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Multigene phylogeny and morphology reveal that the Chinese medicinal mushroom '*Cordyceps gunnii*' is *Metacordyceps neogunnii sp. nov.*

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Abstract

Morphological and molecular phylogenetic analyses of an entomogenous fungus associated with larvae of Lepidoptera in Guizhou and Anhui, China showed it to be a new species, *Metacordyceps neogunnii.* It differs from similar species in having longer asci and wider ascospores. Multigene analysis of ITS, 18S, *TEF*1 and *RPB*1 sequence data also confirmed the distinctiveness of this species. This species has been wrongly regarded in China as '*Cordyceps gunnii*' for more than 30 years. *Cordyceps gunnii* from Tasmania is considered to be in the family Ophiocordycipitaceae based on its multigene phylogeny and morphological analysis.

Key words: Chinese medicinal mushroom, controversial taxa, molecular phylogeny, new species, revision

Introduction

Metacordyceps G.H. Sung, *et al.* introduced by Sung *et al.* (2007a), and previously treated as a member of *Cordyceps* Fr. (Hypocreales), is characterized by solitary or grouped stromata that are simple or branched, with fleshy or tough whitish stipe, a greenish yellow to greenish cylindrical to enlarged fertile part, and perithecia partially or completely immersed in stromata (Sung *et al*. 2007a). This genus comprises five species after transfer of 11 species to *Metarhizium* Sorokīn (Sung *et al.* 2007a, Kepler *et al.* 2012b, 2013, Quandt *et al.* 2014, Wen *et al.* 2015, Index Fungorum, 2017). In China, *Cordyceps sensu lato* has been extensively studied, and more than 140 species have been reported (Song *et al.* 2006, Liang 2007, Li *et al.* 2008a, 2008b, 2010, Lin *et al.* 2008, Yang *et al.* 2009, Gao *et al.* 2010, Zhang *et al.* 2010, Chen *et al.* 2011, 2013, Wen *et al.* 2013, 2014, 2015, Yan & Bau 2015, Yang *et al.* 2015). Of these species, the Chinese '*Cordyceps gunnii*' used as a medicinal mushroom by local people, currently sells for about US$2000/kg, its annual yield from nature is about ten tonnes and the species is only distributed in southern provinces. It has been long recognized as a prized medicinal mushroom and a desirable alternative for natural *Ophiocordyceps sinensis*. This mushroom contains many bioactive chemicals with a high biological activity (Xiao *et al.* 2004, Zhu *et al*. 2011, 2012a, b, 2013). Experiments towards its fermentation have been made in recent years (Fu *et al.* 2004, Sun *et al*. 2012, Wei *et al*. 2012). This species has been wrongly regarded in China as *Cordyceps gunnii* for more than 30 years. Its asexual morph was described in China as *Paecilomyces gunnii* Z.Q. Liang (Liang 1985).

Cordyceps gunnii (Berk.) Berk. is known only from Australia (Berkeley 1848, Hooker 1860). A similar species, *Cordyceps hawkesii* Gray has been reported from Australia (Saccardo 1891) and Japan (Kobayasi & Shimizu 1983). More recently, Chen *et al.* (1984) reported *C. hawkesii* and its anamorph (*Paecilomyces hawkesii*) from China based on morphological observations. Tian *et al.* (2010) added two sequences for *C. hawkesii* to GenBank that had been collected in China, but their study lacked morphological description. Liu *et al.* (2002) and Liang (2007) reported that *C. hawkesii* is a synonym of *C. gunnii* (all specimens used for study were collected in south China). Saccardo (1891) mentioned that it is easy to distinguish between *C. gunnii* and *C. hawkesii* even though they both occur in Tasmania, Australia. Chan *et al.* (2011) revealed that collections of *C. gunnii* from China and Tasmania are not conspecific. Thus, there is a need to re-investigate the classification of these two taxa to avoid unnecessary misidentification.

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In this study, collections of *C. gunnii* from China and Tasmania were restudied using multi-gene analysis and morphology. The collections of '*Cordyceps gunnii*' from China are morphologically different to *C. gunnii* of Tasmania, and combined multi-gene phylogeny also shows them to differ and to belong in a different genus. The fungus named '*Cordyceps gunnii*' in China, which has been regarded as a Traditional Chinese Medicine (TCM) for more than 1000 years is herein recognized as a new species, *Metacordyceps neogunnii*. Our results are reported herein.

Material and methods

Specimens and host

Collections were made in Yuntai Mountain, Shibin, Guizhou Province in April 2013 and in Huangshan Mountain, Anhui Province in June 2014. Specimens were stored in plastic containers at low temperature and transported to the laboratory for identification.

Morphological studies

Specimens 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 fungi were examined using an Olympus CX31 compound microscope and photographed (Wen *et al.* 2013, 2014). Herbarium materials are deposited in the Herbarium of Guizhou University (GZUH). Facesoffungi numbers and Index Fungorum numbers are provided as detailed in Jayasiri *et al.* (2015) and Index Fungorum (2016).

