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Cordyceps yinjiangensis, a new ant-pathogenic fungus

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Abstract

Ant-pathogenic fungi are mainly found in the Ophiocordycipitaceae, rarely in the Cordycipitaceae. During a survey of entomopathogenetic fungi from Southwest China, a new species, *Cordyceps yinjiangensis*, was isolated from the ponerine. It differs from other *Cordyceps* species by its ant host, shorter phialides, and smaller septate conidia formed in an imbricate chain. Phylogenetic analyses based on the combined datasets of (*LSU+RPB2+TEF*) and (*ITS+TEF*) confirmed that *C. yinjiangensis* is distinct from other species. The new species is formally described and illustrated, and compared to similar species.

Keywords: 1 new species, *Cordyceps*, morphology, phylogeny, ponerine

Introduction

The ascomycete genus *Cordyceps sensu lato* (sl) consists of more than 600 fungal species. Sung *et al.* (2007) divided *Cordyceps* sl into four genera, *i.e.*, *Cordyceps sensu stricto* (ss, belonging to the family Cordycipitaceae), *Ophiocordyceps* (Ophiocordycipitaceae), *Metacordyceps* (Clavicipitaceae), and *Elaphocordyceps* (Ophiocordycipitaceae). At that time, *Cordyceps* comprised about 50 species of fungi that mainly parasitize insects (Sung *et al*. 2007). Some new *Cordyceps* species were reported later (Li *et al*. 2008, Yang *et al*. 2009, Palfner *et al*. 2012, Negi *et al*. 2012, Sanjuan *et al*. 2014, Yan & Bau 2015, Tasanathai *et al*. 2016, Chirivi *et al*. 2017). Kepler *et al*. (2017) proposed the rejection of *Isaria* in favour of *Cordyceps*, owing to the confusion surrounding the application of *Isaria*, and combined *Isaria* species into *Cordyceps*. Three new species, *Cordyceps kuiburiensis* Himaman, Mongkols., Noisrip. & Luangsa-ard, *C. jakajanicola* Luangsa-ard, Tasan., Noisrip. & Hywel-Jones and *C. qingchengensis* L.S. Zha & T.C. Wen were reported by Crous *et al*. (2019) and Zha *et al*. (2019). Thus, *Cordyceps* now consists of about 80 species.

The name *Cordyceps sinensis* (Berk.) Sacc. was initially recorded by Saccardo (1878), and was the earliest study on *Cordyceps* in China. Teng *et al*. (1934) summarized the species of *Cordyceps* in China. Song *et al*. (2006) reported the known taxa of *Cordyceps* from China and their distribution (125 species and 3 varieties). Some new *Cordyceps* species were reported later from China (Li *et al*. 2008, 2010, Liang *et al*. 2008, Yang *et al*. 2009, Cao *et al*. 2010, Chen *et al*. 2013b, Wen *et al*. 2013, 2014, 2015, 2016, 2017, Yan & Bau 2014, 2015, Yang *et al*. 2015, Zhou *et al*. 2015, Yu *et al*. 2017, Qu *et al*. 2018a, 2018b, Wang *et al*. 2018, Zha *et al*. 2019). Furthermore, some anamorphic species of *Cordyceps* were also reported (Huang *et al*. 2007, Zou *et al*. 2010, Zhang *et al*. 2013, Chen *et al*. 2013a, 2017a, 2017b, 2018).

Currently, there are more than 800 *Cordyceps* species in the world (containing the name *Cordyceps* or *Ophiocordyceps*). However, fewer than 200 species have been reported in China (http://www.indexfungorum.org/Names/Names.asp, 1 Jun 2020). During a survey of entomopathogenetic fungi from Southwest China, a new *Cordyceps* species was found. It is described here as *Cordyceps yinjiangensis sp. nov*. and is supported by morphological characters and a phylogenic analysis.

