



# *Metarhizium puerense* (Hypocreales, Clavicipitaceae): a new species from Yunnan, south-western China

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## Abstract

## Background

As a genus within the Clavicipitaceae, *Metarhizium* exhibits rich morphological and ecological diversity, with a wide distribution and a variety of hosts. Currently, sixty-eight species of *Metarhizium* have been described.

## New information

A new species of *Metarhizium*, *M. puerense* (Hong Yu bis), was described in Pu'er City, Yunnan Province, south-western China. Based on morphological characteristics and multilocus phylogenetic analyses, *Metarhizium puerense* was confirmed to be phylogenetically related to *M. album*, but was clearly separated and formed a distinct branch. In contrast, the host of *Metarhizium album* was plants and leafhoppers and that lepidopteran larvae were the host of *M. puerense*. The diagnostic features of *M. puerense* were solitary to multiple stromata and smooth-walled, cylindrical with rounded apices conidia.

## Keywords

*Metarhizium*, morphology, phylogenetic analyses

## Introduction

*Metarhizium*, as a group with rich morphological and ecological diversity in Clavicipitaceae, is very rich in widely distributed and complex habitats (Bischoff et al. 2009). The type species *Metarhizium anisopliae* (Metschn 1879) was used by Sorokīn (1883) to establish the new asexual genus *Metarhizium* (Tulloch 1976). Advances in molecular systematics have led to the emergence of multigene systematic analysis as a new technical method for the taxonomic identification of *Metarhizium* sp., combining functional protein genes with rDNA gene fragments (Sung et al. 2007 and Kepler et al. 2012 and Kepler et al. 2014). In their study of genetic diversity within *Metarhizium* species, Driver et al. (2000) were the first to utilise molecular biology techniques. They solved the problem of classification at the species and varietal levels by identifying four variants in the *Metarhizium anisopliae* complex groups, five variants in the *M. flavoviride* (Gams 1973) complex groups and delineating *M. album* (Petch 1931). In a multi-gene phylogenetic study of *Metarhizium anisopliae* and *M. flavoviride* lineages, Bischoff et al. 2006 and Bischoff et al. 2009 elevated and accepted *Metarhizium* varieties to species rank by using additional protein-coding genes (*EF-1 $\alpha$* , *RPB1*, *RPB2* and *TUB*). The re-examination of *Metarhizium* and related genera led to the establishment of six new genera: *Keithomyces*, *Marquandomyces*, *Papiliomyces*, *Purpureomyces*, *Sungia* and *Yosiokobayasia* (Mongkolsamrit et al. 2020). *Chamaeleomyces* (Samson 1974) and *Nomuraea* spp. (Samson 1974), excluding *N. atypicola* (Samson 1974) and *Paecilomyces viridis* (Segretain 1964), were transferred to *Metarhizium*, and 19 new species of *Metarhizium* were reported. Other new species have been reported by Chen et al. 2018a and Chen et al. 2018b and Chen et al. 2018c and Chen et al. 2023 and Li et al. (2023). Currently, sixty-eight species of *Metarhizium* have been described.

According to the latest classification system, the *Metarhizium* genus belongs to the Fungi, Ascomycota, Sordariomycetes, Hypocreales and Clavicipitaceae. Its typical morphological characteristics are: Sexual form: Stromata single or multiple, unbranched or irregularly branched, mostly fleshy, with the main colours being pale yellow, green to greenish-brown or dark purple; fertile parts columnar or rod-shaped; perithecia partially or completely immersed; asci mainly columnar, ascospores linear, fusiform, breaking into secondary ascospores upon maturity or not. Asexual form: Growing rapidly on PDA medium, the colonies are flat and velvety, initially white and turn yellow-green or green after sporulation; phialides are morphologically diverse, single on the aerial hyphae or verticillate on the conidiophores; conidia are smooth, oval to columnar, spherical to subspherical, ovoid, aggregated in chains or clusters (Liang 2007). The typical characteristics of *M. puerense* were solitary to multiple stromata and smooth-walled, brownish in colour and producing a large number of green powdery conidia at the tip.

*Metarhizium* species that parasitise lepidopteran larvae were collected from Yunnan for this investigation. Phylogenetic location was elucidated, based on Bayesian Inference (BI) and Maximum Likelihood (ML) analyses, which involved concatenating sequences of the six loci. The results revealed that the species in question belong to the genus *Metarhizium*, specifically *Metarhizium puerense*.

## Materials and methods

### Collection and isolation of strains

Specimens were collected from the broad-leaved evergreen forest of Pu'er, Yunnan Province, China, 2 August 2023, 22°71.33'N, 100°95.57'E, alt. 1358 m. The samples were preserved in sterile tubes and stored at 4°C. To obtain pure cultures, fresh specimens were cleaned and surface-sterilised by soaking in 30% hydrogen peroxide for approximately one minute. The samples were then washed with sterile water to remove residual hydrogen peroxide and the residual water was aspirated with a sterile filter paper. The worms were dissected on an ultra-clean bench, picked up with a sterilised scalpel with an appropriate amount of white tissue in the sclerotium centre, inoculated on potato dextrose agar medium (PDA: fresh potato 200 g/l, dextrose 20 g/l and agar 18 g/l) (Wang et al. 2020) and allowed to incubate at room temperature. The collected specimens were placed in the Yunnan Herbarium of the Yunnan University (YHH). The obtained strains were preserved at the Yunnan Fungal Culture Conservation Center (YFCC).

