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Ophiocordyceps xuefengensis sp. nov. from larvae of *Phassus nodus* (Hepialidae) in Hunan Province, southern China

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Abstract

An entomogenous taxon, associated with larvae of *Phassus nodus* (Hepialidae) collected from Xuefeng Mountains, Hunan Province, China, was found to be a new species, *Ophiocordyceps xuefengensis sp. nov.* It differs from similar species in having long stromata, without a sterile apex, narrow asci, long ascospores and by its occurrence on *Phassus nodus* in living root or trunk of *Clerodendrum cyrtophyllum*. Combined sequence data from the 5.8S-ITS rDNA, nrSSU, EF-1α, and RPB1 gene loci also confirmed the distinctiveness of this new species. It is presently the world's largest known *Cordyceps sensu lato* species.

Key words: entomogenous fungi, new species, phylogenetic analyses, traditional Chinese medicine

Introduction

The genus *Cordyceps* Fr. (*Clavicipitaceae*, *Hypocreales*, *Ascomycota*) has been recently divided and placed into three families and four genera—*Metacordyceps* (*Clavicipitaceae*), *Elaphocordyceps* (*Ophiocordycipitaceae*), *Ophiocordyceps* (*Ophiocordycipitaceae*) and *Cordyceps* (*Cordycipitaceae*) (Sung *et al.* 2007a). Most species in *Cordyceps sensu lato* are pathogenic on insects and spiders, although a few grow on *Elaphomyces* spp. (soil fungi). Many *Cordyceps* species are used in traditional Chinese medicines in China, Japan, Korea and other eastern Asian countries (Wen *et al.* 2012).

Cordyceps sensu lato is one of the most important genera of invertebrate pathogens (Hywel-Jones 2001) with more than 530 species (www. Indexfungorum.org, December 25, 2012). Although many *Cordyceps* species have been transferred to *Ophiocordyceps*, many species have yet to be restudied. Kirk *et al.* (2008) suggested that there are 140 *Ophiocordyceps* species, and 153 species were listed by Sung *et al.* (2007a). There are more than 175 epithets assigned to *Ophiocordyceps* in Index Fungorum (www. Indexfungorum.org, December 25, 2012), however, some of them have been synonymsed with other genera. Most species of *Cordyceps sensu lato* have been identified from insects on leaves or in soil, 50 species are known to parasitize insects in dead wood, while a few species are known from insects in living tree trunks (Kobayasi & Shimizu 1983, Samson *et al.* 1985).

In this study, a new *Ophiocordyceps* species was found parasitizing *Phassus nodus* Chu & Wang collected from the living root or trunk of the medicinal plant *Clerodendrum cyrtophyllum* Turcz. in the Xuefeng Mountains of Hunan Province in south China. This species is morphologically distinct from all other *Cordyceps sensu lato* species and combined multi-gene analysis also shows it to differ. The new species, *Ophiocordyceps xuefengensis*,

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which is described below, currently sells for about US$20/g. It has been long recognized as a desirable alternative for natural *O. sinensis* in Hunan Province and it is also the world's largest known *Cordyceps* species.

Material & methods

Specimens and host

Collections were made in the Xuefeng Mountains, Dongkou County of Hunan Province between August 2011 and November 2012. Soil, tree parts, living larvae and adults, infected and dead insects, and insects from within the host trees were collected. Specimens were stored in plastic containers and transported to the laboratory for identification. The host insects were identified by Dr Xinjun Hu, Sun Yat-sen University and Prof. Qiong Zhou, Hunan Normal University.

Morphological studies

Fungal fruiting bodies were examined under an Optec SZ660 stereo dissecting microscope (Chongqing Optec Instrument Co., Chongqing, China). Hand sections of the fruiting structures were mounted in water for microscopic studies and photomicrography. The microcharacters of the fungus was examined under an Olympus CX31 compound microscope and photographed.

