



## *Metacordyceps shibinensis* sp. nov. from larvae of Lepidoptera in Guizhou Province, southwest China

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### Abstract

A new entomogenous taxon, *Metacordyceps shibinensis* sp. nov., associated with a larva of Lepidoptera was found in Yuntai Mountains, Guizhou Province, China. It differs from similar species in its white to faint yellow stromata, short ascomata, and very short asci and ascospores. Combined sequence analyses of 5.8S-ITS rDNA, nrSSU, EF-1 $\alpha$  and RPB1 gene-loci also confirmed the distinctiveness of this new species.

**Key words:** *Metacordyceps*, morphology, new species, phylogenetic analyses

### Introduction

*Cordyceps sensu lato* is regarded as one of the most important genera of invertebrate pathogens (Hywel-Jones 2001) with more than 540 species (Index Fungorum, 2015). About 140 species have been reported from China (Song *et al.* 2006, Liang 2007, Li *et al.* 2008, Gao *et al.* 2010, Zhang *et al.* 2010, Yang *et al.* 2009, Li *et al.* 2008, Lin *et al.* 2008, Li *et al.* 2010, Chen *et al.* 2011, Chen *et al.* 2013, Wen *et al.* 2013, 2014, Yan & Bau 2015, Yang *et al.* 2015). *Cordyceps* has been recently divided and placed in three families and six genera—*Polycephalomyces* (*Ophiocordycipitaceae*) (Kepler *et al.* 2013), *Tyrannicordyceps* (*Clavicipitaceae*) (Kepler *et al.* 2012b), *Metacordyceps* (*Clavicipitaceae*), *Elaphocordyceps* (*Ophiocordycipitaceae*), *Ophiocordyceps* (*Ophiocordycipitaceae*) and *Cordyceps* (*Cordycipitaceae*) (Sung *et al.* 2007a). More than 240 *Cordyceps* species have been placed into these six genera, but about half of *Cordyceps sensu lato* species need to be restudied and re-arranged (Sung *et al.* 2007a). Sung *et al.* (2007a) and (Kepler *et al.* 2012a) suggested that there are 15 *Metacordyceps* species, while 14 epithets are assigned to *Metacordyceps* in Index Fungorum (2015).

In this study, a new *Metacordyceps* species was found parasitizing larvae of Lepidoptera collected from the Yuntai Mountains of Guizhou Province in southwest China. This species is morphologically and phylogenetically distinct from all other *Cordyceps sensu lato* species. The purpose of this paper is to introduce the new species with micrographs and a description, and to compare it with related taxa.

### Material and methods

#### *Specimens and host*

Collection was made in the Yuntai Mountains, Shibin County of Guizhou Province in May 2013. Specimens were stored in plastic containers at low temperature and transported to the laboratory for identification.

#### *Morphological studies*

Fungal fruiting bodies were examined using an Olympus stereo dissecting microscope (Olympus Optec Instrument Co.,

Japan). Hand sections of the fruiting structures were mounted in water for microscopic studies and photomicrography. The microcharacters of the fungus were examined using an Olympus CX31 compound microscope (Olympus Optec Instrument Co., Japan) and photographed.

#### *DNA extraction, PCR amplification and determination of DNA sequences*

The total genomic DNA was extracted from rear segment of the host of dried specimens using E.Z.N.A.<sup>TM</sup> Fungal DNA MiniKit (Omega Biotech, CA, USA) according to the manufacturer's protocols and the extracted DNA was stored at -20 °C. Two nuclear (5.8S-ITS rDNA, nrSSU) and two protein genes (EF-1 $\alpha$ , RPB1) loci were amplified and sequenced (Sung *et al.* 2007b).

The PCR amplification and sequencing of ITS1-5.8S-ITS2 rDNA were conducted as described in Wen *et al.* (2012). The ITS1-5.8S-ITS2 rDNA was amplified and sequenced with the primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') (White *et al.* 1990). The PCR amplification and sequencing of nrSSU were conducted as described in Sung *et al.* (2007b). The nrSSU was amplified and sequenced with the primers NS1 (5'-GTAGTCATATGCTTGTCTC-3') and NS4 (5'-CTTCCGTCAATTCCTTTAAG-3') (White *et al.* 1990). In the amplification of EF-1 $\alpha$  and RPB1, we followed Sung *et al.* (2007b) and Castlebury *et al.* (2004). For the amplification of EF-1 $\alpha$ , the primers 983F (5'-GCYCCYGGHCAYCGTGAYTTYAT-3') and 2218R (5'-ATGACACCRACRGRACRGTGTG-3') (Sung *et al.* 2007b) were used. For RPB1, the primers CRPB1A (5'-CAYCCWGGYTTYATCAAGAA-3') and RPB1Cr (5'-CCNGCDATNTRTTRTCCATRTA-3') (Castlebury *et al.* 2004) were used in PCR amplification and sequencing procedure.

All PCR products were sequenced by Life Biotechnology Co., Shanghai, China.

#### *Sequence alignment and phylogenetic analysis*

Recent publications were accessed and a Blast search made to reveal the closest matches in GenBank for the phylogenetic analyses. The taxon information and GenBank accession numbers used in the molecular analyses are listed in Table 1. The four gene datasets (5.8S-ITS rDNA, nrSSU, EF-1 $\alpha$ , RPB1) from specimen GZUH SB13050311, plus datasets obtained from GenBank, including *Cordyceps sensu lato* and related species were aligned using MEGA5.05 (Tamura *et al.* 2011). Alignments were manually adjusted to allow maximum sequence similarity. Gaps were treated as missing data. Unweighted Maximum Parsimony (MP) analysis was performed using PAUP\* 4.0b10 (Swofford 2002). Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Maxtrees of 5,000 branches of zero length were collapsed and all multiple parsimonious trees were saved. Clade stability of the trees resulting from the parsimony analyses were assessed by bootstrap analysis with 1,000 replicates, each with 10 replicates of random stepwise addition of taxa (Felsenstein 1985). Trees were viewed in Treeview and exported to graphics programs (Page 1996); PowerPoint was used for further editing.

