



Multigene phylogeny and morphology reveal a new species, *Ophiocordyceps tettigonia*, from Guizhou Province, China

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Abstract

A species of *Cordyceps sensu lato* associated with an adult of *Tettigonia* was collected in Chishui Danxia natural world heritage site, Guizhou Province, China. Following morpho-phylogenetic studies it was found to be a new species and is described as *Ophiocordyceps tettigonia* sp. nov. It differs from similar *Ophiocordyceps* species in having a unique host insect, and wide secondary ascospores. Combined sequence data from the ITS, SSU, TEF, and RPB1 gene loci also confirmed the distinctiveness of this new species.

Key words: *Cordyceps sensu lato*, new species, phylogenetic analyses, *Tettigonia*

Introduction

Cordyceps sensu lato is one of the most important genera of invertebrate pathogens (Hywel-Jones 2001) with more than 540 epithets (Index Fungorum, 2016). The genus *Cordyceps* Fr. (*Clavicipitaceae*, *Hypocreales*, *Ascomycota*) has been recently divided and placed into three families and more than six genera—*Polycephalomyces* Kobayasi (*Ophiocordycipitaceae* G.H. Sung *et al.*) (Kepler *et al.* 2013), *Tyrannicordyceps* Kepler & Spatafora (*Clavicipitaceae* Earle) (Kepler *et al.* 2012b), *Metacordyceps* G.H. Sung *et al.* (*Clavicipitaceae*), *Tolypocladium* W. Gams (formerly *Elaphocordyceps* G.H. Sung & Spatafora, *Ophiocordycipitaceae*) (Quandt *et al.* 2014), *Ophiocordyceps* Petch (*Ophiocordycipitaceae*) and *Cordyceps* (*Cordycipitaceae* Kreisel ex G.H. Sung *et al.*) (Sung *et al.* 2007a). Most species in *Cordyceps sensu lato* are pathogenic on insects and spiders, although a few grow on species of *Elaphomyces* Nees (soil fungi). 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) suggested that there are 153 *Ophiocordyceps* species. There are 166 epithets assigned to *Ophiocordyceps* in Index Fungorum (2016). Many *Cordyceps* species such as *C. militaris* (L.) Fr., *C. bassiana* Z.Z. Li *et al.*, *Isaria cicada* Miq. and *O. sinensis* (Berk.) G.H. Sung *et al.* are used in traditional medicines in China, Japan, Korea and other east Asian countries (Mortimer *et al.* 2012).

Chishui Danxia is a natural world heritage site in Guizhou Province, south China. This is a special karst landform preserving rare plants since the Jurassic. *Cordyceps chishuiensis* Z.Q. Liang & A.Y. Liu, *C. stipillata* Z.Q. Liang & A.Y. Liu and *C. suoluensis* Z.Q. Liang & A.Y. Liu have been described from this area (Liang 2007).

In this study, a new species, *Ophiocordyceps tettigonia*, was found parasitizing an adult of a *Tettigonia* species collected from soil in Chishui Danxia natural world heritage site. This species is morphologically distinct from all other *Cordyceps sensu lato* species and a combined multi-gene analysis also showed it to differ from species of *Ophiocordyceps*.

Material and methods

Specimens and host

The collections were made in the Chishui Danxia natural world heritage site in June 2014. 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 micro-characters of the fungus were examined using an Olympus CX31 compound microscope and photographed (Wen *et al.* 2013, 2014). Dried material is deposited in the herbarium of Guizhou University. 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 the rear segment of the host of dried specimens using E.Z.N.A.TM Fungal DNA MiniKit (Omega Biotech, CA, USA) according to the manufacturer's protocols and the extracted DNA was stored at -20 °C. The internal transcribed spacer region of ribosomal DNA (ITS1-5.8S-ITS2), the small subunits of the rDNA (SSU) and two protein genes the transcription elongation factor-1 α (TEF), and the first largest subunits of RNA polymerase II (RPB1) loci were amplified and sequenced (Sung *et al.* 2007b).

