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Multigene phylogeny and morphology reveal a new species, *Ophiocordyceps vespulae*, from Jilin Province, China

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Abstract

Ophiocordyceps is entomopathogenic and is the best studied genus in Ophiocordycipitaceae. Members of *Ophiocordyceps* and ants form sophisticated interactions. However, taxonomy and evolutionary relationships of this group of pathogens remain unclear. During a survey in Changbai Mountains, Jiling Province, China, a new entomogenous species, *Ophiocordyceps vespulae sp. nov.* was found as a parasite on wasps (Hymenoptera). The new species is introduced with evidence from morphology and molecular analysis. This species is distinguished from closely related species by white to faint yellow stromata, shorter ascomata and asci, and smaller ascospores. We provide a phylogeny for *Ophiocordyceps* based on combined LSU, ITS, TEF1α and RPB2 DNA sequence data and the taxonomic status of the species is briefly discussed.

Keywords: 1 new species, Changbai Mountains, molecular phylogeny, *Ophiocordyceps*, taxonomy

Introduction

Ophiocordyceps species associated with insects comprise one of the most remarkable and interesting relationships between microbes and animals (Araújo *et al*. 2018). They are commonly found in tropical forests worldwide, with relatively few records from temperate ecosystems (Araújo *et al*. 2018). The genus *Ophiocordyceps* was described by Petch (1931) to accommodate a species of *Cordyceps* having clavate thick-walled asci and ascospores that do not disarticulate into part-spores. *Ophiocordyceps* is the type genus of Ophiocordycipitaceae (Hypocreales), which was separated from Cordycepitaceae based on the morphology and phylogenetic analyses (Sung *et al.* 2007a). Most species of this genus are parasitic on insects (Sung *et al*. 2007a, Maharachchikumbura *et al*. 2015, Wijayawardene *et al*. 2017). *Ophiocordyceps* is estimated to have arisen about 100 million years ago (Sung *et al*. 2008) and since then has colonized ten orders of insects (Sanjuan *et al*. 2015, Araújo & Hughes 2016). *Ophiocordyceps* is the most speciose genus in Ophiocordycipitaceae with over 200 accepted species (Index Fungorum, accessed 14 November 2019), with more than 30 species described recently (Wen *et al.* 2013, 2014, 2016, Ban *et al.* 2015, Sanjuan *et al.* 2015, Khonsanit *et al.* 2019, Araújo *et al.* 2018, Xiao *et al.* 2018, 2019).

Most *Ophiocordyceps* species have darkly pigmented or brightly coloured stromata that are pliant to wiry or fibrous, with immersed, ordinal or obliquely arranged perithecia (Sung *et al.* 2007a). The asexual morphs were reported as *Hirsutella* Pat. 1892, *Hymenostilbe* Petch 1931 and *Paraisari* Samson & B.L. Brady 1983 (Quandt *et al.* 2014,

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Maharachchikumbura *et al*. 2015, 2016). The asexual morphs in most species have hirsutella-like and hymenostilbe-like features (Kepler *et al*. 2013, Maharachchikumbura *et al*. 2015, 2016).

The present study introduces a new species, *Ophiocordyceps vespulae* with a description, photographs, illustrations and a multigene phylogeny and compares it with similar taxa.

Materials and methods

Sample collection and morphological characteristic examination

Two fresh specimens were collected from the Changbai Mountain, China in July 2017. Macro-morphological characters were described based on fresh material, and on the photographs provided here. Fresh specimens were used to isolate the fungus by tissue culture method in potato dextrose agar (PDA) medium. Specimens were dried and placed separately in plastic bags. The materials and living culture were deposited at Guizhou University (GACP) and Kunming Institute of Botany, Chinese Academy of Sciences (KUN). For micro-morphological examination fruiting bodies and living culture mycelium were examined with a stereo dissecting microscope (Motic SMZ 168 series). Sections were cut with a razor blade, mounted in water, and fungal structures were observed, measured, and illustrated using a compound microscope (Nikon ECLIPSE 80i) equipped with a camera (Canon 600D). Measurements were made using Tarosoft (R) Image Frame Work v. 0.9.7. The Facesoffungi number is provided as explained in Jayasiri *et al*. (2015).

DNA extraction, PCR amplification and determination of DNA sequences

Dried samples of fruiting bodies were used to extract genomic DNA using an EZgene TM Fungal gDNA Kit (Biomiga, CA, USA) according to the manufacturer instructions. DNA concentrations were estimated visually in agarose gel by comparing band intensity with a DNA ladder 1Kb (Invitrogen Biotech). Reaction mixtures (50 µl) contained 2 µl template DNA (ca. 10 ng), 19 µl distilled water, and 2 µl (10 µM) of each primer and 25 µl 2x BenchTopTM Taq Master Mix (Biomigas). Amplification conditions were 40 cycles of 95 °C for 30 s, 59 °C for 30 s and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min for all DNA fragments. The primers used in PCR amplification were: ITS4/ITS5 for internal transcribed spacer gene region (ITS) (White *et al.* 1990), LROR/LR5 for partial large subunit rDNA gene region (LSU) (Vilgalys & Hester 1990). 983F/2218R for partial translation elongation factor 1-alpha gene region (TEF-1α) (Sung *et al.* 2007b), RPB2-5F/RPB2-5R for partial RNA polymerase II second largest subunit gene region (RPB2) (Castlebury *et al.* 2004). Amplified PCR products were verified by 1% agarose gel electrophoresis stained with ethidium bromide in 1x TBE. The PCR products were sequenced with primers mentioned above by GenScript Biotechnology Co., Nanjing, China.