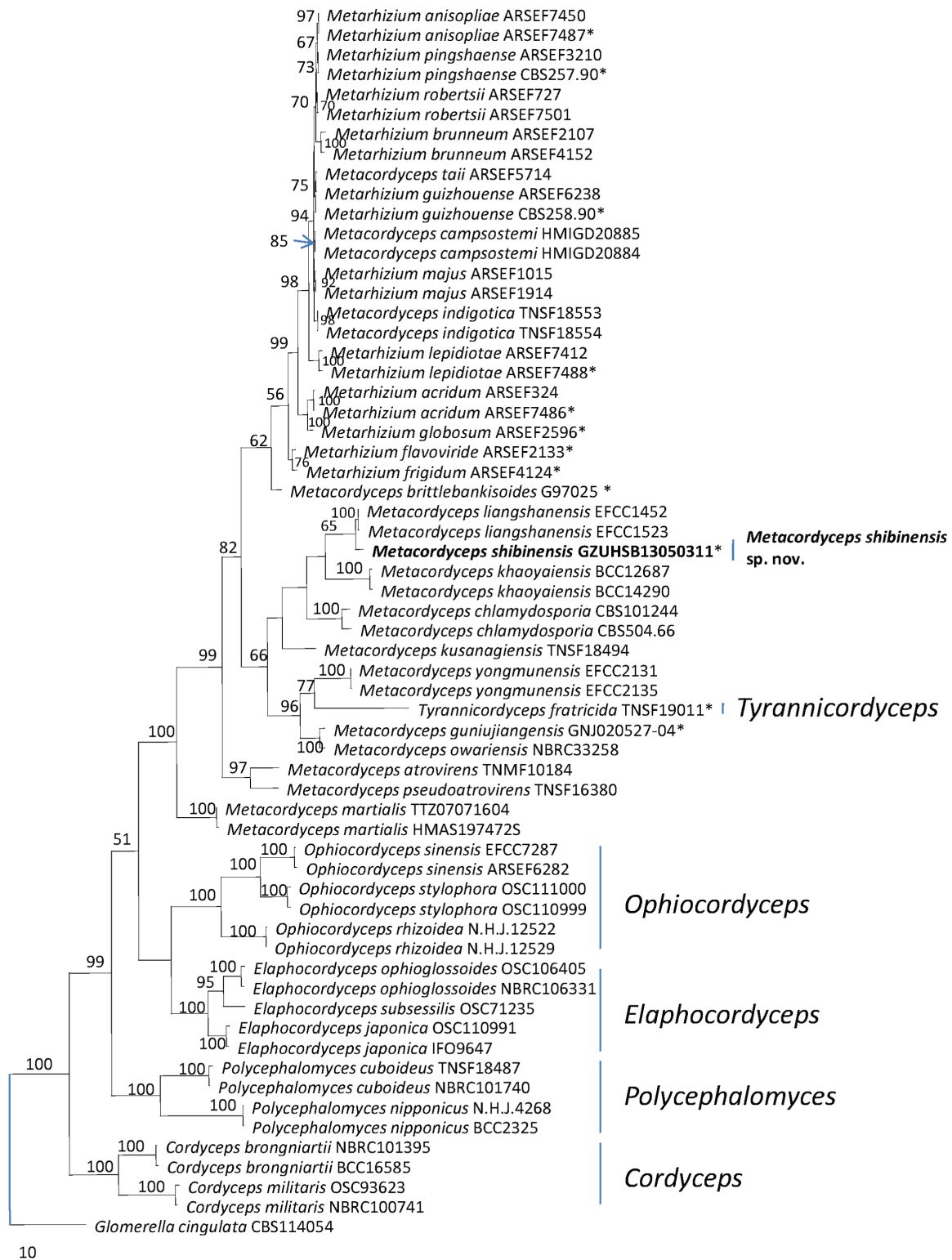
## Results

#### *Phylogenetic analyses*

The partition homogeneity test ( $P = 0.01$ ) suggested that the individual gene partitions were not highly incongruent (Farris *et al.* 1994, Cunningham 1997). The combined datasets comprised 3,566 characters after alignment, of which 1,394 characters were parsimony-informative, 1,751 constant, and 421 parsimony-uninformative. Parsimony analysis generated 5,000 trees; the SH test verified that they were similar, one of which (tree length = 1,309 steps, CI = 0.523, RI = 0.755, RC = 0.395, HI = 0.477), the most parsimonious tree, is shown in Fig. 1.

The data set comprises 35 species (Fig. 1) including the new species *Metacordyceps shibinensis* which formed a separate clade from other species of *Metacordyceps* with credible bootstrap support; thus the new species is introduced.

*Metacordyceps shibinensis* differs from the other species of *Metacordyceps* in having white to faint yellow stromata, short ascomata, and very short asci and ascospores (Fig. 2). *Metacordyceps chlamydosporia* (H.C. Evans) G.H. Sung *et al.* (2007a: 35), *M. guniujiangensis* C.R. Li *et al.* (2010: 221), *M. khaoyaiensis* (Hywel-Jones) Kepler *et al.* (2012a: 182), *M. liangshanensis* (M. Zang, D. Liu & R. Hu) G.H. Sung *et al.* (2007a: 35) and *M. owariensis* (Kobayasi) Kepler *et al.* (2012a: 182) are sister taxa to *M. shibinensis*, but differ in their host and morphology (Tab. 2). Combined sequence phylogeny from the 5.8S-ITS rDNA, nrSSU, EF-1 $\alpha$ , and RPB1 gene-loci also confirmed the distinctiveness of the new species.



