospores* absent.

Etymology:—Referring to the irregular colony.

Additional strains examined:—CHINA. Gansu Province: Dunhuang City, Yumenguan, soil, N24°18'', E09°45'', August 2017, J.J. Wang, GZAC.J102, living culture GZU.J102.

Known distribution:—Yumenguan, Gansu Province, China.

Chrysosporium jiangsuense Y.F. Han, W.H. Chen, J.D. Liang & Z.Q. Liang, *sp. nov*. (Fig. 6)
Mycobank No.: MB 838867

Type:—CHINA. Jiangsu Province: Yangzhou City, N32°24′, E119°26′, from soil, August 2017, Y.F. Han, holotype GZAC.I10; ex-type culture GZU.I10.

Colonies on PDA attaining about 40 mm diam. at 26 °C after 14 days, short densely villiform, margin sparsely villiform, white; reverse white to yellowish. *Hyphae* septate, smooth, hyaline, 1.0–3.5 μm thick. *Racquet hyphae* absent. *Conidia* hyaline, rough, mostly lateral or terminal, arising from aerial hyphae directly, unicellular, solitary, obovoid, 3.5–6.0 × 1.5–2.5 μm, or ellipsoidal, 1.5–3.0 × 1.5–2.5 μm, with truncate base, basal scars 0.5–1.0 μm; intercalary conidia absent. *Chlamydospores* absent.

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

Known distribution:—Yangzhou city, Jiangsu Province, China.