The PCR amplification and sequencing of ITS were conducted as described in White *et al.* (1990). The ITS 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 SSU were conducted as described in Sung *et al.* (2007b). The SSU was amplified and sequenced with the primers NS1 (5'-GTAGTCATATGCTTGTCTC-3') and NS4 (5'-CTTCCGTCAATTCCTTTAAG-3') (White *et al.* 1990). In the amplification of TEF and RPB1, we followed Sung *et al.* (2007b) and Castlebury *et al.* (2004). For the amplification of TEF, the primers 983F (5'-GCYCCYGGHCAYCGTGAYTTYAT-3') and 2218R (5'-ATGACACCRACRGCRCRGTGTG-3') (Rehner & Buckley 2005) were used. For RPB1, the primers CRPB1A (5'-CAYCCWGGYTTYATCAAGAA-3') and RPB1Cr (5'-CCNGCDATNTCRTRTCCATRTA-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

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, SSU, TEF and RPB1) from the *Ophiocordyceps* species, plus datasets obtained from GenBank 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 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 ten 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 datasets comprised 3,507 characters after alignment (ITS 698 characters, SSU 1346 characters, TEF 852 characters, RPB1 611 characters), of which 1,048 characters were parsimony-informative, 2,051 constant, and 408 parsimony-uninformative. Parsimony analysis generated 5,000 trees; SH test verified that they were similar, one of which (tree length = 4,628 steps, CI = 0.493, RI = 0.650, RC = 0.320, HI = 0.507) and the most parsimonious tree is shown in Fig. 1.

The data set comprises 27 species (Fig. 1) including the new species *Ophiocordyceps tettigonia*, which formed a separate clade from other species of *Ophiocordyceps* with strong bootstrap support (100%); thus the new species is introduced.

