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***Full Length Article***

# A New Fungus *Metarhizium gaoligongense* from China

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## Abstract

A new species namely *Metarhizium gaoligongense* collected from Gaoligong mountains, Yunnan Province, China, was described based on both morphology and multilocus (ITS, nrSSU, nrLSU, EF-1α, RPB1 and RPB2) phylogeny. *M. gaoligongense* was clustered in *M. flavoviride* complex which had 6 clearly separated individuals and it developed a well-supported separate clade from other 5 allied species, being more close to *M. koreanum* and *M. minus* and closest to *M. pemphigi*. The microscopic characters of *M. gaoligongense* were also very similar to *M. pemphigi*, in accordance with phylogenic affiliation, while the culture characteristics on potato dextrose agar medium could obviously distinguish the two sister species. *M. pemphigi* colonies often developed radial constriction, showed emerald green, and diffused yellow-green pigmentation into medium. However, *M. gaoligongense* colonies were flat, green, without pigment diffusion into medium, often guttulated condensation on conidia layer surface. 

**Keywords:** *Metarhizium*; Morphology; Multilocus phylogeny; Taxonomy

## Introduction

Species in the cosmopolitan genus *Metarhizium* Sorokin are entomopathogens (Roberts and Leger, 2004) active in regulating insect populations in nature (Lacey *et al.*, 2001). With the rapid advance of genome technologies, multiple species of *Metarhizium* have been model systems to answer the basic questions in parasitology, entomology and speciation (Wang *et al.*, 2016). Three species namely *M. anisopliae*, *M. flavoviride* and *M. album* were recognized based on morphological characteristics in the early classification (Rombach *et al*., 1987). More and more new species and varieties of *Metarhizium* were discovered in the following decades and morphological characteristics showed to be of limited utility for defining similar species (Sung *et al*., 2007; Kepler *et al*., 2014). Multi-gene (EF-1α, RPB1, RPB2 and β-tubulin) phylogenetic analyses had revealed that some recognized *Metarhizium* species were complex lineages consisting of multiple cryptic lineages with similar morphology (Bischoff *et al*., 2006, 2009). Based on the same four-gene analysis, Kepler *et al*. (2014) newly revised *Metarhizium* as including the major species formerly in *Metacordyceps*, green-spored species in *Nomuraea* and *Chamaeleomyces*, and a few species formerly in *Paecilomyces*.

The species resource of *Metarhizium* was diverse in China and recently abundant new species were reported, such as *M. brittlebankisoides* (Liu *et al*., 2001; Kepler *et al*., 2014), *M. campsosterni* (Zhang *et al*., 2004; Kepler *et al*., 2014), *M. guniujiangensis* (Li *et al*., 2010; Kepler *et al*., 2014). Gaoligong mountains are located in the southwestern of China and had complex ecological environment and very high biodiversity (Kavanaugh *et al*., 2014). However, few reports were about the entomopathogens fungi in the famous mountains. Here a new *Metarhizium* species from Gaoligong mountains in China was morphologically described and phylogenetically analyzed.

## Materials and Methods

### Fungal Isolation and Morphological Evaluation

Soil specimens were collected from a coffee farm in the dry-hot valley of Gaoligong mountains in China. *Metarhizium* strains were isolated from soil with a method of insect baiting according to Keyser *et al*. (2015). Soil sample was moistened to slightly damp and 6–7$^{th}$ instar healthy *Tenebrio molitor* larvae were cultured on the soil. The infection status was checked every two days. Dead insects were removed and maintained on sterile moist filter paper at 25°C for about 10 days to produce conidia. *Metarhizium* colonies were subcultured on PDA. Pure (axenic) cultures were incubated at 25°C for 2 weeks and were identified morphologically and evaluated microscopically under a motic BA410 microscope. The new species were morphologically compared with its mostly related species, *M. pemphigi*. *M. pemphigi* isolate BUM 39.4 was also isolated from Gaoligong mountains.