DNA extraction, PCR amplification and determination of DNA sequences

The total genomic DNA was extracted from dried specimens using E.Z.N.A.™ Fungal DNA MiniKit (Omega Biotech, CA, USA) according to the manufacturer's protocols and the extracted DNA was stored at -20 °C. Primes for amplifying and sequencing were: ITS5 and ITS4 for the internal transcribed spacer (ITS) (White *et al*. 1990), NS1 and NS4 for the ribosomal small subunit (18S) (Vilgalys & Hester 1990), 983F and 2218R for partial elongation Factor 1-Alpha (*TEF*1) (Sung *et al.* 2007b) and CRPB1A and RPB1Cr (Castlebury *et al.* 2004) for partial second largest RNA polymerase subunit I (*RPB*1). PCR conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles at 95 °C for 50s, 50–53 °C for 50 s, 72 °C for 80 s, and a final extension of 72 °C for 10 min. All PCR products were directly sequenced by Life Biotechnology Co., Shanghai, China.

Sequence alignment and phylogenetic analysis

Blast searches were made to reveal the closest matches in GenBank for phylogenetic analysis. The taxa information and GenBank accession numbers used in the molecular analysis are listed in Table 1. The four gene datasets (ITS, 18S, *TEF*1 and *RPB*1) from the *Ophiocordyceps* species, plus datasets obtained from GenBank were aligned using MEGA6.06 (Tamura *et al.* 2013). Alignments were manually adjusted to allow maximum sequence similarity. Gaps were treated as missing data. Unweighted Maximium 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 were 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).

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 ITS, 18S, *TEF*1 and *RPB*1 datasets, included 227 sequences (including 206 from GenBank, Table 1), representing 38 species, and comprised 3,427 characters after alignment, of which 961 characters were parsimony-informative, 2,213 constant, and 253 parsimony-uninformative. Parsimony analysis generated 5,000 trees; SH test verified that they were similar, one of which (tree length = 2,289 steps, CI = 0.464, RI = 0.751, RC = 0.348, HI = 0.536) and the most parsimonious tree is shown in Fig. 1. The materials collected