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 manufacturers protocols and the extracted DNA was stored at -20 °C. Two nuclear (5.8S-ITS rDNA, nrSSU) and two protein genes (EF-1α, 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-1α and RPB1, we followed Sung *et al.* (2007b) and Castlebury *et al.* (2004). For the amplification of EF-1α, the primers 983F (5'-GCYCCYGGHCAYCGTGAYTTYAT-3') and 2218R (5'-ATGACACCRACRGCRACRGTYTG-3') (Sung *et al.* 2007b) were used. For RPB1, the primers CRPB1A (5'-CAYCCWGGYTTYATCAAGAA-3') and RPB1Cr (5'-CCNGCDATNTCRTTRTCCATRTA-3') (Castlebury *et al.* 2004) were used in PCR amplification and sequencing.

All PCR products were sequenced by GenScript Biotechnology Co., Nanjing, China.

Sequence alignment and phylogenetic analysis

Blast searches were made to reveal the closest matches in GenBank for phylogenetic analysis. The taxon information and GenBank accession numbers used in the molecular analysis are listed in Table 1. The four gene datasets (5.8S-ITS rDNA, nrSSU, EF-1α, 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 Maximium Parsimony (MP) analysis was performed using PAUP* 4.0b10 (Swofford 1998). 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).

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

Species	Voucher [1]	Host/Substratum	5.8 ITS	nrSSU	tef1	rpb1	Reference
O. xuefengensis	GZUHHN13	*Phassus nodus* larvae	KC631804	KC631785	KC631790	KC631795	This study
O. xuefengensis	GZUH2012HN11	*Phassus nodus* larvae	KC631800	KC631786	KC631791	KC631796	This study
O. xuefengensis	GZUH2012HN13	*Phassus nodus* larvae	KC631801	KC631787	KC631792	KC631797	This study
O. xuefengensis	GZUH2012HN14[T]	*Phassus nodus* larvae	KC631802	KC631789	KC631793	KC631798	This study
O. xuefengensis	GZUH2012HN19	*Phassus nodus* larvae	KC631803	KC631788	KC631794	KC631799	This study
O. acicularis	OSC 110987	Coleopteran larva		EF468950	EF468744	EF468852	Sung *et al.* (2007)
O. acicularis	OSC 110988	Coleopteran larva		EF468951	EF468745	EF468853	Sung *et al.* (2007)
O. agriotidis	ARSEF 5692	Coleoptera	JN049819	DQ522540	DQ522322	DQ522368	Sung *et al.* (2007)
O. aphodii	ARSEF 5498[T]	*Aphodius hewitti* (Coleoptera)		DQ522541	DQ522323		Sung *et al.* (2007)
O. appendiculata	NBRC 106959	Coleopteran larva	JN943325	JN941729		JN992463	Schoch *et al.* (2012)
O. appendiculata	NBRC 106960	Coleopteran larva	JN943326	JN941728		JN992462	Schoch *et al.* (2012)
O. brunneipunctata	OSC 128576[AUT]	Coleoptera		DQ522542	DQ522324	DQ522369	Sung *et al.* (2007)
O. clavata	NBRC 106961	Coleopteran larva	JN943327	JN941727		JN992461	Schoch *et al.* (2012)
O. clavata	NBRC 106962	Coleopteran larva	JN943328	JN941726		JN992460	Schoch *et al.* (2012)
O. cuboidea	NBRC 100941	Beetle larva	JN943329	JN941725		JN992459	Schoch *et al.* (2012)
O. cuboidea	NBRC 101740	Beetle larva	JN943331	JN941724		JN992458	Schoch *et al.* (2012)
O. dipterigena	MY621	Fly (Diptera)	GU723764		GU797126		Luangsa-ard *et al.* (2011)
O. dipterigena	N.H.J. 12170.02	Fly (Diptera)	GU723771		GU797127		Luangsa-ard *et al.* (2011)
O. entomorrhiza	KEW 53484	Coleopteran larva	JN049850	EF468954	EF468749	EF468857	Sung *et al.* (2007)
O. gracilis	EFCC 3101	Lepidopteran larva		EF468955	EF468750	EF468858	Sung *et al.* (2007)
O. gracilis	EFCC 8572	Lepidopteran larva	JN049851	EF468956	EF468751	EF468859	Sung *et al.* (2007)
O. halabalaensis	MY1308[T]	Ant (Hymenoptera)	GU723758		GU797109		Luangsa-ard *et al.* (2011)
O. halabalaensis	MY5151	Ant (Hymenoptera)	GU723763		GU797110		Luangsa-ard *et al.* (2011)
O. heteropoda	EFCC 10125	Cicada nymph (Hemiptera)	JN049852	EF468957	EF468752	EF468860	Sung *et al.* (2007)
O. heteropoda	OSC 106404	Cicada nymph (Hemiptera)		AY489690	AY489617	AY489651	Sung *et al.* (2007)
O. irangiensis	OSC 128577	Ant (Hymenoptera)	JN049823	DQ522546	DQ522329	DQ522374	Sung *et al.* (2007)
O. irangiensis	OSC 128579	Ant (Hymenoptera)		EF469123	EF469060	EF469089	Sung *et al.* (2007)
O. melolonthae	OSC 110993	Scarabaeid larva (Coleoptera)		DQ522548	DQ522331	DQ522376	Spatafora *et al.* (2007)