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Materials & methods

Specimen collection and isolation

Three infected insect specimens were collected from a pinewood in Yinjiang, Tongren city (N 26°21′27.96″, E 107°22′48.22″), in October 2019. Isolation of strains was done as described by Chen *et al*. (2019). The surface of the specimens was rinsed with sterile water, followed by surface sterilization with 75% ethanol for 3–5 s. A part of the insect body was cut off and inoculated with haemocoel on potato dextrose agar (PDA), to which 1% w/v peptone (PDAP) had been added. Fungal colonies emerging from specimens were 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). Strains YJ06021, YJ06022, YJ06221, YJ06222, YJ06311 and YJ06312 were obtained. The specimens and the isolated strains were deposited in the Institute of Fungus Resources, Guizhou University (formally Herbarium of Guizhou Agricultural College; code, GZAC), Guiyang City, Guizhou, China.

Macroscopic and microscopic morphological characteristics of the fungi were examined and growth rates determined from PDA cultures incubated at 25 °C for 14 d. Hyphae and conidiogenous structures were mounted in lactophenol cotton blue or 20% lactate solution and observed with an optical microscope (OM, DM4 B, Leica, Germany).

DNA extraction, PCR amplification and nucleotide sequencing

DNA extraction was carried out according to Liang *et al*. (2011). The extracted DNA was stored at −20 °C. Translation elongation factor 1 alpha (*TEF*) and RNA polymerase II largest subunit 2 (*RPB2*) were amplified according to van den Brink *et al*. (2012). The internal transcribed spacer (*ITS*) region and large subunit ribosomal RNA (*LSU*) genes were amplified by PCR according to the procedures described by White *et al*. (1990). PCR products were purified using the UNIQ-10 column PCR products purification kit [no. SK1141; Sangon Biotech (Shanghai) Co., Shanghai, China] according to the manufacturer's protocol and sequenced at Sangon Biotech (Shanghai) Co. The resulting sequences were submitted to GenBank.

Sequence alignment and phylogenetic analyses

The DNA sequences generated in this study were assembled and edited using Lasergene software (version 6.0, DNASTAR). Sequences of *ITS*, *LSU* rRNA, *RPB2* and *TEF* were selected based on Tasanathai *et al*. (2016), Kepler *et al*. (2017), Mongkolsamrit *et al*. (2018) and the result of a Blast search in GenBank. Two sequences of *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson (isolates CBS 284.36 and CBS 431.87) were chosen as outgroup taxa. Multiple datasets of *ITS*, *LSU*, *RPB2* and *TEF* were aligned using MAFFT v7.037b (Katoh & Standley 2013) and alignments were edited with MEGA6 (Tamura *et al*. 2013), Sequences were concatenated with SequenceMatrix v.1.7.8 (Vaidya *et al*. 2011). Partition homogeneity test in PAUP4.0b10 (Swofford 2002) was performed by using the command 'hompart'.

Two different analyses were carried out using Bayesian inference (BI) and maximum likelihood (ML) methods. The aim of the first analysis was to determine the relationship between *Cordyceps* species and closely-related species in Cordycipitaceae based on the combined dataset of (*LSU*+*RPB2*+*TEF*). The aim of the second analysis was to determine the relationship among *Cordyceps yinjiangensis* and its allies based on the combined dataset of (*ITS*+*TEF*). For BI, a Markov chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities using MrBayes v.3.2 (Ronquist *et al*. 2012) for the combined sequence datasets. The Bayesian analysis resulted in 20,001 trees after 10,000,000 generations. The first 4,000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 16,001 trees were used for calculating posterior probabilities in the majority rule consensus tree. After the analysis was finished, each run was examined using the program Tracer v1.5 (Drummond & Rambaut 2007) to determine burn-in and confirm that both runs had converged. ML analyses were constructed with RAxMLGUI (Silvestro & Michalak 2012). The GTRGAMMA model was used for all partitions, in accordance with recommendations in the RAxML manual against the use of invariant sites. The final alignment is available from TreeBASE under submission ID: 26424 (http://www.treebase.org).