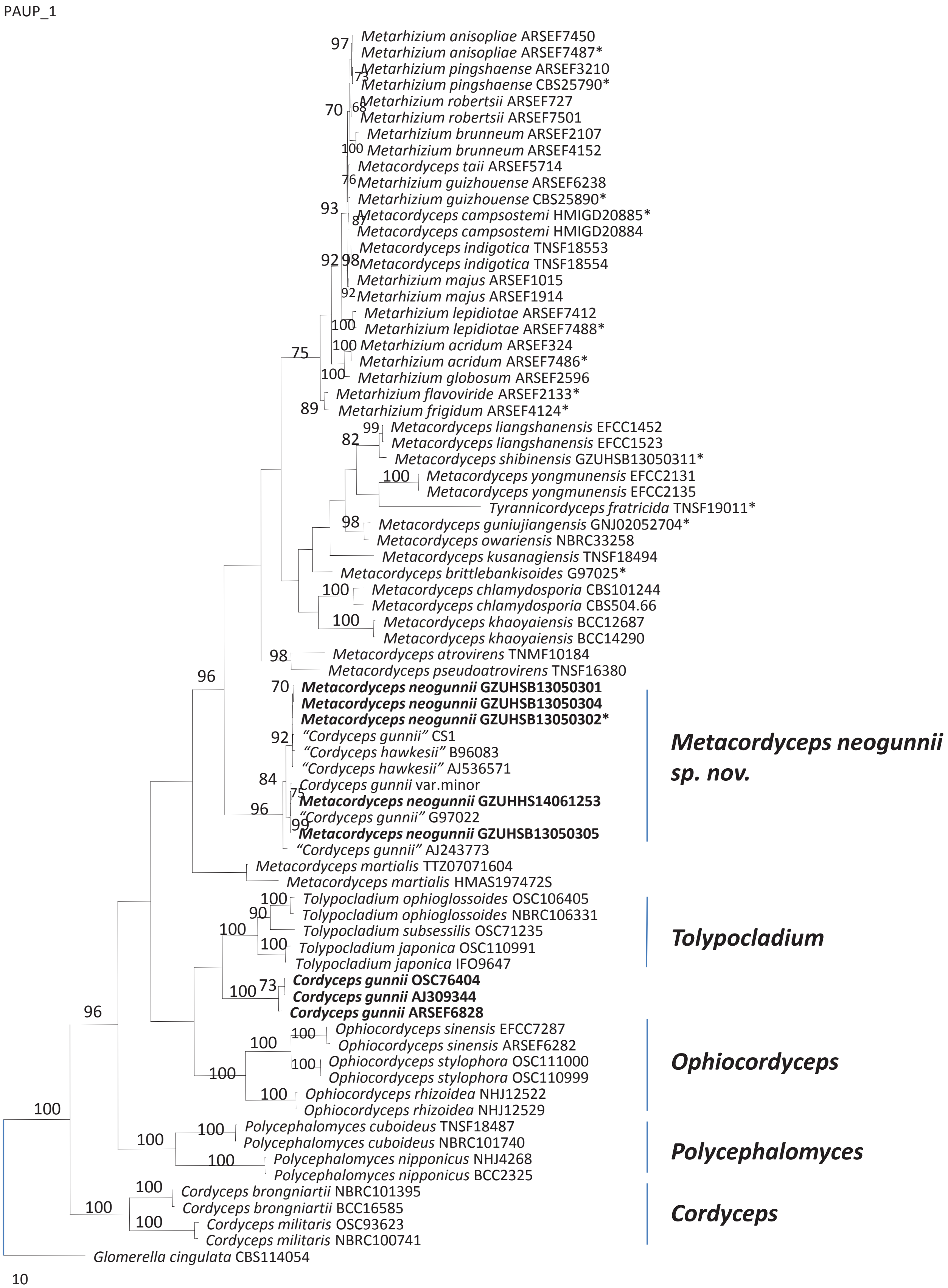

FIGURE 1. Phylogenetic relationships among *Metacordyceps neogunnii* and related species based on combined analysis of ITS, 18S, *TEF*1 and *RPB*1 sequence data. Bootstrap values (1,000 replicates) are indicated above the nodes. The tree is rooted to *Glomerella cingulata*. Ex-type culture or holotype has an asterisk.

from Guizhou and Anhui, China formed a separate clade from other species of *Metacordyceps* with credible bootstrap support (96%), but the relationship with other species within the genus was unresolved. Three specimens of *Cordyceps gunnii* from Tasmania, Australia formed a well-supported clade (100%), closely related to genus *Tolypocladium* in the family Ophiocordycipitaceae (Fig. 1).

Morphologically, *Metacordyceps neogunnii* differs from other species of *Metacordyceps* in having longer asci and wider ascospores (Fig. 2, Table 2), so the new species is introduced.

We consider *Cordyceps gunnii* from Tasmania belongs to Ophiocordycipitaceae based on its multigene phylogeny and morphology analysis. '*Cordyceps gunnii*' from China, used under the wrong name for 30 years, is now recognized as a new species, *Metacordyceps neogunnii* (Liang 1983). *Cordyceps gunnii* var. *minor* from Anhui Province also belongs to *M. neogunnii* (Li *et al.* 1999, Liang 2007) based on phylogeny (ITS tree not show) and its morphology analysis. This study was hindered by a lack of type species and molecular data, so *Cordyceps hawkesii* remains undetermined.

TABLE 1. Taxa used in molecular analyses.

Species	Vouchers [1]	Host/ Source location	GenBank Accession Number				
			ITS	**18S**	***TEF*1**	***RPB*1**	References
Metacordyceps neogunnii	GZUH SB13050301	Lepidopteran larva / Guizhou, China	KU729715	KU729721	KU729726	KU729731	This study
Metacordyceps neogunnii	GZUH SB13050302*	Lepidopteran larva / Guizhou, China	KU729716	KU729722	KU729727	KU729732	This study
Metacordyceps neogunnii	GZUH SB13050304	Lepidopteran larva / Guizhou, China	KU729717	KU729723	KU729728	KU729733	This study
Metacordyceps neogunnii	GZUH SB13050305	Lepidopteran larva / Guizhou, China	KU729718	KU729724	KU729729	KU729734	This study
Metacordyceps neogunnii	GZUH HS14061253	Lepidopteran larva / Anhui, China	KU729719	KU729725	KU729730	KU729735	This study
'Cordyceps gunnii'	CS1	China	HM149352		HM149362	HM149367	Chan *et al.* (2011)
'Cordyceps gunnii'		Guangdong, China	AJ243773				Chan *et al.* (2011)
'Cordyceps gunnii'	G97022	Yunnan, China	AJ309340				Chan *et al.* (2011)
'Cordyceps hawkesii'		China	AJ536571	DQ838790			Tian *et al.* (2010)
'Cordyceps hawkesii'	B96083	Hunan, China	AJ309341				Chan *et al.* (2011)
Cordyceps brongniartii	NBRC 101395		JN943298	JN941759		JN992493	Schoch *et al.* (2012)
Cordyceps brongniartii	BCC 16585		JN049867	JF415951	JF416009	JN049885	Kepler *et al.* (2012a)
Cordyceps gunnii	OSC 76404	Lepidopteran larva / Tasmania, Australia	JN049822	AF339572	AY489616	AY489650	Sung *et al.* (2007a)
Cordyceps gunnii	ARSEF 6828	Tasmania, Australia	HM140630	KU729720	HM140636	HM140639	Chan *et al.* (2011), This study
Cordyceps gunnii	K(M)	Tasmania, Australia	AJ309344				Liu *et al.* (2002)
Cordyceps gunnii var. *minor*		Anhui, China	AF368803				GenBank
Cordyceps militaris	OSC 93623	Lepidopteran pupa	JN049825	AY184977	DQ522332	DQ522377	Sung *et al.* (2007a)
Cordyceps militaris	NBRC 100741		JN943437	JN941755		JN992489	Schoch *et al.* (2012)
Metacordyceps atrovirens	TNM-F 10184	Coleoptera	JN049882	JF415950		JN049884	Kepler *et al.* (2012a)
Metacordyceps brittlebankisoides	G97025*		AJ309332				Liu *et al.* (2002)
Metacordyceps campsosterni	HMIGD 20885*		DQ150247				Zhang *et al.* (2005)
Metacordyceps campsosterni	HMIGD 20884		DQ150246				Zhang *et al.* (2005)
Metacordyceps chlamydosporia	CBS 101244		JN049821	DQ522544	DQ522327	DQ522372	Kepler *et al.* (2012a)
Metacordyceps chlamydosporia	CBS 504.66	Nematode	AJ292398	AF339593	EF469069	EF469098	Sung *et al.* (2007a)