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TABLE 1. (Continued)

Species	Voucher [1]	Host/Substratum	5.8 ITS	nrSSU	tef1	rpb1	Reference
O. myrmecophila	MY163	Ant (Hymenoptera)	GU723759		GU797132		Luangsa-ard *et al.* (2011)
O. paracuboidea	NBRC 100942	Beetle larva	JN943337	JN941711		JN992445	Schoch *et al.* (2012)
O. paracuboidea	NBRC 101742[T]	Beetle larva	JN943338	JN941710		JN992444	Schoch *et al.* (2012)
O. prolifica	NBRC 101750	Larva of *T. japonensis*	JN943340	JN941708		JN992442	Schoch *et al.* (2012)
O. prolifica	NBRC 103839	Larva of *T. japonensis*	JN943342	JN941706		JN992440	Schoch *et al.* (2012)
O. ravenelii	OSC 110995	Coleopteran larva		DQ522550	DQ522334	DQ522379	Sung *et al.* (2007)
O. rhizoidea	N.H.J. 12522	Termite (Isoptera)	JN049857	EF468970	EF468764	EF468873	Sung *et al.* (2007)
O. rhizoidea	N.H.J. 12529	Termite (Isoptera)		EF468969	EF468765	EF468872	Sung *et al.* (2007)
O. robertsii	KEW 27083	Lepidoptera	AJ309335		EF468766		Sung *et al.* (2007)
O. rubiginosiperitheciata	NBRC 100946	Beetle larva	JN943341	JN941705		JN992439	Schoch *et al.* (2012)
O. rubiginosiperitheciata	NBRC 106966	Beetle larva	JN943344	JN941704		JN992438	Schoch *et al.* (2012)
O. ryogamiensis	NBRC 103837	Beetle larva	JN943346	JN941702		JN992436	Schoch *et al.* (2012)
O. ryogamiensis	NBRC 103842	Beetle larva	JN943345	JN941701		JN992435	Schoch *et al.* (2012)
O. sinensis	EFCC 7287	Lepidopteran pupa	JN049854	EF468971	EF468767	EF468874	Sung *et al.* (2007)
O. sinensis	ARSEF 6282		HM595981		HM595918	HM595952	Chan *et al.* (2011)
O. sobolifera	KEW 78842	Cicada nymph (Hemiptera)	JN049855	EF468972		EF468875	Sung *et al.* (2007)
O. sobolifera		Cicada nymph	AB027374	AB027328			Nikoh & Fukatsu (2000)
O. speciesone	N.H.J. 01157		JN942622	JN940997		JN987877	Schoch *et al.* (2012)
O. speciesone	N.H.J. 01164		JN942621	JN940996		JN987878	Schoch *et al.* (2012)
O. sphecocephala	OSC 110998	Wasp (Hymenoptera)		DQ522551	DQ522336	DQ522381	Sung *et al.* (2007)
O. stylophora	OSC 111000	Elaterid larva (Coleoptera)	JN049828	DQ522552	DQ522337	DQ522382	Sung *et al.* (2007)
O. unilateralis	KT3307	Ant (Hymenoptera)	GU723756		GU797111		Luangsa-ard *et al.* (2011)
O. variabilis	ARSEF 5365	Dipteran larva		DQ522555	DQ522340	DQ522386	Spatafora *et al.* (2007)
O. variabilis	OSC 111003	Dipteran larva		EF468985	EF468779	EF468885	Spatafora *et al.* (2007)
Aschersonia placenta	BCC 7869	Scale insect (Hemiptera)	JN049842	EF469121	EF469056	EF469085	Sung *et al.* (2007)

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; 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.