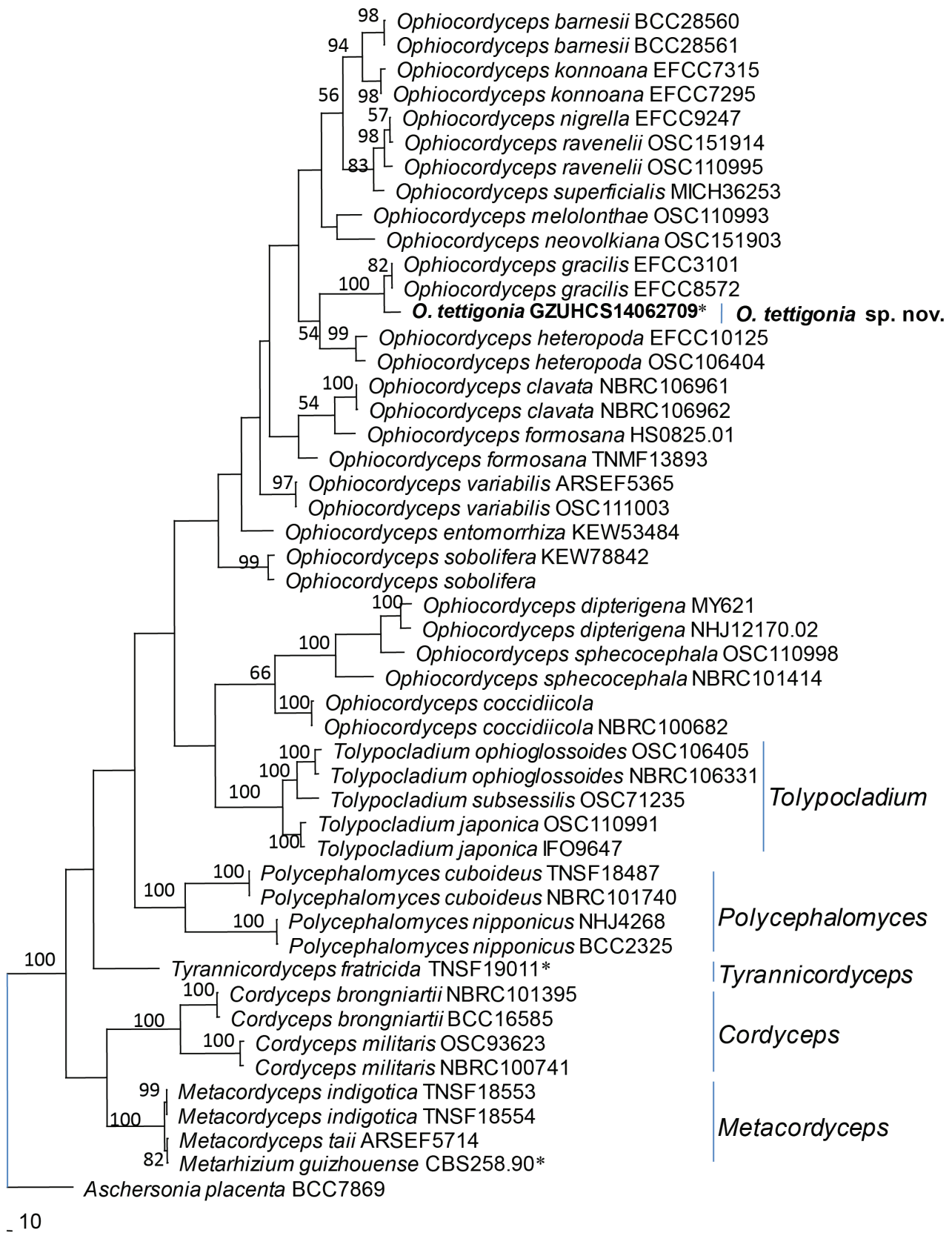


FIGURE 1. Phylogenetic relationships among *Ophiocordyceps tettigonia* and related species based on combined analysis of ITS, SSU, TEF, and RPB1 sequence data. Bootstrap values (1,000 replicates) are indicated above the nodes. Ex-type cultures or holotypes have an asterisk. The tree is rooted to *Aschersonia placenta* Berk.

TABLE 1. Data for taxa used in the sequence analyses.

Species	Vouchers ¹	Hosts/ Substrata	ITS	SSU	TEF	RPB1	References
<i>Ophiocordyceps tettiagoni</i>	GZUH CS14062709 ^T	<i>Tettiagonia</i> adult	KT345954	KT345955	KT375440	KT375441	This study
<i>Aschersonia placenta</i>	BCC 7869	Scale insect (Hemiptera)	JN049842	EF469121	EF469056	EF469085	Sung <i>et al.</i> (2007a)
<i>Cordyceps brongniartii</i>	NBRC 101395		JN943298	JN941759		JN992493	Schoch <i>et al.</i> (2012)
<i>C. brongniartii</i>	BCC 16585		JN049867	JF415951	JF416009	JN049885	Kepler <i>et al.</i> (2012a)
<i>C. militaris</i>	OSC 93623	Lepidopteran pupa	JN049825	AY184977	DQ522332	DQ522377	Sung <i>et al.</i> (2007a)
<i>C. militaris</i>	NBRC 100741		JN943437	JN941755		JN992489	Schoch <i>et al.</i> (2012)
<i>Metacordyceps indigotica</i>	TNS-F18553	Lepidoptera	JN049874	JF415952	JF416010	JN049886	Kepler <i>et al.</i> (2012a)
<i>M. indigotica</i>	TNS-F18554	Lepidoptera	JN049875	JF415953	JF416011	JN049887	Kepler <i>et al.</i> (2012a)
<i>M. taii</i>	ARSEF 5714	Lepidoptera	JN049829	AF543763	AF543775	DQ522383	Sung <i>et al.</i> (2007a)
<i>Metarhizium guizhouense</i>	CBS 258.90 ^T	Lepidoptera	HQ331448		EU248862	EU248914	Bischoff <i>et al.</i> (2009), Schneider <i>et al.</i> (2011)
<i>Ophiocordyceps barnesii</i>	BCC28560	Coleoptera		EU408776		EU408773	Luangsa-ard <i>et al.</i> (2010)
<i>O. barnesii</i>	BCC28561	Coleoptera		EU408775		EU408774	Luangsa-ard <i>et al.</i> (2010)
<i>O. clavata</i>	NBRC 106961	Coleopteran larva	JN943327	JN941727		JN992461	Schoch <i>et al.</i> (2012)
<i>O. clavata</i>	NBRC 106962	Coleopteran larva	JN943328	JN941726		JN992460	Schoch <i>et al.</i> (2012)
<i>O. coccidiicola</i>		Scale insect (Hemiptera)	AB031196	AB031195			Nikoh & Fukatsu (2000)
<i>O. coccidiicola</i>	NBRC 100682		AB968404	AB968391	AB968583		Ban <i>et al.</i> (2015)
<i>O. dipterigena</i>	MY621	Fly (Diptera)	GU723764		GU797126		Luangsa-ard <i>et al.</i> (2011)
<i>O. dipterigena</i>	N.H.J. 12170.02	Fly (Diptera)	GU723771		GU797127		Luangsa-ard <i>et al.</i> (2011)
<i>O. entomorrhiza</i>	KEW 53484	Coleopteran larva	JN049850	EF468954	EF468749	EF468857	Sung <i>et al.</i> (2007a)
<i>O. formosana</i>	HS0825.01		EF689044				Zuo <i>et al.</i> (2008)