Sequence alignment and phylogenetic analyses

The taxon information and GenBank accession numbers used in the molecular analyses are listed in Table 1. Quality of the newly obtained sequences for the *Ophiocordyceps* specimens from Jilin Province, China was checked by observing the chromatogram with BioEdit (Hall *et al.* 2011) and by examining BLAST search results according to Nilsson *et al*. (2012). All the other sequences were retrieved from GenBank (Table 1) based on ITS BLAST searches (Benson *et al*. 2018) and recently published data. Sequences that had possibly been contaminated by micro-fungi or other unnamed species (such as those with aff. in the species name) were discarded, ambiguous regions were excluded and gaps were treated as missing data in the analysis (Nilsson *et al*. 2012). Eighty-seven nucleotide sequences representing 81 species of Ophiocordycipitaceae from worldwide were retrieved from GenBank and those of the newly generated sequences were aligned with MAFFT v.7 (Katoh & Standley 2013) onlined at (http://mafft.cbrc.jp/alignment/server/). The resulting alignment was improved manually when necessary using BioEdit (Hall *et al.* 2011). BioEdit 7.2.0 software was used to combine datasets of multiple genes. The maximum likelihood (ML) analyses were performed using RAxML-HPC2 (Stamatakis 2014) on the CIPRES Science Gateway V. 3.3 (Miller & Blair 2009), with default settings except that the number of bootstrap replicates was set to 1,000. For Bayesian analysis (BY), the GTR+I+G model of nucleotide evolution was selected with the help of MrModeltest 2.2 (Nylander 2004) as the best-fit model and posterior probabilities (PP) (Rannala & Yang 1996) were determined by Markov chain Monte Carlo sampling (BMCMC) using MrBayes v3.1.2 (Ronquist *et al*. 2012). BY analyses were conducted with six simultaneous Markov chains and trees

were summarized every 100th generation. The analyses were stopped after 5,000,000 generations when the average standard deviation of split frequencies was below 0.01. The convergence of the runs was checked using TRACER v1.6 (Rambaut *et al*. 2013). The first 25% of the resulting trees were discarded as burn-in, and PP were calculated from the remaining sampled trees. In both ML and BY analyses, *Tolypocladium inflatum* and *T. ophioglossoides* were selected as outgroup taxa (Kepler *et al.* 2012, Schoch *et al.* 2012). ML bootstrap values and BY posterior probabilities greater than or equal to 50% and 0.95, respectively, were considered as significant support. The phylogenetic tree was visualized with FigTree version 1.4.0 (Rambaut 2012) available at http://tree.bio.ed.ac.uk/software/figtree/.

Results

Phylogeny

The combined ITS, LSU, TEF1α and RPB2 dataset consisted of 183 taxa with 3035 characters (801 for LSU, 526 for ITS, 888 for TEF1α, and 820 for RPB2) (Table 1). Tree topology of the RAxML analysis was similar to the Bayesian analysis. Therefore, only the ML tree is shown (Fig. 1). The best scoring RAxML tree with a final likelihood value of -60426.083693 is presented (Fig. 1). The matrix had 1,908 distinct alignment patterns, with 40.28% of undetermined characters or gaps. Parameters for the GTR model of the concatenated data set were as follows: estimated base frequencies; A = 0.228877, C = 0.289881, G = 0.287771, T = 0.193471; substitution rates AC = 1.175441, AG = 3.584449, AT = 1.200512, CG = 1.1165013, CT = 6.539708, GT = 1.000000; gamma distribution shape parameter α = 0.331985.

Taxonomy

Ophiocordyceps vespulae F.Y. Long, Y.P. Xiao & T.C. Wen, *sp. nov*. (Fig. 2)
Index Fungorum number: IF556626; *Facesoffungi number*: FoF 06237

Etymology:—The specific epithet refers to the host (*Vespula*, Hymenoptera).
Holotype:—GACP2017079