2. [AUT] Authentic material, [T] ex-type culture or holotype.

Results

Phylogenetic analyses

The partition homogeneity test (P = 0.01) suggested that the individual gene partitions were not highly incongruent (Farris *et al.* 1995). 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; SH test verified that they were similar, one of which (tree length = 5,173 steps, CI = 0.567, RI = 0.742, RC = 0.420, HI = 0.443) and the most parsimonious tree is shown in Fig. 1.

The data set comprises 30 species (Fig. 1) including the new species *O. xuefengensis* which formed a separate clade from other species of *Ophiocordyceps* with credible bootstrap support (100%); thus the new species is introduced.

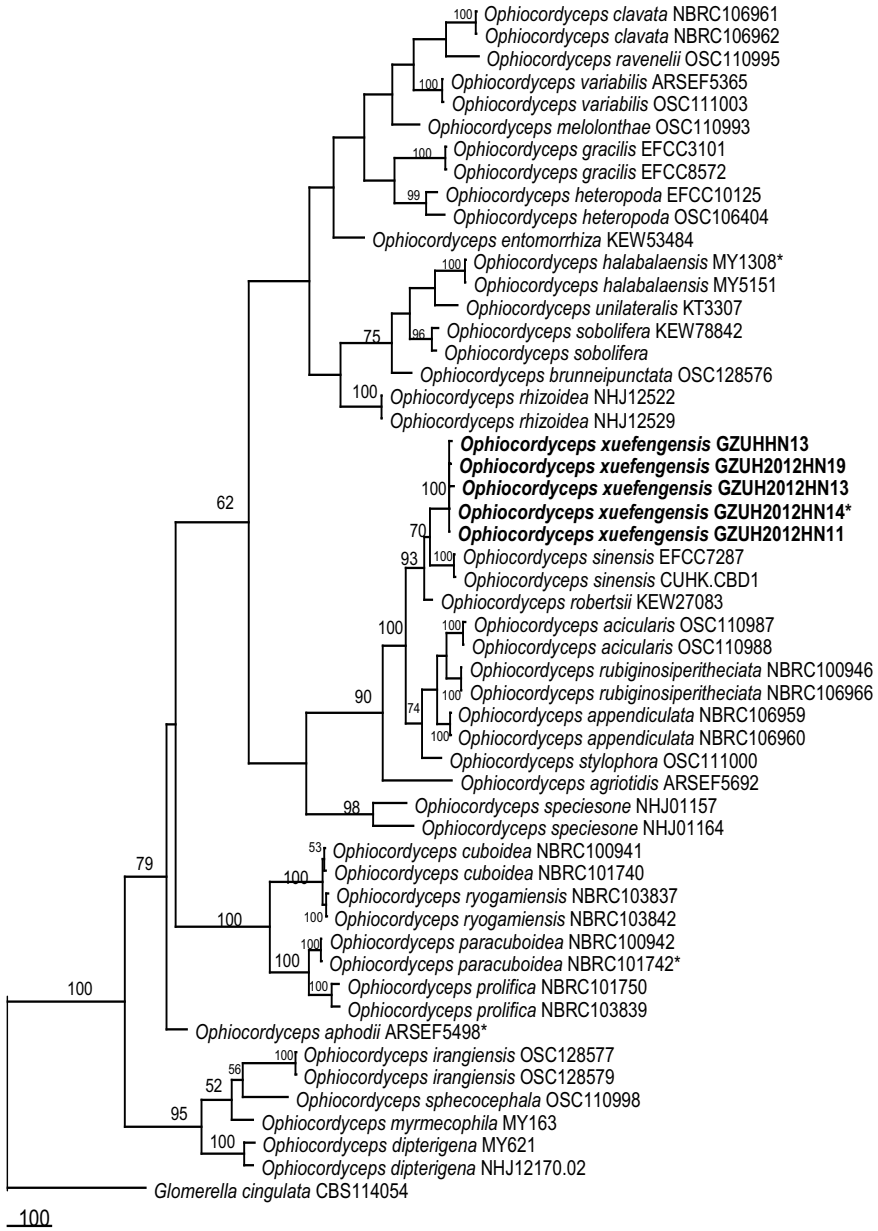

FIGURE 1. Phylogenetic relationships among *Ophiocordyceps xuefengensis* and related species based on four genes (5.8S-ITS rDNA, nrSSU, EF-1α, RPB1) combination. Bootstrap values (1,000 replicates) are indicated above the nodes. Type species have an asterisk. The tree is rooted to *Glomerella cingulata.*

TABLE 2. Synopsis of the characteristics of *Cordyceps* species related to *Ophiocordyceps xuefengensis*.

Species	Host	Habit	Stromata	Ascomata	Asci	Ascospores	Reference
O. xuefengensis	Hepialidae larva	Living trunk or upper root near soil	1–4 arising from head or other part of host, cylindrical, 140–460 × 2–7 mm; fertile part 100–200 × 2–4 mm, cylindrical, yellow-brown, covered with superficial ascomata; sometimes with many branches	Superficial, long ovoid, 416–625 × 161–318 µm. Perdium 20–74 µm wide	Cylindrical, 191–392 × 4.5–8.9 µm, with a 4.2–6.1 µm wide × 3.8–5.8 µm high hemiglobose cap	Thread-like, with many septa, not breaking into secondary ascospores, 130–380 × 1.4–5.2 µm	This study
O. sinensis	Hepialidae larva	Soil	Single, occasionally 2–3, 40–110 mm long, with sterile apex	Nearly superficial, ellipsoidal to ovate, 380–550 × 140–240 mm	Slender, long, 240–485 × 12–16 µm	Usually 2–4 mature ascospores, multiseptate, not breaking into secondary ascospores, 160–470 × 5–6 µm	Liang *et al.* (2007)
O. stylophora	Elateridae larva	Dead wood	Single, occasionally 2, caespitous, arising from head of host, 15–45 mm long, with short sterile tip. Cylindrical, capitate, twisted-rounded apex, 15 × 1.5–2 mm	Entirely embedded to the surface or at right angles to the surface, narrowly flask-shaped or ovoid, 240–420 × 144–240 µm	Cylindric-clavate, somewhat attenuated below, slightly narrowed above, 170–220 × 8–10 µm	Fusoid-cylindric, 102–164 × 2–3 µm, overlapping in the ascus, multiseptate, the cells 12–29 × 2–3 µm, not breaking into secondary ascospores	Mains (1941)
