



Ophiocordyceps aphrophoridarum sp. nov., a new entomopathogenic species from Guizhou, China

Yu Yang^{‡,§,¶}, Yuanpin Xiao^{¶,§}, Gangjiang Yu^{‡,§,¶}, TingChi Wen^{‡,§,¶,¶}, ChunYing Deng[¶], Juan Meng^{§,¶,¶}, Zhenghua Lu^{‡,§,¶}

‡ School of liquor and food engineering, Guizhou University, Guiyang, China

§ The Engineering Research Center of Southwest Bio-Pharmaceutical Resources, Ministry of Education, Guizhou University, Guiyang, China

¶ Mushroom Research Center, School of agriculture, Guizhou University, Guiyang, China

¶ Mae Fah Luang University, Chiang Rai, Thailand

State Key Laboratory Breeding Base of Green Pesticide and Agricultural Bioengineering, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Guiyang, China

¶ Guizhou Institute of Biology, Guizhou Academy of Sciences, Guiyang, China

¶ Mushroom Research Center, School of agriculture, Guiyang, China

Corresponding author: TingChi Wen (tingchiwen@yahoo.com)

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Abstract

Background

Ophiocordyceps is the largest genus in the family Ophiocordicipitaceae, including many entomopathogenic species. In recent years, many species have been described in this genus, with a wide range of host insects. Entomopathogenic fungi include ecologically, economically and medicinally important species, but a large portion of their diversity remains to be discovered and described.

New information

In this study, a new species, *Ophiocordyceps aphrophoridarum* sp. nov, parasitising *Aphrophoridae* sp. (Hemiptera) is proposed from China, based on evidence from

morphology and molecular phylogenetic analyses. This species is characterised by fibrous, pigmented stromata, cylindrical asci and filiform ascospores. Compared to its closest relative, *O. tricentri*, the new species has wider perithecia and longer asci. Molecular phylogenetic analyses of a multilocus dataset (consisting of SSU, ITS, LSU, TEF1, RPB1 and RPB2) confirm its placement in *Ophiocordyceps*. *Ophiocordyceps aphrophoridarum* is morphologically described and illustrated with colour photographs. Morphological comparisons with closely-related species are also presented in tabulated format.

Keywords

one new taxon, morphology, *Ophiocordyceps*, multilocus phylogeny, taxonomy

Introduction

Insect-associated fungi represent a largely unknown and undescribed group; only 1.5% of these fungi have been reported (Mueller and Schmit 2007). In 2019, scientists determined 48 new species of animal-associated Sordariomycetes, including eight species of *Ophiocordyceps*, one of the best-known entomopathogenic genera (Cheek et al. 2020). The following year, 12 new species of *Ophiocordyceps* were described (Araujo et al. 2020, Tasanathai et al. 2020).

The genus *Ophiocordyceps* was proposed by Petch (1931) and was originally considered as a subgenus of *Cordyceps* (Kobayasi 1941, Kobayasi and Shimizu 1983). Sung et al. (2007b) established Ophiocordycipitaceae as a new family in Hypocreales with *Ophiocordyceps* as type genus. Due to the polyphyletic nature of *Cordyceps*, species formerly assigned to this genus had to be recombined in *Ophiocordyceps* (Sung et al. 2007a, Johnson et al. 2009). To date, *Ophiocordyceps* is the most speciose genus in Ophiocordycipitaceae with 289 accepted species (Index Fungorum, accessed 11 March 2021). Species of *Ophiocordyceps* are characterised by producing fibrous, hard, flexible, pigmented stromata and cylindrical asci with apical caps (Sung et al. 2007a, Ban et al. 2015, Maharachchikumbura et al. 2015, Wijayawardene et al. 2017, Xiao et al. 2019). The asexual morph of *Ophiocordyceps* is linked to *Hirsutella*, *Hymenostilbe*, *Paraisaria*, *Stibella* and *Syngliocladium* (Sung et al. 2007a, Thanakitpipattana et al. 2020) and known as *Hirsutella*-like and *Hymenostilbe*-like (Kepler et al. 2013, Maharachchikumbura et al. 2016, Maharachchikumbura et al. 2015).

Species in Ophiocordycipitaceae are found on a wide range of insect hosts; some taxa are host specific, such as *Ophiocordyceps unilateralis* sensu lato (De Bekker et al. 2014, Kobmoo et al. 2019). Blattaria, Coleoptera, Dermaptera, Diptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Megaloptera, Neuroptera, Odonata and Orthoptera are the insect orders most commonly reported to be associated with *Ophiocordyceps* (Evans et al. 2011, Luangsa-Ard et al. 2018, Araujo and Hughes 2019). The functional morphology of *Ophiocordyceps* is diverse and considered to be exclusively related to the host's ecology and biology (Evans et al. 2011).