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**DNA Extraction, PCR and Sequencing**

DNA was extracted from mycelia and conidia cultured on PDA medium for 20 d with the Plant Genomic DNA Purification Kit (Qiagen). The partial sequences of 6 genes including 5.8S-ITS, nrSSU, nrLSU, EF-1α, RPB1 and RPB2were amplified. Their primer pairs were referred to Chen *et al*. (2013). PCR reactions were conducted in 20 $\mu$L mixture composed of 10 $\mu$L 2×EasyTaq PCR Supermix (TransGen Biotech, Beijing, China), 1 $\mu$L of each primer (10 $\mu M$), 1 $\mu$L of template DNA (1–2 ng) and 7 $\mu$L sterile water. PCR programs of ITS-5.8S, nrSSU and nrLSU were referred to Chen *et al*. (2013), and EF-1α, RPB1 and RPB2 were according to Bischoff *et al*. (2006). DNA purification was performed with Gel Purification Kit (Bioteke, Beijing, China) and the genes cloning were carried out with TaKaRa PMD$^{TM}$18-T vector system (TaKaRa Bio, Dalian, China). DNA sequencing was performed at SinoGenoMax Co., Ltd. and the acquired sequences were submitted to the database of GenBank.

**Phylogenetic Analysis**

DNA sequences of 6–locus were retrieved from GenBank, incluing 33 taxa (*M. gaoligongense*, 30 *Metarhizium* species used by Kepler *et al*. (2014), *Metacordyceps shibinensis* used by Wen *et al*. (2015) and *Beauveria bassiana* as outgroup). Theirtaxonomies and GenBank accession numbers were shown in Table 1.

The 6-locus sequences were individually aligned using Clustal X2.0 (Larkin *et al*., 2007). Ambiguous regions in two terminals were removed in the subsequent phylogenetic analyses and gaps were treated as missing data. Phylogenic consensus tree was analyzed using MEGA6 program (Tamura *et al*., 2013). Maximum Likelihood (ML) estimation was carried out with 1000 bootstrap replicates. Clades supported with ML values ≥70% were regarded as significantly supported by the data.

## Results

**Taxonomy**

*Metarhizium gaoligongense* Z.H. Chen & L. Xu, spp. Nov. MycoBank no.: MB 818981.

Colonies on PDA medium being white at first, then green at maturity, often prominently guttulate, and reaching 40 mm in diameter for 14 days of cultivation at 25°C. Hyphae beinghyaline, septate, branched, smooth-walled, 2.1–3.3 ($\overline{X}$ = 2.8 ± 0.3) µm wide. Conidiophores solitary or branched, 1-6 phialides for each. Phialides cylindrical, 5.6–18.4×1.6–3.4 ($\overline{X}$ = 11.3 ± 3.2×2.5±0.4) $\mu$m. Conidia forming columns in culture and hyaline (green en masse), aseptate, smooth, cylindrical, 5.4–7.7×1.9–2.8 ($\overline{X}$ = 6.7 ± 0.9×2.3 ± 0.3) µm.

Holotype:- China. Yunnan Province: Gaoligong mountains, altitude 1120 m, 12 May 2015, Zi-Hong Chen (CCTCC M 2016588).

Sexual state:- Unknown.

Host:- Unknown.

Type locality:- Soil of a coffee farmland in the dry-hot valley of Gaoligong mountains, Yunnan Province, China.

Etymology:- *gaoligongense*, referring to the location where the type material was collected.

Deposition:- China Center for type Culture Collection, deposition No.: CCTCC M 2016588.

**Phylogenetic Analyses**

Concatenated alignments of the combined 6-locus of *Metarhizium* contained 5 045 base pairs, therein 713 from 5.8S-ITS, 848 bp from nrLSU, 1 022 bp from nrSSU, 917 bp from EF-1α, 713 bp from RPB1, and 832 bp from RPB2.

Phylogenetic tree was built based on the combined data set of 6 loci for *M. gaoligongense* and other 31 *Metarhizium* species, and *B. bassiana* as an outgroup (Fig. 1). It showed 10 clearly separated species in the *M. anisopliae* complex, and 6 clearly separated species in the *M. flavoviride* complex. The new species, *M. gaoligongense* was clustered in *M. flavoviride* complex and formed a well-supported separate clade from other 5 allied individuals, being more close to *M. koreanum* and *M. minus*, and most closely related to*M. Pemphigi* (Fig. 1). *M. pemphigi* isolate, BUM 39.4 from Gaoligong mountains, was clustered together with other two known isolates of *M. pemphigi*, confirmed its classification status.