**FIGURE 1.** Phylogenetic relationships among *Metacordyceps shibinensis* and related species based on four combined 5.8S-ITS rDNA, nrSSU, EF-1 $\alpha$ , RPB1 gene data. Bootstrap values (1,000 replicates) are indicated above the nodes. The tree is rooted to *Glomerella cingulata*. Type species have an asterisk.

TABLE 1. Taxa used in molecular analyses.

Species	Voucher <sup>1,2</sup>	Host/ Substratum	GenBank Accession Number				References
			5.8S ITS	nrSSU	EF-1 $\alpha$	RPB1	
<i>Metacordyceps shibinensis</i>	GZUH SB13050311*	Lepidopteran pupa	KR153585	KR153588	KR153589	KR153590	In this study
<i>Cordyceps brongniartii</i>	NBRC 101395		JN943298	JN941759		JN992493	Schoch <i>et al.</i> (2012)
<i>Cordyceps brongniartii</i>	BCC 16585		JN049867	JF415951	JF416009	JN049885	Kepler <i>et al.</i> (2012a)
<i>Cordyceps militaris</i>	OSC 93623	Lepidopteran pupa	JN049825	AY184977	DQ522332	DQ522377	Sung <i>et al.</i> (2007a)
<i>Cordyceps militaris</i>	NBRC 100741		JN943437	JN941755		JN992489	Schoch <i>et al.</i> (2012)
<i>Elaphocordyceps japonica</i>	OSC 110991	<i>Elaphomyces</i> sp.	JN049824	DQ522547	DQ522330	DQ522375	Sung <i>et al.</i> (2007a)
<i>Elaphocordyceps japonica</i>	IFO 9647		AB027366	AB027320			Nikoh & Fukatsu (2000)
<i>Elaphocordyceps ophioglossoides</i>	OSC 106405	<i>Elaphomyces</i> sp.		AY489691	AY489618	AY489652	Sung <i>et al.</i> (2007a)
<i>Elaphocordyceps ophioglossoides</i>	NBRC 106331		JN943320	JN941733		JN992467	Schoch <i>et al.</i> (2012)
<i>Elaphocordyceps subsessilis</i>	OSC 71235	Scarabaeid larva	JN049844	EF469124	EF469061	EF469090	Sung <i>et al.</i> (2007a)
<i>Metacordyceps atrovirens</i>	TNM-F 10184	Coleoptera	JN049882	JF415950		JN049884	Kepler <i>et al.</i> (2012a)
<i>Metacordyceps brittlebankisoides</i>	G97025*		AJ309332				Liu <i>et al.</i> (2002)
<i>Metacordyceps campsosterni</i>	HMIGD 20885*		DQ150247				Zhang <i>et al.</i> (2005)
<i>Metacordyceps campsosterni</i>	HMIGD 20884		DQ150246				Zhang <i>et al.</i> (2005)
<i>Metacordyceps chlamydosporia</i>	CBS 101244		JN049821	DQ522544	DQ522327	DQ522372	Kepler <i>et al.</i> (2012a)
<i>Metacordyceps chlamydosporia</i>	CBS 504.66	Nematode	AJ292398	AF339593	EF469069	EF469098	Sung <i>et al.</i> (2007a)
<i>Metacordyceps guniujiangensis</i>	GNJ020527-04*		AY913757				Li <i>et al.</i> (2010)
<i>Metacordyceps indigotica</i>	TNS-F18553	Lepidoptera	JN049874	JF415952	JF416010	JN049886	Kepler <i>et al.</i> (2012a)
<i>Metacordyceps indigotica</i>	TNS-F18554	Lepidoptera	JN049875	JF415953	JF416011	JN049887	Kepler <i>et al.</i> (2012a)
<i>Metacordyceps khaoyaiensis</i>	BCC 12687	Lepidoptera	JN049868		JF416013	JN049889	Kepler <i>et al.</i> (2012a)
<i>Metacordyceps khaoyaiensis</i>	BCC 14290	Lepidoptera	JN049869		JF416012	JN049888	Kepler <i>et al.</i> (2012a)
<i>Metacordyceps kusanagiensis</i>	TNS F18494	Coleoptera	JN049873	JF415954	JF416014	JN049890	Kepler <i>et al.</i> (2012a)
<i>Metacordyceps liangshanensis</i>	EFCC 1452	Lepidopteran pupa		EF468962	EF468756		Sung <i>et al.</i> (2007a)

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TABLE 1. (Continued)