Results

Phylogenetic analyses

The phylogenetic tree of *Cordyceps* species and closely-related species in Cordycipitaceae (analysis 1) and *Cordyceps yinjiangensis* and its allies (analysis 2) were generated from the ML and BI analysis on the (*LSU+RPB2+TEF*) and (*ITS+TEF*) datasets, respectively. Statistical support (≥ 50%/0.5) is shown at the nodes for ML bootstrap support/ BI posterior probabilities (Figs 1, 2). The strain numbers are noted after the name of each species. The tree of *Cordyceps* species and closely-related species in Cordycipitaceae and *C. yinjiangensis* and its allies were rooted with *Purpureocillium lilacinum* (CBS 284.36 and CBS 431.87). The concatenated sequences of analysis 1 and analysis 2 included 69 and 20 taxa, and consisted of 1,611 (*LSU*: 389, *RPB2*: 541 and *TEF*: 681) and 1,314 (*ITS*: 521 and *TEF*: 793) characters with gaps, respectively.

Analysis 1: *Cordyceps* species and closely-related species in Cordycipitaceae. The RAxML analysis of the combined dataset (*LSU+RPB2+TEF*) yielded the highest scoring tree (Fig. 1) with a final ML optimization likelihood value of–20,068.990080. Parameters for the GTR model of the concatenated data set was as follows: estimated base frequencies; A = 0.241732, C = 0.280519, G = 0.268489, T = 0.209260; substitution rates AC = 0.861138, AG = 2.825605, AT = 0.837813, CG = 0.777712, CT = 6.085726, GT = 1.000000; gamma distribution shape parameter α = 0.326462. In the phylogenetic tree (Fig. 1), *C. yinjiangensis* clustered with *C. morakotii* in a subclade and formed an independent branch.

Analysis 2: *Cordyceps yinjiangensis* and its allies. The RAxML analysis of the combined dataset (*ITS+TEF*) yielded the highest scoring tree (Fig. 2) with a final ML optimization likelihood value of–8,051.357083. Parameters for the GTR model of the concatenated data set was as follows: estimated base frequencies; A = 0.223851, C = 0.322850, G = 0.259791, T = 0.193508; substitution rates AC = 1.063551, AG = 1.739521, AT = 1.082774, CG = 0.809050, CT = 3.237928, GT = 1.000000; gamma distribution shape parameter α = 0.657732. In the phylogenetic tree (Fig. 2), *C. yinjiangensis* also clustered with *C. morakotii* in a subclade and formed an independent branch.

Taxonomy

Cordyceps yinjiangensis Y.P. Li, W.H. Chen, Y.F. Han & Z.Q. Liang, *sp. nov.* (Fig. 3)

Mycobank No.: MB 835788

Type:—CHINA. Guizhou Province: Tongren City, Yinjiang (N 27°55'17.1", E 108°41'25.2"), on a ponerine, 1 October 2019, Yuping Li, holotype GZAC YJ0622; ex-type culture GZAC YJ06221. Sequences from the strain YJ06221 have been deposited in GenBank with accession numbers: *ITS*=MT560349, *LSU*=MT560352, *RPB2*=MT577002, *TEF*=MT577003.

Colonies on PDA reaching ca. 3.2–3.5 cm diam in 14 d at 25 ℃, at first white with basal felt, becoming cream in the center within 14 d, cottony, with abundant conidial density, reverse unpigmented, yellowish. *Prostrate hyphae* smooth, septate, hyaline, 1.5–2.4 μm diam. *Conidiophores* erect arising from prostrate hyphae, or on aerial hyphae, verticillate with phialides in whorls of two to three. *Phialides* with a cylindrical basal portion, tapering into a distinct neck, 11.6–18.9 × 1.7–2.2 μm. *Conidia* formed in imbricate chain, hyaline, cylindrical, some curved, multiple-septate, ranging from 1 to 3 septations, (3.1–)5.3–7.2(–16.1) × (1.6–)2.1(–4.4) μm. Chlamydospores and synnemata not observed. In culture both phialides and conidia are of similar general shape and size to those found on the ponerine.

