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A new entomopathogenic fungus, *Ophiocordyceps ponerus* sp. nov., from China

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Abstract

A new species, *Ophiocordyceps ponerus*, is reported from a survey of invertebrate-associated fungi in Xiaochehe Wetland Park, Guiyang City, China. Evidence for the new species is provided by morphological and molecular characters. Synnemata of this species emerged from cadavers of soldier ants of *Ponera* sp. (Hymenoptera). It differs from similar species mainly in having verticillate phialides on the upper portions of the synnemata with septa, 112–158 × 7.5–10 µm; cylindrical or oval conidiogenous cells, which are inflated at the base, and suddenly tapering to a short, thin and verrucose neck, 13–25 × 2.5–5 µm; solitary, smooth conidia nearly oval or forming curved orange segments, 7.5–10 × 3.8–5 µm. Phylogenetic analysis using combined ITS, SSU, RPB2 and TEF sequence data support its systematic position in *Ophiocordyceps* and as a new species.

Key words: 1 new species, entomopathogenic fungi, *Ophiocordyceps*, multiple genes, taxonomy

Introduction

The entomopathogenic genus *Ophiocordyceps* (= *Hymenostilbe*) belongs to Ophiocordycipitaceae, Hypocreales (Wijayawardene *et al.* 2017). *Hymenostilbe* was first proposed based on the type species *H. muscarium*, a species parasitic on dipteran insects that was later found to be an asexual morph of *Ophiocordyceps forquignonii* (Quél) Sung (Petch, 1931; Sung *et al*., 2007). As the oldest asexual generic name associated with the '*O. sphecocephala* clade', *Hymenostilbe* was synonymized under *Ophiocordyceps*, most species of which sporulate from adult insects (Sung *et al*., 2007; Luangsa-ard *et al*., 2011; Maharachchikumbura *et al*., 2015, 2016 ; Wijayawardene *et al.* 2017). Species of *Ophiocordyceps* have unusual cylindrical synnemata and smooth conidia that are very similar to those of *Akanthomyces* (Lebert, 1858) and are parasitic on insects of Diptera, Orthoptera, Hymenoptera and spiders (Wijayawardene *et al.* 2017). The main identifying characteristics of this asexual *Ophiocordyceps* are solitary conidia, which are polyblastic and form on a denticle, whereas those of *Akanthomyces* form in chains on phialides (Petch, 1932; Mains, 1950; Huang *et al*., 1998). Petch questioned the acceptability of the genus *Hymenostilbe* when he reported the species *Akanthomyces aculeatus* Lebert (Petch, 1933). Subsequently, Kobayasi (1941) and Mains (1950) found that it was very difficult to delimit *Hymenostilbe* from *Akanthomyces*, especially for species producing synnemata which covers with a hymenial layer at surface. After comprehensive studies, Samson and Evans (1975) proposed polyphialides as a species-specific character of *Hymenostilbe*. On the basis of this, they re-described nine taxa and established the taxonomic status of *Hymenostilbe*. Sung suggested that *Hymenostilbe* asexual morphs might be derived from within *Hirsutella* because of the close phylogenetic relationship between *Hirsutella* and *Hymenostilbe* asexual morphs, but more evidence is needed to support this (Sung *et al*., 2007).

In the Index Fungorum (http://www.indexfungorum.org), there are 26 asexual morph species listed in *Hymenostilbe*, including 24 formally described species. Among them, *O. aranearum* Petch was originally classified in *Akanthomyces* (Mains, 1950) and *Hymenostilbe sphecophila* Petch (as *Isaria sphecophila* Ditmar) was later merged with *Hirsutella* (Van *et al.*, 2005). However, there has been debate about the attribution of *Hymenostilbe sphecophila*, and some scholars believe that it is an asexual morph of *Ophiocordyceps sphecocephala* and should be placed in *Hymenostilbe* (Nigel, 1995). In the last two decades, a small number of Chinese and international studies have addressed the

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taxonomy, molecular evolution and phylogeny of *Hymenostilbe* asexual morphs, with sporadic reports of six new species, including *O. ventricosa* Hywel-Jones (1995), *O. aurantiaca* Hywel-Jones (1996), *O. ichneumonophila* Van Vooren & Audibert (2005), *O. furcata* Aung *et al*. (2006), *O. spiculata* Huang *et al*. (1998) and *O. verrucosa* Peng *et al*. (2008).