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TABLE 1. (Continued)

Species	Vouchers [1]	Host/ Source location	GenBank Accession Number				
			ITS	**18S**	***TEF*1**	***RPB*1**	References
Metacordyceps guniujiangensis	GNJ020527-04*		AY913757				Li *et al.* (2010)
Metacordyceps indigotica	TNS-F18553	Lepidoptera	JN049874	JF415952	JF416010	JN049886	Kepler *et al.* (2012a)
Metacordyceps indigotica	TNS-F18554	Lepidoptera	JN049875	JF415953	JF416011	JN049887	Kepler *et al.* (2012a)
Metacordyceps khaoyaiensis	BCC 12687	Lepidoptera	JN049868		JF416013	JN049889	Kepler *et al.* (2012a)
Metacordyceps khaoyaiensis	BCC 14290	Lepidoptera	JN049869		JF416012	JN049888	Kepler *et al.* (2012a)
Metacordyceps kusanagiensis	TNS F18494	Coleoptera	JN049873	JF415954	JF416014	JN049890	Kepler *et al.* (2012a)
Metacordyceps liangshanensis	EFCC 1452	Lepidopteran pupa		EF468962	EF468756		Sung *et al.* (2007a)
Metacordyceps liangshanensis	EFCC 1523	Lepidopteran pupa		EF468961	EF468755		Sung *et al.* (2007a)
Metacordyceps martialis	TTZ070716-04	Lepidoptera	JN049871	JF415955		JN049891	Kepler *et al.* (2012a)
Metacordyceps martialis	HMAS 197472(S)	Lepidoptera	JN049881	JF415956	JF416016	JN049892	Kepler *et al.* (2012a)
Metacordyceps owariensis	NBRC 33258	Hemiptera	JN049883		JF416017		Kepler *et al.* (2012a)
Metacordyceps pseudoatrovirens	TNSF 16380	Coleoptera	JN049870			JN049893	Kepler *et al.* (2012a)
Metacordyceps shibinensis	GZUH SB13050311*	Lepidopteran larva	KR153585	KR153588	KR153589	KR153590	Wen *et al.* (2015)
Metacordyceps taii	ARSEF 5714	Lepidoptera larva	JN049829	AF543763	AF543775	DQ522383	Sung *et al.* (2007a)
Metacordyceps yongmunensis	EFCC 2131	Lepidoptera	JN049856	EF468977	EF468770	EF468876	Kepler *et al.* (2012a)
Metacordyceps yongmunensis	EFCC 2135	Lepidoptera		EF468979	EF468769	EF468877	Kepler *et al.* (2012a)
Metarhiziu manisopliae	ARSEF 7450	Coleoptera	HQ331464		EU248852	EU248904	Schneider *et al.* (2011), Bischoff *et al.* (2009)
Metarhizium acridum	ARSEF 324	Orthoptera	HQ331457		EU248844	EU248896	Schneider *et al.* (2011), Bischoff *et al.* (2009)
Metarhizium acridum	ARSEF 7486*	Orthoptera	HQ331458		EU248845	EU248897	Schneider *et al.* (2011), Bischoff *et al.* (2009)
Metarhizium anisopliae	ARSEF 7487*	Orthoptera	HQ331446		DQ463996	DQ468355	Schneider *et al.* (2011), Bischoff *et al.* (2009)
Metarhizium brunneum	ARSEF 2107{	Coleoptera	KC178691		EU248855	EU248907	Kepler *et al.* (2013), Bischoff *et al.* (2009)
Metarhizium brunneum	ARSEF 4152	Soil	HQ331452		EU248853	EU248905	Schneider *et al.* (2011), Bischoff *et al.* (2009)
Metarhizium flavoviride	ARSEF 2133*	Coleoptera			DQ463999	DQ468358	Bischoff *et al.* (2009)
Metarhizium frigidum	ARSEF 4124*	Coleoptera			DQ464002	DQ468361	Bischoff *et al.* (2009)
Metarhizium globosum	ARSEF 2596*	Lepidoptera	HQ331459		EU248846	EU248898	Schneider *et al.* (2011), Bischoff *et al.* (2009)
Metarhizium guizhouense	CBS 258.90*	Lepidoptera	HQ331448		EU248862	EU248914	Schneider *et al.* (2011), Bischoff *et al.* (2009)
Metarhizium guizhouense	ARSEF 6238	Lepidoptera	HQ331447		EU248857	EU248909	Schneider *et al.* (2011), Bischoff *et al.* (2009)

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TABLE 1. (Continued)