Sexual morph:—*Host* 1.5–2 × 0.5–1 cm, brown. *Stromata* 3–7 cm long, 0.3–1 mm diam., single or double, stipitate, unbranched or branched into 2 fertile heads, arising from the head and thorax of insect. *Stipe* 2–6.5 cm long, 0.3–0.5 mm diam., yellow, fibrous, cylindrical, often flexuous, with a fertile apex. *Fertile head* 0.5–1 cm long, 0.5–1 mm diam., single, cylindrical or elliptical, pale yellow. *Ascomata* 520–720 × 200–380 µm ($\bar{x}$ = 596 × 260 µm, n = 30), immersed, pale to yellowish, elongated flask-shaped. *Asci* 320–570 × 5.3–7.5 µm ($\bar{x}$ = 454 × 5.8 µm, n = 60), narrow cylindrical, apex thickened, hyaline; apical cap 6.2–7.9 × 3.4–5.4 µm ($\bar{x}$ = 7.0 × 4.0 µm, n = 60), hyaline. *Ascospores* almost as long as asci, filiform, hyaline, easily breaking into part-spores. *Secondary ascospores* 7.5–11.5 × 1.5–3 µm ($\bar{x}$ = 9.1 × 2.1 µm, n = 90), fusiform, hyaline, smooth. **Asexual morph:—**undetermined.

Culture characteristics:—on PDA reaching 5 cm diam. after 6 weeks at 25 °C, superficial cottony, white, reverse yellow; after 10 weeks at 25 °C, reaching 6 mm diam., no conidia observed.

Material examined:—CHINA. Jilin Province: Changbai Mountain, parasitic on wasps (*Vespula* sp., Hymenoptera), collected from the underside of leaf litter, 30 July 2013, Fan YG (GACP2017079, holotype); ex-type living culture GACP2017064.

Discussion

Our study on entomopathogenic fungi led to the discovery of a new species *Ophiocordyceps vespulae* in Jilin Province, China. This species lies in a phylogenetic clade with *O. tricentri* and *O. sphecocephala*. However, in pairwise nucleotide sequence comparison, there are sufficient differences to justify *O. vespulae* as an independent taxon. *Ophiocordyceps vespulae* differs from *O. tricentri* by 24 bp differences in ITS, 23 bp differences in LSU, 29 bp differences in TEF1α,

FIGURE 1. Phylogram of *Ophiocordyceps vespulae* generated from maximum likelihood (RAxML) analysis of combined ITS, LSU, TEF1α and RPB2 sequence data. *Tolypocladium inflatum* and *T. ophioglossoides* were the outgroup taxa. Maximum likelihood bootstrap values greater than 75% and posterior probabilities from Bayesian inference ≥ 0.90 are given above the nodes as bootstrap values/Bayesian posterior probabilities. The new species is in red and bold.

52 bp differences in RPB2, and differs from *O. sphecocephala* by 23 bp differences in ITS, 34 bp differences in LSU, 41 bp differences in TEF1α, 58 bp differences in RPB2. Furthermore, *O. vespulae* differs from *O. oxycephala* by 67 bp differences in ITS and 48 bp differences in RPB2.

Morphologically, *Ophiocordyceps vespulae* differs from *O. tricentri* in having a smaller fertile head (3–7 cm long × 0.3–1 mm diam. vs. 5–6 cm long × 1–1.5 mm diam.), wider ascomata (520–720 × 200–380 µm vs. 550–650 × 110–120 µm), and longer and wider asci (320–570 × 5.3–7.5 µm vs. 300–320 × 5 µm).

The host of *Ophiocordyceps tricentri* is Cercopidae (Hemiptera), while for *O. vespulae, O. sphecocephala* and *O. oxycephala* it is *Vespula* spp. (Hymenoptera). *Ophiocordyceps vespulae* differs from *O. sphecocephala* in having a smaller fertile head (3–7 cm long × 0.3–1 mm diam. vs. 5–6 cm long × 1–1.5 mm diam.), smaller ascomata (520–720 × 200–380 µm vs. 880–1000 × 200–260µm), shorter asci (320–570 × 5.3–7.5 µm vs. 700 × 7 µm), and shorter part-spores (7.5–11.5 × 1.5–3 µm vs. 10–14 × 1.5–2.5 µm) (Hywel-Jones 1995, Shrestha & Sung 2005; Table 2).

Ophiocordyceps oxycephala differs from *O. cylindrospora*, *O. vespulae* and *O. sphecocephala*, in having longer and thinner secondary spore (Shimizu 1997; Table 2). *Ophiocordyceps oxycephala* differs from *O. fulgoromorphila* by producing smaller perithecia and secondary ascospores without oil drops (Sanjuan *et al.* 2015; Table 2). There is no molecular data reported in GenBank for *O. elongatistromata*, *O. humbertii* and *O. smithii*, three other species recorded on *Vespula* spp. *Ophiocordyceps vespulae* produces a shorter stipe, longer ascomata and longer asci than these three species (Penzig & Saccardo 1897, Mains 1939, Kobayasi 1983; Table 2). Hence, both morphological and molecular data strongly support *O. vespulae* as a separate taxonomic entity in *Ophiocordyceps*. *Ophiocordyceps* species parasites in wasps are listed in Table 2.