Ophiocordyceps has a pan-global distribution, but is most species-rich in the tropics and subtropics (Petch 1933, Petch 1937, Kobayasi 1941, Tzean et al. 1997, Ban et al. 2015). The Yuntai Mountain Nature Reserve, China, a dolomite karst landform, has become a hotspot for fungal diversity (Luo et al. 2013, Wen et al. 2015, Wen et al. 2017) and, in 2019, samples of *Ophiocordyceps* were collected that proved to be an undescribed species. Here, we formally describe this species, based on morphological study and the phylogenetic analysis of a multilocus dataset.

Materials and methods

Collection and morphological characteristics examination

Two fresh samples of *Ophiocordyceps*, parasitising *Aphrophoridae* sp. (Hemiptera), were collected in June 2019 from the broad-leaved forest in Yuntai Mountain Nature Reserve, Guizhou Province, China. The samples were dried with silica gel and then stored in plastic boxes in the Herbarium of Mae Fah Luang University (MFLU). For micro-morphological observations, ascomata were examined using a Motic SMZ 168 Series stereomicroscope (Motic, Xiamen, China). Structures were observed and measured after being sliced with a double-sided blade and placed into water. Microphotographs were taken using an Eclipse 80i compound microscope (Nikon, Tokyo, Japan), fitted with an EOS 600D camera (Canon, Tokyo, Japan). Measurements were made using the Tarosoft (R) Image Frame software v. 0.9.7.

DNA extraction, PCR amplification and determination of DNA sequences

DNA was extracted from dried fruiting bodies using the Fungal gDNA Kit (Biomiga, Sang Diego, CA, USA). We amplified the small and large subunits (SSU, LSU) of the ribosomal RNA gene, internal transcribed spacer region (ITS), translation elongation factor-1 α (TEF1) and the largest and second-largest subunit of RNA polymerase II gene (RPB1, RPB2). The following primer pairs were used: NS1/NS4 for SSU, ITS4/ITS5 for ITS, LR0R/LR5 for LSU (Hopple and Vilgalys 1994, Vilgalys and Hester 1990, White et al. 1990), EF1-983F/EF1-2218R for TEF1 (Sung et al. 2007b), CRPB1A/RPB1Cr for RPB1 and fRPB2-6f/RPB2-7CR for RPB2 (Castlebury et al. 2004). The 25- μ l PCR reaction volume contained 2 μ l of DNA template, 8.5 μ l of H₂O, 1 μ l of each forward reverse primer and 12.5 μ l of 2 \times benchtopm Taq Master Mix (Biomiga, San Diego, CA, USA). Cycling conditions were as follows: for SSU and LSU: initial denaturation at 94°C for 3 min; followed by 33 cycles at 94°C for 30 s, 51°C for 30 s and 72°C for 2 min; and final extension at 72°C for 10 min. For ITS: initial denaturation at 94°C for 3 min; followed by 33 cycles of 94°C for 30 s, 51°C for 50 s and 72°C for 45 s; and final extension at 72°C for 10 min. For TEF1: initial denaturation at 94°C for 3 min; followed by 33 cycles of 94°C for 30 s, 58°C for 50 s and 72°C for 1 min; and final extension at 72°C for 10 min. For RPB1: initial denaturation at 94°C for 3 min; followed by 33 cycles of 94°C for 1 min, 52°C for 1 min and 72°C for 1 min; and final extension at 72°C for 10 min. Lastly, for RPB2: initial denaturation at 94°C for 3 min; followed by 33 cycles of 94°C for 30 s, 54°C for 40 s and 72°C for 80 s; and final extension at 72°C for 10 min. Amplified PCR products were verified by 1% agarose gel

electrophoresis, stained with ethidium bromide in 1× TBE. The PCR products were sequenced by Shanghai Shengong Biological Engineering Co. (Hangzhou, Shanghai, China). Forward and reverse sequence reads were assembled and edited by BioEdit v. 7.0.9 (Hall et al. 2011).

Sequence alignment and phylogenetic analyses

Reference sequences (Suppl. materials 1, 2) were downloaded from NCBI GenBank, based on previous studies (Suppl. material 2, Ban et al. 2015, Crous et al. 2018, Kepler et al. 2012, Long et al. 2021, Sanjuan et al. 2015, Sung et al. 2007a, Xiao et al. 2019, Araujo et al. 2015, Araujo et al. 2018). Sequences were aligned with MAFFT v.7 (Kato and Standley 2013, <http://mafft.cbrc.jp/alignment/server/>). TrimAl v.1.3 (Capella-Gutiérrez et al. 2009) was used for automated alignment trimming for poorly-aligned regions of each locus. *Tolypocladium inflation* and *T. ophiogloides* (Kepler et al. 2012, Schoch et al. 2012) were selected as outgroup taxa.

Maximum Likelihood (ML) analyses were performed using IQ-TREE 2 (Minh et al. 2020) under partitioned models; the built-in ModelFinder (Kalyaanamoorthy et al. 2017) was used to select appropriate models for each of the six loci. Branch support was estimated using 1000 ultrafast bootstrap (UFBoot2) replicates (Hoang et al. 2018). Bayesian Inference (BI) was determined by Markov Chain Monte Carlo (MCMC) sampling using MrBayes v.3.1.2 (Ronquist et al. 2012). The six loci were concatenated into a single dataset. BI was performed with six independent MCMC runs and trees were sampled 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 v.1.6 (Rambaut et al. 2018). The first 25% of the resulting trees were discarded as burn-in and posterior probabilities (PP) were calculated from the remaining sampled trees. The ML tree was visualised with FigTree v.1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Taxon treatment

Ophiocordyceps aphrophoridarum Y. Yang, Y.P. Xiao & T.C. Wen, sp. nov.