Species	Vouchers [1]	Host/ Source location	GenBank Accession Number				
			ITS	**18S**	***TEF*1**	***RPB*1**	References
Metarhizium lepidiotae	ARSEF 7412	Coleoptera	HQ331455		EU248864	EU248916	Schneider *et al.* (2011), Bischoff *et al.* (2009)
Metarhizium lepidiotae	ARSEF 7488*	Coleoptera	HQ331456		EU248865	EU248917	Schneider *et al.* (2011), Bischoff *et al.* (2009)
Metarhizium majus	ARSEF 1015	Lepidoptera	HQ331444		EU248866	EU248918	Schneider *et al.* (2011), Bischoff *et al.* (2009)
Metarhizium majus	ARSEF 1914{	Coleoptera	HQ331445		EU248868	EU248920	Schneider *et al.* (2011), Bischoff *et al.* (2009)
Metarhizium pingshaense	ARSEF 3210	Isoptera	HQ331449		DQ463995	DQ468354	Schneider *et al.* (2011), Bischoff *et al.* (2009)
Metarhizium pingshaense	CBS 257.90*	Coleoptera	HQ331450		EU248850	EU248902	Schneider *et al.* (2011), Bischoff *et al.* (2009)
Metarhizium robertsii	ARSEF 727	Orthoptera	HQ331453		DQ463994	DQ468353	Schneider *et al.* (2011), Bischoff *et al.* (2009)
Metarhizium robertsii	ARSEF 7501				EU248849	EU248901	Bischoff *et al.* (2009)
Ophiocordyceps rhizoidea	N.H.J. 12522	Termite (Isoptera)	JN049857	EF468970	EF468764	EF468873	Sung *et al.* (2007a)
Ophiocordyceps rhizoidea	N.H.J. 12529	Termite (Isoptera)		EF468969	EF468765	EF468872	Sung *et al.* (2007a)
Ophiocordyceps sinensis	EFCC 7287	Lepidopteran pupa	JN049854	EF468971	EF468767	EF468874	Sung *et al.* (2007a)
Ophiocordyceps sinensis	ARSEF 6282		HM595981		HM595918	HM595952	Chan *et al.* (2011)
Ophiocordyceps stylophora	OSC 111000	Elaterid larva	JN049828	DQ522552	DQ522337	DQ522382	Sung *et al.* (2007a)
Ophiocordyceps stylophora	OSC 110999	Coleopteran larva		EF468982	EF468777	EF468882	Sung *et al.* (2007a)
Polycephalomyces cuboideus	TNS F18487			KF049609	KF049683		Quandt *et al.* (2014)
Polycephalomyces cuboideus	NBRC 101740		JN943331	KF049610	KF049684	KF049646	Quandt *et al.* (2014)
Polycephalomyces nipponicus	N.H.J. 4268			KF049621	KF049695	KF049654	Quandt *et al.* (2014)
Polycephalomyces nipponicus	BCC 2325		KF049665	KF049622	KF049696	KF049655	Quandt *et al.* (2014)
Tolypocladium japonica	OSC110991	*Elaphomyces* sp.	JN049824	DQ522547	DQ522330	DQ522375	Sung *et al.* (2007a)
Tolypocladium japonica	IFO9647		AB027366	AB027320			Nikoh & Fukatsu (2000)
Tolypocladium ophioglossoides	OSC106405	*Elaphomyces* sp.		AY489691	AY489618	AY489652	Sung *et al.* (2007a)
Tolypocladium ophioglossoides	NBRC106331		JN943320	JN941733		JN992467	Schoch *et al.* (2012)
Tolypocladium subsessilis	OSC71235	Scarabaeid larva	JN049844	EF469124	EF469061	EF469090	Sung *et al.* (2007a)
Tyrannicordyceps fratricida	TNS 19011*	Fungi	JQ349068	JQ257022	JQ257028	JQ257016	Kepler *et al.* (2012b)
Glomerella cingulata	CBS 114054	*Fragaria* sp.	DQ286202	AF543762	AF543773	AY489659	Sung *et al.* (2007b)

1 **ARSEF**, USDA-ARS Collection of Entomopathogenic Fungal cultures, Ithaca, NY; **BCC**, BIOTEC Culture Collection, KlongLuang, 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 neogunnii T.C. Wen & K.D. Hyde, *sp. nov.* (Figs. 3–4)
Index Fungorum no IF551950; Facesoffungi number FoF 01915.
'*Paecilomyces gunnii*' sensu Z.Q. Liang, Acta Mycol. Sin. 4(3): 163 (1985)
= *Cordyceps gunnii* var. *minor* Z.Z. Li, C.R. Li, B. Huang, M.Z. Fan & M.W. Lee, Korean J. Mycol. 27(3): 232 (1999)
= *Paecilomyces gunnii* var. *minor* Z.Z. Li, C.R. Li, B. Huang, M.Z. Fan & M.W. Lee, Korean J. Mycol. 27(3): 233 (1999)

Differs from related *Metacordyceps* species mainly in having longer asci and wider ascospores.

Type:—CHINA. Guizhou Province: Shibin County, Yuntai Mountain, on larvae of Lepidoptera in the soil, 23 April 2013, *Li-Ping Chen* SB13050302 (GZUH SB13050302, holotype!), ex-type living culture GZUCCSB130503021!