O. acicularis	Elateridae larvae	Soil	Single, cylindrical, 70 × 1 mm, with a sterile appendage	Superficial, long ovoid, 360–420 × 200–240 µm	Cylindrical, 7–7.4 µm wide, with a 4.8–5.4 µm wide × 3.6–4.8 µm high hemi-globose cap	Thread-like, multiseptate, 34.5–48 × 2–2.4 µm, not breaking into secondary ascospores	Liang *et al.* (2007)
O. robertsii	Hepialidae larva	Soil	Single, cylindrical, 100–380 × 3–4 mm, with sterile apex	Ascomata superficial, elongate-obvate or elliptical, 600–880 × 300–400 µm	Narrowly cylindrical, 280–400 × 9–10 µm	In parallel fascicles, filiform, multiseptate, 280 × 3 µm, breaking into secondary ascospores, 5–6 × 3 µm	Cunningham (1921)
O. cylindrostromata	lepidoptera larva	Soil	Binate, cylindrical, simple, 33–40 × 1–1.5 mm. Stipe short, 8–10 × 1–1.2 mm, dark brown. Fertile part cylindrical, yellow brown, 25–30 × 1.2–1.5 mm	Superficial, subpyriform, (255–)375–405 × 150–225 µm	Thread-like, 1600 × 4.2 µm, with subglobose cap, 3 µm high, 3.6–4.2 µm thick	Multiseptate, septate cells 6–8 µm long and 1.5–2 µm thick, not breaking into secondary ascospores	Liang *et al.* (2003)
O. gryllotalpae	*Cryllotalpa africana*	Soil	Binate, cylindrical, arise from the chest parts of host, 35–70 × 1.8–2 mm. Fertile part cylindrical, black, 15–20 × 2 mm with sterile apex	Densely superficial, elliptical, 210–155 × 130–140 µm	Cylindrical, 50–70 × 7–9 µm	Thread-like, 8-spored, multiseptate (7–8), 40–63 × 2–2.5 µm, not breaking into secondary ascospores	Kobayasi (1941)
O. jiangxiensis	*Campsosternus auratus* larva	Soil	Single, caespitous or fasciculate, cylindrical, 40–90 × 5 mm, sometimes ramified, commonly without tail-like sterile tip. Endosclerotia white	Superficial, compactly aggregated on middle to upper part of stroma, elongated-ovate, 520–600 × 300 µm	6 µm diameter, with a flattened-globose to conical cap, 2.4–3.0 × 1.8–2.2 µm	Long cylindrical, 1.0–1.2 µm thick, multiseptate, each cell 5.5–7.5 µm long, not breaking into secondary ascospores	Liang *et al.* (2001)
C.aeruginosclerota	Cockchafer larva	Soil	Cylindrical, 3 arising from nearly tail of host, 100–140 × 4–5 mm. Fertile part cylindric, distinct from stalk, without sterile tip, dark brown. Endosclerotia green	Densely superficial, short ovoid, 260–300 × 160–240 µm	Cylindrical or long clavate, 150 × 4.5–7.5 µm, with a short cylindrical cap, mostly 3.6 µm high, 3 µm thick	Filiform, multiseptate, attenuated toward both ends, not breaking into secondary ascospores, septate cells 6–14 × 1.2–1.8 µm	Liang *et al.* (1997)