- IndexFungorum [IF558176](#)

Materials

Holotype:

- scientificName: *Ophiocordyceps aphrophoridarum*; country: China; stateProvince: Guizhou; locality: Qiandongnan, Shibing, Yuntaishan; verbatimElevation: 854m; locationRemarks: label transliteration: "Guizhou, Qiandongnan, Shibing, Yuntaishan, on *Aphrophoridae* sp., 19 June 2019, Yu Yang; verbatimCoordinates: 27°06'28.28"N, 108°06'32.15"E; decimalLatitude: 27.107858; decimalLongitude: 108.108932; georeferenceProtocol: label; lifeStage: Telemorph; catalogNumber: MFLU 20-0641; recordedBy: Yu Yang; identifiedBy: Yuan-pin Xiao; dateIdentified: 2020

Paratype:

- a. scientificName: *Ophiocordyceps aphrophoridarum*; country: China; stateProvince: Guizhou; locality: Qiandongnan, Shibing, Yuntaishan; verbatimElevation: 859m; locationRemarks: label transliteration: "Guizhou, Qiandongnan, Shibing, Yuntaishan, on *Aphrophoridae* sp., 19 June 2019, Yu Yang; verbatimCoordinates: 27°06'30.44"N, 108°06'27.15"E; decimalLatitude: 27.108457; decimalLongitude: 108.107542; georeferenceProtocol: label; lifeStage: Teleomorph; catalogNumber: MFLU 20-0642; recordedBy: Yu Yang; identifiedBy: Yuan-pin Xiao; dateIdentified: 2020

Description

Facesoffungi number: FoF09653

Sexual morph: **Stromata** 8–10 cm long, 0.5–3 mm diam., solitary, yellow, fibrous, unbranched, stipitate, slender. **Stipe** 7–8 cm long, 0.1–0.8 mm diam., cylindrical, with a fertile apex, yellow. **Fertile head** 1–2 cm long, 2–5 mm diam., cylindrical to fusiform, differs from the stipe, yellow, single. **Perithecia** 638–798 × 108–178 µm (= 718 × 143 µm, n = 40), obliquely immersed, flask-shaped to elongated obpyriform. **Peridium** 26–68 µm (= 47 µm, n = 50) divided into two layers, hyaline, outer layer textura prismatica, inner layer textura porrecta. **Asci** 337–445 × 6.1–8.7 µm (= 391 × 7.4 µm, n = 60), 8-spored, hyaline, filiform, with a thick apex. **Apical cap** 5.1–8.2 × 3.6–5.2 µm (= 6.7 × 4.4 µm, n = 40), thick, with a small channel in the centre. **Ascospores** 258–315 × 3.1–5.5 µm (= 286.5 × 4.3 µm, n = 50), filiform, hyaline, multiseptate, easily breaking into secondary ascospores. **Partspore** 6.4–8.8 × 1.4–2.4 µm (= 7.6 × 1.9 µm, n = 90), fusoid, 1-celled, hyaline, smooth-walled. **Asexual morph:** Undetermined (Fig. 1)

Etymology

Referring to the host, *Aphrophoridae* sp.

Distribution

Thus far only known from China.

Host

Aphrophoridae sp. (Hemiptera), collected from the underside of leaves litter, stromata growing from the prothorax.

Analysis**Phylogenetic analyses**

A total of 185 sequences, representing 128 species of Ophiocordycipitaceae, were downloaded from GenBank. The final alignment length was 4412 characters, representing 185 taxa (822 for LSU, 481 for ITS, 919 for SSU, 918 for TEF1, 536 for RPB1 and 736 for RPB2) (Suppl. materials 1, 2). Tree topology of the IQ-TREE analysis was similar to the

one from the Bayesian analyses. The best-scoring ML ($-\ln L = 81595.8951$) is shown in Fig. 2.