**Morphological Comparison of *M. gaoligongense* with Related Species**

*M. gaoligongense* was groupedin *M. flavoviride* complex and had closest evolution relationship with *M. pemphigi* by phylogenetic analyses. Morphological comparison of *M. gaoligongense* with its related species in *M. flavoviride* also agreed with the affinity (Table 2). Conidia of *M. gaoligongense* ($\overline{X}$ =2.3 ± 0.3 µm wide for CCTCC M 2016588) and *M. pemphigi* ($\overline{X}$ =2.4±0.43 $\mu$m wide for DAR74295 and $\overline{X}$ =2.2 ± 0.3 $\mu$m wide for BUM 39.4) were much narrower than other known species of *M. flavoviride* complex. The phialides of *M. gaoligongense* were $\overline{X}$ =11.3 ± 3.2×2.5 ± 0.4 $\mu$m, also more being closely resembled that of *M. pemphigi* ($\overline{X}$ =10.1 ± 3.1×2.4 ± 0.4 µm for BUM 39.4).

Morphological comparison was further conducted between the two sister species, *M. gaoligongense* and *M. pemphigi*. Their microscopic characters were very similar, except that conidia of *M. gaoligongense* ($\overline{X}$ =6.7±0.9 $\mu$m long for CCTCC M 2016588) were a little longer than *M. pemphigi* ($\overline{X}$ =6.2 ± 1.2 $\mu$m long for BUM 39.4). However, their culture character had significant difference. *M. gaoligongense* colonies on PDA medium had flat surface