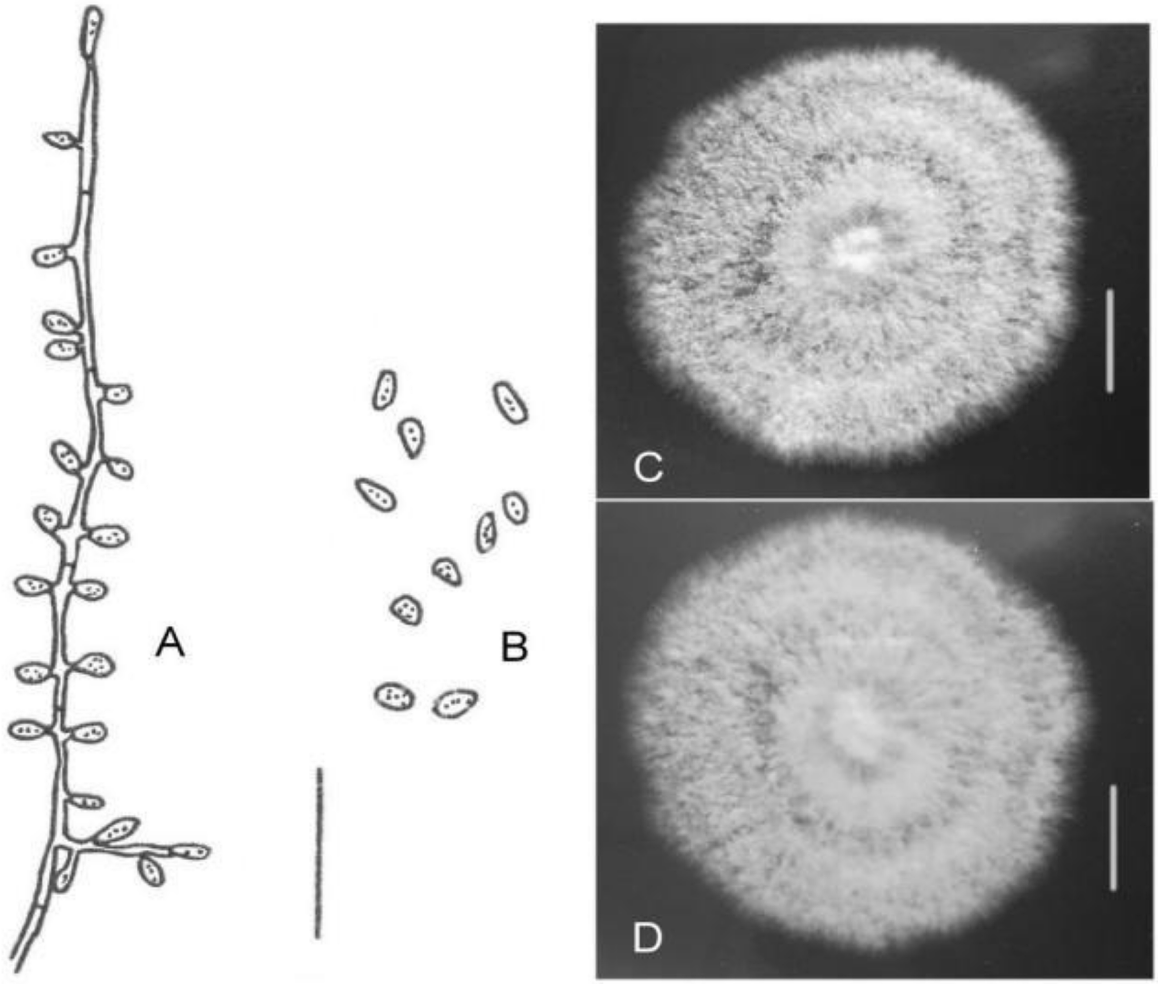

FIGURE 6. *Chrysosporium jiangsuense* (holotype). A. Conidiogenous structures. B. Conidia. C–D. Colonies (front and reverse) on PDA. Bars: A–B = 20 μm; C–D = 10 mm.

Chrysosporium kaiyangense Y.F. Han, W.H. Chen, J.D. Liang & Z.Q. Liang, *sp. nov*. (Fig. 7)
Mycobank No.: MB 838868

Type:—CHINA. Guizhou Province: Kaiyang City, N27°06′, E107°09′, from soil, August 2017, Yanfeng Han, holotype GZAC.EB0702M; ex-type culture GZU.EB0702M.