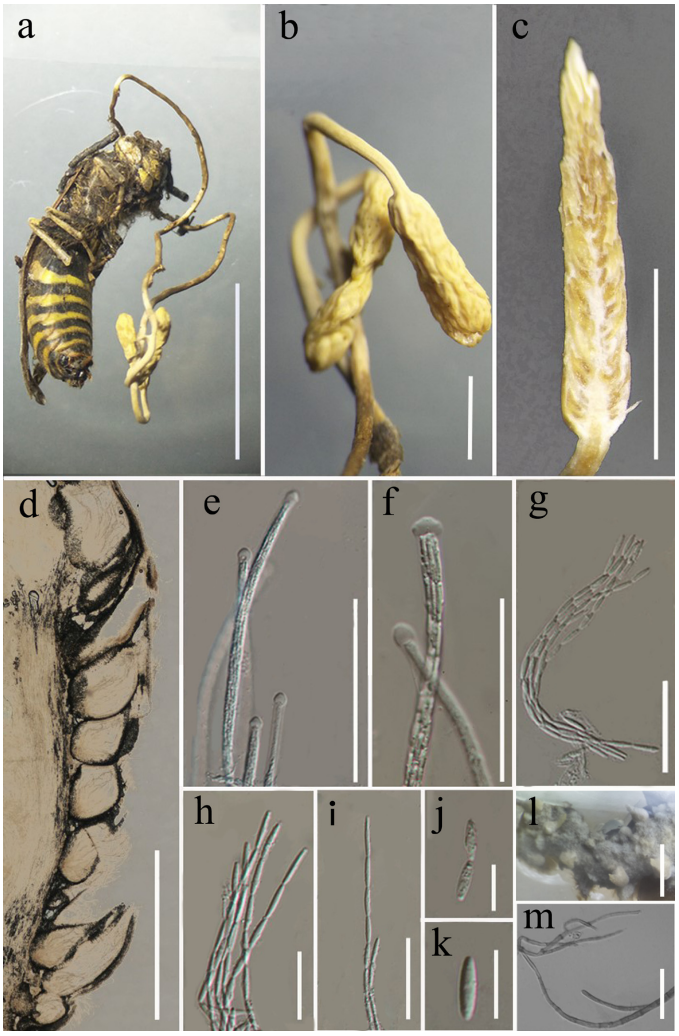

FIGURE 2. a. Overview of the stromata and the host. b. Fertile head. c. Longitudinal section showing the complete immersed perithecia. d. Ascomata. e–f. Part asci with apical cap. g–i. Part of ascospores. j, k. Secondary ascospores. l. Upper side of PDA culture. m. Hyphae in PDA culture. Scale bars a = 1 cm, b, c, l = 2 mm, d = 1 mm, e = 100 µm, f, m = 50 µm, g–i = 20 µm, j–k =10 µm.

TABLE 1. Sources of isolates and GenBank accession numbers.

Species	Voucher	ITS	LSU	TEF1α	RPB2	References
Ophiocordyceps acicularis	OSC 110987		EF468805	EF468744		Sung *et al*. 2007a
O. acicularis	OSC 128580	JN049820	DQ518757	DQ522326	DQ522423	Kepler *et al*. 201
O. agriotidis	ARSEF 5692	JN049819	DQ518754	DQ522322	DQ522418	Ban *et al*. 2015
O. albacongiuae	RC20			KX713670		Araújo *et al*. 2018
O. amazonica	HUA 186113		KJ917572		KM411980	Sanjuan *et al*. 2015
O. amazonica	HUA 186143		KJ917571	KM411989	KM411982	Sanjuan *et al*. 2015
O. annulata	CEM 303			KJ878962		Quandt *et al*. 2014
O. aphodii	ARSEF 5498		DQ518755	DQ522323	DQ522419	Spatafora *et al*. 2007
O. appendiculata	NBRC 106959	JN943325	JN941412	AB968578	AB968540	Ban *et al*. 2015
O. araracuarensis	HUA 186135		KC610769	KC610738	KC610716	Sanjuan *et al*. 2015
O. arborescens	NBRC 105891	AB968398	AB968414	AB968572	AB968534	Ban *et al*. 2015
O. asiatica	BCC 30516	MH754722	MH753675	MK284263	MK214091	Tasanathai *et al*. 2019
O. australis	HUA 186147	KF937351	KC610764	KC610734		Sanjuan *et al*. 2015
O. australis	HUA 186104		KC610763	KC610733	KC610713	Sanjuan *et al*. 2015
O. barnesii	BCC28560				EU418599	Luangsa-ard *et al*. 2010
O. bispora	KVL 606		AF009654			Suhet *et al*. 1998
O. blakebarnesii	MISSOU4		KX713609	KX713685		Araújo *et al*. 2018
O. blattarioides	HUA186093		KJ917570	KM411992		Sanjuan *et al*. 2015
O. blattarioides	HUA 186108		KJ917569		KM411984	Sanjuan *et al*. 2015
O. brunneinigra	BCC 69015		MF614653	MF614637	MF614680	Luangsa-ard *et al*. 2018
O. brunneiperitheciata	BCC 49312		MF614660	MF614642	MF614686	Luangsa-ard *et al*. 2018
O. brunneipunctata	OSC 128576		DQ518756	DQ522324	DQ522420	Spatafora *et al*. 2007
O. brunneirubra	BCC 14384	MH754736	MH753690	GU797121	MK751468	Tasanathai *et al*. 2019
O. buquetii	HMAS 199613		KJ878904	KJ878984		Quandt *et al*. 2014
O. camponoti-atricipis	ATRI3			KX713677		Araújo *et al*. 2018
O. camponoti-balzani	G143		KX713595	KX713690		Araújo *et al*. 2018
O. camponoti-bispinosi	OBIS5		KX713616	KX713693		Araújo *et al*. 2018
O. camponoti-femorati	FEMO2		KX713590	KX713678		Araújo *et al*. 2018
O. camponoti-floridani	Flx2		KX713592	KX713674		Araújo *et al*. 2018
O. camponoti-hippocrepidis	HIPPOC		KX713597	KX713673		Araújo *et al*. 2018
O. camponoti-indiani	INDI2		KX713598			Araújo *et al*. 2018
O. camponoti-nidulantis	NIDUL2		KX713611	KX713669		Araújo *et al*. 2018
O. camponoti-novogranadensis	Mal63		KX713603			Araújo *et al*. 2018
O. camponoti-renggeri	ORENG		KX713617	KX713671		Araújo *et al*. 2018
O. camponoti-rufipedis	G177		KX713596	KX713680		Araújo *et al*. 2018
O. cf acicularis	NHJ10418 01	GU723765		GU797116		Luangsa-ard *et al*.2011