During a survey of invertebrate-associated fungi in natural forests near Guiyang City in China, a fungus parasitic on soldier ants of *Ponera* sp. (Hymenoptera) was found in a nest. Attempts to identify the fungus showed that neither the gene sequences nor morphological traits matched any known *Hymenostilbe* asexual morphs. To conform to Article 59 of the International Code of Botanical Nomenclature, some mycologists have proposed suppressing the use of some asexual names proposed for taxa in this '*O. sphecocephala* clade', including *Hymenostilbe*, in favor of *Ophiocordyceps* as the genus name for the entire clade (Sung *et al*., 2007, Kepler *et al*., 2013, Quandt *et al*., 2014, Simmons *et al*., 2015, Spatafora *et al*., 2015). In light of these recommendations by respected researchers of these fungi, we describe the fungus represented by GZUIFR–2012xch03 as *Ophiocordyceps ponerus*. Based on morphological character comparisons and phylogenetic analyses, the aim of the present study is to introduce the new species and to investigate its biology and phylogenetic position.

Materials and methods

Specimen

The specimen was collected from Xiaochehe Wetland Park, Guiyang City, Guizhou Province, China (26°32' N, 106°40' E, approximately 1100 m above sea level) in November 2012 by J. J. Qu & Y. M. Zhou, on cadavers of soldier ant of *Ponera* sp. (Hymenoptera) from rotting wood. Holotypus: GZUIFR–2012xch03 and an isolated strain of its asexual stage GZUIFR xch03 were deposited at the Institute of Fungal Resources of Guizhou University (GZUIFR); the isolated strain was also deposited at the China General Microbiological Culture Collection Center (CGMCC), CGMCC 3.18756.

Fungal isolation and culture

The surface of specimen was rinsed with sterile water, followed surface sterilization with 75% ethanol for 3–5 s. The part of the insect body was cut off and inoculated a piece of tissue in haemocoel on the potato dextrose (PDA) agar. Then, the strain was isolated and cultured at 22°C for 14 d under 12-h light/12-h dark conditions following protocols described by Zou *et al.* (2010).

OM and SEM observations

For optical microscopy (OM) observations and imaging, the fresh hyphae were stained with lactic acid phenol cotton blue solution and observed with optical microscope (OM, BK5000, OPTEC, USA). The captured images were edited and digitally contrasted with Paint Shop Pro v. 5.0.1 (Corel, Ottawa, Canada).

Electron microscopy was carried out following to Qu *et al*. (2017). Briefly, 1 cm wide agar blocks with hyphae of the fungus were cut from PDA cultures, and the collected samples were fixed with 4% glutaraldehyde at 4°C overnight, then washed three times with phosphate buffer solution (PBS) (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4) three times, 10 min/times. Fixed hyphae and conidia were dehydrated using 50%, 70%, 90% and 100% alcohol, 10 min/each level; dehydrated with supercritical carbon dioxide at last. Placed the samples to spray gold. Conidia and mucilage were examined with scanning electron microscope (SEM, S-3400N, HITACHI, Japan) and photographed.

DNA extraction, PCR amplification and sequencing

To construct a phylogeny of major lineages, representative taxa of members from the major species were chosen based on previous phylogenetic studies (Sung *et al.*, 2007; Quandt *et al.*, 2014). A total of 45 taxa were selected to represent the morphological and ecological diversity of *Ophiocordyceps*, including outgroup taxon *Colletotrichum gloeosporioides*, which is classified within Glomerellaceae (Sung *et al.*, 2007). Axenic mycelia (0.05–0.1g) of tested fungi which needed to extract molecular data were harvested from PDA plates and transferred into 1.5 ml eppendorf tubes for genomic DNA extraction and PCR amplification, which were carried out as previously described (White *et al.*, 1990; Rehner *et al.*, 1994; Rehner *et al.*, 2005; Sung *et al.*, 2007; van den Brink *et al.*, 2012; Simmons *et al.*, 2015). Sequences from four nuclear loci, including the small subunit ribosomal RNA (SSU), the transcription elongation

factor-1 alpha (TEF), the largest and second largest subunits of RNA polymerase II (RPB2) and the first and the internal transcribed spacers (ITS1–5.8S rDNA–ITS2 region, ITS) were used for phylogenetic analyses. All other sequences were collected from GenBank. Efforts were made for all species to have data for at least two genes to be considered in our analyses. Sequences used in this study were combined with published data on species of *Hymenostilbe* asexual morphs, *Polycephalomyces* and *Ophiocordyceps*. The GenBank accession numbers are shown in Table 1.