### Morphological characterization

Fresh specimens, including the stromata and hosts, were photographed using a Canon 750D camera. For descriptions of colony appearance and microscopic features, the colonies on PDA plates were cultured for two weeks and the colony characteristics (size, texture and colour) were photographed with a Canon 700D camera to characterise the morphology of the colonies. Observations, measurements and photographs of the phialides and conidia were obtained using a light microscope (Olympus BX53).

### DNA extraction, PCR and sequencing

DNA extraction was performed using a ZR Fungal DNA kit (Zymo, California, USA). DNA was preserved at -20°C and used as a template for PCR amplification of the six loci. To amplify the largest and second-largest subunit sequences of RNA polymerase II (*RPB1* and *RPB2*), the primer pair RPB1-5'F and RPB1-5'R, as well as the primer pair RPB2-5'F and RPB2-5'R, were applied (Bischoff et al. 2006). The nuclear ribosomal small and large subunits (SSU and LSU) were amplified using the primer pairs used by 18S-CoF and 18S-CoR (Wang et al. 2015), as well as LR5 and LR0R (Vilgalys and Hester 1990 and Rehner and Samuels 1994). The translation elongation factor 1 $\alpha$  (*EF-1 $\alpha$* ) gene was amplified using the primer pair *EF1 $\alpha$ -EF* and *EF1 $\alpha$ -ER* (Bischoff et al. 2006 and Sung et al. 2007). PCR primers used to amplify the internal transcribed spacers were ITS4 and ITS5 (White et al. 1990). All PCR reactions were performed in a final volume of 50  $\mu$ l and

contained 25 µl of 2 × Taq PCR Master Mix (Tiangen, Beijing, China), 0.5 µl forward and reverse primers (10 µM), 1 µl template DNA (1 ng/µl) and 23 µl sterile distilled water. The polymerase chain reaction (PCR) was performed as described by Wang et al. (2015).

### Phylogenetic analysis

The data matrix included 72 sequences from 48 species in *Metarhizium* and two outgroup taxa. Sequences of six loci (ITS, SSU, LSU, *EF-1α*, *RPB1* and *RPB2*) were retrieved from GenBank. Sequences were aligned using MUSCLE software (Tamura et al. 2013). After alignment, the gene sequences were concatenated. *Clonostachys rosea* (GJS 90-227) and *Hydropisphaera peziza* (CBS 102038) were designated as the outgroup taxa. Phylogenetic analyses were conducted using BI and ML methods with MrBayes v.3.1.2 and RaxML 7.0.3, respectively (Ronquist and Huelsenbeck 2003 and Stamatakis et al. 2008). The GTR+G+I model was determined using jModelTest version 2.1.4 (Darriba et al. 2012) with five million generations for the BI analysis. GTR+I was selected as the optimal model for the ML analysis and 1,000 rapid bootstrap replicates were performed on the dataset.

### Taxon treatment

#### *Metarhizium puerense* Hong Yu bis, J. M. Ma & Z.Q. Wang, sp. nov.

- MycoBank [852903](https://www.ncbi.nlm.nih.gov/GenBank/852903)

#### Materials

##### *Holotype*:

- scientificName: *Metarhizium puerense* sp. nov.; country: China; stateProvince: Yunnan; locality: Pu'er City, Simao District; verbatimElevation: 1358 m; verbatimLatitude: 22°71.33'N; verbatimLongitude: 100°95.57'E; year: 2023; month: August; day: 2; identifiedBy: Hong Yu bis; institutionID: YHH MP2308031; collectionID: YFCCMP 9458; occurrenceID: 5C808899-7FA6-5C36-8FA2-DBD6D69DBD82

##### *Other materials*:

- scientificName: *Metarhizium puerense* sp. nov.; country: China; stateProvince: Yunnan; locality: Pu'er City, Simao District; verbatimElevation: 1358 m; verbatimLatitude: 22°71.33'N; verbatimLongitude: 100°95.57'E; year: 2023; month: August; day: 2; identifiedBy: Hong Yu bis; institutionID: YHHMP 2308032; collectionID: YFCCMP 9459; occurrenceID: E7C934B3-11EC-5ED2-A85F-CF9975A5C5E4
- scientificName: *Metarhizium puerense* sp. nov.; country: China; stateProvince: Yunnan; locality: Pu'er City, Simao District; verbatimElevation: 1359 m; verbatimLatitude: 22°71.33'N; verbatimLongitude: 100°95.57'E; year: 2023; month: August; day: 2; identifiedBy: Hong Yu bis; institutionID: YHHMP 2308033; occurrenceID: D72AACD2-AF02-550B-8D65-667FC7E27EB7