Etymology:—*yingjiangensis* named after the place, Yinjiang from which the fungus was collected.

Additional materials examined:—CHINA. Guizhou Province: Tongren City, Yinjiang (N 27°55'17.1", E 108°41'25.2"), on a ponerine, 1 October 2019, Yuping Li (YJ0602) and Tangyan Ao (YJ0631). Sequences from strains YJ06222, YJ06021, YJ06022, YJ06311 and YJ06312 have been deposited in GenBank with accession numbers: *ITS*= MT560354, MT560344, MT560354, MT560357 and MT560361, *LSU*= MT560355, MT560345, MT560348 and MT560358, *RPB2*= MT577004, MT576999 and MT577001, *TEF*= MT577005, MT577000, MT577008, MT577006 and MT577007.

Known distribution:—Yinjiang, Tongren, Guizhou Province, China.

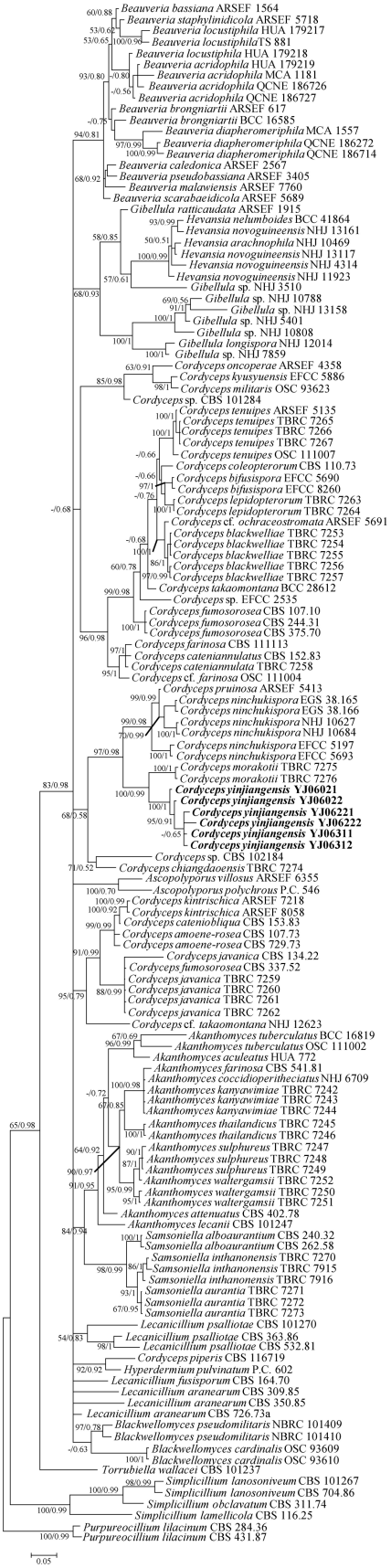

FIGURE 1. Phylogenetic relationships among *Cordyceps* species and closely-related species in Cordycipitaceae based on multigene dataset (*LSU*, *RPB2* and *TEF*). Statistical support values (≥ 0.5/50%) are shown at the nodes for ML bootstrap support/BI posterior probabilities. The new species is in bold.