Sexual morph: *Stromata* arising from head of host (larvae of Lepidoptera), solitary or in groups of 2–3, rarely branched, 40–80 mm long, 2–6 mm wide, pallid, fleshy. *Stipe* cylindrical 2–4 mm wide, white to grey, fleshy, glabrous, enlarging abruptly at fertile portion. *Fertile portion* round head-shape, 10–12 mm long, 3–6 mm wide, white to grey (fresh specimen). *Ascomata* crowded, completely immersed, ampuliform, ovoid to oblong, 630–830 × 240–340 µm ($\bar{x}$ = 732 × 278, n = 10), curved, with the ostioles opening on the surface of the fertile head. *Peridium* 24–38 µm ($\bar{x}$ = 29, n = 15) wide, comprising three layers. *Asci* 250–480 × 3–5 µm ($\bar{x}$ = 347 × 3.8, n = 10), 8-spored, hyaline, narrowly cylindrical, possessing a prominent apical cap. *Apical cap* 5–8 × 3–6 µm ($\bar{x}$ = 7.2 × 4.7, n = 15). *Ascospores* 330–460 × 2–3 µm ($\bar{x}$ = 397 × 2.6, n = 15), hyaline, filiform, multi-septate, disarticulating into secondary ascospores. *Secondary ascospores* 2.5–4 × 1.5–2 µm ($\bar{x}$ = 3 × 1.9, n = 15), cylindrical, hyaline. **Asexual state**: *Paecilomyces*-like.

Asexual morph: *Colonies* on Czapek agar, attaining a diameter of 25–30 mm within 14 d at 25 °C, dense, white at first, becoming pale to grey; brownish or dark brown at center, reddish brown at margin, slightly penetrating medium, reverse similar in colour (Fig. 2). *Conidiophores* short, hyaline, smooth, up to 60 µm long, mostly arising from aerial hyphae. *Conidiogenous cells* swollen globose at basal portion, tapering into thin and long cylindrical neck, smooth, hyaline, 12–21.5 × 2–3 µm, sometimes proliferating. *Conidia* ellipsoidal, ovoid or fusiform, 1-celled, spinulose conidia, 3.5–5 × 2–3 µm, in long divergent, dry chains.

Distribution:—Anhui, Guangdong, Guizhou, Henan, Hunan, Jiangxi, Sichuan and Yunnan Province, China.

Host:—On larvae of Lepidoptera living in soil.

Other material studied (paratypes):——CHINA. Guizhou Province: Shibin County, Yuntai Mountain, on lepidopteran larva, 23 April 2013, *Li-Ping Chen SB13050301* (GZUHSB13050301!), *Li-Ping Chen SB13050304* (GZUHSB13050304!), *Li-Ping Chen SB13050305* (GZUHSB13050305!); Anhui Province: Huangshan Mountain, on lepidopteran larva, 12 June 2014, *Ting-Chi Wen HS14061253* (GZUHHS14061253!).

Etymology:—Refers to previous, incorrect name used for this species in China, *Cordyceps gunnii* (Berk.) Berk.

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

Species	Host	Stromata (mm)	Ascomata(µm)	Asci (µm)	Ascospores (µm)	References
Metacordyceps neogunnii	Larvae of Lepidoptera	40–80 × 2–6, white to gray	Embedded, 630–830 × 240–340	Cylindrical, 250–480 × 3–5	Ascospores 330–460 × 2–3, breaking into 2.5–4 × 1.5–2 secondary ascospores	This study
Cordyceps consumpta (= *Cordyceps gunnii*)	*Porina* sp.	40–30 long, head 8–10 × 2–3, black	Embedded, 1000–1200 × 200–500	Cylindrical, 250 × 7	Ascospores 180–220 long, breaking into 4–5 × 1–1.5 secondary ascospores	Cunningham (1921)
Cordyceps gunnii		40–80 long	Embedded	Cylindrical	Ascospores 155–165 × 2.5–3, with 4–5 septet	Massee (1895)
Cordyceps gunnii var. *minor*	Larvae of *Endoclita excrescens*	Stipe 36.6–52.3 × 4.8–8.6, head 18.5–19.3 × 4–9.4	Embedded, 870–920 × 260–320	Cylindrical, 345–530 × 4.4–6.9	Ascospores breaking into 2–4.3 × 1–1.8 secondary ascospores	Li *et al.* (1999)
Cordyceps hawkesii			Embedded, 500–550 × 170–200	Cylindrical, 230–300 × 7–9		Shimizu (1994)