Ophiocordyceps xuefengensis differs from the other species of *Ophiocordyceps* in having long stromata without a sterile apex, narrow asci, long ascospores and its occurrence on *Phassus nodus* in living root or trunk (Fig. 2). *Ophiocordyceps xuefengensis* and *O. sinensis* are sister taxa, but differ in their host, morphology and ecology (Table 2).

Taxonomy

Ophiocordyceps xuefengensis T.C. Wen, R.C. Zhu, J.C. Kang & K.D. Hyde, *sp. nov.* (Fig. 2) MycoBank MB803424

Type:—CHINA. Hunan Province: Dongkou County, Xuefeng Mountains, on *Phassus nodus* in root of living *Clerodendrum cyrtophyllum,* 20 October 2012, *Ru-Cai Zhu 2012HN14* (GZUH 2012HN14, holotype!).

Differs from related *Ophiocordyceps* species mainly by its long stromata, without a sterile apex, narrow asci, long ascospores and by its occurrence on *Phassus nodus* in living root or trunk of *Clerodendrum cyrtophyllum*.

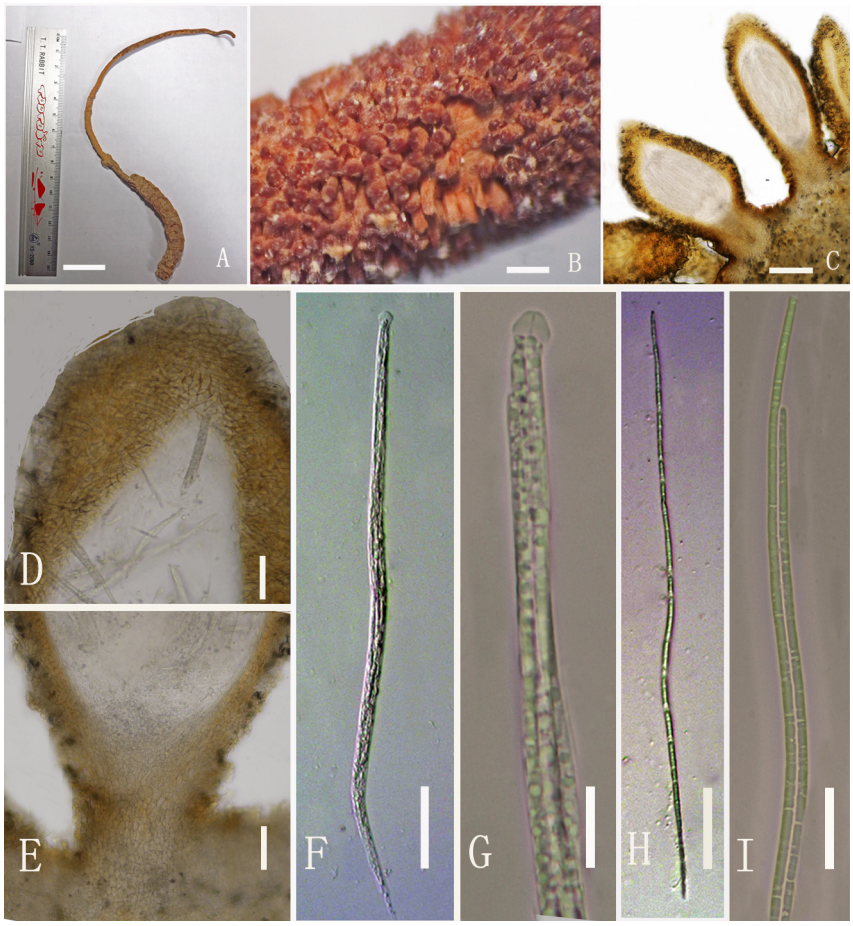

FIGURE 2. *Ophiocordyceps xuefengensis*. A. Overview of stroma and the host. B. Brown, superficial ascomata on stroma. C. Section of ascomata. D. Apical peridium. E. Basal peridium formed from stroma. F. Mature ascus with ascospores. G. Ascus cap. H, I. Hyaline and filliform ascospores with many septa. Scale bars: A = 20 mm, B = 0.5 mm, C = 100 µm, D, E = 50 µm, F, H = 40 µm, G = 10 µm, I = 20 µm.