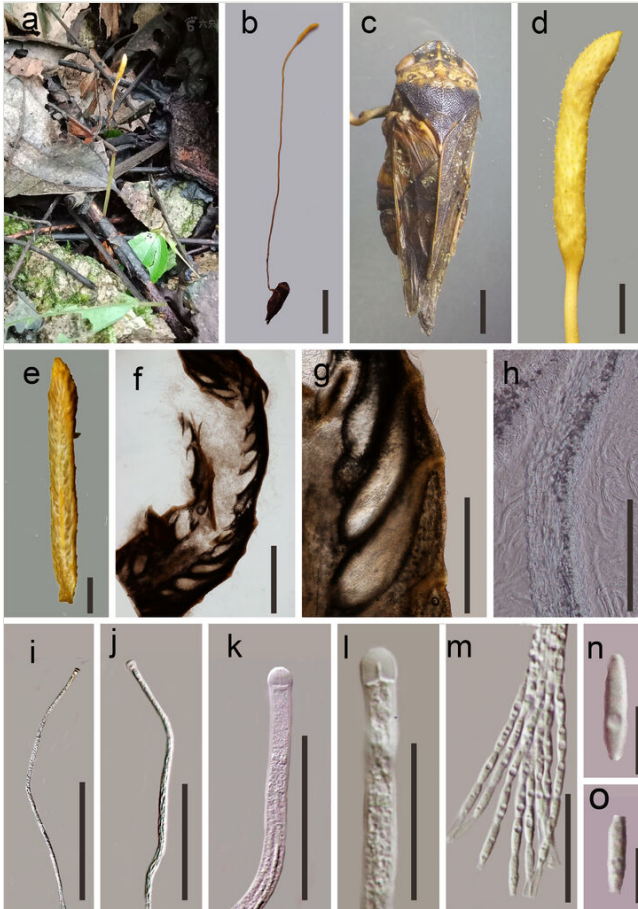


Figure 1. [doi](#)

Ophiocordyceps aphrophidarum (MFLU 20–0641, holotype): **a** Habitat **b** Overview of the host and stromata **c** Host **d** Stromata **e** Vertical section of the stroma **f–g** Section of ascomata **h** Peridium **i–j** Immature to mature asci **k–l** Apical cap of asci **m** Part of ascospores **n–o** Partspore. Scale bars: **b** = 10 mm, **c–d** = 5 mm, **e–f** = 1000 μm , **g** = 500 μm , **i** = 200 μm , **j** = 100 μm , **k–m** = 30 μm , **n–o** = 5 μm .

Discussion

The Yuntai Mountain Nature Reserve, situated in Shibing County, Guizhou Province, China, is a dolomite karst landform. The Reserve is home to 106 species of macrofungi (Luo et al. 2013), including two species of *Metacordyceps* that are currently only known from the holotype locality (Wen et al. 2015, Wen et al. 2017) Here, we present a new entomopathogenic species, *O. aphrophidarum*, from the same Reserve.

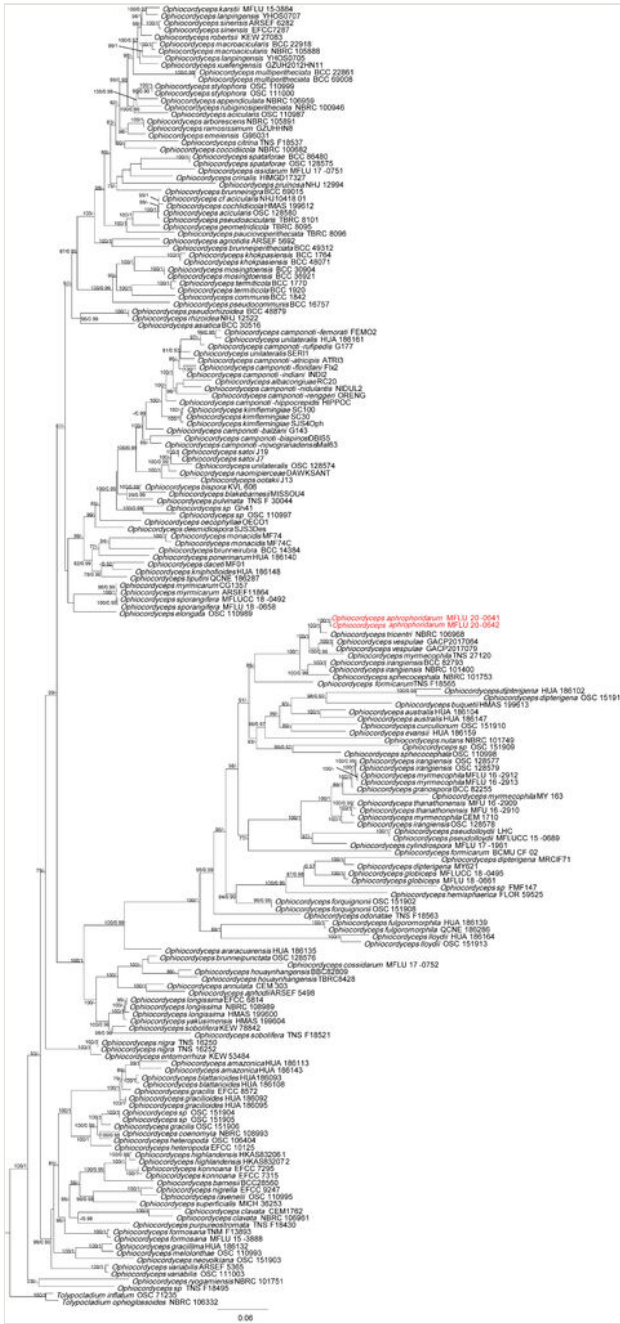


Figure 2. [doi](#)

Phylogeny of *Ophiocordyceps* reconstructed from a six-locus dataset (ITS, SSU, LSU, TEF1, RPB2 and RPB1). The topology is the result of ML inference performed in IQ-TREE. The tree is rooted with both *Tolypocladium inflatum* and *T. ophioglossoides*. MLBS ≥ 75 and BIPP ≥ 0.90 are presented above branches. The new species is highlighted in red.