Species	Voucher <sup>1,2</sup>	Host/ Substratum	GenBank Accession Number				References
			5.8S ITS	nrSSU	EF-1 $\alpha$	RPB1	
<i>Metacordyceps liangshanensis</i>	EFCC 1523	Lepidopteran pupa		EF468961	EF468755		Sung <i>et al.</i> (2007a)
<i>Metacordyceps martialis</i>	TTZ070716-04	Lepidoptera	JN049871	JF415955		JN049891	Kepler <i>et al.</i> (2012a)
<i>Metacordyceps martialis</i>	HMAS 197472(S)	Lepidoptera	JN049881	JF415956	JF416016	JN049892	Kepler <i>et al.</i> (2012a)
<i>Metacordyceps owariensis</i>	NBRC 33258	Hemiptera	JN049883		JF416017		Kepler <i>et al.</i> (2012a)
<i>Metacordyceps pseudoatrovirens</i>	TNSF 16380	Coleoptera	JN049870			JN049893	Kepler <i>et al.</i> (2012a)
<i>Metacordyceps taii</i>	ARSEF 5714	Lepidoptera	JN049829	AF543763	AF543775	DQ522383	Sung <i>et al.</i> (2007a)
<i>Metacordyceps yongmunensis</i>	EFCC 2131	Lepidoptera	JN049856	EF468977	EF468770	EF468876	Kepler <i>et al.</i> (2012a)
<i>Metacordyceps yongmunensis</i>	EFCC 2135	Lepidoptera		EF468979	EF468769	EF468877	Kepler <i>et al.</i> (2012a)
<i>Metarhizium acridum</i>	ARSEF 324	Orthoptera	HQ331457		EU248844	EU248896	Schneider <i>et al.</i> (2011), Bischoff <i>et al.</i> (2009)
<i>Metarhizium acridum</i>	ARSEF 7486*	Orthoptera	HQ331458		EU248845	EU248897	Schneider <i>et al.</i> (2011), Bischoff <i>et al.</i> (2009)
<i>Metarhizium anisopliae</i>	ARSEF 7450	Coleoptera	HQ331464		EU248852	EU248904	Schneider <i>et al.</i> (2011), Bischoff <i>et al.</i> (2009)
<i>Metarhizium anisopliae</i>	ARSEF 7487*	Orthoptera	HQ331446		DQ463996	DQ468355	Schneider <i>et al.</i> (2011), Bischoff <i>et al.</i> (2009)
<i>Metarhizium brunneum</i>	ARSEF 2107{	Coleoptera	KC178691		EU248855	EU248907	Kepler <i>et al.</i> (2013), Bischoff <i>et al.</i> (2009)
<i>Metarhizium brunneum</i>	ARSEF 4152	Soil	HQ331452		EU248853	EU248905	Schneider <i>et al.</i> (2011), Bischoff <i>et al.</i> (2009)
<i>Metarhizium flavoviride</i>	ARSEF 2133*	Coleoptera			DQ463999	DQ468358	Bischoff <i>et al.</i> (2009)
<i>Metarhizium frigidum</i>	ARSEF 4124*	Coleoptera			DQ464002	DQ468361	Bischoff <i>et al.</i> (2009)
<i>Metarhizium globosum</i>	ARSEF 2596*	Lepidoptera	HQ331459		EU248846	EU248898	Schneider <i>et al.</i> (2011), Bischoff <i>et al.</i> (2009)
<i>Metarhizium guizhouense</i>	CBS 258.90*	Lepidoptera	HQ331448		EU248862	EU248914	Schneider <i>et al.</i> (2011), Bischoff <i>et al.</i> (2009)
<i>Metarhizium guizhouense</i>	ARSEF 6238	Lepidoptera	HQ331447		EU248857	EU248909	Schneider <i>et al.</i> (2011), Bischoff <i>et al.</i> (2009)

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TABLE 1. (Continued)

Species	Voucher <sup>1,2</sup>	Host/ Substratum	GenBank Accession Number				References
			5.8S ITS	nrSSU	EF-1 $\alpha$	RPB1	
<i>Metarhizium lepidiotae</i>	ARSEF 7412	Coleoptera	HQ331455		EU248864	EU248916	Schneider <i>et al.</i> (2011), Bischoff <i>et al.</i> (2009)
<i>Metarhizium lepidiotae</i>	ARSEF 7488*	Coleoptera	HQ331456		EU248865	EU248917	Schneider <i>et al.</i> (2011), Bischoff <i>et al.</i> (2009)
<i>Metarhizium majus</i>	ARSEF 1015	Lepidoptera	HQ331444		EU248866	EU248918	Schneider <i>et al.</i> (2011), Bischoff <i>et al.</i> (2009)
<i>Metarhizium majus</i>	ARSEF 1914{	Coleoptera	HQ331445		EU248868	EU248920	Schneider <i>et al.</i> (2011), Bischoff <i>et al.</i> (2009)
<i>Metarhizium pingshaense</i>	ARSEF 3210	Isoptera	HQ331449		DQ463995	DQ468354	Schneider <i>et al.</i> (2011), Bischoff <i>et al.</i> (2009)
<i>Metarhizium pingshaense</i>	CBS 257.90*	Coleoptera	HQ331450		EU248850	EU248902	Schneider <i>et al.</i> (2011), Bischoff <i>et al.</i> (2009)
<i>Metarhizium robertsii</i>	ARSEF 727	Orthoptera	HQ331453		DQ463994	DQ468353	Schneider <i>et al.</i> (2011), Bischoff <i>et al.</i> (2009)
<i>Metarhizium robertsii</i>	ARSEF 7501				EU248849	EU248901	Bischoff <i>et al.</i> (2009)
<i>Ophiocordyceps rhizoidea</i>	N.H.J. 12522	Termite (Isoptera)	JN049857	EF468970	EF468764	EF468873	Sung <i>et al.</i> (2007a)
<i>Ophiocordyceps rhizoidea</i>	N.H.J. 12529	Termite (Isoptera)		EF468969	EF468765	EF468872	Sung <i>et al.</i> (2007a)
<i>Ophiocordyceps sinensis</i>	EFCC 7287	Lepidopteran pupa	JN049854	EF468971	EF468767	EF468874	Sung <i>et al.</i> (2007a)
<i>Ophiocordyceps sinensis</i>	ARSEF 6282		HM595981		HM595918	HM595952	Chan <i>et al.</i> (2011)
<i>Ophiocordyceps stylophora</i>	OSC 111000	Elaterid larva	JN049828	DQ522552	DQ522337	DQ522382	Sung <i>et al.</i> (2007a)
<i>Ophiocordyceps stylophora</i>	OSC 110999	Coleopteran larva		EF468982	EF468777	EF468882	Sung <i>et al.</i> (2007a)
<i>Polycephalomyces cuboideus</i>	TNS F18487			KF049609	KF049683		Quandt <i>et al.</i> (2014)
<i>Polycephalomyces cuboideus</i>	NBRC 101740		JN943331	KF049610	KF049684	KF049646	Quandt <i>et al.</i> (2014)
<i>Polycephalomyces nipponicus</i>	N.H.J. 4268			KF049621	KF049695	KF049654	Quandt <i>et al.</i> (2014)
<i>Polycephalomyces nipponicus</i>	BCC 2325		KF049665	KF049622	KF049696	KF049655	Quandt <i>et al.</i> (2014)
<i>Tyrannicordyceps fraticida</i>	TNS 19011*	Fungi	JQ349068	JQ257022	JQ257028	JQ257016	Kepler <i>et al.</i> (2012b)
<i>Glomerella cingulata</i>	CBS 114054	<i>Fragaria</i> sp.	DQ286202	AF543762	AF543773	AY489659	Sung <i>et al.</i> (2007b)