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TABLE 1. (Continued**)**

Species	Voucher	ITS	LSU	TEF1α	RPB2	References
O. citrina	TNS F18537		KJ878903	KJ878983		Quandt *et al*. 2014
O. clavata	NBRC 106961	JN943327	JN941414	AB968586	AB968547	Schoch *et al*. 2012
O. clavata	CEM1762		KJ878882	KJ878963		Quandt *et al*. 2014
O. coccidiicola	NBRC 100682	AB968404	AB968419	AB968583	AB968545	Ban *et al*. 2015
O. cochlidiicola	HMAS 199612		KJ878884	KJ878965		Quandt *et al*. 2014
O. coenomyia	NBRC 108993	AB968396	AB968412	AB968570	AB968532	Ban *et al*. 2015
O. communis	BCC 1842	MH754726	MH753680	MK284266	MK214096	Tasanathai *et al*. 2019
O. cossidarum	MFLU 17 0752		MF398187			Hyde *et al*. 2017
O. crinalis	HIMGD17327	EU149926				Zhang *et al*. 2007
O. curculionum	OSC 151910		KJ878885			Quandt *et al*. 2014
O. cylindrospora	MFLU 17 1961	MG553635	MG553652		MG647029	Hyde *et al*. 2018
O. daceti	MF01		KX713604	KX713667		Araújo *et al*. 2018
O. desmidiospora	SJS3Des		MH536514	MN785129		Saltamachia *et al*.2020
O. dipterigena	MRCIF71	EU573346				Freire 2015
O. dipterigena	OSC 151912		KJ878887	KJ878967		Quandt *et al*. 2014
O. dipterigena	HUA 186102		KJ917568		KC610715	Quandt *et al*. 2014
O. dipterigena	MY621	GU723764		GU797126		Luangsa-ard *et al*. 2011
O. elongata	OSC 110989		EF468808	EF468748		Sung *et al*. 2007a
O. emeiensis	G96031	AJ309347				Liu *et al*. 2002
O. entomorrhiza	KEW 53484	JN049850	EF468809	EF468749	EF468911	Quandt *et al*. 2014
O. evansii	HUA 186159	KP200889	KC610770	KC610736		Sanjuan *et al*. 2015
O. formicarum	TNS F18565		KJ878888	KJ878968	KJ878946	Quandt *et al*. 2014
O. formicarum	BCMU CF 02	AB222679				Freire 2015
O. formosana	TNM F13893			KJ878956	KJ878943	Quandt *et al*. 2014
O. formosana	MFLU 15 3888			KU854949		Li *et al*. 2016
O. forquignonii	OSC 151902		KJ878876		KJ878945	Quandt *et al*. 2014
O. forquignonii	OSC 151908		KJ878889		KJ878947	Quandt *et al*. 2014
O. fulgoromorphila	QCNE 186286		KC610759			Luangsa-ard *et al*. 2011
O. fulgoromorphila	HUA 186139		KC610760	KC610729	KC610719	Sanjuan *et al*. 2015
O. geometridicola	TBRC 8095		MF614648	MF614632	MF614679	Luangsa-ard *et al*. 2018
O. globiceps	MFLUCC 18 0495	MH725815	MH725829	MH727387		Xiao *et al*. 2019
O. globiceps	MFLU 18 0661	MH725816	MH725830	MH727388		Xiao *et al*. 2019
O. gracilioides	HUA 186095			KM411994		Araújo *et al*. 2018
O. gracilioides	HUA 186092		KJ130992			Araújo *et al*. 2018
O. gracilis	OSC 151906		KJ878890	KJ878969		Quandt *et al*. 2014
O. gracilis	EFCC 8572	JN049851	EF468811	EF468751	EF468912	Kepler *et al*. 2012
O. gracillima	HUA 186132	KF937353	KC610768	KC610744		Sanjuan *et al*. 2015