**Table 1:** Voucher information for the six loci used in this study

| Species | Strain code | GenBank Accession Number | | | | | |
|---|---|---|---|---|---|---|---|
| | | nrSSU | nrLSU | EF-1α | RPB1 | RPB2 | 5.8S-ITS |
| *M. acridum* | ARSEF 324 | data missing | data missing | EU248844.1 | EU248896.1 | EU248924.1 | HM055449.1 |
| *M. acridum* | ARSEF 7486 | data missing | data missing | EU248845.1 | EU248897.1 | EU248925.1 | NR132019.1 |
| *M. album* | ARSEF 2082 | DQ518775.1 | DQ522560.1 | DQ522352.1 | DQ522398.1 | DQ522452.1 | data missing |
| *M. album* | ARSEF 2179 | data missing | data missing | KJ398807.1 | KJ398618.1 | data missing | HM055452.1 |
| *M. anisopliae* | ARSEF 7450 | data missing | data missing | EU248852.1 | EU248904.1 | EU248932.1 | HQ331464.1 |
| *M. anisopliae* | ARSEF 7487 | data missing | data missing | DQ463996.2 | DQ468355.1 | DQ468370.1 | NR132017.1 |
| *M. brasiliense* | ARSEF 2948 | data missing | data missing | KJ398809.1 | KJ398620.1 | data missing | data missing |
| *M. brunneum* | ARSEF 2107 | data missing | data missing | EU248855.1 | EU248907.1 | EU248935.1 | NR132023.1 |
| *M. brunneum* | ARSEF 4179 | data missing | data missing | EU248854.1 | EU248906.1 | EU248934.1 | HQ331451.1 |
| *M. carneum* | CBS 239.32 | EF468843.1 | EF468988.1 | EF468789.1 | EF468894.1 | EF468938.1 | NR131993.1 |
| *M. cylindrosporum* | ARSEF 6926 | data missing | data missing | KJ398814.1 | KJ398625.1 | data missing | AF368270.1 |
| *M. flavoviride* | ARSEF 2133 | data missing | data missing | DQ463999.1 | DQ468358.1 | DQ468373.1 | data missing |
| *M. flavoviride* | ARSEF 2025 | data missing | AF138269.1 | KJ398804.1 | KJ398614.1 | DQ468374.1 | AF138269.1 |
| *M. frigidum* | ARSEF 7445 | data missing | data missing | KJ398818.1 | KJ398628.1 | data missing | data missing |
| *M. frigidum* | ARSEF 4124 | data missing | data missing | DQ464002.1 | DQ468361.1 | DQ468376.1 | NR132012.1 |
| *M. gaoligongense* | CCTCC M 2016588 | KY087812 | KY087816 | KY087820 | KY087824 | KY087826 | KY087808 |
| *M. gaoligongense* | BUM 3.5 | KY087810 | KY087814 | KY087818 | KY087822 | data missing | KY087806 |
| *M. gaoligongense* | BUM 1.4 | KY087811 | KY087815 | KY087819 | KY087823 | data missing | KY087807 |
| *M. globosum* | ARSEF 2596 | data missing | data missing | EU248846.1 | EU248898.1 | EU248926.1 | NR132020.1 |
| *M. granulomatis* | UAMH 11176 | HM635078.1 | data missing | KJ398782.1 | KJ398593.1 | data missing | HM195306.1 |
| *M. granulomatis* | UAMH 11028 | HM195304.1 | HM635076.1 | KJ398781.1 | data missing | data missing | NR132013.1 |
| *M. guizhouense* | CBS 258.90 | data missing | data missing | EU248862.1 | EU248914.1 | EU248942.1 | HQ331448.1 |
| *M. guizhouense* | ARSEF 6238 | data missing | data missing | EU248857.1 | EU248909.1 | EU248937.1 | HQ331447.1 |
| *M. indigoticum* | TNS-F 18553 | JF415968.1 | JF415952.1 | JF416010.1 | JN049886.1 | JF415992.1 | JN049874.1 |
| *M. indigoticum* | TNS-F 18554 | JF415952.1 | JF415969.1 | JF416011.1 | JN049887.1 | JF415993.1 | JN049875.1 |
| *M. khaoyaiense* | BCC 12687 | JF415971.1 | data missing | KJ398796.1 | JN049889.1 | data missing | data missing |
| *M. khaoyaiense* | BCC 14290 | JF415970.1 | data missing | KJ398797.1 | JN049888.1 | data missing | JN049869.1 |
| *M. koreanum* | ARSEF 2039 | data missing | data missing | KJ398806.1 | KJ398616.1 | data missing | data missing |
| *M. koreanum* | ARSEF 2038 | data missing | data missing | KJ398805.1 | KJ398615.1 | data missing | data missing |
| *M. kusanagiensis* | TNS-F 18494 | JF415972.1 | JF415954.1 | JF416014.1 | JN049890.1 | data missing | JN049873.1 |
| *M. lepidiotae* | ARSEF 7412 | data missing | data missing | EU248864.1 | EU248916.1 | EU248944.1 | HQ331455.1 |
| *M. majus* | ARSEF 1946 | data missing | data missing | EU248867.1 | EU248919.1 | EU248947.1 | HM055450.1 |
| *M. majus* | ARSEF 1914 | data missing | data missing | KJ398801.1 | KJ398610.1 | data missing | HQ331445.1 |
| *M. marquandii* | CBS 182.27 | EF468845.1 | EF468990.1 | EF468793.1 | EF468899.1 | EF468942.1 | NR131994.1 |
| *M. minus* | ARSEF 1764 | AF280635.1 | AF280632.1 | DQ464006.1 | KJ398609.1 | DQ468380.1 | HM055453.1 |
| *M. minus* | ARSEF 2037 | AF339531.1 | AF339580.1 | DQ464007.1 | DQ468366.1 | DQ468381.1 | AF138271.1 |
| *M. novozealandicum* | ARSEF 4661 | data missing | data missing | KJ398811.1 | KJ398622.1 | data missing | data missing |
| *M. novozealandicum* | ARSEF 4674 | data missing | data missing | KJ398812.1 | KJ398623.1 | data missing | data missing |
| *M. owariense* | NBRC 33258 | HQ165730.1 | HQ165669.1 | HQ165689.1 | HQ1665747.1 | data missing | HQ165712.1 |
| *M. pemphigi* | ARSEF 7491 | data missing | data missing | KJ398819.1 | KJ398629.1 | DQ468379.1 | data missing |
| *M. pemphigi* | ARSEF 6569 | data missing | data missing | KJ398813.1 | KJ398624.1 | DQ468378.1 | data missing |
| *M. pemphigi* | BUM 39.4 | KY087813 | KY087817 | KY087821 | KY087825 | KY087827 | KY087809 |
| *M. pinghaense* | CBS 257.90 | data missing | data missing | EU248850.1 | EU248902.1 | EU248930.1 | NR077205.1 |
| *M. pinghaense* | ARSEF 4342 | data missing | data missing | EU248851.1 | EU248903.1 | EU248931.1 | HQ331454.1 |
| *M. pseudoatrovirens* | TNSF 16380 | JF415977.1 | data missing | data missing | JN049893.1 | JF415997.1 | JN049870.1 |
| *M. rileyi* | ARSEF 1972 | data missing | data missing | KJ398803.1 | KJ398613.1 | data missing | data missing |
| *M. rileyi* | CBS 806.71 | data missing | AY526491.2 | EF468787.1 | EF468893.1 | EF468937.1 | NR119513.1 |
| *M. robertsii* | ARSEF 727 | data missing | data missing | DQ463994.1 | DQ468353.1 | DQ468368.1 | HQ331453.1 |
| *M. viride* | ARSEF 2456 | data missing | data missing | KJ398808.1 | KJ398619.1 | data missing | EU553291.1 |
| *M. viride* | CBS 659.71 | HQ165735.1 | HQ165673.1 | HQ165692.1 | data missing | HQ165652.1 | HQ165714.1 |
| *M. viridulum* | ARSEF 6927 | data missing | data missing | KJ398815.1 | KJ398626.1 | data missing | data missing |
| *M. yongmunense* | EFCC 2135 | data missing | EF468979 | EF468834 | EF468769 | EF468877 | data missing |
| *M. yongmunense* | EFCC 2131 | EF468833.1 | EF468977.1 | EF468770.1 | EF468876.1 | data missing | JN049856.1 |
| *M. shibinensis* | GZUHSB 13050311 | KR153588.1 | data missing | KR153589.1 | KR153590.1 | data missing | KR153585.1 |
| *B. bassiana* | ARSEF 7518 | HQ880975.1 | HQ880975.1 | HQ880975.1 | HQ880834.1 | HQ880834.1 | HQ880762.1 |