1 **ARSEF**, USDA-ARS Collection of Entomopathogenic Fungal Cultures, Ithaca, NY; **BCC**, BIOTEC Culture Collection, Klong Luang, Thailand; **CBS**, Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; **EFCC**, Entomopathogenic Fungal Culture Collection, Chuncheon, Korea; **GZUH**, Guizhou University Herbarium, Guiyang, Guizhou, China; **HKAS**, Herbarium of Herbarium of Cryptogams, Kunming Institute of Botany, Academia Sinica (HKAS), Kunming, China; **N.H.J.**, Nigel Hywel-Jones personal collection; **OSC**, Oregon State University Herbarium, Corvallis, OR.

2. \* Denotes an ex-type isolate. { Denotes an ex-epitype isolate.

## Taxonomy

*Metacordyceps shibinensis* T.C. Wen, J.C. Kang & K.D. Hyde, *sp. nov.* (Fig. 2) MycoBank MB812302; Facesoffungi number FoF 00646.

Differs from related *Metacordyceps* species mainly by its white stromata, short ascomata, and very short asci and ascospores.

**Type:**—CHINA. Guizhou Province: Shibin County, Yuntai Mountains, on larvae of Lepidoptera in the soil, 3 May 2013, *Li-Ping Chen* SB13050311 (GZUH SB13050311, holotype!).

*Stromata* 42 mm long, 2–3 mm wide, growing from the head of Lepidoptera larva, simple. *Stipe* 17–20 mm long, 2–3 mm wide, flexuous, white to faint yellow. Fertile part on the upper 50% of the stromata, 18–22 mm long, 2–3 mm wide, cylindrical or obtuse, faint yellow, differentiated from stipe, without a sterile apex. *Ascomata* 400–475 × 135–195 µm ( $\bar{x}$  = 439 × 164, n = 30), completely immersed, elongated or ampulliform, with the ostioles opening on the surface. *Peridium* 20–33 µm wide ( $\bar{x}$  = 26, n = 60), comprising three layers. *Asci* 130–200 × 5.1–8.3 µm ( $\bar{x}$  = 165 × 6.7, n = 60), 8-spored, hyaline, cylindrical, with a prominent apical cap; apical cap 4.7–5.9 × 2.8–3.5 µm ( $\bar{x}$  = 5.3 × 3.2, n = 60). *Ascospores* 120–170 × 1.4–2.1 µm ( $\bar{x}$  = 144 × 1.8, n = 60), hyaline, filiform, smooth-walled, multiseptate with cells 4–5.6 µm long, not breaking into secondary ascospores. Asexual state: Undetermined.

**Etymology:**—Refers to the type collecting site, Shibin County.

**Distribution:**—Shibin County, Guizhou Province, China.

**Host:**—On larvae of Lepidoptera living in soil.

## Discussion

*Metacordyceps shibinensis* is a remarkable new species for several reasons: 1) it differs from similar species in having a unique lepidopterous larvae host, rather than cicada nymphs; 2) it has white pigments in the stromata when dry, while in similar species pigment in dried specimens is always black; 3) its ascomata are relatively short and; 4) it has the shortest asci and ascospores in the *Metacordyceps* group (Table 2).

The 5.8S-ITS rDNA sequence of *M. shibinensis* (KR153585) differs from any known sequences of *Metacordyceps* and related species. A Blast search of GenBank reveals it is similar to *Metarhizium flavoviride* W. Gams & Rozsypal (HM583833) and *Metacordyceps owariensis* MY03260 (HQ165699), the maximal identities are 95% and 90%, respectively. For EF-1 $\alpha$  gene, the maximal identities with *Metacordyceps liangshanensis* EFCC1452 (EF468756) and *M. chlamydosporia* JCM18608 (AB758481) are both 97%. For RPB1 gene, the maximal identities with *M. yongmunensis* CEM1961 (KJ398612) and *M. brittlebankisoides* ARSEF1914 (KJ398610) are 91% and 90%, respectively. Therefore, *M. shibinensis*, a new species belonging to *Metacordyceps* is introduced based on both morphological and molecular characters.

*Cordyceps sensu lato* is regarded as one of the most important genera of invertebrate pathogens (Hywel-Jones 2001) with more than 140 species found in China (www. Indexfungorum.org, March 9, 2015), of which more than 40 species were reported in Guizhou Province, China. More than 240 *Cordyceps* species have been placed into the six genera, but about half of *Cordyceps sensu lato* species need to be restudied to naturally rearrange them in other genera (Sung *et al.* 2007a, Quandt *et al.* 2014). Many previous studies were restricted by both limited taxon sampling and the inadequate resolution power of ribosomal DNA, resulting in limited conclusions regarding systematics of *Cordyceps sensu lato* (Sung *et al.* 2007a). There are only 100 species with 5.8 S ITS sequence data and only 80 species have

been studied using multi-gene phylogeny. Recent phylogenetic studies (Spatafora *et al.* 2007, Sung *et al.* 2007a, b, Kepler *et al.* 2012a, b, 2013), based on multiple independent loci, have provided a greater level of resolution for this complex group of fungi. Therefore, a detailed systematic classification of *Cordyceps* and the family *Clavicipitaceae* based on multigene phylogenies is necessary to reflect the natural placement in Hypocreales (Maharachchikumbura *et al.* 2015).