#### Description

**Sexual morph:** Sexual morphs were not found.

**Asexual morph:** Stroma arising from the larvae of Lepidoptera larva buried in soil, solitary or multiple, brownish in colour and producing a large number of green powdery conidia at the tip. Colonies on PDA grew at 25°C, reaching 25-28 mm diam. in 14 days, cottony with high mycelium density, white to light yellow and reverse yellow; 45-52 mm in diameter in 30 days at 25°C, first white turning to green, powdery while sporulating, white mycelium at the margin. Hyphae septate, smooth-walled. Conidiophores smooth, cylindrical and erect. Phialides cylindrical, borne singly on aerial mycelium or whorled on conidial peduncle,  $6.1-17.6 \times 1.5-2.9 \mu\text{m}$ . Conidia were smooth-walled, ellipsoid to columnar, rounded at the tip, aggregated into chains or clusters,  $3.8-7.1 \times 1.3-2.1 \mu\text{m}$  (Fig. 1).

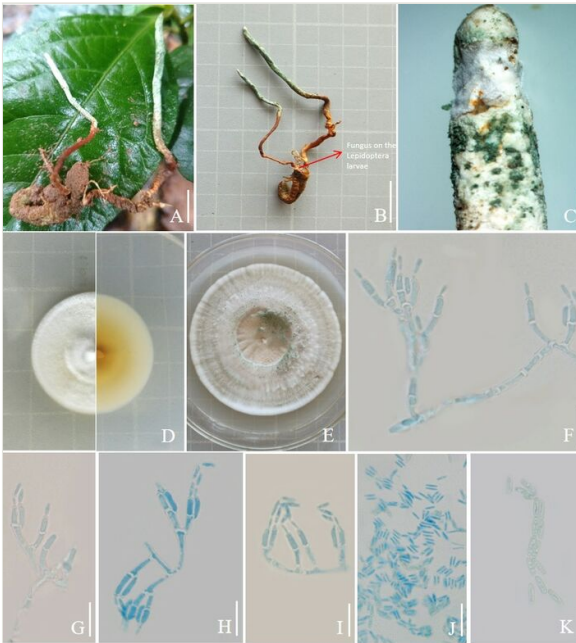


Figure 1. [doi](#)

**Figure 1.** *Metarhizium puerense* (YFCCMP 9458). **A.** Stromata arising from hosts buried in soil. **B.** Fungus on the larvae of Lepidoptera. **C.** Apical part of stromata **D-E.** Culture characters on PDA (**D** = after 14 days, **E** = after 30 days). **F-I.** Conidiophores, phialides and conidia. **J-K.** Conidia. Scale bars: **A-E** = 1 cm. **F-J** = 10  $\mu\text{m}$ . **K** = 5  $\mu\text{m}$ .

**Notes:** Phylogenetically, *Metarhizium puerense* is closely related to *M. album*, but differs in morphological characteristics. The morphological characteristics of *M. puerense* are as follows: stroma arising from the larva of Lepidoptera buried in soil,

solitary or multiple, brownish in colour and producing a large number of green powdery conidia at the tip. *Metarhizium album* was collected from plants and leafhoppers (Homoptera, Auchenorrhyncha) from rice. Moreover, *M. puerense* was indicated by its conidia size ( $3.8\text{-}7.1 \times 1.3\text{-}2.1 \mu\text{m}$ ), which was smaller than that of *M. album* ( $5\text{-}8 \times 2\text{-}2.5 \mu\text{m}$ ). The phialides of *M. puerense* ( $6.1\text{-}17.6 \times 1.5\text{-}2.9 \mu\text{m}$ ) was more slender than *M. album* ( $10\text{-}12.5 \times 2\text{-}3.5 \mu\text{m}$ ) (Michiel et al. 1987). Morphological comparisons of *Metarhizium puerense* with its related species (Table 2).

### Etymology

Named after Pu'er City, where the species were first collected.

### Analysis

These 49 taxa were used for phylogenetic analyses (Table 1). The combined six-locus dataset contained 4862 base pairs (bp) of sequences after alignment: 607 bp for ITS, 914 bp for SSU, 802 bp for LSU, 902 bp for *EF-1 $\alpha$* , 688 bp for *RPB1* and 1101 bp for *RPB2*. *Clonostachys rosea* (GJS 90227) and *Hydropisphaera peziza* (CBS 102038) were designated as outgroup taxa in the phylogenetic tree. In phylogenetic trees based on both Bayesian Inference (BI) and Maximum Likelihood (ML) analyses, the samples collected in Yunnan formed a strongly-supported clade that was sister to *M. album* (BI posterior probability = 1.00, ML bootstrap =100%). This result indicates that it is a new species of *Metarhizium*, named *M. puerense* (Fig. 2).

Table 1.

GenBank accession numbers of materials used in this study.