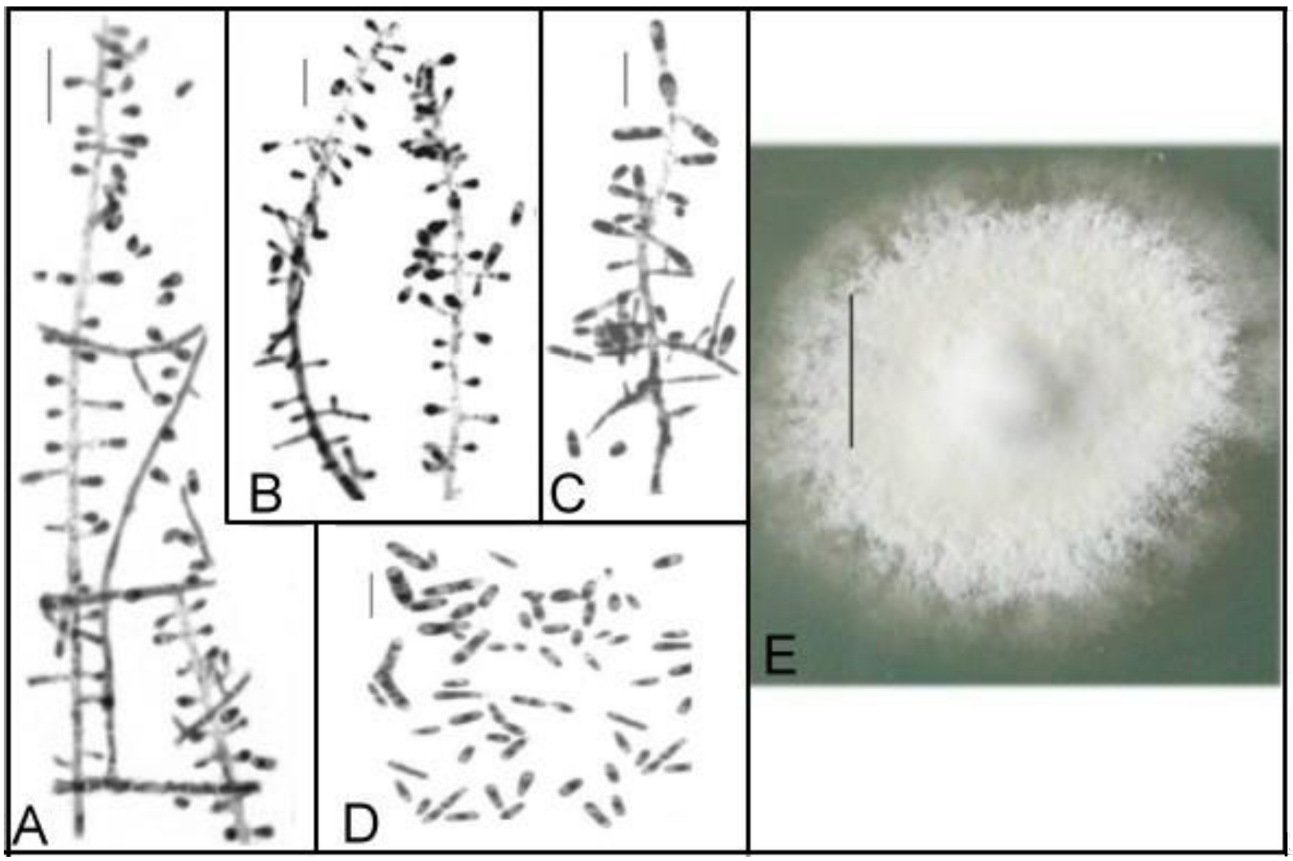

FIGURE 7. *Chrysosporium kaiyangense* (holotype). A–C. Conidiogenous structures. D. Conidia. E. Colony on PDA media. Bars: A–D = 10 μm, E = 10 mm.

Colonies on PDA attaining about 35 mm diam. at 26 °C after 14 days, raised in the center, felty, white, margin sparsely villiform; reverse yellowish. *Hyphae* septate, smooth, hyaline, 2.0–3.0 μm thick. *Racquet hyphae* absent. *Terminal and lateral conidia* hyaline, smooth, arising from aerial hyphae directly or on short protrusions, 1- or 3-celled, solitary, obovoid, 2.0–3.5 × 1.0–2.5 μm, or cylindrical to clavate, 4.0–10.5 × 2.0–3.0 μm, with truncate base, basal scars 1.5–2.0 μm; intercalary conidia absent. *Chlamydospores* absent.

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

Known distribution:—Kaiyang city, Guizhou Province, China.

Chrysosporium multiforme Y.F. Han, W.H. Chen, J.D. Liang & Z.Q. Liang, *sp. nov*. (Fig. 8)
Mycobank No.: MB 838869

Type:—CHINA. Gansu Province: Lanzhou City, N36°03′, E103°40′, from soil, August 2017, J.J. Wang, holotype GZAC.U3; ex-type culture GZU.U3.

Colonies on PDA attaining about 45–50 mm diam. at 26 °C after 14 days, sparsely villiform, flat, yellowish, with obvious conidial powder; reverse yellowish. *Hyphae* septate, smooth or rough, hyaline, 2.0–3.0 μm thick. *Racquet hyphae* absent. *Conidia* hyaline, smooth, abundant, arising from aerial hyphae directly or on short protrusions, unicellular, solitary, pyriform, 9.5–15.5 × 7.0–8.0 μm, or obovate or ellipsoidal, 8.5–10.0 × 7.5–9.0 μm, with truncate base, basal scars 1.5–3.0 μm; intercalary conidia long oval, solitary or 2–4 in a bunch, 7.5–13.5 × 4.0–8.0 μm, or fusiform, 9.0–24.5 × 5.5–8.0 μm. *Chlamydospores* absent.

Etymology:—Referring to the various shape of conidia.

Additional strains examined:—CHINA. Gansu Province: Lanzhou, soil, N36°03′, E103°40′, August 2017, J.J. Wang, GZAC.U302, living culture GZU.U302.

Known distribution:—Lanzhou city, Gansu Province, China.