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TABLE 1. (Continued)

Species	Vouchers ¹	Hosts/ Substrata	ITS	SSU	TEF	RPB1	References
<i>O. formosana</i>	TNM F13893			KJ878908	KJ878956	KJ878988	Quandt <i>et al.</i> (2014)
<i>O. gracilis</i>	EFCC 3101	Lepidopteran larva		EF468955	EF468750	EF468858	Sung <i>et al.</i> (2007a)
<i>O. gracilis</i>	EFCC 8572	Lepidopteran larva	JN049851	EF468956	EF468751	EF468859	Sung <i>et al.</i> (2007a)
<i>O. heteropoda</i>	EFCC 10125	Cicada nymph (Hemiptera)	JN049852	EF468957	EF468752	EF468860	Sung <i>et al.</i> (2007a)
<i>O. heteropoda</i>	OSC 106404	Cicada nymph (Hemiptera)		AY489690	AY489617	AY489651	Sung <i>et al.</i> (2007a)
<i>O. konnoana</i>	EFCC7315	Coleopteran larva		EF468959	EF468753	EF468861	Sung <i>et al.</i> (2007a)
<i>O. konnoana</i>	EFCC7295	Coleopteran larva		EF468958		EF468862	Sung <i>et al.</i> (2007a)
<i>O. melolonthae</i>	OSC 110993	Scarabaeid larva Coleoptera		DQ522548	DQ522331	DQ522376	Spatafora <i>et al.</i> (2007)
<i>O. neovolkiana</i>	OSC 151903			KJ878930	KJ878976	KJ879010	Quandt <i>et al.</i> (2014)
<i>O. nigrella</i>	EFCC9247	Lepidopteran larva	JN049853	EF468963			Kepler <i>et al.</i> (2012a)
<i>O. ravenelii</i>	OSC110995	Coleopteran larva		DQ522550	DQ522334	DQ522379	Sung <i>et al.</i> (2007a)
<i>O. ravenelii</i>	OSC151914			KJ878932	KJ878978	KJ879012	Quandt <i>et al.</i> (2014)
<i>O. sobolifera</i>	KEW 78842	Cicada nymph (Hemiptera)	JN049855	EF468972		EF468875	Sung <i>et al.</i> (2007a)
<i>O. sobolifera</i>		Cicada nymph	AB027374	AB027328			Nikoh & Fukatsu (2000)
<i>O. sphecocephala</i>	OSC110998	Wasp (Hymenoptera)		DQ522551	DQ522336	DQ522381	Sung <i>et al.</i> (2007a)
<i>O. sphecocephala</i>	NBRC101414		JN943443	JN941700		JN992434	Schoch <i>et al.</i> (2012)
<i>O. superficialis</i>	MICH36253	Coleopteran larva		EF468983			Sung <i>et al.</i> (2007a)
<i>O. variabilis</i>	ARSEF 5365	Dipteran larva		DQ522555	DQ522340	DQ522386	Spatafora <i>et al.</i> (2007)
<i>O. variabilis</i>	OSC 111003	Dipteran larva		EF468985	EF468779	EF468885	Spatafora <i>et al.</i> (2007)
<i>Polycephalomyces cuboideus</i>	TNS F18487			KF049609	KF049683		Quandt <i>et al.</i> (2014)