**FIGURE 2.** *Metacordyceps shibinensis* (holotype). **a.** Overview of stroma and host. **b.** Infected host. **c, d.** Pale yellow, superficial ascomata on stroma. **e.** Cross section showing the stroma and ascomata. **f, g.** Sections of ascomata. **h, i.** Part of ascoma. **j, m.** Ascus. **k, m.** Ascus stained in methylene blue. **l.** Mature ascospores in methylene blue. **n.** Section of stipe. Scale bars: f = 1000  $\mu$ m, g = 200  $\mu$ m, h = 100  $\mu$ m, i = 50  $\mu$ m, j–l = 40  $\mu$ m, m = 50  $\mu$ m, n = 20  $\mu$ m.



**TABLE 2.** Synopsis of the characteristics of *Cordyceps* species related to *Metacordyceps shibinensis*.

Species	Host	Habit	Stromata	Ascomata ( $\mu\text{m}$ )	Asci ( $\mu\text{m}$ )	Ascospores ( $\mu\text{m}$ )	References
<i>M. shibinensis</i>	Lepidopteran larva	Soil	Single, stipe 17–20 $\times$ 2–3 mm, fertile part 18–22 $\times$ 2–3 mm	Embedded, long ovoid, 402–476 $\times$ 136–193	Cylindrical, 132–198 $\times$ 5.1–8.3	122–166 $\times$ 1.4–2.1, multiseptate with cells 4–5.6 long, not breaking into secondary ascospores	This study
<i>M. chlamydosporia</i>	Mollusc eggs	Forest litter	Single, 12–16 $\times$ 0.5–1.25 mm, fertile area terminal, 2–3.5 mm in length.	Superficial, 600–650 $\times$ 250–300	Cylindrical, 200–500 $\times$ 3–4	150–350 $\times$ 1, not breaking into secondary ascospores	Zare <i>et al.</i> (2001)
<i>M. guniujiangensis</i>	Cicada nymph	Soil	40.3–42.5 mm long, with yellow, acute, glabrous sterile tip	Embedded, 640–770 $\times$ 240–320	Cylindrical, 310–380 $\times$ 4–4.8	240–330 $\times$ 0.8–1, multiseptate, the cells 8–17 $\mu\text{m}$ long, not breaking into secondary ascospores	Li <i>et al.</i> (2010)
<i>M. khaoyaiensis</i>	Lepidopteran larva	Soil	Single, 55 $\times$ 4 mm wide, fertile part 15–20 $\times$ 2–4 mm, with obtusely rounded apex	Embedded, 335–410 $\times$ 200–270 m	Cylindrical, 215–340 $\times$ 5–6	165–240 $\times$ 1, multiseptate, not breaking into secondary ascospores	Hywel Jones (1994)
<i>M. liangshanensis</i>	Lepidopteran larva	Soil	Single, 20–30 $\times$ 1.5–2.5 mm	Superficial, 400–750 $\times$ 300–450	Cylindrical, 260–480 $\times$ 8–12	160–350 $\times$ 2.5–3.5, secondary ascospores 10–20 $\times$ 2.5–3.5	Zang <i>et al.</i> (1982)
<i>M. owaritensis</i>	Cicada nymph	Soil	Single or 3–4 branched, 30–45 mm long; fertile part 16–25 $\times$ 2–4 mm	Embedded, 440–640 $\times$ 180–320	Cylindrical, 180–300 $\times$ 5–6.5	150–280 $\times$ 1–2, multiseptate, not breaking into secondary ascospores	Uchiyama & Udagawa (2002)