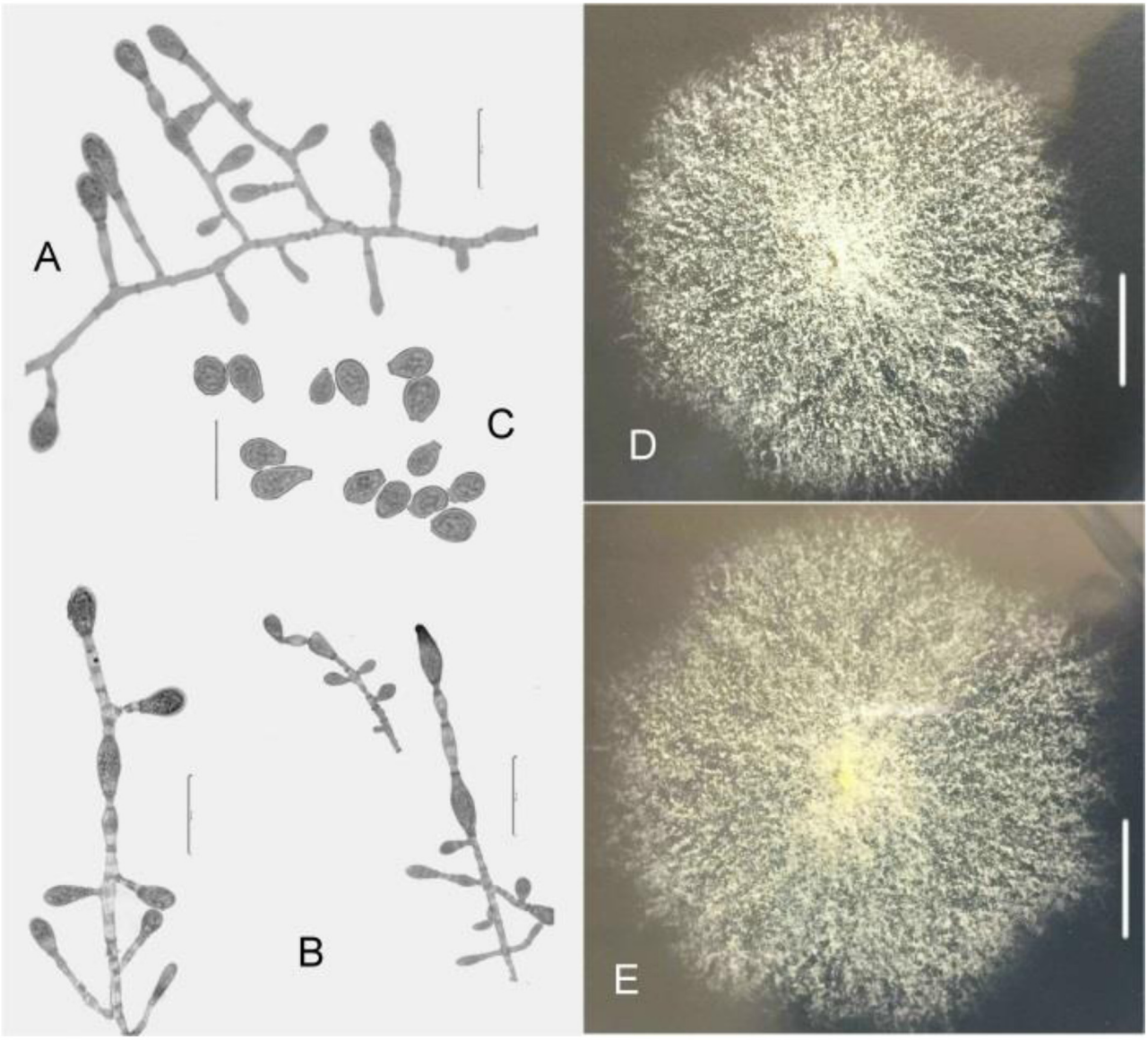

FIGURE 8. *Chrysosporium multiforme* (holotype). A. Conidiogenous structures. B. Intercalary conidia. C. Conidia. D–E. Colony (front and reverse) on PDA. Bars: A–C = 20 μm, D–E = 10 mm.