TABLE 1. Specimen information and GenBank accession numbers for sequences used in this study

Species	Voucher Information	ITS	RPB2	SSU	TEF
Ophiocordyceps irangiensis	OSC 128577	JN049823	DQ522427	DQ522546	DQ522329
Ophiocordyceps nutans	OSC 110994	AF224274	EF495090	DQ522549	DQ522333
Ophiocordyceps sphecocephala	OSC 110998	AJ786597	DQ522432	DQ522551	DQ522336
Colletotrichum gloeosporioides	FAU 513	EU358953	DQ858455	JN940361	AF543772
Colletotrichum gloeosporioides	FAU 553	EU358952	DQ522441	JN940359	AF543773
Ophiocordyceps aurantiaca	OSC 128578		DQ522445	DQ522556	DQ522345
Ophiocordyceps dipterigena	OSC 151912	GU723771	KC610712	KJ878920	KJ878967
Ophiocordyceps muscaria	OSC 151902		KJ878945	KJ878912	
Ophiocordyceps odonatae	TNS F18563	AB104725	KJ878992	D86055	
Ophiocordyceps ponerus	**CGMCC 3.18756**	**KP890688**	**KY953145**	**KY953152**	**KY953153**
Ophiocordyceps acicularis	OSC 128580	JN049820	DQ522423	DQ522543	DQ522326
Ophiocordyceps agriotidis	ARSEF 5692	JN049819	DQ522418	DQ522540	DQ522322
Ophiocordyceps annulata	CEM 303			KJ878915	KJ878962
Ophiocordyceps brunneipunctata	OSC 128576	GU723777	DQ522420	DQ522542	DQ522324
Ophiocordyceps curculionum	OSC 151910		KJ878999	KJ878918	
Ophiocordyceps dipterigena	OSC 151911	EU573346	KC610715	KJ878919	KJ878966
Ophiocordyceps entomorrhiza	KEW 53484	AJ786561	EF468911	EF468954	EF468749
Ophiocordyceps formicarum	TNSF 18565	AB222679	KJ878946	KJ878921	KJ878968
Ophiocordyceps forquignonii	OSC 151908	HQ662164	KJ878947	KJ878922	
Ophiocordyceps gracilis	EFCC 8572	HM142942	EF468912	EF468956	EF468751
Ophiocordyceps heteropoda	EFCC 10125	JN049852	EF468914	EF468957	EF468752
Ophiocordyceps irangiensis	OSC 128579	GU723767	EF469107	EF469123	EF469060
Ophiocordyceps lloydii	OSC 151913	KP200892	KJ878948	KJ878924	KJ878970
Ophiocordyceps longissima	EFCC 6814	AB968406	AB968546	AB968392	EF468757
Ophiocordyceps myrmecophila	CEM 1710			KJ878927	KJ878973
Ophiocordyceps myrmecophila	HMAS 199620	EU573350		KJ878929	KJ878975
Ophiocordyceps nigrella	EFCC 9247	JN049853	EF468920	EF468963	EF468758
Ophiocordyceps nutans	NBRC 100944	AB544486	AB968549	DQ522549	DQ522333
Ophiocordyceps oxycephala	MRCIF53	EU573348	EF495091	DQ838794	
Ophiocordyceps ravenelii	OSC 110995		DQ522430	DQ522550	DQ522334
Ophiocordyceps rhizoidea	NHJ 12522	GU723769	EF468923	EF468970	EF468764
Ophiocordyceps sobolifera	KEW 78842	AB027374	EF468925	EF468972	AB968590
Ophiocordyceps sphecocephala	NBRC 101753	JN943351	AB968553	JN941700	AB968592
Ophiocordyceps stylophora	OSC 111000	JN049828	DQ522433	DQ522552	DQ522337
Ophiocordyceps tricentri	NBRC 106968	AB968410	AB968554	AB968393	AB968593
Ophiocordyceps unilateralis	OSC 128574	AY494596	DQ522436	DQ522554	DQ522339
Ophiocordyceps buquetii	HMAS 199613			KJ878939	KJ878984
Ophiocordyceps buquetii	HMAS 199617			KJ878940	KJ878985
Polycephalomyces formosus	ARSEF 1424	KF049661	KF049671	KF049615	DQ118754
Polycephalomyces nipponica	BCC 2325	KF049665	KF049677	KF049622	KF049696
Polycephalomyces paracuboidea	NBRC 101742	AB925954	KF049669	KF049611	KF049685
Polycephalomyces prolifica	TNSF 18547	KF049660	KF049670	KF049613	KF049687
Polycephalomyces tomentosus	BL 4	KF049666	KF049678	KF049623	KF049697
Polycephalomyces sp.	RMK 2013	KF049662	KF049672	KF049616	KF049690