Ophiocordyceps aphrophoridarum was phylogenetically retrieved as a sister species of *O. tracentri*, in a maximum supported clade with *O. irangiensis*, *O. myrmecophila*, *O. sphecocephala* and *O. vespulae* (Fig. 2). The sequences of six loci of *O. tracentri*, *O. irangiensis*, *O. sphecocephala* and *O. vespulae* share between 86–94% identity with *O. aphrophoridarum* in their ITS, 94–99% in SSU, 99–100% in LSU, 97–99% in TEF1, 94–95% in RPB1 and 95–97% in RPB2.

Both *O. irangiensis* and *O. myrmecophila* have Formicinae spp. (Hymenoptera) as host (Hywel-Jones 1996), whereas the host of *O. aphrophoridarum* is *Aphrophoridae* sp. (Hemiptera). Morphologically, *O. aphrophoridarum* differs from *O. irangiensis* in its smaller ascomata, shorter asci and shorter partspores (Hywel-Jones 1996). *Ophiocordyceps aphrophoridarum* differs from *O. myrmecophila* in terms of having smaller ascomata, shorter asci and longer partspores (Hywel-Jones 1996). The host of *O. sphecocephala* is *Vespula* sp. (Hymenoptera) (Hywel-Jones 1995). This species produces larger ascomata, longer asci and longer partspores compared to *O. aphrophoridarum* (Shrestha and Sung 2005). Additionally, *O. vespulae* has *Vespula* sp. as host (Hymenoptera) and is distinct from the new species by its longer asci and partspores (Long et al. 2021).

Ophiocordyceps tracentri is phylogenetically most closely related to the new species and it has similar morphological characters. *Ophiocordyceps tracentri* was initially described as *Cordyceps tracentri* from Japan. It is characterised by stipitate stroma with a yellow fusoid fertile head (Yasuda 1922, Table 1). The host of *C. tracentri* was initially identified as *Tricentrus* sp. (Hemiptera, Membracidae), but later corrected to *Aphrophora intermedia* (Hemiptera, *Aphrophoridae*) (Yasuda 1922). Later, *Aphrophora flavomaculata*, *Aphrophora rugosa* and *Peuceptyelus medius* were reported as the hosts of *C. tracentri* (Kobayasi 1941, Shrestha 2017). Additionally, another species, *Cordyceps aphrophorae*, was synonymised with *C. tracentri* (Yasuda 1922, Lim and Kim 1973). Shrestha and Sung 2005 recorded *Cordyceps tracentri* obtained from Nepal, but presented no molecular data (Table 1). Following molecular phylogenetic analyses, *C. tracentri* was transferred to *Ophiocordyceps* (Sung et al. 2007b). Ban et al. 2015 presented sequence data of *O. tracentri* from strain NBRC 106968, but did not provide morphological information. It is clear that more data are needed to fully understand the species limits with regards to *O. tracentri*. The new species, *O. aphrophoridarum*, is morphologically similar to *O. tracentri*, but can be recognised by its longer and finer stromata and much longer asci (Yasuda 1922, Shrestha and Sung 2005, Table 1).

Table 1.

Synopsis of closely-related *Ophiocordyceps* species. Measurements in μm .

Species	<i>O. aphrophoridarum</i>	<i>O. tracentri</i> (holotype)	<i>O. tracentri</i> (EFCC 7251, 7252)
Distribution	China	Japan	Nepal
Stromata (mm)	Clavated, branched or unbranched, 80–100 × 0.5–1.2	Fusoid, yellow, unbranched	Solitary, yellow, 50–60 × 1–1.5

Fertile heads (mm)	Yellow single, allantoideus, 10–20 × 2–5		Ovoid, 50–60 × 1–1.5
Perithecia (µm)	Obliquely buried, ovoid to elongated pyriform, 638–798 × 108–178		Immersed, ovoid, 550–650 × 110–120
Asci (µm)	8-spored, hyaline, filiform, 337–445 × 6.1–8.7	Cylindrical, 120 × 5–6	300–320 × 5
Partspores (µm)	Fusoid, 1-celled, straight, hyaline, 6.4–8.8 × 1.4–2.4	Fusoid, smooth, 1-celled, hyaline, 8–10 × 1.5	
References	This study	Yasuda 1922	Shrestha and Sung 2005

In conclusion, there is sufficient evidence from both morphology and molecular phylogenetic analyses to support *O. aphrophoridarum* as a new species of *Ophiocordyceps*.