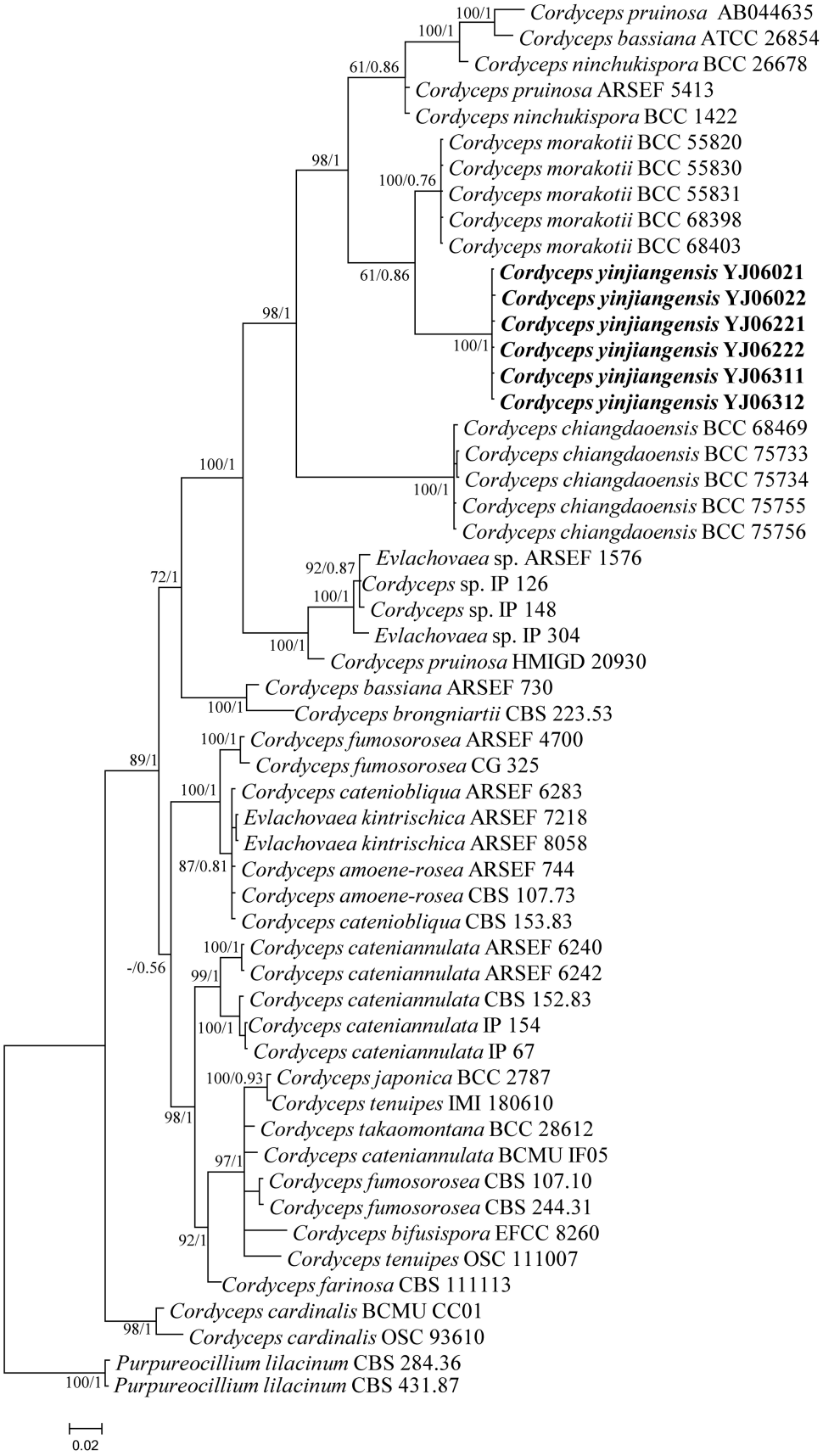

FIGURE 2. Phylogenetic relationships among the new taxon *Cordyceps yinjiangensis* and its allies by *ITS+TEF* sequences. Statistical support values (≥ 0.5/50%) are shown at the nodes for ML bootstrap support/BI posterior probabilities. The new species is in bold.