Molecular phylogeny

Sequences were aligned and edited manually using the BioEdit Sequence Alignment Editor ver. 7.0.5.3 (Hall, 1999) with the Clustal X ver. 1.83 software (Thompson *et al.*, 1999) package. Gaps were excluded from the phylogenetic analysis. The data set contained 45 taxa and consisted of 574 bp for SSU, 508 bp for TEF, 512 bp for RPB2 and 306 bp for

ITS. A combined data included 1900 characters set of the four regions was analyzed. The Akaike information criterion (AIC) in jModeltest 0.1.1 (Guindon & Gascuel, 2003; Posada, 2008) was used to select the nucleotide substitution model for each partition. Maximum likelihood (ML) phylogenetic analyses were conducted in RAxML (Stamatakis *et al.*, 2008) with the recommended partition parameters to determine the best tree topology and bootstrap support values from 500 search replicates, which were summarized in FigTree. Bayesian posterior probabilities (BPP) were estimated with the same partition parameters in an analysis conducted in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003), in which two runs of four chains each were executed simultaneously for 5 000 000 generations, with sampling every 500 generations. TreeGraph was used to compute BPP from a summary of 7501 trees retained after a burn-in of the first 2500 trees collected.

Results

Taxonomy

Ophiocordyceps ponerus X. Zou & Y. F Han, *sp. nov*. Fig. 1.
MycoBank no.: MB 814427; Facesoffungi number: FoF03305.

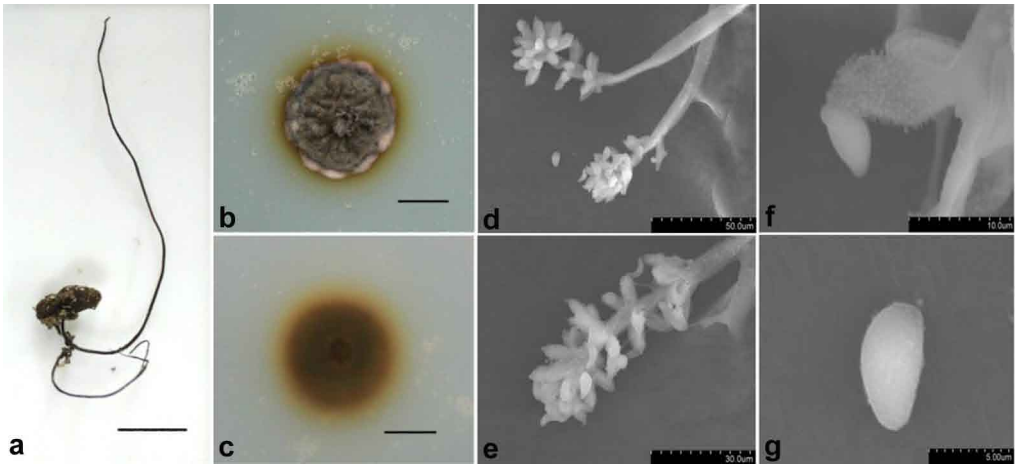

FIGURE 1. *Ophiocordyceps ponerus* (GZUIFR–2012xch03, holotype). a. The Infection ant specimen with long and black synnemata; b, c. Front and rear morphology of colonies formed on PDA medium after 20 d; d, e. SEM images showing the synnemata and whorled conidiophores; f, g. SEM images showing the phialides and conidia. Scale bars: a–c = 10 mm, d = 50 μm, e = 30 μm, f = 10 μm, g = 5 μm.

Etymology: the species epithet refers to the genus name of the host, *Ponera* sp. (Lat."ponerus"). Host was approximately 5 mm long × 3 mm wide. Synnemata approximately 20–50 mm long × 0.2 mm wide, several, cylindrical, tapering at the end, arising from the thorax of the insect, sometimes simple-branched, black, fawn brown near the apex. Mycelium spreading slowly 20–30 mm diam. after 20 d, on PDA medium under 20–22°C.; Colony circular, center of surface with dark brown dense bulges, pink sparse flocculent aerial hyphae on colonies margins; much brown pigment secreting into the media making the back of the colonies show dark brown. Conidiophores 112–158 × 7.5–10 μm, formed outside the synnemata with septa, verticillate phialides on the upper portions of the synnemata, asperulate; conidiogenous cells 13–25 × 2.5–5 μm, forming on conidiophores or side branches, cylindrical or oval inflated at the base, suddenly tapering to the short and thin neck, verrucose; conidia 7.5–10 × 3.8–5 μm, solitary, smooth, hyaline, nearly oval or shaped like curved orange segments.