Chrysosporium sichuanense Y.F. Han, W.H. Chen, J.D. Liang & Z.Q. Liang, *sp. nov*. (Fig. 9)
Mycobank No.: MB 838870

Type:—CHINA. Sichuan Province: Bazhong City, N31.15°, E106.21°, from soil, August 2017, Y.F. Han, holotype GZAC.FX8; ex-type culture GZU.FX8.

Colonies on PDA attaining about 30 mm diam. at 26 °C after 7 days, villiform, light raised, white; reverse white. *Hyphae* septate, smooth, hyaline, 1.5–3.5 μm thick, sometimes having inflated structure. *Racquet hyphae* present, 34.0–129.0 × 4.5–7.0 μm. *Conidia* hyaline, smooth, abundant, arising from aerial hyphae directly or on short protrusions, unicellular or bicellular, solitary or 2–3 in short chain, clavate, 6.5–8.5 × 2.0–2.5 μm, or obovate, 4.0–6.5 × 2.0–2.5 μm, or pyriform, 5.0–9.5 × 2.5–4.5 μm, with truncate base, basal scars 1.0–2.0 μm; intercalary conidia ellipsoidal, 4.5–5.5 × 2.0–4.5 μm; arthroconidia hyaline, smooth, cylindrical, 4.5–7.5 × 2.0–2.5 μm. *Chlamydospores* absent.

Etymology:—Referring to the region from which the holotype was isolated.

Additional strains examined:—CHINA. Shanxi Province: Jinzhong, soil, N37.35°, E 112.33°, August 2017, Y.F. Han, GZAC.I17 and GZAC.I18, living cultures GZU.I17 and GZU.I18.

Known distribution:—Bazhong city, Sichuan Province; Jinzhong City, Shanxi Province, China.