*Metarhizium* was abbreviated to *M*; *Beauveria* was abbreviated to *B*

and often prominently guttulate on the surface of conidia layer (Fig. 2A and B), while *M. pemphigi* colonies formed radial constriction in central region (Fig. 2C and D). The conidia cultures were green for *M. gaoligongense* and emerald green for *M. pemphigi* (Fig. 2B and C).

*M. pemphigi* could diffuse yellow-green pigmentation into PDA medium, while *M. gaoligongense* hardly produced pigment diffusion (Fig. 2C and D).

## Discussion

Most species in *Metarhizium* are biological insecticides and have significant ecological and economic values (Kepler *et al*., 2014). New species of *Metarhizium* were continuously revealed and enriched the biocontrol fungal resource (Wen *et al*., 2015). Herein a new species of *Metarhizium*, *M. gaoligongense*, was proposed and described from morphological characters and phylogenetic analysis. Six

**Table 2:** Morphological characters comparison among *Metarhizium flavoviride* complex

| Species | Strain | Habitat | Host | Phialides ($\mu$m) | Conidia ($\mu$m) | Reference |
|---|---|---|---|---|---|---|
| *M. minus* | ARSEF 2037 | Philippines | Soil | | 4.5–7.0×2.0–3.0 | Rombach *et al*., 1986 |
| *M. pemphigi* | DAR 74295 | Britain | Pemphigus treherni | | 5.4 ± 0.47×2.4 ± 0.43 | Driver *et al*., 2000 |
| *M. pemphigi* | BUM39.4 | China | Soil | 6.9-16.3×2.1-2.9 (10.1 ± 3.1×2.4 ± 0.4) | 4.6-8.6×1.9-2.7 (6.2 ± 1.2×2.2 ± 0.3) | This study |
| *M. gaoligongense* | CCTCC M 2016588 | China | Soil | 5.6-18.4×1.6-3.4 (11.3 ± 3.2×2.5 ± 0.4) | 5.4-7.7×1.9-2.8 (6.7 ± 0.9×2.3 ± 0.3) | This study |
| *M. frigidum* | ARSEF 4124 | Australia | Coleoptera | | 4.5–7.5×2.5–3.5 | Bischoff *et al*., 2006 |
| | ARSEF 4561 | Australia | Soil | 6.5–12.5×2.5–4.0 | 4.5–7.5 (–9)×3.0–4.0 | Bischoff *et al*., 2006 |
| *M. koreanum* | ARSEF 2039 | Korean | Planthoppers | 9.0–17.5×3.0–5.0 | 6.0–9.0×3.0–4.0 | Kepler *et al*., 2014 |
| *M. flavoviride* | ARSEF 2025 | Germany | Agricultural Soil | 11.5–17.0×2.5–4.5 | 8.0–11.0×3.5–4.5 | Bischoff *et al*., 2006 |