Ophiocordyceps ponerus differs from related species mainly in having verticillate phialides on the upper portions of the synnemata; conidiogenous cells that are cylindrical or oval, inflated at the base, and verrucose; and conidia that are solitary, smooth, and nearly oval or forming curved orange segments (7.5–10 × 3.8–5 μm).

Holotype: CHINA. Guizhou Prov.: elev. 1100m, Xiaochehe Wetland Park, Guiyang City, on cadavers of soldier ant of

Ponera sp. (Hymenoptera) from rotting wood, 16 November 2012, GZUIFR–2012xch03. Isotype: KUN–F0085001. GenBank: ITS = KP890688; RPB2= KY953145; SSU= KY953152; TEF= KY953153.

Known distribution: Xiaochehe Wetland Park, Guiyang, Guizhou Province, China.

Phylogenetic analysis

The tree was regenerated with maximum likelihood analysis and Bayesian posterior probabilities with *Colletotrichum gloeosporioides* as the outgroup taxon (Fig. 2). The tree could be broadly divided into three clades: *Hymenostilbe*, *Hirsutella* and *Polycephalomyces* clades. In the phylogenetic tree, *Ophiocordyceps ponerus* cluster with *Hymenostilbe* asexual morphs species and formed a separate branch from other species with credible bootstrap support (87/92%). Within a separate branch, *Ophiocordyceps ponerus* and *Ophiocordyceps odonatae* clustered together closely, suggesting that these species were truly related. The molecular phylogenetic analysis confirmed that there were differences between *Ophiocordyceps ponerus* and other related species.