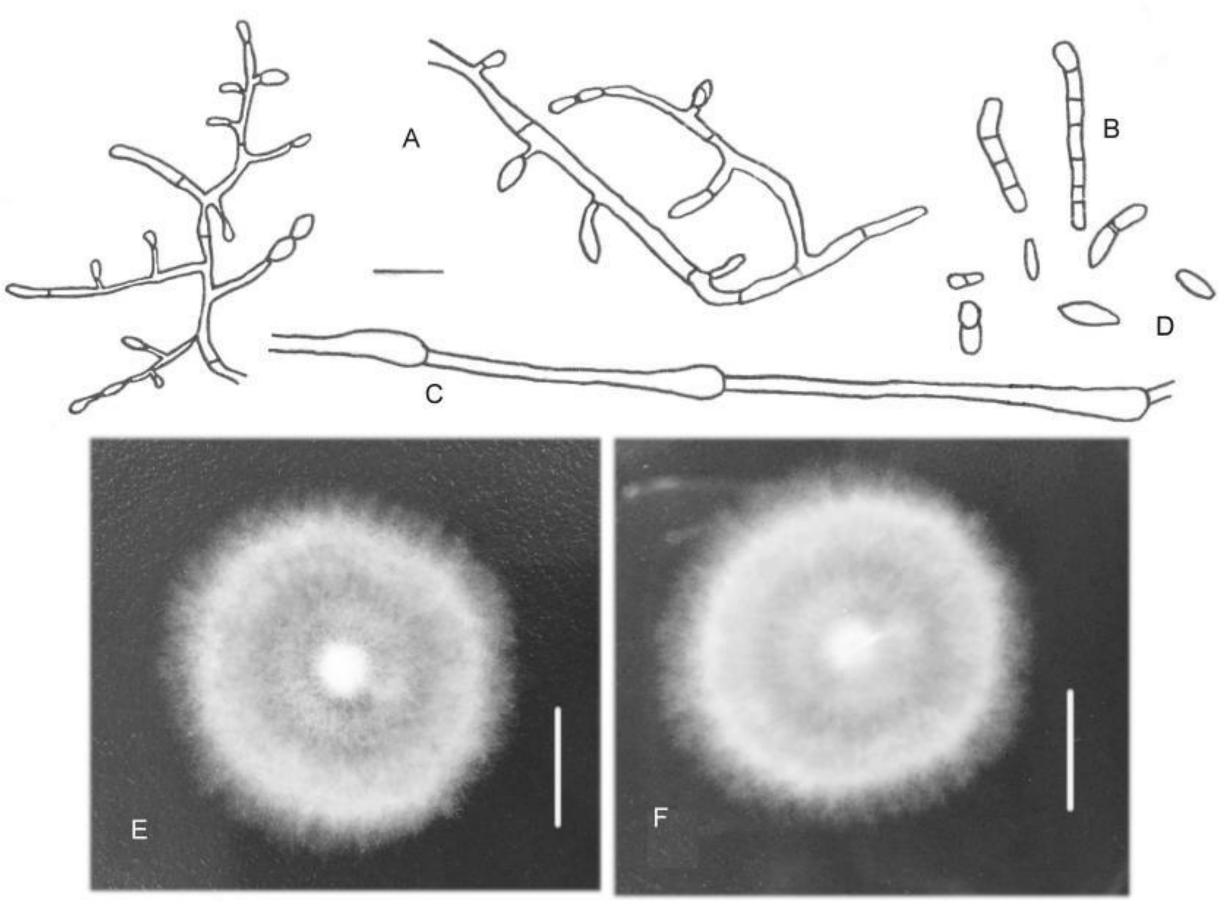

FIGURE 9. *Chrysosporium sichuanense* (holotype). A. Conidiogenous structures. B. Arthroconidia. C. Racquet hyphae. D. Conidia. E–F. Colony (front and reverse) on PDA. Bars: A–D = 20 µm, E–F = 10 mm.

Chrysosporium villiforme Y.F. Han, W.H. Chen, J.D. Liang & Z.Q. Liang, *sp. nov*. (Fig.10)
Mycobank No.: MB 838871

Type:—CHINA. Shanxi Province: Linfen City, Ji county, N36.09°, E110.68°, from soils, August 2017, Y.F. Han, holotype GZAC.L19.4; ex-type culture GZU.L19.4.

Colonies on PDA attaining about 40 mm diam. at 26 °C after 7 days, densely villiform in the center, sparsely villiform in the margin, creamy, margin irregular; reverse white to creamy. *Hyphae* septate, smooth, hyaline, 1.5–3.0 µm thick. *Racquet hyphae* absent. *Conidia* hyaline, smooth, abundant, arising from aerial hyphae directly or on short protrusions or side branches, unicellular, solitary or 2–3 in a short chain, ovoid, 4.0–7.5 × 2.0–2.5 µm, or pyriform,4.0–5.5 × 2.5–3.0 µm, or clavate, 4.5–7.0 × 2.0–2.5 µm, with truncate base, basal scars 0.5–1.5 µm; intercalary conidia ellipsoidal, 2.0–3.0 × 1.0–2.5 µm. *Chlamydospores* absent.

Etymology:—Referring to the villiform texture of colony on PDA media.

Additional strains examined:—CHINA. Shanxi Province: Linfen, soil, N36.09°, E 110.68°, August 2017, Y.F. Han, GZAC.L19.5, living culture GZU.L19.5.

Known distribution:—Linfen City, Shanxi Province, China.

Discussion

In this study, the phylogenetic analyses showed that there were several clades, indicating that the genus *Chrysosporium* is polyphyletic, and consistent with previous results based on phylogenetic analysis of *ITS* sequences (Vidal *et al*. 2000, Zhao *et al*. 2018). Recent studies demonstrated that *ITS* rDNA sequences alone were able to successfully distinguish *Chrysosporium* species (Zhang *et al*. 2020a, Li *et al*. 2019).