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## References

- Bischoff, J.F., Rehner, S.A. & Humber, R.A. (2009) A multilocus phylogeny of the *Metarhizium anisopliae* lineage. *Mycologia* 101 (4): 512–530.  
<http://dx.doi.org/10.3852/07-202>
- Castlebury, L.A., Rossman, A.Y., Sung, G.H., Hyten, A.S. & Spatafora, J.W. (2004) Multigene phylogeny reveals new lineage for *Stachybotrys chartarum*, the indoor air fungus. *Mycological Research* 108: 864–872.  
<http://dx.doi.org/10.1017/S0953756204000607>
- Chan, W.H., Ling, K.H., Chiu, S.W., Shaw, P.C. & But, P.P.H. (2011) Molecular analyses of *Cordyceps gunnii* in China. *Journal of Food and Drug Analysis* 19 (1): 18–25.
- Chen, J.Y., Cao, Y.Q., Yang, D.R. & Li, M.H. (2011) A new species of *Ophiocordyceps* (Clavicipitales, Ascomycota) from southwestern China. *Mycotaxon* 115: 1–4.  
<http://dx.doi.org/10.5248/115.1>
- Chen, Z.H., Dai, Y.D., Yu, H., Yang, K., Yang, Z.L., Yuan, F. & Zeng, W.B. (2013) Systematic analyses of *Ophiocordyceps lanpingensis* sp. nov., a new species of *Ophiocordyceps* in China. *Microbiological Research* 168: 525–532.  
<http://dx.doi.org/10.1016/j.micres.2013.02.010>
- Cunningham, C.W. (1997) Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* 14: 733–740.
- Farris, J.S., Källersjö, M., Kluge, A.G. & Bult, C. (1994) Testing significance of incongruence. *Cladistics* 10: 315–319.  
<http://dx.doi.org/10.1111/j.1096-0031.1994.tb00181.x>
- Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.  
<http://dx.doi.org/10.2307/2408678>
- Gao, S., Zhang, Q., Li, C.R., Huang, B., Fan, M.Z., Spatafora, J.W. & Li, Z.Z. (2010) *Cordyceps cardinalis*, a new species in China and its *Isaria* anamorph. *Journal of Anhui agricultural University* 37 (4): 712–715.
- Hywel-Jones, N.L. (1994) *Cordyceps khaoyaiensis* and *C. pseudomilitaris*, two new pathogens of lepidopteran larvae from Thailand. *Mycological Research* 98 (8): 939–942.
- Hywel-Jones, N.L. (2001) The biological diversity of invertebrate pathogenic fungi. In: Hyde, K.D. (Ed.) *Biodiversity of tropical microfungi*. Hong Kong University Press, Hong Kong, pp. 107–120.
- Kepler, R.M., Sung, G.H., Ban, S., Nakagiri, A., Chen, M.J., Huang, B., Li, Z.Z. & Spatafora, J.W. (2012a) New teleomorph combinations in the entomopathogenic genus *Metacordyceps*. *Mycologia* 104 (1): 182–197.  
<http://dx.doi.org/10.3852/11-070>
- Kepler, R.M., Sung, G.H., Harada, Y., Tanaka, K., Tanaka, E., Hosoya, T., Bischoff, J.F. & Spatafora, J.W. (2012b) Host jumping onto close relatives and across kingdoms by *Tyrannicordyceps* (Clavicipitaceae) gen. nov. and *Ustilaginoidea* (Clavicipitaceae). *American Journal of Botany* 99 (3): 552–561.  
<http://dx.doi.org/10.3732/ajb.1100124>
- Kepler, R., Ban, S., Nakagiri, A., Bischoff, J., Hywel-Jones, N.L., Owensby, C.A. & Spatafora, J.W. (2013) The phylogenetic placement of hypocrealean insect pathogens in the genus *Polycephalomyces*: an application of One Fungus One Name. *Fungal Biology* 117: 611–622.  
<http://dx.doi.org/10.1016/j.funbio.2013.06.002>
- Li, C.R., Chen, A.H., Zuo, D.P., Fan, M.Z. & Li, Z.Z. (2008) Species of *Cordyceps* and *Shimizuomyces* new to China. *Mycosystema* 27 (3): 464–468.
- Li, C.R., Huang, B., Fan, M.Z. & Lin, Y.R. (2010) *Metacordyceps guniujiangensis* and its *Metarhizium* anamorph: a new pathogen on cicada nymphs. *Mycotaxon* 111: 221–231.  
<http://dx.doi.org/10.5248/111.221>
- Li, T.H., Deng, C.Y. & Song, B. (2008) A distinct species of *Cordyceps* on coleopterous larvae hidden in twigs. *Mycotaxon* 103: 365–369.