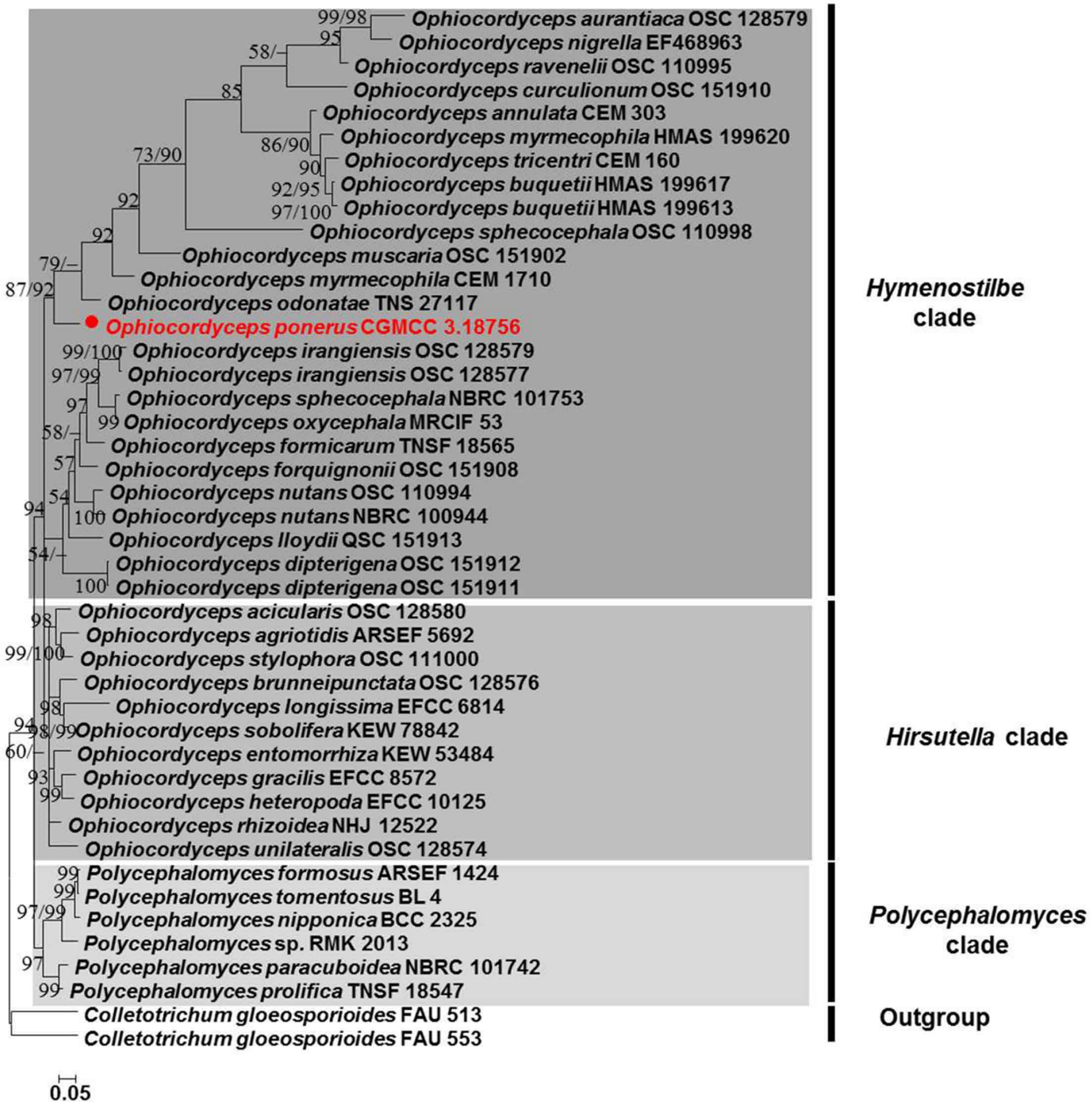

FIGURE 2. Phylogenetic tree of *Ophiocordyceps ponerus* and related species using combined DNA sequences of the RPB2, TEF, ITS and SSU datasets obtained with maximum likelihood method. Numbers below the branches are bootstrap percentage values based on 10,000 replicates, ML/BPP, maximum likelihood bootstrap support values greater than 50% and Bayesian posterior probabilities above 90%. The placement of *O. ponerus* is indicated in red.

Key

1. Host is an ant, phialides verrucose or not 2
1. Host is not an ant, phialides verrucose 3
2. Phialides verrucose, synnemata cylindrical, tapering to the end, 6–11 × 1–1.2 µm ..*O. lloydii* (Fawc) Sung (*H. formicarum* Petch)
2. Phialides verrucose; conidia nearly oval, or orange segments, 7.5–10 × 3.8–5 µm..... ***O. ponerus* X. Zou & Y. F Han**
2. Conidia with smooth surface, narrowly terete, slightly curved..... *O. longispora* Samson & Evans
3. Host is orthopteran or an arachnid..... 4
3. Host is a dipteran 5
4. Conidia cylindrical, round at both ends, 6.5–9 × 1.5–1.8 µm ..... *O. fragilis* Petch
4. Conidia 6–8 × 2–3.5 µm, host is a spider ..... *O. verrucosa* Mains
5. Conidia 4–13.5 × 2–4 µm ..... *O. dipterigena* Petch
5. Conidia 3–7 × 1.5–3.5 µm ..... *O. muscaria* Petch

Discussion

Both morphological and phylogenetic analysis showed that *Ophiocordyceps ponerus* is a new taxon. *Ophiocordyceps* species produce conidia singly from multiple denticles on conidiogenous cells that form a palisade-like layer along the entire outer surface of the synnemata (Mains, 1950). However, the new species has verticillate and verrucose phialides in whorls on the synnemata, distinguishing it from the vast majority of species in the genus. Other reported similar species parasitic on ants include *O. aurantiaca* Hywel-Jones (1996), *O. australiensis* Mains (1948), *O. camponoti* Mains (1950), *O. lloydii* Sung *et al.* (2007) (*Hymenostilbe formicarum* Mains), *O. longispora* Samson *et al.* (1975) and *O. melanopoda* Petch (1932). Unlike the solitary conidia of *O. ponerus*, *O. aurantiaca* and *O. longispora* are polyblastic, and the others apparently form a palisade layer over the synnemata that clearly distinguishes them from *O. ponerus*. In addition, other similar species, such as *O. aphidis* Petch (1942), *O. lecaniicola* Mains (1950), *O. spiculata* Huang *et al.* (1998), *O. ventricosa* Hywel-Jones (1995) and *O. verrucosa* Mains (1950), differ from the new species in the shape of their phialides and conidia. For more details see the Key and Supplementary Table S1.

As the asexual species associated with *Ophiocordyceps*, *Hymenostilbe* and *Hirsutella* were synonymized under *Ophiocordyceps* (Maharachchikumbura *et al*. 2015). In this study, multiple gene sequences of related *Ophiocordyceps* species were used for the phylogenetic analysis despite the fact that the available genetic data in public databases are limited for a number of this species and include mainly partial sequences (Aung *et al.*, 2006; Sung *et al.*, 2007; Quandt *et al.*, 2014). In our phylogenetic tree, the close phylogenetic relationship with *Ophiocordyceps* asexual morphs is exemplified by the close positions of the two separate clades. Furthermore, *Ophiocordyceps ponerus* and *Ophiocordyceps odonatae* cluster closely, but the phialides of the latter are cylindrical (9.7–14 × 3.2 µm) and the conidia are narrower and cylindrical (6.5–9.7 × 1.1–2.2 µm).