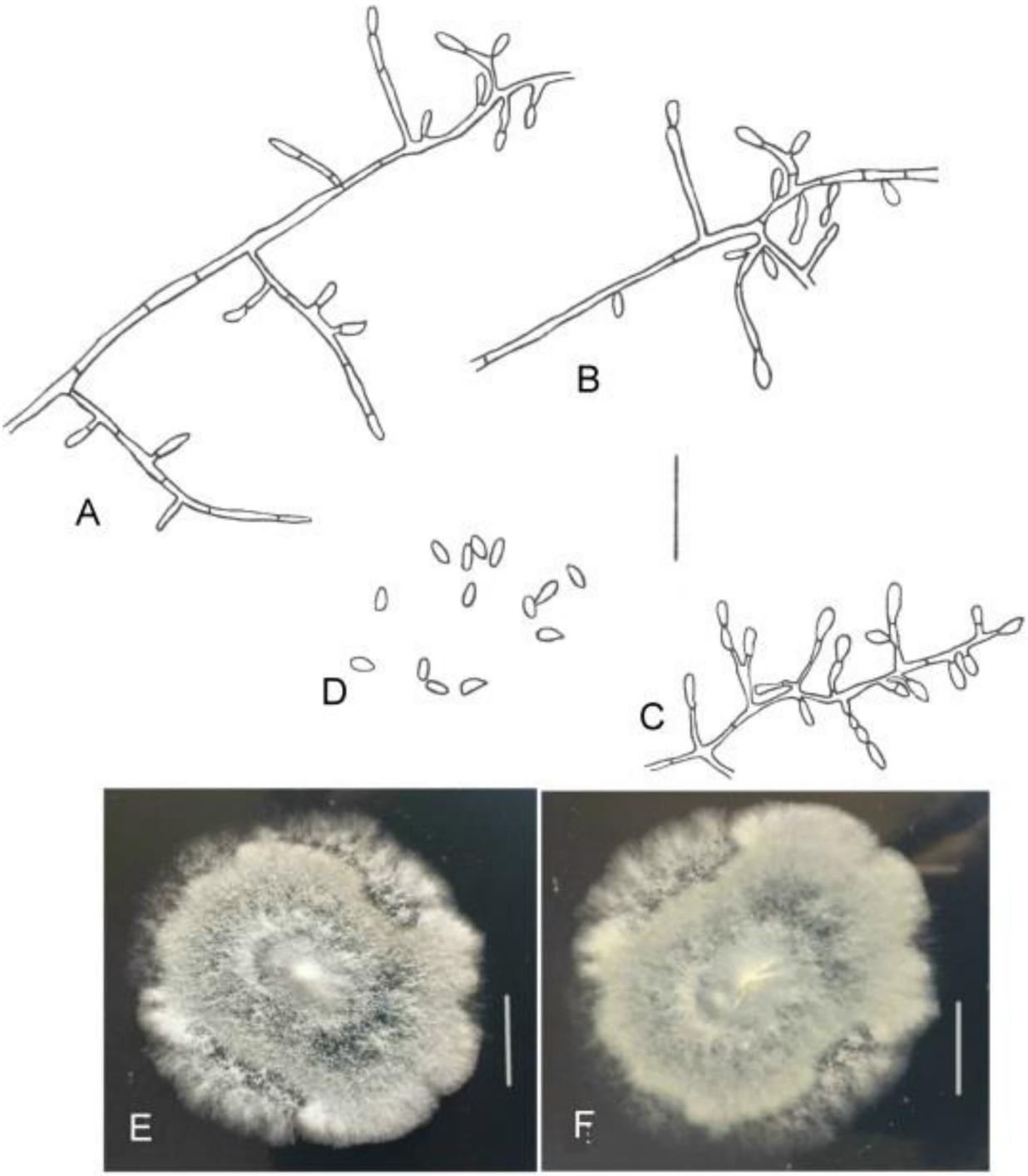

FIGURE 10. *Chrysosporium villiformum* (holotype). A–C. Conidiogenous structures. D. Conidia. E–F. Colony (front and reverse) on PDA. Bars: A–D = 20 μm, E–F = 10 mm.