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References

- Araujo JPM, Evans HC, Mackay WP, Geiser DM, Hughes DP (2015) Unravelling the diversity behind the *Ophiocordyceps unilateralis* (*Ophiocordycipitaceae*) complex: Three new species of zombie-ant fungi from the Brazilian Amazon. *Phytotaxa* 220 (3): 224–238. <https://doi.org/10.11646/phytotaxa.220.3.2>
- Araujo JPM, Evans HC, Kepler R, Hughes DP (2018) Zombie-ant fungi across continents: 15 new species and new combinations within *Ophiocordyceps*. I. *Myrmecophilous hirsutelloid* species. *Studies in Mycology* 90: 119–160. <https://doi.org/10.1016/j.simyco.2017.12.002>
- Araujo JPM, Hughes DP (2019) Zombie-ant fungi emerged from non-manipulating, beetle-infecting ancestors. *Current Biology* 29 (21): 3735–3738. <https://doi.org/10.1016/j.cub.2019.09.004>
- Araujo JPM, Evans HC, Fernandes IO, Ishler MJ, Hughes DP (2020) Zombie-ant fungi cross continents: II. *Myrmecophilous hymenostilboid* species and a novel zombie lineage. *Mycologia* 112 (6): 1138–1170. <https://doi.org/10.1080/00275514.2020.1822093>
- Ban S, Sakane T, Nakagiri A (2015) Three new species of *Ophiocordyceps* and overview of anamorph types in the genus and the family *Ophiocordycipitaceae*. *Mycological Progress* 14 (1): 1–12. <https://doi.org/10.1007/s11557-014-1017-8>
- Capella-Gutiérrez S, Silla-Martínez J, Gabaldón T (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25 (15): 1972–1973. <https://doi.org/10.1093/bioinformatics/btp348>

- Castlebury L, Rossman A, Sung G, Hyten A, Spatafora J (2004) Multigene phylogeny reveals new lineage for *Stachybotrys chartarum*, the indoor air fungus. *Mycological Research* 108 (8): 864-872. <https://doi.org/10.1017/S0953756204000607>
- Cheek M, Lughadha EN, Kirk PM, Lindon H, Niskanen T (2020) New scientific discoveries: Plants and fungi. *Plants People Planet* 2 (5): 371-388.
- Crous P, Luangsa-Ard J, Wingfield MJ, Carnegie A, Hernández-Restrepo M, Lombard L, Roux J, Barreto R, Baseia I, Cano J, Martín M, Morozova O, Stchigel AM, Summerell B, Brandrud TE, Dima B, García D, Giraldo López A, Guarro J, Groenewald JZ (2018) Fungal Planet description sheets- 785 – 867. *Persoonia - Molecular Phylogeny and Evolution of Fungi* 41 <https://doi.org/10.3767/persoonia.2018.41.12>
- De Bekker C, Quevillon L, Smith P, Fleming K, Ghosh D, Patterson A, Hughes D (2014) Species-specific ant brain manipulation by a specialized fungal parasite. *BMC Evolutionary Biology* 14 <https://doi.org/10.1186/PREACCEPT-8313271611363357>
- Evans H, Elliot S, Hughes D (2011) Hidden diversity behind the zombie-ant fungus *Ophiocordyceps unilateralis*: Four new species described from carpenter ants in Minas Gerais, Brazil. *PLOS One* 6 <https://doi.org/10.1371/journal.pone.0017024>
- Hall T, Biosciences I, Carlsbad C (2011) BioEdit: An important software for molecular biology. *GERF Bull. Biosci.* 2: 60-61.
- Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2018) UFBot2: Improving the Ultrafast Bootstrap Approximation. *Molecular Biology and Evolution* 35 (2): 518-522. <https://doi.org/10.1093/molbev/msx281>
- Hopple J, Vilgalys R (1994) Phylogenetic relationships among coprinoid taxa and allies based on data from restriction site mapping of nuclear rDNA. *Mycologia* 86: 96-107. <https://doi.org/10.1080/00275514.1994.12026378>
- Hywel-Jones N (1995) *Cordyceps sphecocephala* and a *Hymenostilbe* sp. infecting wasps and bees in Thailand. *Mycological Research* 99 (2): 154-158. [https://doi.org/10.1016/S0953-7562\(09\)80879-4](https://doi.org/10.1016/S0953-7562(09)80879-4)
- Hywel-Jones N (1996) *Cordyceps myrmecophila*-like fungi infecting ants in the leaf litter of tropical forest in Thailand. *Mycological Research* 100 (5): 613-619. [https://doi.org/10.1016/S0953-7562\(96\)80017-7](https://doi.org/10.1016/S0953-7562(96)80017-7)
- Johnson D, Sung GH, Hywel-Jones NL, Luangsa-Ard JJ, Bischoff JF, Kepler RM, Spatafora JW (2009) Systematics and evolution of the genus *Torrubiella* (Hypocreales, Ascomycota). *Mycological Research* 113 (3): 279-289. <https://doi.org/10.1016/j.mycres.2008.09.008>
- Kalyaanamoorthy S, Minh BQ, Wong T, Haeseler AV, Jermini LS (2017) ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates. *Nature Methods* 14 (6). <https://doi.org/10.1038/nmeth.4285>
- Katoh K, Standley D (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772-780. <https://doi.org/10.1093/molbev/mst010>
- Kepler R, Sung GH, Ban S, Nakagiri A, Chen MJ, Huang B, Li Zz, Spatafora J (2012) New teleomorph combinations in the entomopathogenic genus *Metacordyceps*. *Mycologia* 104: 182-197. <https://doi.org/10.3852/11-070>
- Kepler R, Ban S, Nakagiri A, Bischoff J, Hywel-Jones N, Owensby C, Spatafora J (2013) The phylogenetic placement of hypocrealean insect pathogens in the genus *Polycephalomyces*: An application of one fungus one name. *Fungal Biology* 117: 611-622. <https://doi.org/10.1016/j.funbio.2013.06.002>