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TABLE S1 Morphological comparison among *Ophiocordyceps ponerus* and its similar species

species	host	synnemata	conidia	phialides	refrence
Ophiocordyceps ponerus	ant	cylindrical, 2.0–5.0 cm long × 0.2 mm wide, black, fawn brown near the apex;	nearly oval, sometimes curved orange segment, 7.5–10 × 3.8–5 µm;	on conidiophores or side branches, cylindrical or oval inflated at the base, with thin tumors, 13–25 × 2.5–5 µm;	**This study**
O. ampullifera	gnats	very slender, up to 3 mm long, brown, white from pulverulent masses of adhering spores;	narrowly cylindric 5.2–9 ×1.1–2 µm slightly narrowing but rounded at the ends, smooth, catenulate;	ellipsoid to short cylindric, 10–15 × 3–5 µm, narrowing above into an acuminate apex with a short sterigma, smooth;	Mains, 1950
O. aphidis	aphids	solitary, rufous brown, up to 2 mm high, 0.1 mm diameter, terete, minutely pruinose;	narrow oval or fusoid, hyaline, smooth, 9–15 × 4–5 µm;	conoid, narrow flask-shaped or subcylindrical, 12–18 × 4–6 µm; cylindrical sterigma, 3–6 × 1 µm;	Petch, 1942
O. aranearum	spiders	cylindric to clavate, 0.8–10 mm long × 0.1–0.2 mm thick, brown, flexuous, asperulate hyphae;	narrowly obclavate, 8–14 × 1.5–3 µm, often acute at the lower end, narrowing upward, hyaline, smooth, catenulate;	obovoid or ellipsoid, 6–12 × 4–8 µm, rounded above and abruptly narrowing into a short sterigma, asperulate;	Mains, 1950
O. aurantiaca	ants	usually single, slender, cylindrical, up to 150 mm long, 150–200 µm diam, apricot orange to orange;	solitary, cymbiform to obclavate to strongly obclavate, single-celled, smooth-walled, orange, 5.3–17.0 ×1.3–3. 0 µm;	orange, clavate, 9.3–25.0 × 3–6 µm, polyblastic, sympodial succession, denticles stout;	Hywel-jones, 1996
O. australiensis	ants	linear with the apices acute, obtuse or slightly inflated into a head;	clavate or obovate, 6–9 × 2.5–4 µm;	cylindric or clavate, 15–18 × 3 µm, apparently forming a palisade layer over the synnemata;	Mains, 1948
O. camponoti	ant	cylindric, 8 mm long × 0.3 mm thick below, furcate above into two short branches, grayish brown;	broadly fusoid, 4–6 × 2 µm, acute at the ends, hyaline;	subcylindric, 6–10 × 3–3.5 µm; narrowing to an acute apex terminated by a sterigma to 4µm long;	Mains, 1950
O. dipterigena	muscidae	Subcylindric, 4–12 mm long × 0.2–0.5 mm thick;	obovoid, 4–9 × 2–4 µm, hyaline, single;	terminating lateral branches covering the synnema;	Mains, 1950
***O. lloydii* (*H. formicarum*)**	ant	simple, terete, up to 14 mm. long × 0.2 mm thick, enlarged to 0.3 mm above or furcate, pale brown ashy, fibrillose below, pruinose above;	conidia narrowly clavate or subcylindric, 6–11 × 1–1.2 µm, one end acute, the other truncate or rounded;	cylindric, 24 × 4 µm, verrucose above, with one or two broad truncate sterigmata;	Mains, 1950
O. fragilis	orthopterous larva	clavate, 0.7–1.5 mm long; the upper portion sporogenous, subgloboid to obovoid, 130–300 µm long × 130–250 µm thick, white;	subcylindric 6.5–9 × 1.5 µm somewhat narrowed and rounded at the ends, catenulate;	subcylindric to narrowly clavate, 7–10 × 2.5–3 µm, verrucose in the upper portions;	Mains, 1950
O. furcata	Hemipteran nymph	slender, 10–14 mm long, 94–120 µm wide, cylindrical, white; central core of parallel hyphae composed of cells 3–55 × 2.5–4 µm;	solitary, smooth, hyaline, fusiform, 8.5–15 × 3–4.5 µm;	polyblastic, clavate or cylindrical, 5–18 × 3.5–6.5 µm, apically with 2–7 furcellate denticles, 0.6–2.4 µm;	Aung *et al*., 2006
O. ghanensis	spider	cylindrical to clavate, grey to lilac, 3–25 mm long, 50–125 µm wide;	solitary, pyriform, apiculate, smooth–walled to finely roughened, hyaline, 4.5–6.5 × 2.7–3.8 µm;	polyblastic, clavate or cylindrical, apically crowed with denticles 0.5 µm;	Samson *et al.*, 1975
O. ichneumonophila	*Ichneumon* sp.	cylindric, filiform, 12–15 × 0.2 mm, solitary or furcal, gray to white;	round or ellipsoidal, smooth-walled, hyaline, 6–9 (10) × 3.5–5 µm	claviform, with anobtuse apex, 12–24 × 3.5–4 µm,	Van Vooren *et al*., 2006
O. lecaniicola	scale insect	cylindric to slightly clavate, up to 3 mm long × 0.1–0.5 mm thick, gray to brownish, pruinose;	ellipsoid to broadly fusoid, 4–8 × 2–2.5 µm, hyaline single;	arising laterally from outer hyphae, subcylindric 10–30 × 4–5 µm;	Mains, 1950
O. longispora	ant	cylindrical to club-shaped, black, near the fertile apex pink, red or white, 6–20 mm long × 50–125 µm wide;	solitary, narrowly clavate, occasionally slightly curved, smooth-walled, hyaline, 11–24 × 1.5–2.3 µm;	3–13 × 4.5–6 µm, polyblastic, cylindrical to clacate, apically with crowded denticles, 2 µm long;	Samson *et al.*,1975