Stromata 140–460 mm long, 2–7 mm wide, cylindrical, yellow-brown, 1–4 arising mainly from the head or other part of host; living on larvae of *Phassus nodus* (Hepialidae) in the trunk or upper root near the soil of *Clerodendrum cyrtophyllum* (*Verbenaceae*). Stipe 40–260 mm long, 2–7 mm diam., and sometimes covered with beige, thick, dense, loosely woven mycelium. Fertile part 100–200 mm long × 2–4 mm diam., cylindrical, yellow-brown. Cells of stromata thin-walled, globose, hyaline, becoming brown-walled towards the outside. Host 60–110 mm long, 7–12 mm wide, yellow-brown to black-brown, with white endosclerotia.

Ascomata 416–625 × 161–318 μm ($\bar{x}$ = 520 × 243, n = 20), superficial, long ovoid, with a basal stipe connected to the stromata. Peridium 20–74 μm wide ($\bar{x}$ = 39, n = 20), comprising three layers; hamathecium of paraphyses. Asci 191–392 × 4.5–8.9 μm ($\bar{x}$ = 277 × 7.5, n = 20), 8-spored, cylindrical, pedicellate not clear, with a 4.2–6.1 μm wide × 3.8–5.8 μm high ($\bar{x}$ = 5.1 × 4.7, n = 20) hemiglobose cap. Ascospores 130–380 × 1.4–5.2 μm ($\bar{x}$ = 256 × 3.2, n = 20), fasciculate, thread-like, slender and long, with many septa, not breaking into secondary ascospores.

Asexual state:—*Hirsutella.*

Etymology:—Refers to the type collecting site "Xuefeng Mountains".

Distribution:—Dongkou County, Hunan Province, China.

Host:—On larvae of *Phassus nodus* Chu & Wang living in the root or trunk of *Clerodendrum cyrtophyllum* Turcz.