- Kepler RM, Sung GH, Ban S, Nakagiri A, Chen MJ, Huang B, Li Z, Spatafora JW (2012) New teleomorph combinations in the entomopathogenic genus *Metacordyceps*. *Mycologia* 104 (1): 182-197. <https://doi.org/10.3852/11-070>
- Kobayasi Y (1941) The genus *Cordyceps* and its allies. Report of the Tokyo Bunrika Daigaku Section B 5 (84): 53-260.
- Kobayasi Y, Shimizu D (1983) Iconography of vegetable wasps and plant worms. Hoikusha Publishing Co Ltd, Osaka, 280 pp.
- Kobmoo N, Mongkolsamrit S, Arnarnart N, Luangsa-ard JJ, Giraud T (2019) Population genomics revealed cryptic species within host-specific zombie-ant fungi (*Ophiocordyceps unilateralis*). *Molecular Phylogenetics and Evolution* 140 <https://doi.org/10.1016/j.ympev.2019.106580>
- Lim JH, Kim BK (1973) Taxonomic Investigations on Korean Higher Fungi(II) -Two Unrecorded Species of the Genus *Cordyceps* in Korea. *The Korean Journal of Mycology* 1 (1): 13-16.
- Long FY, Qin LW, Xiao YP, Hyde K, Wang SX, Wen TC (2021) Multigene phylogeny and morphology reveal a new species, *Ophiocordyceps vespulae*, from Jilin Province, China. *Phytotaxa* 478: 33-48. <https://doi.org/10.11646/phytotaxa.478.1.2>
- Luangsa-Ard J, Tasanathai K, Thanakitpipattana D, Khonsanit A, Stadler M (2018) Novel and interesting *Ophiocordyceps* spp. (Ophiocordycipitaceae, Hypocreales) with superficial perithecia from Thailand. *Studies in Mycology* 89 <https://doi.org/10.1016/j.simyco.2018.02.001>
- Luo GT, Wang JX, Wen TC (2013) Resource investigation of macrofungi in Yuntai Mountain in Shibing County, Guizhou Province. *Guizhou Science* 31 (4).
- Maharachchikumbura S, Hyde K, Jones E, McKenzie E, Huang SK, Abdel-Wahab M, Daranagama D, Dayarathne M, D'souza M, Goonasekara I, Hongsanan S, Jayawardena R, Kirk P, Konta S, Liu JK, Liu ZY, Norphanphoun C, Pang KL, Perera R, Xu J (2015) Towards a natural classification and backbone tree for Sordariomycetes. *Fungal Diversity* 72: 199-301. <https://doi.org/10.1007/s13225-015-0331-z>
- Maharachchikumbura S, Hyde K, Jones E, McKenzie E, Bhat DJ, Dayarathne M, Huang SK, Norphanphoun C, Senanayake I, Perera R, Shang QJ, Xiao YP, D'souza M, Hongsanan S, Jayawardena R, Daranagama D, Konta S, Goonasekara I, Zhuang WY, Wijayawardene N (2016) Families of Sordariomycetes. *Fungal Diversity* 79: 1-317. <https://doi.org/10.1007/s13225-016-0369-6>
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams M, Von Haeseler A, Lanfear R (2020) IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution* (5). <https://doi.org/10.1093/molbev/msaa015>
- Mueller GM, Schmit JP (2007) Fungal biodiversity: what do we know? What can we predict? *Biodiversity and Conservation* 16 <https://doi.org/10.1007/s10531-006-9117-7>
- Petch T (1931) Notes on entomogenous fungi. *Transactions of the British Mycological Society* 16 (1): 55-75. [https://doi.org/10.1016/S0007-1536\(31\)80006-3](https://doi.org/10.1016/S0007-1536(31)80006-3)
- Petch T (1933) Notes on entomogenous fungi. *Transactions of the British Mycological Society* 18 (1): 48-75. [https://doi.org/10.1016/S0007-1536\(33\)80026-X](https://doi.org/10.1016/S0007-1536(33)80026-X)
- Petch T (1937) Notes on entomogenous fungi. *Transactions of the British Mycological Society* 21 (1): 34-67. [https://doi.org/10.1016/S0007-1536\(37\)80005-4](https://doi.org/10.1016/S0007-1536(37)80005-4)

- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67 (5): 901-904. <https://doi.org/10.1093/sysbio/syy032>
- Ronquist F, Teslenko M, Mark P, Ayres D, Darling A, Höhna S, Larget B, Liu L, Suchard M, Huelsenbeck J (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539-542. <https://doi.org/10.1093/sysbio/sys029>
- Sanjuan TI, Franco–Molano AE, Kepler RM, Spatafora JW, Tabima J, Vasco–Palacios AM, Restrepo S (2015) Five new species of entomopathogenic fungi from the Amazon and evolution of neotropical *Ophiocordyceps*. *Fungal Biology* 119 (10): 901-916.
- Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding C, Fungal Barcoding Consortium Author L (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proceedings of the National Academy of Science* 109 (16): 6241-6246. <https://doi.org/10.1073/pnas.1117018109>
- Shrestha B, Sung JM (2005) Notes on *Cordyceps* species collected from the central region of Nepal. *Mycobiology* 33 (4): 235-239. <https://doi.org/10.4489/MYCO.2005.33.4.235>
- Shrestha B (2017) Mycosphere Essay 19. *Cordyceps* species parasitizing hymenopteran and hemipteran insects. *Mycosphere* 8 (9): 1424-1442. <https://doi.org/10.5943/mycosphere/8/9/8>
- Sung GH, Sung JM, Hywel-Jones N, Spatafora J (2007a) A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): Identification of localized incongruence using a combinational bootstrap approach. *Molecular Phylogenetics and Evolution* 44: 1204-1223. <https://doi.org/10.1016/j.ympev.2007.03.011>
- Sung GH, Hywel-Jones NL, Sung JM, Luangsa-ard JJ, Shrestha B, Spatafora JW (2007b) Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. *Studies in Mycology* 57 [https://doi.org/10.1016/S0166-0616\(14\)60128-7](https://doi.org/10.1016/S0166-0616(14)60128-7)
- Tasanathai K, Thanakitpipattana D, Himaman W, Phommavong K, Dengkhhamounh N, Luangsa-ard J (2020) Three new *Ophiocordyceps* species in the *Ophiocordyceps pseudoacicularis* species complex on Lepidoptera larvae in Southeast Asia. *Mycological Progress* 19 (10): 1043-1056. <https://doi.org/10.1007/s11557-020-01611-6>
- Thanakitpipattana D, Tasanathai K, Mongkolsamrit S, Khonsanit A, Lamlerthton S, Luangsa-Ard JJ (2020) Fungal pathogens occurring on Orthoptera in Thailand. *Persoonia - Molecular Phylogeny and Evolution of Fungi* 44: 140-160. <https://doi.org/10.3767/persoonia.2020.44.06>
- Tzean SS, Hsieh LS, Wu WJ (1997) Atlas of entomopathogenic fungi from Taiwan. Council of Agriculture, Executive Yuan, Taiwan.
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172 (8): 4238-4246. <https://doi.org/10.1128/jb.172.8.4238-4246.1990>
- Wen TC, Zha LS, Xiao YP, Wang Q, Kang JC, Hyde K (2015) *Metacordyceps shibinensis* sp. nov. from larvae of Lepidoptera in Guizhou Province, Southwest China. *Phytotaxa* 226 <https://doi.org/10.11646/phytotaxa.226.1.5>
- Wen TC, Xiao YP, Han Yf, Huang SK, Zha LS, Hyde K, Kang JC (2017) Multigene phylogeny and morphology reveal that the Chinese medicinal mushroom ‘*Cordyceps*

gunnii is *Metacordyceps neogunnii* sp. nov. Phytotaxa 302 <https://doi.org/10.11646/phytotaxa.302.1.2>

- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics: PCR–protocols and applications—A laboratory manual. In: Innis M, Gelfand D, Sninsky J, White T (Eds) PCR Protocols. Academic Press, San Diego, 315–322 pp. [ISBN 978-0-12-372180-8]. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Wijayawardene N, Hyde K, Rajeshkumar KC, Hawksworth D, Madrid H, Kirk P, Braun U, Singh R, Crous P, Kukwa M, Lücking R, Kurtzman C, Yurkov A, Haelewaters D, Aptroot A, Lumbsch T, Timdal E, Ertz D, Etayo J, Karunarathna S (2017) Notes for genera: Ascomycota. Fungal Diversity 86: 1-594. <https://doi.org/10.1007/s13225-017-0386-0>
- Xiao YP, Hongsanan S, Hyde K, Brooks S, Xie N, Long FY, Wen TC (2019) Two new entomopathogenic species of *Ophiocordyceps* in Thailand. MycoKeys 47: 53-74. <https://doi.org/10.3897/mycokeys.47.29898>
- Yasuda (1922) The botanical magazine = Shokubutsugaku zasshi. v.36 (1922). Tokyo Botanical Society, Tokyo :. URL: <https://www.biodiversitylibrary.org/item/130630> [ISBN 0006-808X]

Supplementary materials

Suppl. material 1: Sources of isolates and GenBank accession numbers [doi](#)

Authors: Yu Yang, Yuan-pin Xiao, Gang-jiang Yu, Juan Meng, Zheng-hua Lu, Chun-ying Deng, Ting-chi Wen

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Authors: Yu Yang

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