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TABLE 1. (Continued)

Species	Vouchers ¹	Hosts/ Substrata	ITS	SSU	TEF	RPB1	References
<i>P. cuboideus</i>	NBRC 101740		JN943331	KF049610	KF049684	KF049646	Quandt <i>et al.</i> (2014)
<i>P. nipponicus</i>	N.H.J. 4268			KF049621	KF049695	KF049654	Quandt <i>et al.</i> (2014)
<i>P. nipponicus</i>	BCC 2325		KF049665	KF049622	KF049696	KF049655	Quandt <i>et al.</i> (2014)
<i>Tolypocladium japonica</i>	OSC110991	<i>Elaphomyces</i> sp.	JN049824	DQ522547	DQ522330	DQ522375	Sung <i>et al.</i> (2007a)
<i>T. japonica</i>	IFO9647		AB027366	AB027320			Nikoh & Fukatsu (2000)
<i>T. ophioglossoides</i>	OSC106405	<i>Elaphomyces</i> sp.		AY489691	AY489618	AY489652	Sung <i>et al.</i> (2007a)
<i>T. ophioglossoides</i>	NBRC106331		JN943320	JN941733		JN992467	Schoch <i>et al.</i> (2012)
<i>T. subsessilis</i>	OSC71235	Scarabaeid larva	JN049844	EF469124	EF469061	EF469090	Sung <i>et al.</i> (2007a)
<i>Tyrannicordyceps fratricida</i>	TNS 19011 [†]	Fungus	JQ349068	JQ257022	JQ257028	JQ257016	Kepler <i>et al.</i> (2012b)

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; KEW, Mycology Collection of Royal Botanical Garden, Kew, Surrey, UK; N.H.J., Nigel Hywel-Jones personal collection; OSC, Oregon State University Herbarium, Corvallis, OR; GZUH, Herbarium of Guizhou University, Guiyang, Guizhou, China.

[†] ex-type culture or holotype.

Ophiocordyceps tettigonia *sp. nov.* differs from the other species of *Ophiocordyceps* in having a unique host insect, and wide secondary ascospores (Table 2). *Ophiocordyceps entomorrhiza* (Dicks.) G.H. Sung *et al.*, *O. gracilis* (Grev.) G.H. Sung *et al.*, *O. heteropoda* (Kobayasi) G.H. Sung *et al.*, and *O. neovoliana* (Kobayasi) G.H. Sung *et al.* are sister taxa to *O. tettigonia*; they all have a rounded fertile part, but differ in their hosts and morphology (Table 2). The nrDNA-ITS sequence (513 bp) of *O. tettigonia* (KT345954) differs from any known sequences of *Ophiocordyceps*, and a Blast search of GenBank reveals it similar to *Bionectria* sp. (KF367477, KU377273) and the maximal identities are 99%. The TEF sequence (734 bp) of *O. tettigonia* (KT375440) differs from any known sequences of *Ophiocordyceps*. A Blast search of GenBank reveals it is similar to *O. gracilis* (EF468750, EF468751), and the maximal identities are 99%. For the RPB1 sequence (637 bp) of *O. tettigonia* (KT375441), it differs from any known sequences of *Ophiocordyceps*. A Blast search of GenBank reveals it is similar to *O. gracilis* (EF468858, EF468859), and the maximal identities are 98% and 99%, respectively. Combined sequence phylogeny from the ITS, SSU, TEF and RPB1 gene-loci also confirmed the distinctiveness of the new species.