<i>Metarhizium acidum</i>	ARSEF 7486	Orthoptera	<a href="#">HQ331458</a>			<a href="#">EU248845</a>	<a href="#">EU248897</a>	<a href="#">EU248925</a>
<i>Metarhizium album</i>	ARSEF 2082	Hemiptera	<a href="#">AY375446</a>	<a href="#">DQ522560</a>	<a href="#">DQ518775</a>	<a href="#">DQ522352</a>	<a href="#">KJ398617</a>	<a href="#">KJ398715</a>
<i>Metarhizium alvesii</i>	CG 1123	Soil				<a href="#">KY007614</a>	<a href="#">KY007612</a>	<a href="#">KY007613</a>
<i>Metarhizium anisopliae</i>	ARSEF 7487	Orthoptera	<a href="#">HQ331446</a>			<a href="#">DQ463996</a>	<a href="#">DQ468355</a>	<a href="#">DQ468370</a>
<i>Metarhizium anisopliae</i>	BUM 1900	Soil	<a href="#">MH143803</a>	<a href="#">MH143837</a>	<a href="#">MH143820</a>	<a href="#">MH143854</a>	<a href="#">MH143869</a>	<a href="#">MH143884</a>
<i>Metarhizium argentinense</i>	CEP 414	Blattodea: Blaberidae ( <i>Epilampra</i> sp.)	<a href="#">MF784813</a>			<a href="#">MF966620</a>	<a href="#">MF966621</a>	<a href="#">MF966622</a>
<i>Metarhizium argentinense</i>	CEP 424	Blattodea: Blaberidae ( <i>Epilampra</i> sp.)				<a href="#">MF966624</a>	<a href="#">MF966625</a>	<a href="#">MF966626</a>
<i>Metarhizium baoshanense</i>	BUM 63.4	Soil	<a href="#">KY264173</a>	<a href="#">KY264178</a>	<a href="#">KY264175</a>	<a href="#">KY264170</a>	<a href="#">KY264181</a>	<a href="#">KY264184</a>
<i>Metarhizium baoshanense</i>	CCTCCM2016589	Soil	<a href="#">KY264172</a>	<a href="#">KY264177</a>	<a href="#">KY264174</a>	<a href="#">KY264169</a>	<a href="#">KY264180</a>	<a href="#">KY264183</a>
<i>Metarhizium bionidarum</i>	CBS 648.67	Coleoptera: Scarabaeidae ( <i>Cetonia aurata</i> )				<a href="#">LC126075</a>	<a href="#">LC125907</a>	<a href="#">LC125923</a>