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TABLE 1. (Continued**)**

Species	Voucher	ITS	LSU	TEF1α	RPB2	References
O. granospora	BCC 82255	MH028143	MH028156	MH028183	MH028177	Araújo *et al*. 2018
O. hemisphaerica	FLOR 59525	KX197233				Hyde *et al*. 2016
O. heteropoda	EFCC 10125	JN049852	EF468812	EF468752	EF468914	Kepler *et al*. 2012
O. heteropoda	OSC 106404		AY489722	AY489617		Castlebury *et al*. 2004
O. highlandensis	HKAS83207 2				KM581281	Yang *et al*. 2015
O. highlandensis	HKAS83206 1				KM581278	Yang *et al*. 2015
O. houaynhangensis	BBC82809	MH092892	MH092908	MH092899		Crous *et al*. 2018
O. houaynhangensis	TBRC8428	MH092891	MH092902	MH092894		Crous *et al*. 2018
O. irangiensis	BCC 82793	MH028141		MH028185	MH028173	Araújo *et al*. 2018
O. irangiensis	OSC 128578	JN049833	DQ518770	DQ522345	DQ522445	Spatafora *et al*. 2007
O. irangiensis	OSC 128577	JN049823	DQ518760	DQ522329	DQ522427	Spatafora *et al*. 2007
O. irangiensis	OSC 128579		EF469076	EF469060	EF469107	Sung *et al*. 2007a
O. irangiensis	NBRC 101400	JN943335	JN941426			Schoch *et al*. 2012
O. issidarum	MFLU 17 0751	MF398185	MF398188			Hyde *et al*. 2017
O. karstii	MFLU 15 3884			KU854945		Li *et al*. 2016
O. khokpasiensis	BCC 48071	MH754728	MH753682	MK284269		Tasanathai *et al*. 2019
O. khokpasiensis	BCC 1764	MH754730	MH753684	MK284271	MK214098	Tasanathai *et al*. 2019
O. kimflemingiae	SC30		KX713622	KX713699		Araújo *et al*. 2018
O. kimflemingiae	SC100		KX713624	KX713696		Araújo *et al*. 2018
O. kimflemingiae	SJS4Oph		MH536516	MN785130		Saltamachia *et al*. 2020
O. kniphofioides	HUA 186148			KC610739	KC610717	Sanjuan *et al*. 2015
O. konnoana	EFCC 7295				EF468915	Sanjuan *et al*. 2015
O. konnoana	EFCC 7315			EF468753	EF468916	Sung *et al*. 2007a
O. lanpingensis	YHOS0707		KC417461	KC417463		Chen *et al*. 2013
O. lanpingensis	YHOS0705		KC417460	KC417462	KC456333	Chen *et al*. 2013
O. lloydii	HUA 186164	KP200892		KC610741		Sanjuan *et al*. 2015
O. lloydii	OSC 151913		KJ878891	KJ878970	KJ878948	Quandt *et al*. 2014
O. longissima	EFCC 6814		EF468817	EF468757		Kepler *et al*. 2012
O. longissima	NBRC 108989	AB968407	AB968421	AB968585		Sanjuan *et al*. 2015
O. longissima	HMAS 199600			KJ878972	KJ878949	Quandt *et al*. 2014
O. macroacicularis	BCC 22918		MF614655	MF614639	MF614675	Araújo *et al*. 2018
O. macroacicularis	NBRC 105888	AB968401	AB968417	AB968575	AB968537	Ban *et al*. 2015
O. melolonthae	OSC 110993		DQ518762	DQ522331		Spatafora *et al*. 2007
O. monacidis	MF74C		KX713606			Araújo *et al*. 2018
O. monacidis	MF74		KX713605			Araújo *et al*. 2018
O. mosingtoensis	BCC 30904	MH754732	MH753686	MK284273	MK214100	Tasanathai *et al*. 2019
O. mosingtoensis	BCC 36921	MH754731	MH753685	MK284272	MK214099	Tasanathai *et al*. 2019