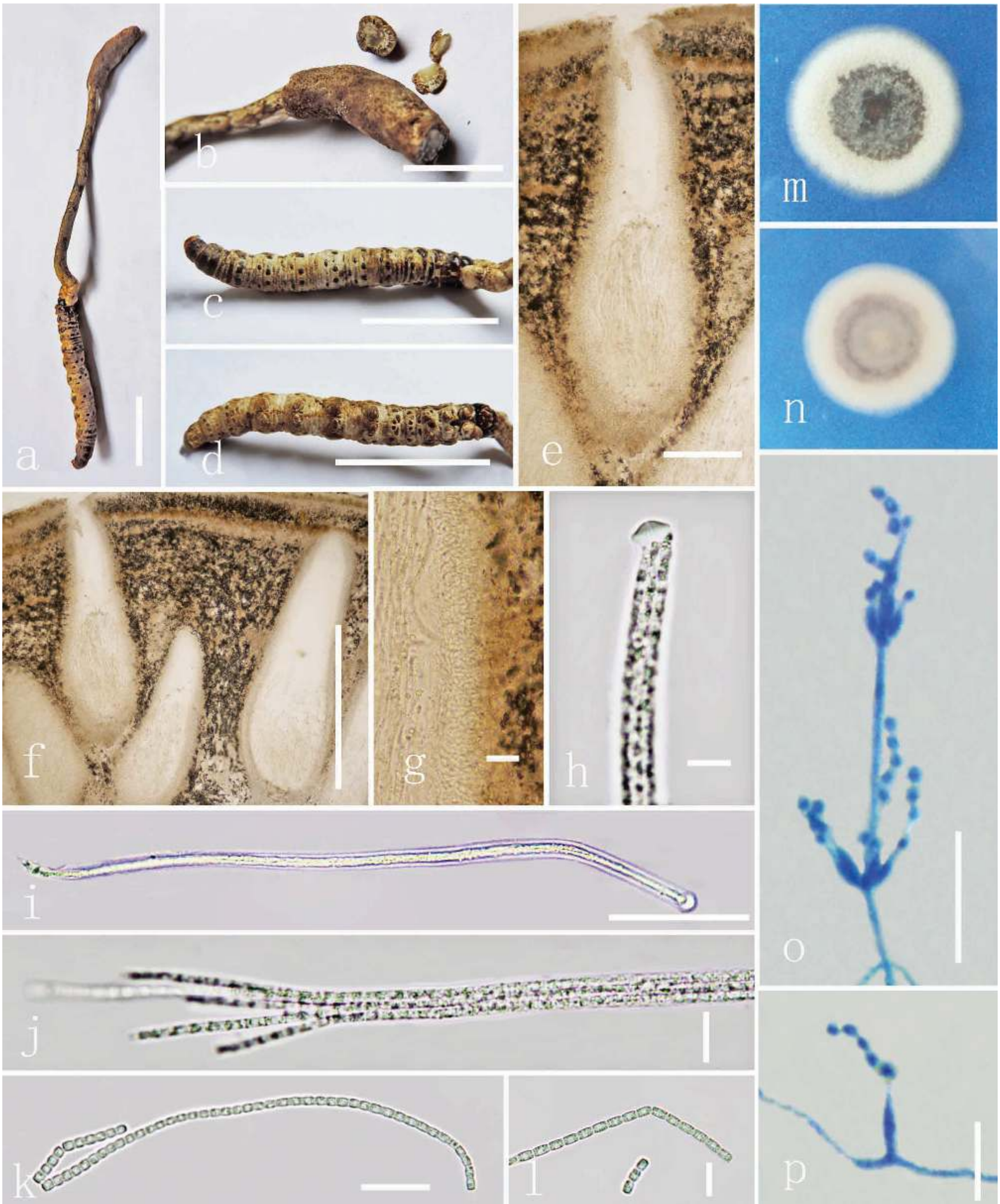

FIGURE 2. ***Metacordyceps neogunnii*** (holotype) **a.** Overview of stroma and host (dry specimen). **b.** Stroma. **c.** Dorsal view of the host. **d.** Ventral view of the host. **e, f.** Sections of ascomata. **g.** Section of a peridium. **h.** Ascus with apical cap. **i.** Immature ascus. **j.** Mature asci. **k.** Ascospore. **l.** Secondary ascospores. **m.** Czapek agar culture viewed from above. **n.** Czapek agar culture viewed from below. **o, p.** Conidiogenous cells, conidiophores and developing conidia, stained with cotton blue. Scale bars: f = 400 μm, e, i = 100 μm, k, o = 50 μm, j, h, l = 10 μm, g, p = 20 μm.