<i>Metarhizium bionidarum</i>	NBRC 112661	Diptera (March fly larva)				<a href="#">LC126076</a>	<a href="#">LC125908</a>	<a href="#">LC125924</a>
<i>Metarhizium blattodeae</i>	ARSEF 12850	Blattodea: Ectobiidae	<a href="#">KU182915</a>			<a href="#">KU182917</a>	<a href="#">KU182918</a>	<a href="#">KU182916</a>
<i>Metarhizium blattodeae</i>	MY00896	Blattodea	<a href="#">HQ165697</a>	<a href="#">HQ165657</a>	<a href="#">HQ165719</a>	<a href="#">HQ165678</a>	<a href="#">HQ165739</a>	<a href="#">HQ165663</a>
<i>Metarhizium brachyspermum</i>	CM1	Coleoptera	<a href="#">LC469747</a>		<a href="#">LC469749</a>	<a href="#">LC469751</a>		
<i>Metarhizium brasiliense</i>	ARSEF 2948	Hemiptera	<a href="#">AF139854</a>			<a href="#">KJ398809</a>	<a href="#">KJ398620</a>	<a href="#">KJ398718</a>
<i>Metarhizium brittlebankisoides</i>	Hn1	Coleoptera				<a href="#">AB778556</a>	<a href="#">AB778555</a>	<a href="#">AB778554</a>
<i>Metarhizium brunneum</i>	ARSEF 2107T	Coleoptera	<a href="#">KC178691</a>		<a href="#">MH868397</a>	<a href="#">EU248855</a>	<a href="#">EU248907</a>	<a href="#">EU248935</a>
<i>Metarhizium campostemi</i>	BUM 10	Soil	<a href="#">MH143798</a>	<a href="#">MH143832</a>	<a href="#">MH143815</a>	<a href="#">MH143849</a>	<a href="#">MH143864</a>	<a href="#">MH143879</a>
<i>Metarhizium chaiyaphumense</i>	BCC 19020	Hemiptera: Cicadidae (Cicada adult)	<a href="#">HQ165695</a>	<a href="#">HQ165654</a>	<a href="#">HQ165716</a>	<a href="#">HQ165675</a>	<a href="#">HQ165737</a>	<a href="#">HQ165635</a>
<i>Metarhizium chaiyaphumense</i>	BCC 19021	Hemiptera: Cicadidae (Cicada nymph)	<a href="#">HQ165696</a>	<a href="#">HQ165655</a>	<a href="#">HQ165717</a>	<a href="#">HQ165676</a>	<a href="#">HQ165738</a>	<a href="#">HQ165636</a>
<i>Metarhizium chaiyaphumense</i>	BCC 78198	Hemiptera: Cicadidae (Cicada nymph)		<a href="#">KX369596</a>	<a href="#">KX369593</a>	<a href="#">KX369592</a>	<a href="#">KX369594</a>	<a href="#">KX369595</a>
<i>Metarhizium cylindrosporium</i>	ARSEF 6926	Hemiptera				<a href="#">KJ398814</a>	<a href="#">KJ398625</a>	<a href="#">KJ398723</a>
<i>Metarhizium cylindrosporium</i>	CBS 256.90	Hemiptera	<a href="#">MH862209</a>		<a href="#">MH873892</a>	<a href="#">KJ398783</a>	<a href="#">KJ398594</a>	<a href="#">KJ398691</a>
<i>Metarhizium flavoviride</i>	ARSEF 2025	Soil	<a href="#">AF138269</a>			<a href="#">KJ398804</a>	<a href="#">KJ398614</a>	<a href="#">KJ398712</a>
<i>Metarhizium flavoviride</i>	CBS 218.56	Coleoptera	<a href="#">MH857590</a>		<a href="#">MH869139</a>	<a href="#">KJ398787</a>	<a href="#">KJ398598</a>	<a href="#">KJ398694</a>
<i>Metarhizium frigidum</i>	ARSEF 4124	Coleoptera	<a href="#">HM055448</a>			<a href="#">DQ464002</a>	<a href="#">DQ468361</a>	<a href="#">DQ468376</a>
<i>Metarhizium gaoiigongense</i>	CCTCCM2016588	Soil	<a href="#">KY087808</a>	<a href="#">KY087812</a>	<a href="#">KY087816</a>	<a href="#">KY087820</a>	<a href="#">KY087824</a>	<a href="#">KY087826</a>
<i>Metarhizium globosum</i>	ARSEF 2596	Lepidoptera	<a href="#">HQ331459</a>			<a href="#">EU248846</a>	<a href="#">EU248898</a>	<a href="#">EU248926</a>
<i>Metarhizium granulomatis</i>	UAMH 11028	<i>Chamaeleo calyptratus</i>	<a href="#">HM195305</a>	<a href="#">HM635076</a>	<a href="#">HM195304</a>	<a href="#">KJ398781</a>		<a href="#">KJ398688</a>
<i>Metarhizium granulomatis</i>	UAMH 11176	<i>Chamaeleo calyptratus</i>	<a href="#">HM195306</a>		<a href="#">HM635078</a>	<a href="#">KJ398782</a>	<a href="#">KJ398593</a>	<a href="#">KJ398689</a>
<i>Metarhizium guizhouense</i>	CBS 258.90	Lepidoptera larva	<a href="#">MH862211</a>		<a href="#">MH873894</a>	<a href="#">EU248862</a>	<a href="#">EU248914</a>	<a href="#">EU248942</a>
<i>Metarhizium humberi</i>	IP 46	Soil				<a href="#">MH837574</a>	<a href="#">MH837556</a>	<a href="#">MH837565</a>
<i>Metarhizium humberi</i>	IP 86	Soil				<a href="#">MH837576</a>	<a href="#">MH837558</a>	<a href="#">MH837567</a>
<i>Metarhizium indigoticum</i>	TNS F18553	Lepidoptera larva	<a href="#">JN049874</a>	<a href="#">JF415952</a>	<a href="#">JF415968</a>	<a href="#">JF416010</a>	<a href="#">JN049886</a>	<a href="#">JF415992</a>
<i>Metarhizium kalasinense</i>	BCC 53581	Coleoptera larva	<a href="#">KC011178</a>	<a href="#">KC011174</a>	<a href="#">KC011182</a>	<a href="#">KC011188</a>		
<i>Metarhizium kalasinense</i>	BCC 53582	Coleoptera larva	<a href="#">KC011179</a>	<a href="#">KC011175</a>	<a href="#">KC011183</a>	<a href="#">KC011189</a>		
<i>Metarhizium koreanum</i>	ARSEF 2038	Hemiptera	<a href="#">HM055431</a>			<a href="#">KJ398805</a>	<a href="#">KJ398615</a>	<a href="#">KJ398713</a>