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TABLE 1. (Continued**)**

Species	Voucher	ITS	LSU	TEF1α	RPB2	References
O. multiperitheciata	BCC 22861		MF614656	MF614640	MF614683	Araújo *et al*. 2018
O. multiperitheciata	BCC 69008		MF614657	MF614641	MF614682	Luangsa-ard *et al*. 2018
O. myrmecophila	ARSEF 11864	JX566954	JX566965	JX566973		Simmons *et al*. 2015
O. myrmecophila	MY 163	GU723759		GU797132		Luangsa-ard *et al*. 2011
O. myrmecophila	CEM 1710			KJ878974		Quandt *et al*. 2014
O. myrmecophila	TNS 27120		KJ878895	KJ878975		Quandt *et al*. 2014
O. myrmecophila	MFLU 16 2912	MF351726	MF372585	MF372759		Xiao *et al*. 2017
O. myrmecophila	MFLU 16 2913	MF351727	MF372586			Xiao *et al*. 2017
O. myrmicarum	ARSEF11864	JX566954	JX566965	JX566973		Simmons *et al*. 2015
O. naomipierceae	DAWKSANT		KX713589			Araújo *et al*. 2018
O. neovolkiana	OSC 151903		KJ878896	KJ878976		Quandt *et al*. 2014
O. nigra	TNS 16252		KJ878906	KJ878986		Quandt *et al*. 2014
O. nigra	TNS 16250			KJ878987		Quandt *et al*. 2014
O. nigrella	EFCC 9247	JN049853	EF468818	EF468758	EF468920	Sung *et al*. 2007a
O. nutans	NBRC 101749	AB968408	JN941429	AB968589	AB968550	Ban et. al. 2015
O. odonatae	TNS F18563	AB104725	KJ878877			Ito & Hirano 1997
O. oecophyllae	OECO1					Araújo *et al*. 2018
O. ootakii	J13		KX713600	KX713681		Araújo *et al*. 2018
O. pauciovoperitheciata	TBRC 8096		MF614649	MF614636	MF614672	Luangsa-ard *et al*. 2018
O. ponerinarum	HUA 186140			KC610740		Sanjuan *et al*. 2015
O. pruinosa	NHJ 12994		EU369041	EU369024	EU369084	Johnson *et al*. 2009
O. pseudoacicularis	TBRC 8101		MF614645	MF614629	MF614676	Luangsa-ard *et al*. 2018
O. pseudocommunis	BCC 16757	MH754733	MH753687	MK284274	MK214101	Tasanathai *et al*. 2019
O. pseudolloydii	MFLUCC 15 0689	MF351725		MF372758		Xiao *et al*. 2017
O. pseudolloydii	LHC	KX714602		KX714603		Chung *et al*. 2017
O. pseudorhizoidea	BCC 48879	MH754720	MH753673	MK284261	MK214089	Tasanathai *et al*. 2019
O. pulvinata	TNS F 30044			GU904209		Quandt *et al*. 2014
O. purpureostromata	TNS F18430		KJ878897	KJ878977		Quandt *et al*. 2014
O. ramosissimum	GZUHHN8	KJ028007		KJ028014		Wen *et al*. 2014
O. ravenelii	OSC 110995		DQ518764	DQ522334	DQ522430	Spatafora *et al*. 2007
O. rhizoidea	NHJ 12522	JN049857	EF468825	EF468764	EF468923	Sung *et al*. 2007a
O. robertsii	KEW 27083		EF468826	EF468766		Sung *et al*. 2007a
O. rubiginosiperitheciata	NBRC 100946	JN943341	JN941436	AB968581	AB968543	Ban *et al*. 2015
O. ryogamiensis	NBRC 101751		KF049633	KF049688		Kepler *et al*. 2013
O. satoi	J7		KX713599	KX713683		Araújo *et al*. 2018
O. satoi	J19		KX713601	KX713684		Araújo *et al*. 2018
O. sinensis	ARSEF 6282	KM652173	KM652126	KM652009		Araújo *et al*. 2018