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TABLE S1 (Continued)

species	host	synnemata	conidia	phialides	refrence
O. melanopoda	ant	stalk bifurcate, branch bearing a globose head, red to black;	obovate, hyaline, with a truncate base 5–10 × 3–4 µm;	cylindric, with anobtuse apex, 10–15 × 3 µm;	Petch, 1932
O. nutans	Callibaphus longirostris	stromata up to 10cm in length;	ellipsoide or fusiform, 4.3–7 × 2.7–3.3 µm, hyalina;	polyblastic, cylindrical, 15–24 × 4.5–6.5 µm, pointed apically denticles, 1.5–2.5 µm long;	Hywel-Jones, 1995
O. odonatae	dragonfly	orange, developed from all body internode membrane, 0.4–0.5 cm long × 179–333 µm;	solitary, cylindrical, 6.5–9.7 × 1.1–2.2 µm;	cylindrical, 9.7–14 × 3.2 µm;	Kobayasi, 1941; Zhou *et al*., 2015
O. sphecocephala	wasp	terete, up to 3 cm long, 0.5 mm thick, composed of longitudinal, parallel hyphae;	fusoid, 6–12 × 2.5–3.5 µm;	forming a compact palisade layer, subcylindric to clavate, 16–24 × 4–5 µm, acute at the apices;	Mains, 1950
O. sphingum	moths	terete, narrowing upward, very variable in length, 1–8 mm. long × 0.1–0.5 mm thick, yellowish;	ellipsoid or obovoid often acute at the lower end, 3–6 × 2–3 µm, smooth, hyaline catenulate;	subcylindric or narrowly ellipsoid, 6–16 × 2.5–4 µm, narrowing above to an acute apex terminated by a short sterigma up to 4µm long, smooth;	Mains, 1950
O. spiculata	spider	cylindrical, 0.5–0.3 × 0.05–0.13 mm, cream;	solitary, obovoid, 5.6–8.6 × 1.3–2.7 µm, smooth, hyaline;	forming a compact palisade layer, polyblastic, cylindrical, 10.1–15.6 × 2.7–3.9 µm;	Huang *et al.*, 1998
O. sulphurea	*Homoptera*	cylindrical, sulphur yellow, white and powdery near the apex, 15–27 mm long, 250–400 µm wide;	solitary, subglobose to ellipsoidal, apiculate, rough–walled to finely echinulate, hyaine, 6.5–9.2 × 4.5–5.5 µm;	polyblastic, cylindrical to clavate, 15–25 × 5.0–6.5 µm, apically crowded with denticles, 1.5 µm;	Samson *et al.*,1975
O. ventricosa	cockroach nymphs	several, 6–13 mm long, 200–350 µm wide, cylindrical, very pale pink;	solitary, ventricose, smooth–walled, hyaline, 10.0–14.0 µm long 3.7–5.3 µm,base truncate 2–3 µm wide;	cylindric or clavate, 12–18 × 2–3 µm, verrucose or tuberculate at the apices;	Hywel-jones, 1995
O. verrucosa	spider	narrowly cylindric or slightly clavate, 1–4 mm long, 0.2–0.4 mm thick, pale brown	obovoid, 6–8 × 2–3.5 µm, hyaline;	cylindric or clavate, 12–18 × 2–3 µm, verrucose or tuberculate at the apice.	Mains, 1950

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