Taxonomy

Ophiocordyceps tettigonia T.C. Wen, Y.P. Xiao & K. D. Hyde, *sp. nov.* (Fig. 2)

Index Fungorum number: IF551451; *Facesoffungi* number: FoF01020.

Differs from related *Ophiocordyceps* species mainly by its unique host, and wide secondary ascospores.



FIGURE 2. *Ophiocordyceps tettigonia* (holotype). a. Overview of stromata and the host. b. Pale, superficial ascomata on stroma. c. Cross section showing the complete stroma and perithecia. d–f. Sections of ascomata. g, h. Part of peridium. i–k. Asci. l. Ascus with apical cap. m. Ascus with apical cap stained by iodine solution. n. Ascus stained by iodine solution. o. Secondary ascospores. p. Secondary ascospores stained by iodine solution. Scale bars: d = 1000 μ m, e, f = 200 μ m, g, h = 30 μ m, i–k = 100 μ m, l, m, o, p = 10 μ m, n = 20 μ m.

Type:—CHINA. Guizhou Province: Chishui City, Shizhangdong scenic spot, Chishui Danxia natural world heritage site, on adult of *Tettigonia* in the soil, 9 June 2014, *Ting-Chi Wen* CS14062709 (GZUHCS14062709, holotype).

Parasitic on an adult of *Tettigonia*. Host covered with white, dense mycelium, substratum buried in the soil, red to red-brown. Sexual morph: *Stromata* 35–40 mm long, 2–2.5 mm wide, growing from the head, with two branches. *Stipe* 32.5–37.5 mm long, 2 mm wide, flexuous, white. *Fertile head* 2–2.5 mm wide, globose, differentiated from stipe. *Ascomata* 520–680 × 205–275 µm (\bar{x} = 599 × 242, n = 30), completely immersed, elongated or ampuliform, with the ostioles opening on the surface of the head. *Peridium* 22.8–35.0 µm (\bar{x} = 28.9, n = 30) wide, comprised of three layers. *Asci* 530–615 × 6.5–9.3 µm (\bar{x} = 573.3 × 7.9, n = 30), 8-spored, hyaline, cylindrical, possessing a prominent apical cap. *Apical cap* 3.9–4.8 µm wide (\bar{x} = 4.35, n = 30). *Ascospores* 350–465 × 1.5–2.2 µm (\bar{x} = 407 × 1.8, n = 60), fasciculate, thread-like, slender and long, breaking into secondary ascospores when mature. *Secondary ascospores* 6.7–9.4 × 1.5–2.3 µm (\bar{x} = 8.1 × 1.9, n = 60), hyaline, cylindrical. Asexual morph: Undetermined.

Etymology:—Refers to the unique host insect, *Tettigonia*.

Distribution:—Chishui City, Guizhou Province, China.

Host:—On adult of *Tettigonia* living in the soil.

TABLE 2. Synopsis of the characters of *Cordyceps* species related to *Ophiocordyceps tettigonia*.