<i>Metarhizium lepidotae</i>	ARSEF 7412	Coleoptera	<a href="#">HQ331455</a>			<a href="#">EU248864</a>	<a href="#">EU248916</a>	<a href="#">EU248944</a>
<i>Metarhizium lepidotae</i>	ARSEF 7488	Coleoptera	<a href="#">HQ331456</a>			<a href="#">EU248865</a>	<a href="#">EU248917</a>	<a href="#">EU248945</a>
<i>Metarhizium lymantriidae</i>	BUM 818		<a href="#">OM955147</a>	<a href="#">OM951242</a>	<a href="#">OM951247</a>	<a href="#">OM988196</a>	<a href="#">OM988192</a>	<a href="#">OM988188</a>
<i>Metarhizium lymantriidae</i>	KUNCC 4991		<a href="#">OM955148</a>	<a href="#">OM951243</a>	<a href="#">OM951248</a>	<a href="#">OM988197</a>	<a href="#">OM988193</a>	-
<i>Metarhizium majus</i>	ARSEF 1914	Coleoptera	<a href="#">HQ331445</a>			<a href="#">EU248868</a>	<a href="#">EU248920</a>	<a href="#">EU248948</a>
<i>Metarhizium majus</i>	ARSEF 1946	Coleoptera	<a href="#">HM055450</a>			<a href="#">EU248867</a>	<a href="#">EU248919</a>	<a href="#">EU248947</a>
<i>Metarhizium minus</i>	ARSEF 2037	Hemiptera	<a href="#">AF 138271</a>	<a href="#">AF 339580</a>	<a href="#">AF 339531</a>	<a href="#">DQ522353</a>	<a href="#">DQ522400</a>	<a href="#">DQ522454</a>
<i>Metarhizium novozealandicum</i>	ARSEF 3056	Soil				<a href="#">KJ398810</a>	<a href="#">KJ398621</a>	<a href="#">KJ398719</a>
<i>Metarhizium novozealandicum</i>	ARSEF 4661	Soil				<a href="#">KJ398811</a>	<a href="#">KJ398622</a>	<a href="#">KJ398720</a>
<i>Metarhizium owarfense</i>	NBRC 33258	Hemiptera	<a href="#">JN049883</a>	<a href="#">HQ165669</a>	<a href="#">HQ165730</a>	<a href="#">JF416017</a>	<a href="#">KJ398596</a>	<a href="#">JF415996</a>
<i>Metarhizium pemphigi</i>	ARSEF 6569	Hemiptera: Apididae	-	-	-	<a href="#">KJ398813</a>	<a href="#">KJ398624</a>	<a href="#">KJ398722</a>
<i>Metarhizium pinghaense</i>	CBS 257.90	Coleoptera	<a href="#">HQ331450</a>	-	<a href="#">MH873893</a>	<a href="#">EU248850</a>	<a href="#">EU248902</a>	<a href="#">EU248930</a>
<i>Metarhizium prachinense</i>	BCC 47950	Lepidoptera	<a href="#">KC011176</a>	<a href="#">KC011172</a>	<a href="#">KC011180</a>	<a href="#">KC011186</a>	<a href="#">KC011184</a>	-
<i>Metarhizium prachinense</i>	BCC 47979	Lepidoptera	<a href="#">KC011177</a>	<a href="#">KC011173</a>	<a href="#">KC011181</a>	<a href="#">KC011187</a>	<a href="#">KC011185</a>	-
<i>Metarhizium purpureogenum</i>	ARSEF 12570	Soil				<a href="#">LC126079</a>	<a href="#">LC125911</a>	<a href="#">LC125922</a>
<i>Metarhizium purpureogenum</i>	ARSEF 12571	Soil			<a href="#">AB700552</a>	<a href="#">LC126078</a>	<a href="#">LC125913</a>	<a href="#">LC125920</a>
<i>Metarhizium putuoense</i>	HMAS 285457	Coleoptera (larva)		<a href="#">OQ981977</a>	<a href="#">OQ981970</a>	<a href="#">OQ980403</a>	<a href="#">OQ980411</a>	
<i>Metarhizium putuoense</i>	HMAS 285457	Coleoptera (larva)		<a href="#">OQ981978</a>	<a href="#">OQ981971</a>	<a href="#">OQ980404</a>	<a href="#">OQ980412</a>	
<b><i>Metarhizium puerense</i></b>	<b>YFCCMP 9458</b>	<b>Lepidoptera</b>	<a href="#">PP733948</a>	<a href="#">PP733950</a>	<a href="#">PP733952</a>	<a href="#">PP776150</a>	<a href="#">PP776152</a>	<a href="#">PP776154</a>
<b><i>Metarhizium puerense</i></b>	<b>YFCCMP 9459</b>	<b>Lepidoptera</b>	<a href="#">PP733949</a>	<a href="#">PP733951</a>	<a href="#">PP733953</a>	<a href="#">PP776151</a>	<a href="#">PP776153</a>	<a href="#">PP776155</a>
<i>Metarhizium reniforme</i>	ARSEF 429	Orthoptera	<a href="#">DQ069284</a>	<a href="#">HQ165671</a>	<a href="#">HQ165733</a>	<a href="#">HQ165690</a>		<a href="#">HQ165650</a>
<i>Metarhizium reniforme</i>	ARSEF 577	Orthoptera: Tettigoniidae	<a href="#">DQ069283</a>	<a href="#">HQ165672</a>	<a href="#">HQ165734</a>	<a href="#">HQ165691</a>		<a href="#">HQ165651</a>
<i>Metarhizium rileyi</i>	CBS 806.71	Lepidoptera: Noctuidae ( <i>Trichoplusia ni</i> )	<a href="#">AY624205</a>	<a href="#">AY526491</a>	<a href="#">MH872111</a>	<a href="#">EF468787</a>	<a href="#">EF468893</a>	<a href="#">EF468937</a>
<i>Metarhizium robertsii</i>	ARSEF 727	Orthoptera	<a href="#">HQ331453</a>			<a href="#">DQ463994</a>	<a href="#">DQ468353</a>	<a href="#">DQ468368</a>
<i>Metarhizium samlanense</i>	BCC 17091	Hemiptera: <i>Cicadellidae</i> (adult)	<a href="#">HQ165707</a>	<a href="#">HQ165665</a>	<a href="#">HQ165727</a>	<a href="#">HQ165686</a>		<a href="#">HQ165646</a>
<i>Metarhizium samlanense</i>	BCC 17093	Hemiptera: <i>Cicadellidae</i> (adult)	<a href="#">HQ165709</a>	<a href="#">HQ165666</a>	<a href="#">HQ165728</a>	<a href="#">HQ165687</a>	<a href="#">HQ165746</a>	<a href="#">HQ165647</a>
<i>Metarhizium takense</i>	BCC 30934	Hemiptera: Cicadidae ( <i>nymph</i> )	<a href="#">HQ165698</a>	<a href="#">HQ165658</a>	<a href="#">HQ165720</a>	<a href="#">HQ165679</a>	<a href="#">HQ165740</a>	<a href="#">HQ165639</a>