*Metarhizium* was abbreviated to *M.*

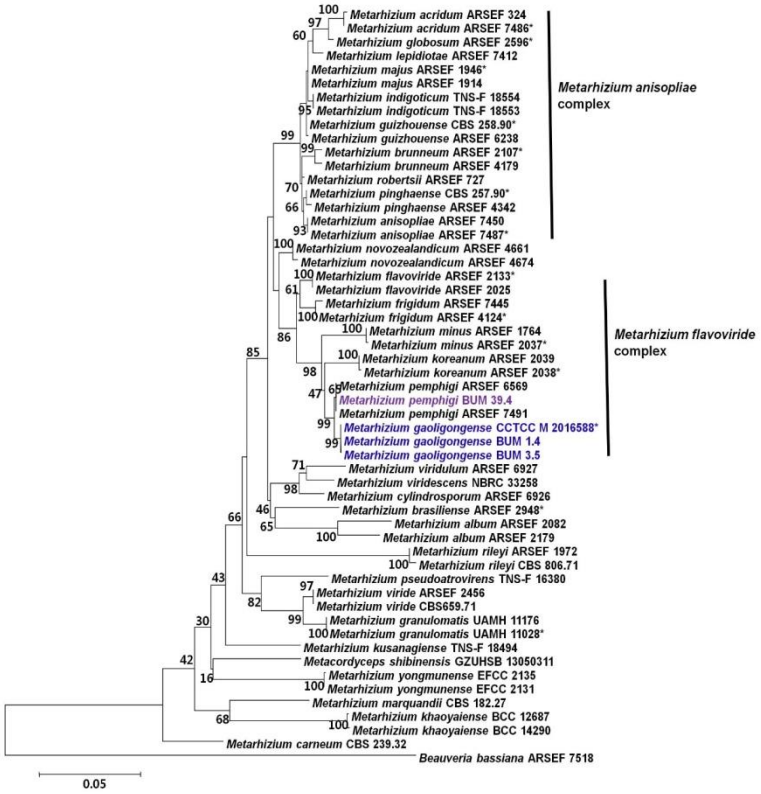


**Fig. 1:** Phylogenetic tree of *Metarhizium* based on ML analysis of 6-locus (5.8S-ITS, nrSSU, nrLSU, EF-1α, RPB1 and RPB2) dataset

*Denotes an ex-type isolate

widely used genes (5.8S-ITS, nrSSU, nrLSU, EF-1α, RPB1 and RPB2) in fungal phylogenetic analysis (Kepler *et al*., 2012; Sanjuan *et al*., 2014; Wen *et al*., 2015) were selected. The dataset of 30 species in *Metarhizium* used by Kepler *et al*. (2014) were included in this study and their phylogenetic affiliation was well congruent, suggesting that our result could depict similar interspecific genetic relationships and the position of *M. gaoligongense* in the tree from 6-locusdata was reasonable and reliable. *M. gaoligongense* was clearly separated from its allied species and well supported as an independent clade, being differed from its closest species, *M. pemphigi*, with credible bootstrap support (99%) (Fig. 1). Morphology also supported the recognition of *M. gaoligongense* as a distinct species. *M. gaoligongense* was quite similar to *M. pemphigi* on thinner conidia and phialide, but its conidia were longer and its culture characteristics on PDA medium had considerable distinction with *M. pemphigi*, including flat colony surface, guttulate on the surface of conidia layer and its conidia being green without pigment diffusion.