Species	Host	Stromata (mm)	Ascomata (µm)	Asci (µm)	Secondary ascospores (µm)	References
<i>O. tettigonia</i>	Adult of <i>Tettigonia</i>	Stipe 32.5–37.5 × 2, fertile part round, white, 2–2.5 diam.	Embedded, 520–680 × 205–275	Cylindrical, 530–615 × 6.5–9.3	6.7–9.4 × 1.5–2.3	This study
<i>C. grylli</i>	Adult of Gryllidae	20–50 × 1–2, fertile part cylindrical, 10–20 × 2–3	Superficial, 650–810 × 270–370	Cylindrical, 300 × 3–4	3.5–5 × 1	Teng (1963), Liang (2007)
<i>O. amazonica</i>	Coleopteran larva	25–30 × 1, fertile part round, red, 2.5–3 wide	Embedded	Cylindrical, 130–150 × 3.5–4	10 × 0.5–0.7	Hennings (1904), Kobayasi (1963)
<i>O. entomorrhiza</i>	Coleopteran larva	25–40 long, fertile part round, 3–4 × 4–7	Embedded	Cylindrical	6–7 × 1	Kobayasi & Shimizu (1983)
<i>O. gracilioides</i>	Coleopteran larva	40–90 × 2.5–3, fertile part round, 5–5.5 diam.	Embedded, 830–900 × 200–300	Cylindrical, 600–700 × 6–6.5	6.3–8.5 × 1.2–1.5	Fan <i>et al.</i> (2001)
<i>O. gracilis</i>	Lepidopteran larva	15 × 90, fertile part round, 2–7 × 2–5	Embedded, 560–840 × 200–360	Cylindrical, 400–528 × 4–6	6–8 × 1–1.5	Brady (1984)
<i>O. heteropoda</i>	Cicada nymph	120 long, fertile part round, 7–9 × 6–7	Embedded, 610–660 × 210	Cylindrical, 250–300 × 5.2–7	6–7.7 × 0.9–1	Kobayasi & Shimizu (1963)
<i>O. neovolkiana</i>	Scarabaeidae larva	13–18 × 2.5–3, fertile part round, 4–6 × 3–6	Embedded 340–460 × 140–165	Cylindrical 230–300 × 9–10	2.8–8 × 1.7–2	Kobayasi (1941)

Discussion

Ophiocordyceps tettigonia is distinctive in that it is associated with a unique host (*Tettigonia* adult), and it has wider secondary ascospores than related species. There are about six *Ophiocordyceps* species with round fertile part (Table 2). Although *O. tettigonia* is similar to *O. amazonica*, *O. tettigonia* has red *Tettigonia* adult host, while *O. amazonica* has yellow adult of Acrididae. *Ophiocordyceps amazonica* has red round fertile part, for *O. tettigonia* it is white. They also differ considerably in size of asci and ascospores (Kobayasi 1983). *Ophiocordyceps tettigonia* is similar to *O. gracilis*, but asci of *O. tettigonia* are longer and wider than those of *O. gracilis*, and secondary ascospores of *O. tettigonia* are much wider than in *O. gracilis* (Brady 1984) (Table 2). *Ophiocordyceps gracilioides* is similar to *O. gracilis* in its appearance, but their host insect is different, and secondary ascospores of *O. gracilioides* are much shorter than in *O. gracilis* (Fan *et al.* 2001) (Table 2). Combined sequence data from the ITS, SSU, TEF, and RPB1 gene loci also confirmed the distinctiveness of the new species.

This is the fourth new species of *Cordyceps sensu lato* from Chishui Danxia natural world heritage site, China, and further studies are likely to reveal other new species because of the special karst landform dating from Jurassic times.

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References

- Ban, S., Sakane, T. & Nakagiri, A. (2015) Three new species of *Ophiocordyceps* and overview of anamorph types in the genus and the family Ophiocordycepsaceae. *Mycological Progress* 14: 1017–1028.
<http://dx.doi.org/10.1007/s11557-014-1017-8>
- 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>
- Brady, B.L.K. (1984) *Cordyceps gracilis*. *CMI Descriptions of Pathogenic Fungi and Bacteria* 813: 1–2.
- 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>
- Cunningham, C.W. (1997) Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* 14: 733–740.
- Fan, M.Z., Li, C.R., Chen, Y.Y. & Li, Z.Z. (2001) *Cordyceps gracilioides*, a new record for China. *Mycosystema* 20 (2): 273–274.
- 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>
- Hennings, P. (1904) Fungi amazonici II. a cl. Ernesto Ule collecti. *Hedwigia* 43: 246–249.
- 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.
- Index Fungorum (2016) www.indexfungorum.org (accessed 1 October 2016)
- Jayasiri, S.C., Hyde, K.D., Ariyawansa, H.A., Bhat, J., Buyck, B., Cai, L., Dai, Y.C., Abd-Elsalam, K.A., Ertz, D., Hidayat, I., Jeewon, R., Gareth Jones, E.B., Bahkali, A.H., Karunarathna, S.C., Liu, J.K., Luangsa-ard, J.J., Lumbsch, H.T., Maharachchikumbura, S.S.N., McKenzie, E.H.C., Moncalvo, J.M., Ghobad-Nejhad, M., Nilsson H., Pang, K.L., Pereira, O.L., Phillips, A.J.L., Raspé, O., Rollins, A.W., Romero, A.I., Etayo, J., Selçuk, F., Stephenson, S.L., Suetrong, S., Taylor, J.E., Tsui, C.K.M., Vizzini, A., Abdel-Wahab, M.A., Wen, T.C., Boonmee, S., Dai, D.Q., Daranagama, D.A., Dissanayake, A.J., Ekanayaka, A.H., Fryar, S.C., Hongsanan, S., Jayawardena, R.S., Li, W.J., Perera, R.H., Phookamsak, R., de Silva, N.I., Thambugala, K.M., Tian, Q., Wijayawardene, N.N., Zhao, R.L., Zhao, Q., Kang, J.C. & Promptutha, I. (2015) The Faces of Fungi database: fungal names linked with morphology, phylogeny