<i>Metarhizium takense</i>	BCC 30939	Hemiptera: Cicadidae (nymph)	<a href="#">HQ165699</a>	<a href="#">HQ165659</a>	<a href="#">HQ165721</a>	<a href="#">HQ165680</a>	<a href="#">HQ165741</a>	<a href="#">HQ165640</a>
<i>Metarhizium viride</i>	CBS 659.71	Hemiptera: Cicadidae (nymph)	<a href="#">HQ165714</a>	<a href="#">HQ165673</a>	<a href="#">HQ165735</a>	<a href="#">HQ165692</a>		<a href="#">HQ165652</a>
<i>Metarhizium viridulum</i>	ARSEF 6927	<i>Chamaeleo lateralis</i>				<a href="#">KJ398815</a>	<a href="#">KJ398626</a>	<a href="#">KJ398724</a>
<i>Metarhizium viridulum</i>	BUM 721	Hemiptera	<a href="#">MH143808</a>	<a href="#">MH143842</a>	<a href="#">MH143825</a>	<a href="#">MH143859</a>	<a href="#">MH143874</a>	<a href="#">MH143889</a>
<i>Metarhizium taii</i>	KS 50	Soil		<a href="#">GU979940</a>	<a href="#">GU979949</a>	<a href="#">GU979958</a>		<a href="#">GU979972</a>
<i>Metarhizium</i> sp.	OSC 110996			<a href="#">EF468974</a>	<a href="#">EF468832</a>	<a href="#">EF468773</a>	<a href="#">EF468880</a>	<a href="#">EF468928</a>
<i>Clonostachys rosea</i>	GJS 90-227			<a href="#">AY489684</a>	<a href="#">AY489716</a>	<a href="#">AY489611</a>		
<i>Hydropisphaera peziza</i>	CBS 102038			<a href="#">AY489698</a>	<a href="#">AY489730</a>	<a href="#">AY489625</a>	<a href="#">AY489661</a>	<a href="#">DQ522444</a>

Table 2.

Morphological comparisons of *Metarhizium puerense* with its related species.

Species	Host	Stromata	Fertile part	Colony on PDA	Anamorph	P hialides (µm)	Conidia (µm)	References
<i>M. puerense</i>	Lepidoptera larva	Solitary or multiple, 2.6–4.7 cm long, 1.2–1.5mm broad	Cylindrical to clavate, contains a large number of green conidia, 1–2 cm long, 1–1.5 mm broad	White dense mycelium, producing green spores later	Chain shape, clumping together	Solitary or in whorls of 2, 6.1–17.6 × 1.5–2.9	Ovoid to elliptical, 3.8–7.1 × 1.3–2.1	This study
<i>M. album</i>	Leafhoppers			Pure white to yellowish white, or greyish white becoming pinkish to fawn to pale brown upon sporulation	Conidial chains	Clavate phialides, solitary or in whorls of 2–5, 10–12.5 × 2–3.5	Narrowly ellipsoid or ovoid, (3–)4–6 × 1.5–2.5	Michiel et al., 1987
<i>M. brasiliense</i>	Leafhoppers (Hemiptera: Cicadellidae)			White to cream, becoming dark green to bluish green			Short conidia, 5.5–9 × 2.5–3.5	Kepler et al., 2014
<i>M. samlanense</i>	Leafhoppers (Hemiptera: Cicadellidae)			At first white turning green due to conidiation	Conidial chains	Phialides are short and cylindrical, 5–7 × 2–3	Green, globose, 3 × 5	Jennifer Luangsa-ard et al., 2016
<i>M. prachinense</i>	Lepidoptera larva	Stromata usually branched, 50–86 × 1–2 mm, broad	Cylindrical with pointed ends, white, pale yellow to grayish yellow, 0.8–1.7 × 1 mm	Initially colorless, turning green due to the production of green conidia	Conidial chains	Ovoid to obpyriform with short distinct neck, 3–5 × 2	Subglobose, green, 3–5 × 1.5–2.5	Jennifer Luangsa-ard et al., 2016