Other material studied (paratypes):—CHINA. Hunan Province: Dongkou County, Xuefeng Mountains, on *Phassus nodus* in root of living *Clerodendrum cyrtophyllum,* 8 October 2011, *Ru-Cai Zhu HN13* (GZUHHN13!); ex-paratype living culture GZUCCHN131!; Hunan Province: Dongkou County, Xuefeng Mountains, on *Phassus nodus* in root of living *Clerodendrum cyrtophyllum,* 20 October 2012, *Ru-Cai Zhu 2012HN11* (GZUH2012HN11!); Hunan Province: Dongkou County, Xuefeng Mountains, on *Phassus nodus* in trunk of living *Clerodendrum cyrtophyllum,* 20 October 2012, *Ru-Cai Zhu 2012HN13* (GZUH2012HN13!); Hunan Province: Dongkou County, Xuefeng Mountains, on *Phassus nodus* in root of living *Clerodendrum cyrtophyllum,* 20 October 2012, *Ru-Cai Zhu 2012HN19* (GZUH2012HN19!).

Discussion

Ophiocordyceps xuefengensis is remarkable for four reasons: 1) it is probably the largest insect inhabiting *Cordyceps sensu lato* species ever discovered (stromata 140–460 mm long); 2) it is associated with *Phassus nodus* larvae on a single tree species; 3) it develops on larvae growing within the trunk or root of a tree and; 4) it lacks a sterile apex as compared with allies having superficial ascomata in the *Ophiocordyceps* group. If this large species, associated with a single tree species, has been previously overlooked by mycologists, especially in China where entomogenous fungi have been well-studied (Song *et al.* 2006), one would predict that further studies will reveal many new species. This may have important implications for the numbers of fungi worldwide.

There are about 90 species with cylindric stromata in *Cordyceps sensu lato* and only a few species (i.e., *O. gryllotalpae* Petch (1942: 255), *O. jiangxiensis* (Z.Q. Liang, A.Y. Liu Yong C. Jiang) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora (2007a: 43), *C. aeruginosclerota* Z.Q. Liang & A.Y. Liu (in Liang *et al*. 1997: 63) and *O. cylindrostromata* (Z.Q. Liang, A.Y. Liu & M.H. Liu) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora (2007a: 42) have stromata lacking a sterile apex, superficial ascomata and ascospores not breaking into secondary ascospores (Kobayasi & Shimizu 1983, Liang *et al.* 1997, 2001, 2003). The new species differs from the above species in host, habit, having long, seldom branched stromata and narrow ascospores.

It is unusual that *O. xuefengensis* lives only on *Phassus nodus* larvae in the living root or trunk of the medicinal plant *Clerodendrum cyrtophyllum* and has a 2–3 year life cycle. In the first year, the stromata are smooth and lack ascomata, while in the second or third year sexual structures with spores develop. The species is used in traditional Chinese medicine as a replacement for *O. sinensis* in Hunan Province of south China, the gathering of the latter is causing substantial reductions in populations (Peter *et al.* 2004). *Ophiocordyceps xuefengensis* is the world's largest known *Cordyceps* species (4.0 g dry weight for one specimen, and 460 mm long, Table 3), perhaps because its large host larvae contain a lot of nutrition.

TABLE 3. Comparison of several macro *Cordyceps sensu lato* related to *Ophiocordyceps xuefengensis*.

Species	Host type	Size of host	Stromata	Whole dry weight (g)	Reference
Ophiocordyceps xuefengensis	Larva of *Phassus nodus*	60–110 × 7–12 mm	1–4 arising from host, 140–460 × 2–7 mm	4.0	This study
Metacordyceps liangshanensis	Larva of Hepialidae	20–50 mm long	Single, 20–30 × 1.5–2.5 mm	No data	Liang *et al.* (2007)
Cordyceps henleyae	Larva of Hepialidae	No data	Single, 180–200 × 7 mm	No data	Massee (1894)
Cordyceps larvarμm	Larva of lepidoptera	50–70 mm long	Single, 90–380 × 2–2.5 mm	No data	Liang *et al.* (2007)
Ophiocordyceps robertsii	Larva of Hepialidae	40–70 mm long	Single, 100–380 × 3–4 mm	No data	Cunningham (1921), Liang *et al.* (2007)

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