- and human impacts, *Fungal Diversity* 74 (1): 3–18.
<http://dx.doi.org/10.1007/s13225-015-0351-8>
- 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>
- Kobayasi, Y. (1941) The genus *Cordyceps* and its allies. *Science Reports of the Tokyo Bunrika Daigaku* 5 (Section B, no. 84): 169–170.
- Kobayasi, Y. & Shimizu, D. (1963) Monographic studies of *Cordyceps* 2. Group parasitic on Cicadae. *Bulletin of the National Science Museum, Tokyo* 6: 286–314.
- Kobayasi, Y. & Shimizu, D. (1983) *Iconography of vegetable wasps and plant worms*. Horkusha Publishing, Tokyo, 280 pp.
- Liang, Z.Q. (2007) *Flora Fungorum Sinicorum, vol. 32, Cordyceps*. Science Press, Beijing, 190 pp.
- Luangsa-ard, J.J., Ridkaew, R., Mongkolsamrit, S., Tسانathai, K. & Hywel-Jones, N.L. (2010) *Ophiocordyceps barnesii* and its relationship to other melolonthid pathogens with dark stromata. *Fungal Biology* 114 (9): 739–745.
<http://dx.doi.org/10.1016/j.funbio.2010.06.007>
- Luangsa-ard, J.J., Ridkaew, R., Tسانathai, K., Thanakitpipattana, D. & Hywel-Jones, N. (2011) *Ophiocordyceps halabalaensis*: a new species of *Ophiocordyceps* pathogenic to *Camponotus gigas* in HalaBala Wildlife Sanctuary, Southern Thailand. *Fungal Biology* 115 (7): 608–614.
<http://dx.doi.org/10.1016/j.funbio.2011.03.002>
- Mortimer, P.E., Karunarathna, S.C., Li, Q.H., Gui, H., Yang, X.Q., Yang, X.F., He, J., Ye, L., Guo, J.Y., Li, H.L., Sysouphanthong, P., Zhou, D.Q., Xu, J.C. & Hyde, K.D. (2012) Prized edible Asian mushrooms: ecology, conservation and sustainability. *Fungal Diversity* 56: 31–47.
<http://dx.doi.org/10.1007/s13225-012-0196-3>
- 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>
- Rehner, S.A. & Buckley, E.P. (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-a sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97 (1): 84–98.
<http://dx.doi.org/10.3852/mycologia.97.1.84>
- 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>
- Spatafora, J.W., Sung, G.H., Sung, J.M., Hywel-Jones, N.L. & White, J.F. Jr. (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>
- Teng, S.C. (1963) *Fungi of China*. Science Press, Beijing, 586 pp.
- 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>
- Zuo, D.P., Li, C.R., Huang, B., He, Y.Q., Fan, M.Z. & Li, Z.Z. (2008) Molecular identification for connection of *Cordyceps formosana* and its *Hirsutella* anamorph. *Mycosystema* 27 (2): 224–229.