From the phylogenetic tree, 23 *Chrysosporium* strains isolated in this study clustered in different clades. Both sequences of *C. multiforme* clustered in a separate subclade related to *C. keratinophilum*, but it can be distinguished from the latter by absence of racquet hyphae and various shaped terminal or lateral conidia (Oorschot 1980). Both sequences of *C. gansuense* were related to *C. shanxiense* and *C. tropicum*, but *C. shanxiense* has obvious inflated collar-shaped structures between conidiogenous cells and conidia (Zhang *et al*. 2016b); *C. tropicum* has cylindrical intercalary conidia and globose chlamydospores (Oorschot 1980), which is different from *C. gansuense* in the morphology. Both sequences of *C. irregularum* were in a separate clade with high bootstrap value and it had an irregular colony. *C. jiangsuense* clustered in a subclade with *C. indicum* and *C. linfenense*, but *C. indicum* has intercalary conidia (Oorschot 1980), the conidia of *C. linfenense* are smooth (Liang *et al*. 2009), while those of *C. jiangsuense* are rough. Three

sequences of *C. fusiforme* clustered in a separate subclade with *C. jiangsuense*, *C. linfenense* and *C. indicum* with high bootstrap value (93/1); *C. linfenense* and *C. indicum* have racquet hyphae (Liang *et al*. 2009, Oorschot 1980), while *C. fusiforme* and *C. jiangsuense* lack such structures. Intercalary conidia were absent in *C. jiangsuense* but present in *C. fusiforme. C. villiforme* was closely related to *C. qinghaiense* in a separate subclade, while it could be distinguished from *C. qinghaiense* by having intercalary conidia and absence of bicellular conidia (Han *et al*. 2013). *C. guangxiense* was in a separate subclade and related to *C. alvearium*, however, *C. alvearium* has chains of up to 10 conidia (Zhao *et al*. 2018), while the conidia of *C.guangxiense* are solitary or in pairs. *C. sichuanense* including three sequences was separated from other *Chrysosporium* spp. with high bootstrap value, and it has obvious arthroconidia which can distinguish it from other species. *C. kaiyangense* was closely related to *C. ovalisporum*, but *C. ovalisporum* has limited colony growth and brown reverse on PDA, and bigger conidia than *C. kaiyangense (*Li *et al*. 2019*)*. Six strains clustered to known species of *Chrysosporium* and were identified as *C. keratinophilum*, *C. alvearium*, *C. hubeiense*, and *C. ovalisporum.* These four species have been recorded in China previously in Tibet, Hubei, and Fujian Province (Geng *et al*. 2008, Zhao *et al*. 2018, Zhang *et al*. 2016a, Li *et al*. 2019).

Key to the 13 *Chrysosporium* species found in this study:

1. Racquet hyphae present 2
1. Racquet hyphae absent 3
2. Arthroconidia present ..... *Chrysosporium sichuanense*
2. Arthroconidia absent 4
3. Intercalary conidia present 5
3. Intercalary conidia absent 6
4. Chlamydospores present ..... *.Chrysosporium alvearium*
4. Chlamydospores absent 7
5. Intercalary conidia ellipsoidal 8
5. Intercalary conidia fusiform, pyriform or long oval 9
6. Conidia smooth 10
6. Conidia rough, obovoid, ellipsoidal ..... *Chrysosporium jiangsuense*
7. Intercalary conidia absent ..... *Chrysosporium hubeiense*
7. Intercalary conidia ellipsoidal ..... *Chrysosporium guangxiense*
8. Conidia rough, pyriform or clavate, ellipsoidal ..... *Chrysosporium gansuense*
8. Conidia smooth 11
9. Conidia hyaline 12
9. Conidia hyaline, pale brown, moderate brown ..... *Chrysosporium keratinophilum*
10. Lateral or terminal conidia ovoid, 2.0–3.5 × 1.0–2.5 µm ..... *Chrysosporium kaiyangense*
10. Lateral or terminal conidia ovoid, 5–15 × 2.5–7 µm ..... *Chrysosporium ovalisporum*
11. Lateral or terminal conidia cylindrical, 3.5–9.5 × 1.0–2.5 µm ..... *Chrysosporium irregularum*
11. Lateral or terminal conidia ovoid, 4.0–7.5 × 2.0–2.5 µm ..... *Chrysosporium villiforme*
12. Intercalary conidia fusiform, 6.5–9.0 × 2.5–3.0 µm ..... *Chrysosporium fusiforme*
12. Intercalary conidia long oval, 7.5–13.5 × 4.0–8.0 µm ..... *Chrysosporium multiforme*

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