The type material for *M. gaoligongense* was isolated from the dry-hot valley of Gaoligong mountains, Yunnan Province, China. The habitat was the soil of a coffee farmland with high temperature (31–35°C), low humidity (30%–34%). The vast zone of dry-hot valley of Gaoligong mountains might hold a treasure of undiscovered and unclassified species of entomopathogenic fungi and should be further investigated. The host and telomorphic stage of

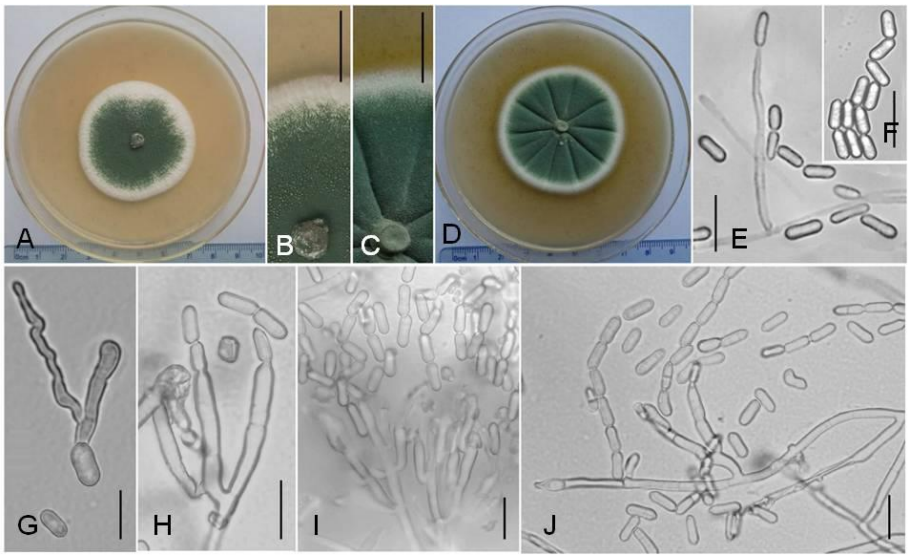


**Fig. 2:** Morphology of *Metarhizium gaoligongense* and *M. pemphigi*
**A, B:** colony of *Metarhizium gaoligongense*; **C, D:** colony of *M. pemphigi*; **E:** phialides and conidia of *M. pemphigi*; **F:** conidial chain of *M. pemphigi*; **G:** sprouting conidia of *M. gaoligongense*; **H:** phialide with budding conidium of *M. gaoligongense*; **I:** phialide with conidial chain of *M. gaoligongense*; **J:** conidial chain and separated conidia of *M. gaoligongense*. Bars: B, C 1 cm; E–J 10 $\mu$m
*M. gaoligongense* Z.H. Chen & L. Xu, spp. Nov, MycoBank no.: MB 818981

*M. gaoligongense* were unknown so far. Additional specimens of this new species were expected to determine the precise anamorph-teleomorph connection.

## Conclusion

*M. gaoligongense* (CCTCC M 2016588) from Gaoligong mountains was confirmed to be a *Metarhizium* species according to morphology and mutilocus molecular evidence (5.8S-ITS, nrSSU, nrLSU, EF-1α, RPB1 and RPB2). Both morphology and phylogenic affiliations of this species was closet to *M. pemphigi*, while the database of its muti-gene sequences formed a dominant independent clade and its colony on PDA medium was obviously distinct from *M. pemphigi*.

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## References

Bischoff, J.F., S.A. Rehner and R.A. Humber, 2009. A multilocus phylogeny of the *Metarhizium anisopliae* lineage. *Mycologia*, 101: 512–530

Bischoff, J.F., S.A. Rehner and R.A. Humber, 2006. *Metarhizium frigidum* spp. nov.: a cryptic species of *M. anisopliae* and a member of the *M. flavoviride* complex. *Mycologia*, 98: 737–745

Chen, Z.H., Y.D. Dai, H. Yu, K. Yang, Z.L. Yang, F. Yuan and W.B. Zeng, 2013. Systematic analyses of *Ophiocordyceps lanpingensis* spp. nov., a new species of *Ophiocordyceps* in China. *Microbiol. Res.*, 168: 525–532