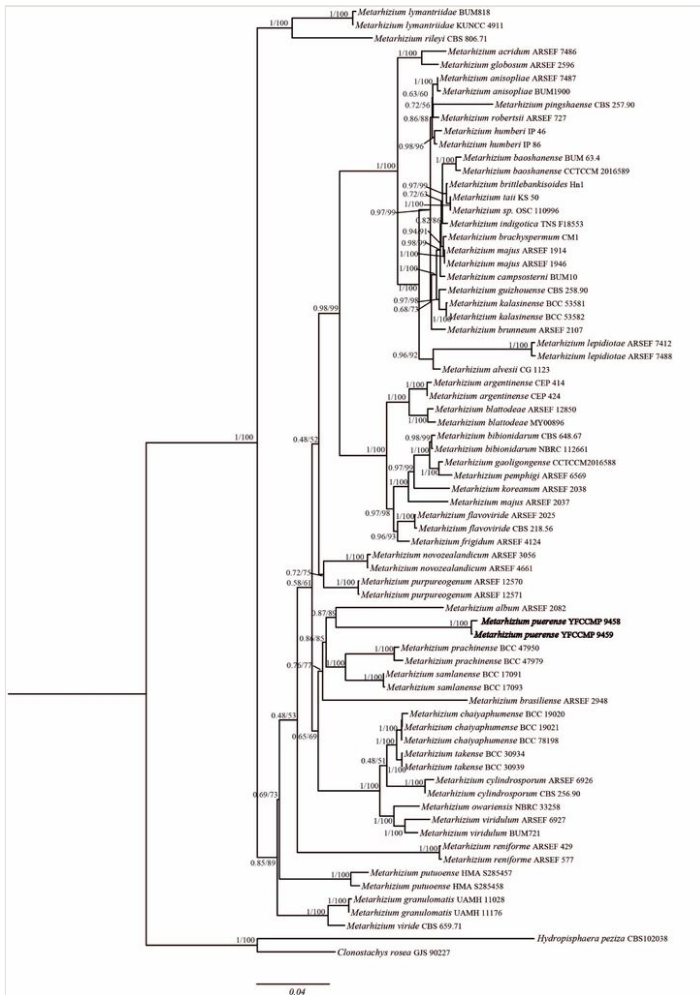


Figure 2. [doi](#)  
**Figure 2.** Phylogenetic placement of *M. puerense* was inferred from Maximum Likelihood (ML) and Bayesian Inference (BI) analyses, based on six loci (ITS, SSU, LSU, *EF-1 $\alpha$* , *RPB1* and *RPB2*).

## Discussion

To date, multi-locus phylogenetics, based on the joint analysis of ribosomal DNA and functional protein-coding genes, have been widely used in the phylogenetic study of fungi and have achieved many results (Sung et al. 2007 and Luangsa-ard et al. 2017 and Mongkolsamrit et al. 2020). In this study, we conducted an investigation, searched for and retrieved the *Metarhizium* nuclear gene sequences from the NCBI database. Subsequently, the sequences were compared with the obtained data. Additionally, a phylogenetic tree was constructed, based on multilocus database analyses (ITS, SSU,

LSU, *EF-1 $\alpha$* , *RPB1* and *RPB2*) to elucidate the phylogenetic position of *M. puerense*. Phylogenetically, *Metarhizium puerense* is closely related to *M. album*. However, in terms of morphological characteristics, *M. puerense* parasitises the larvae of Lepidoptera, either solitary or multiple and produces a large number of green conidia. There were also differences in the sizes of phialides and conidia.

In the forests of Pu'er City, Yunnan Province, China, which are characterised by a warm and humid climate, a diverse array of entomopathogenic fungi thrive. Amongst these, *Metarhizium* is a fungal insecticide with large-scale production capabilities. It offers significant value owing to its environment-friendly nature, extended efficacy period and low resistance potential. This makes it an important asset for pest control. Hence, it is crucial to accurately identify the *Metarhizium* species and determine their host range to facilitate the development and utilisation of this potent insecticidal agent. In the current study, a new species collected from Pu'er City, *Metarhizium puerense*, is described. The phylogenetic and morphological evidence presented in this study supports the classification of the species as a new taxon within the genus *Metarhizium*. This research contributes to the expansion of diversity within *Metarhizium* species, enhances our understanding of host interactions, morphology, distribution and pure culture characteristics and provides valuable taxonomic and phylogenetic information for further detailed investigations of the genus. Additionally, this opens up new possibilities for the development of fungal insecticides.

In the investigation of entomogenous fungi resources in Yunnan, a new species of *Metarhizium* was discovered and identified. This work not only increases the diversity of species in the genus *Metarhizium*, enriches the biological fungal species resource pool in Yunnan Province, but also lays a certain foundation for the distribution of *Metarhizium* species in Yunnan Province and other regions in China. Additionally, it deepened our understanding of the morphology, distribution and pure culture characteristics of the *Metarhizium* genus and provided taxonomic and phylogenetic information for a more detailed study of the genus's systematics.

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