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TABLE 1. (Continued**)**

Species	Voucher	ITS	LSU	TEF1α	RPB2	References
O. sinensis	EFCC7287	JN049854	EF468827	EF468767	EF468924	Sung *et al*. 2007a
O. sobolifera	KEW 78842	JN049855	EF468828		EF468925	Kepler *et al*. 2012
O. sobolifera	TNS F18521		KJ878898	KJ878979		Quandt *et al*. 2014
Ophiocordyceps sp.	Gh41			KX713668		Araújo *et al*. 2018
Ophiocordyceps sp.	TNS F18495		KJ878901			Quandt *et al*. 2014
Ophiocordyceps sp.	OSC 151904		KJ878899	KJ878980		Quandt *et al*. 2014
Ophiocordyceps sp.	OSC 151905			KJ878981	KJ878951	Quandt *et al*. 2014
Ophiocordyceps sp.	OSC 151909		KJ878900	KJ878982	KJ878952	Quandt *et al*. 2014
Ophiocordyceps sp.	FMF147	KX197238				Freire 2015
Ophiocordyceps sp.	OSC 110997			EF468774	EF468929	Quandt *et al*. 2014
O. spataforae	BCC 86480		MG831747	MG831746	MG831749	Luangsa-ard *et al*. 2018
O. spataforae	OSC 128575	JN049845	EF469079	EF469064	EF469110	Sung *et al*. 2007a
O. sphecocephala	OSC 110998		DQ518765	DQ522336	DQ522432	Kepler *et al*. 2012
O. sphecocephala	NBRC 101753	JN943350	JN941446	AB968592	AB968553	Ban *et al*. 2015
O. sporangifera	MFLUCC 18 0492	MH725818	MH725832	MH727390		Xiao *et al*. 2019
O. sporangifera	MFLU 18 0658	MH725817	MH725831	MH727389		Xiao *et al*. 2019
O. stylophora	OSC 110999		EF468837	EF468777	EF468931	Sung *et al*. 2007
O. stylophora	OSC 111000	JN049828	DQ518766	DQ522337	DQ522433	Spatafora *et al*. 2007
O. superficialis	MICH 36253					Sung *et al*. 2007a
O. termiticola	BCC 1920	MH754724	MH753678	MK284265	MK214094	Tasanathai *et al*. 2019
O. termiticola	BCC 1770	GU723780	MH753677	MK284264	MK214093	Tasanathai *et al*. 2019
O. thanathonensis	MFU 16 2910	MF850375	MF850378	MF872614		Xiao *et al*. 2017
O. thanathonensis	MFU 16 2909	MF850376	MF850377	MF872613		Xiao *et al*. 2017
O. tiputini	QCNE 186287		KC610773	KC610745		Kepler *et al*. 2012
O. tricentri	NBRC 106968	AB968410	AB968423	AB968593	AB968554	Ban *et al*. 2015
O. unilateralis	SERI1		KX713626	KX713675		Araújo *et al*. 2018
O. unilateralis	HUA 186161			KC610742	KC610718	Sanjuan *et al*. 2015
O. unilateralis	OSC 128574		DQ518768	DQ522339	DQ522436	Spatafora *et al*. 2007
O. variabilis	ARSEF 5365		DQ518769	DQ522340	DQ522437	Kepler *et al*. 2012
O. variabilis	OSC 111003		EF468839	EF468779	EF468933	Sung *et al*. 2007a
O. vespulae	**GACP2017064**	**MN044857**	**MN044858**	**MN117075**	**MN107547**	**This study**
O. vespulae	**GACP2017079**		**MN044859**	**MN117076**	**MN107548**	**This study**
O. xuefengensis	GZUH2012HN11	KC631800		KC631791		Wen *et al*. 2013
O. yakusimensis	HMAS 199604		KJ878902		KJ878953	Quandt *et al*. 2014
Tolypocladium inflatum	OSC 71235	JN049844	EF469077	EF469061	EF469108	Kepler *et al*. 2012
T. ophioglossoides	NBRC 106332	JN943322	JN941409			Schoch *et al*. 2012

TABLE 2. *Ophiocordyceps* species parasites in *Vespula* spp.

Species	Stromata	Fertile heads	Perithecia (µm)	Asci (µm)	Secondary ascospores (µm)	References
O. cylindrospora	Capitate, 50–90 × 0.5–1.5 mm	Fusiform, yellow, 3–3.2 × 1–1.2 mm	Oblique, flask-shaped, 551–638 × 261–327	Cylindrical, 248–313 × 5–7	Cylindrical, 3.1–3.9 × 1.6–2	Hyde *et al*. 2018
O. elongatistromata	Single or double, pale brown, 6–12 cm	White, 2–3 parts, 15–20 × 1.7–2 mm	Immersed		7–10 × 1.5	Kobayasi & Shimizu 1983
O. fulgoromorphila	Capitate, 87–110 mm, branched in secondary bicolored stroma	Cylindrical, brownish yellow to brownish orange, 7–25 × 2–3.5 mm	Immersed, ellipsoid, 780–1100 × 280–380	Cylindrical, 300–600 × 5–6	9–12 × 1–2 (with oil drops)	Sanjuan *et al.* 2015
O. humbertii	Two, clavate, emerging from host on both sides of thorax		Immersed			Somavilla *et al.* 2020
O. oxycephala	Up to 6 cm long, capitate with cylindric heads, 7-5 mm long, 0.5-1 mm thick, with short acute sterile apices	Thick stipe and central core of the head, 0.2-0.3 mm	Conoid, 800–900 × 170–210, brown walls	Uncertain	Fusoid, 8–10 × 1.5	Mains 1959
O. smithii.	Capitate, 4-5 cm long	Narrow ovoid, reddish brown heads	Ovoid, 240–260 × 140–160	Clavate, 90–120 × 8–9	Absent	Mains 1958
O. sphecocephala	Single stroma, from head and thorax, 45 × 1.4–1.8 mm	Terminal, variable size and shape, cream-yellow	Oblique 880–1000 × 200–260	Filiform, 700 × 7	Fusoid, 10–14 × 1.5–2.5	Hywel-Jones 1995
O. vespulae	Capitate, 30–70 × 0.3–1 mm	Yellow, 5–10 × 0.5–1 mm	Immersed, 520–720 × 200–380	320–570 × 5.3–7.5	7.5–11.5 × 1.5–3	This study

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