Driver, F., R.J. Milner and W.H. Wrueman, 2000. A taxonomic revision *Metarhizium* of based on a phylogenetic analysis of rDNA sequence data. *Mycol. Res.*, 104: 134–150

Kavanaugh, D.H., F. Hieke, H. Liang and D. Dong, 2014. Inventory of the carabid beetle fauna of the Gaoligong Mountains, western Yunnan Province, China: species of the tribe Zabrini (Coleoptera, Carabidae). *Zookeys*, 407: 55–119

Kepler, R.M., R.A. Humber, J.F. Bischoff and S.A. Rehner, 2014. Clarification of generic and species boundaries for *Metarhizium* and related fungi through multigene phylogenetics. *Mycologia*, 106: 811–829

Kepler, R.M., G.H. Sung, S. Ban, A. Nakagiri, M.J. Chen, B. Huang, Z.Z. Li and J.W. Spatafora, 2012. New teleomorph combinations in the entomopathogenic genus *Metacordyceps*. *Mycologia*, 104: 182–197

Keyser, C.A., H.H.D.F. Licht, B.M. Steinwender and N.V. Meyling, 2015. Diversity within the entomopathogenic fungal species *Metarhizium flavoviride* associated with agricultural crops in Denmark. *BMC Microbiol.*, 15: 249

Lacey, L., R. Frutos, H. Kaya and P. Vail, 2001. Insect pathogens as biological control agents: Do they have a future? *Biol. Contr.*, 21: 230–248

Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson and D.G. Higgins, 2007. Clustal W and clustal X version 2.0. *Bioinformatics*, 23: 2947–2948

Li, C., B. Huang, M. Fan, Y. Lin and Z. Li, 2010. *Metacordyceps guniujiangensis* and its *Metarhizium* anamorph: a new pathogen on cicada nymphs. *Mycotaxon*, 111: 221–231

Liu, Z.Y., Z.Q. Liang, A.J.S. Whalley, Y.J. Yao and A.Y. Liu, 2001. *Cordyceps brittlebankisoides*, a New Pathogen of Grubs and Its Anamorph, *Metarhizium anisopliae* var. *majus*. *J. Invertebr. Pathol.*, 78: 178–182

Roberts, D.W. and R.J.S. Leger, 2004. *Metarhizium* spp., cosmopolitan insect-pathogenic fungi: mycological aspects. *Adv. Appl. Microbiol.*, 54: 1–70

Rombach, M.C., R.A. Humber and H.C. Evans, 1987. *Metarhizium album*, a fungal pathogen of leaf- and plant hoppers of rice. *Trans. Brit. Mycol. Soc*., 88: 451–459

Rombach, M.C., R.A. Humber and D.W. Roberts, 1986. *Metarhizium flavoviride* var. *minus* var. nov. a pathogen of plant- and leafhoppers on rice in the philippines and solomon islands. *Mycotaxon*, 27: 87–92

Sanjuan, T., J. Tabima, S. Restrepo, T. Læssøe, J.W. Spatafora and A.E. Franco-Molano, 2014. Entomopathogens of Amazonian stick insects and locusts are members of the *Beauveria* species complex (Cordyceps sensu stricto). *Mycologia*, 106: 260–275

Sung, G.H., N.L. Hywel-Jones, J.M. Sung, J.J. Luangsa-ard, B. Shrestha and J.W. Spatafora, 2007. Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. *Stud. Mycol*., 57: 5–59

Tamura, K., G. Stecher, D. Peterson, A. Filipski, M. Nei and S. Kumar, 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol*., 30: 2725–2729

Wang, J.B., R.J.S. Leger and C. Wang, 2016. Advances in Genomics of Entomopathogenic Fungi. *Adv. Genet*., 94: 1–39

Wen, T.C., L.S. Zha, Y.P. Xiao, Q. Wang, J.C. Kang and K.D. Hyde, 2015. *Metacordyceps shibinensis* sp. nov. from larvae of Lepidoptera in Guizhou Province, southwest China. *Phytotaxa*, 226: 51–62

Zhang, W.M., T.H. Li, Y.Q. Chen and L.H. Qu, 2004. *Cordyceps campsosterna*, a new pathogen of *Campsosternus auratus*. *Fung. Divers*., 17: 239–242

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