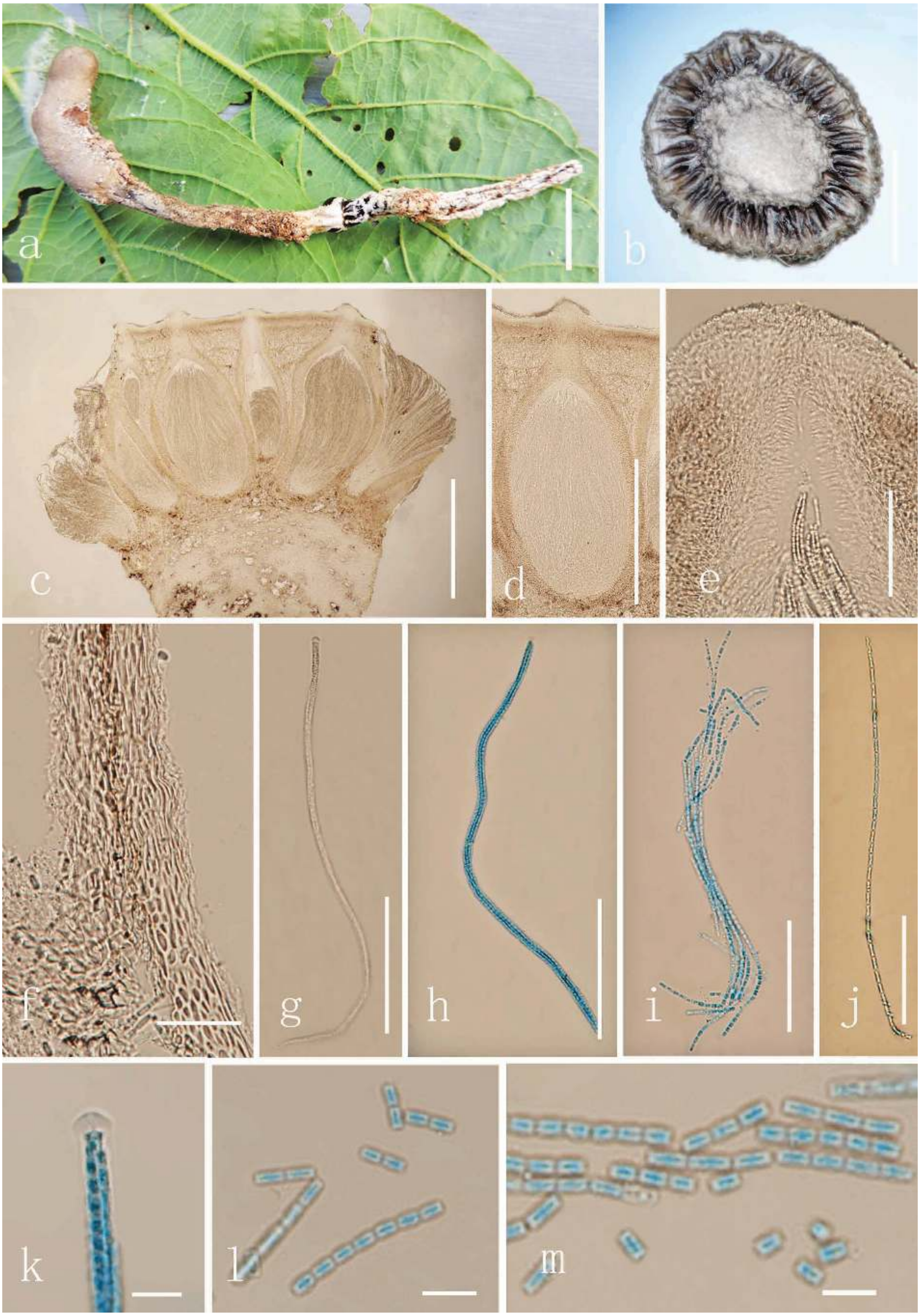

FIGURE 3. ***Metacordyceps neogunnii*** (GZUHHS14061253, overmature) **a.** Overview of the stroma and the host. **b.** Cross section of the stroma. **c, d.** Sections of ascomata. **e.** Section of an ascoma. **f.** Part of a peridium. **g.** An ascus. **h, i.** Asci stained with methylene blue. **j.** Ascospore stained with methylene blue. **k.** Ascus with an apical cap. **l.** Secondary ascospores. Scale bars: c, d = 400 µm, e = 100 µm, f = 50 µm, g–j = 80 µm, k–m = 10 µm.

Discussion

Many cordyceps species, such as *C. militaris* (L.) Fr., *C. bassiana* Z.Z. Li *et al.*, *Isaria cicadae* Miq. and *Ophiocordyceps sinensis* (Berk.) G.H. Sung *et al.*, are widely used as traditional Chinese medicine in China, Japan, Korea and other eastern Asian countries (Mortimer *et al.* 2012) and are listed in Chinese pharmacopoeia. Because of limited wild resources and high prices, many fake cordyceps have been found in markets, so determination of genuine cordyceps and related products is especially important for quality control (Zhu *et al*. 1998, Wen *et al.* 2016). Winkler (2009) estimated that total annual yield of natural *O. sinensis* in the Himalayas and Tibetan plateau is 85 to 185 tonnes, while the total amount emerged in world markets is more than 200 tonnes per year. *Metacordyceps neogunnii*, as a medicinal mushroom with high biological activity, has been also long regarded as a prized medicinal mushroom in China (Xiao *et al.* 2004, Zhu *et al*. 2011).

There has been no thorough study of the collections of *C. gunnii* from China and Tasmania (Stensrud *et al.* 2005, Sung *et al.* 2007a, b). Only one publication (Liu *et al*. 2002) suggested Chinese *C. hawkesii* and *C. gunnii* are conspecific based on 5.8S-ITS rDNA sequences. Lacking both a morphology comparison and sufficient molecular data, many details about these two species remain undetermined. Thus, a taxonomic re-evaluation of these important fungi was urgently needed (Chan *et al.* 2011). This study conclusively demonstrates that the Chinese collections identified as *C. gunnii* are misidentified. Our results also support Quandt *et al.* (2014) and Spatafora *et al.* (2015) that *Cordyceps gunnii* from Tasmania is in the family Ophiocordycipitaceae.

The age of the stromata of a cordyceps species is often reflected by differing morphology, which is illustrated by *Metacordyceps neogunnii* in Fig. 2 and 3. Based on differences in maturity of stromata apparent new species have been described, for example, *C. gansuensis* Zhang, Wang & Yan, *C. multiaxialis* Zang & Kinjo and *O. nepalensis* (M. Zang & Kinjo) G.H. Sung *et al.* are all synonyms of *O. sinensis* (Liu *et al.* 2001, Liang 2007). In pharmaceutical industry and traditional medicine fields, correct identification of source material is essential. Incorrect scientific name of an organism may lead to legal disputes and ineffective conservation, and even misleading information in filing and retrieval (Chan *et al.* 2011). The strategies used for identifying cordyceps species based on combined sequenced data from multi-gene loci could have a wide application in other traditional Chinese medicine and in fungal biotechnology.

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