- Liang, Z.Q. (2007) *Flora Fungorum Sinicorum*, vol. 32, *Cordyceps*. Science Press, Beijing, 190 pp.
- Lin, Q.Y., Li, T.H. & Song, B. (2008) *Cordyceps guangdongensis* sp. nov. from China. *Mycotaxon* 103: 371–376.
- Liu, Z.Y., Liang, Z.Q., Liu, A.Y., Yao, Y.J., Hyde, K.D. & Yu, Z.N. (2002) Molecular evidence for teleomorph-anamorph connections in *Cordyceps* based on ITS-5.8S rDNA sequences. *Mycological Research* 106: 1100–1108.  
<http://dx.doi.org/10.1017/S0953756202006378>
- Maharachchikumbura, S.S.N., Hyde, K.D., Jones, E.G.B., McKenzie, E.H.C., Huang, S.K., Abdel-Wahab, M.A., Daranagama, D.A., Dayaratne, M., J. D'souza, M., Goonasekara, I.D., Hongsanan, S., Jayawardena, R.S., Kirk, P., Konta, S., Liu, J.K., Norphanphoun, C., Pang, K.L., Perera, R.H., Senanayake, I.C., Shang, Q.J., Shenoy, D., Xiao, Y.P., Wen, T.C. & Xu, J.C. (2015) Towards a natural classification and backbone tree for *Sordariomycetes*. *Fungal Diversity* 72: 199–301.  
<http://dx.doi.org/10.1007/s13225-015-0331-z>
- Nikoh, N. & Fukatsu, T. (2000) Interkingdom host jumping underground: phylogenetic analysis of entomoparasitic fungi of the genus *Cordyceps*. *Molecular Biology and Evolution* 17 (4): 629–638.  
<http://dx.doi.org/10.1093/oxfordjournals.molbev.a026341>
- Page, R.D.M. (1996) TreeView: an application to display phylogenetic trees on personal computers. *CABIOS* 12: 357–358.
- Quandt, C.A., Kepler, R.M., Gams, W., Araújo, J.P.M., Ban, S., Evans, H.C., Hughes, D., Humber, R., Hywel-Jones, N., Li, Z.Z., Luangsa-ard, J.J., Rehner, S.A., Sanjuan, T., Sato, H., Shrestha, B., Sung, G.H., Yao, Y.J., Zare, R. & Spatafora, J.W. (2014) Phylogenetic-based nomenclatural proposals for *Ophiocordycipitaceae* (*Hypocreales*) with new combinations in *Tolypocladium*. *IMA Fungus* 5 (1): 121–134.  
<http://dx.doi.org/10.5598/imafungus.2014.05.01.12>
- Schneider, S., Rehner, S.A., Widmer, F. & Enkerli, J. (2011) A PCR-based tool for cultivation-independent detection and quantification of *Metarhizium* clade 1. *Journal of Invertebrate Pathology* 108 (2): 106–114.  
<http://dx.doi.org/10.1016/j.jip.2011.07.005>
- Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Bergeron, M.J., Hamelin, R.C., Vialle, A. & Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Science* 109: 6241–6246.  
<http://dx.doi.org/10.1073/pnas.1117018109>
- Song, Bin., Lin, Q.Y., Li, T.H., Shen, Y.H., Li, J.J. & Luo, D.X. (2006) Known species of *Cordyceps* from China and their distribution. *Journal of Fungal Research* 4 (4): 10–26.
- Spatafora, J.W., Sung, G.H., Sung, J.M., Hywel-Jones, N.L. & White Jr., J.F. (2007) Phylogenetic evidence for an animal pathogen origin of ergot and the grass endophytes. *Molecular Ecology* 16 (8): 1701–1711.  
<http://dx.doi.org/10.1111/j.1365-294X.2007.03225.x>
- Sung, G.H., Hywel-Jones, N.L., Sung, J.M., Luangsa-ard, J.J., Shrestha, B. & Spatafora, J.W. (2007a) Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. *Studies in Mycology* 57: 5–59.  
<http://dx.doi.org/10.3114/sim.2007.57.01>
- Sung, G.H., Sung, J.M., Hywel-Jones, N.L. & Spatafora, J.W. (2007b) A multi-gene phylogeny of *Clavicipitaceae* (*Ascomycota*, Fungi): identification of localized incongruence using a combinational bootstrap approach. *Molecular Phylogenetics and Evolution* 44 (3): 1204–1223.  
<http://dx.doi.org/10.1016/j.ympev.2007.03.011>
- Swofford, D.L. (2002) *PAUP\*: phylogenetic analysis using parsimony (\*and other methods), version 4.0b10*. Sinauer Associates, Sunderland.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739.  
<http://dx.doi.org/10.1093/molbev/msr121>
- Uchiyama, S. & Udagawa, S. (2002) *Cordyceps owariensis* f. *viridescens* and its new *Nomuraea* anamorph. *Mycoscience* 43: 135–141.  
<http://dx.doi.org/10.1007/s102670200020>
- Wen, T.C., Li, M.F., Kang, J.C. & He, J. (2012) A molecular genetic study on fruiting-body formation of *Cordyceps militaris*. *African Journal of Microbiology Research* 6 (24): 5215–5221.  
<http://dx.doi.org/10.5897/AJMR12.522>
- Wen, T.C., Zhu, R.C., Kang, J.C., Huang, M.H., Tan, D.B., Ariyawansa, H., Hyde, K.D. & Liu, H. (2013) *Ophiocordyceps xuefengensis* sp. nov. from larvae of *Phassus nodus* (Hepialidae) in Hunan Province, southern China. *Phytotaxa* 123 (1): 41–50.  
<http://dx.doi.org/10.11646/phytotaxa.123.1.2>
- Wen, T.C., Xiao, Y.P., Li, W.J., Kang, J.C. & Hyde, K.D. (2014) Systematic analyses of *Ophiocordyceps ramosissimum* sp. nov., a new species from a larvae of Hepialidae in China. *Phytotaxa* 161 (3): 227–234.  
<http://dx.doi.org/10.11646/phytotaxa.161.3.6>
- White, T.J., Bruns, T., Lee, S. & Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics.

- In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols: a guide to methods and applications*. Academic Press, New York, pp. 315–322.  
<http://dx.doi.org/10.1016/b978-0-12-372180-8.50042-1>
- Yan, J.Q. & Bau, T. (2015) *Cordyceps ningxiaensis* sp. nov., a new species from dipteran pupae in Ningxia Hui Autonomous Region of China. *Nova Hedwigia* 100 (1–2): 251–258.  
[http://dx.doi.org/10.1127/nova\\_hedwigia/2014/0222](http://dx.doi.org/10.1127/nova_hedwigia/2014/0222)
- Yang, Y.H., Cai, S.X., Zheng, Y.M. & Lu, X.M. (2009) *Metarhizium taii* var. *chongqingensis* nov., anamorph of *Cordyceps chongqingensis* sp. nov. isolated from a low altitude area in Chongqing, China. *Current Microbiology* 58: 635–641.  
<http://dx.doi.org/10.1007/s00284-009-9382-2>
- Yang, Z.L., Qin, J., Xia, C.F., Hu, Q. & Li, Q.Q. (2015) *Ophiocordyceps highlandensis*, a new entomopathogenic fungus from Yunnan, China. *Phytotaxa* 204 (4): 287–295.  
<http://dx.doi.org/10.11646/phytotaxa.204.4.5>
- Zang, M., Liu, D.Q. & Hu, R.Y. (1982) Notes concerning the subdivisions of *Cordyceps* and a new species from China. *Acta Botanica Yunnanica* 4 (2): 173–176.
- Zare, R., Gams, W. & Evans, H.C. (2001) A revision of *Verticillium* section Prostrata. V. The genus *Pochonia*, with notes on *Rotiferophthora*. *Nova Hedwigia* 73 (1–2): 51–86.
- Zhang, Q., Zhu, C.L., Li, C.R., Huang, B., Fan, M.Z., Spatafora, J.W. & Li, Z.Z. (2010) A species of *Ophiocordyceps* new to China. *Journal of Anhui Agricultural University* 37 (4): 709–711.
- Zhang, W.M., Hu, B., Zhong, H., Chen, Y.Q., Tao, M.H. & Qu, L.H. (2005) Study on the anamorph of *Cordyceps campsosterna*. *Proceedings of the First Symposium on Development of China's Medicinal Fungi Industry* 81–82.