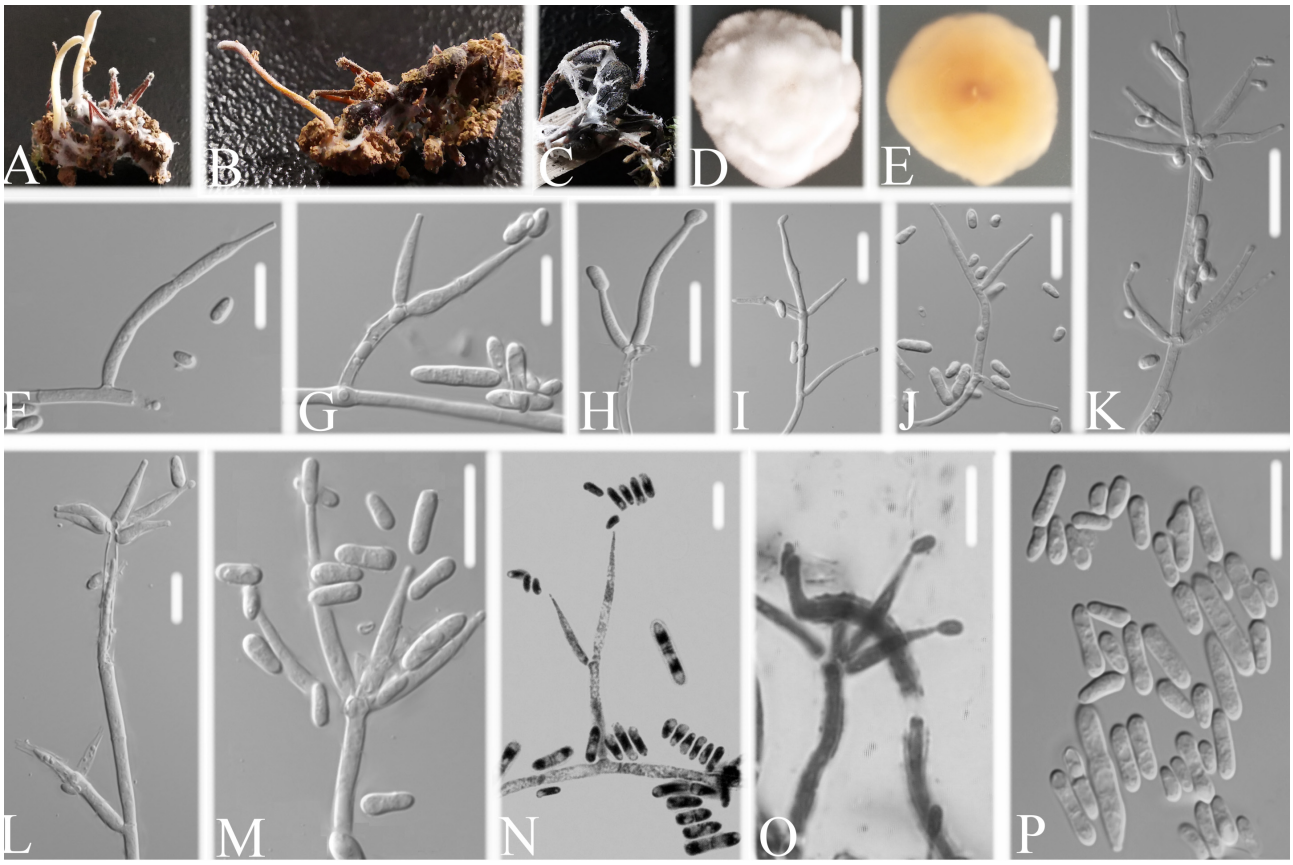

FIGURE 3. *Cordyceps yinjiangensis*. **A**–**C**. Infected ponerine. **D**–**E.** Culture on PDA, showing the top (D) and the underside (E). **F–N.** Phialides and conidia in chains formed on PDA. **O.** Phialides and conidia arising on the natural substrate. **P.** Conidia formed on PDA. Scale bars: D, E = 10 mm, F–P = 10 µm.

Discussion

Phylogenetic analyses based on the combined datasets of (*LSU+RPB2+TEF*) and (*ITS+TEF*) suggests that strains YJ06021, YJ06022, YJ06221, YJ06222, YJ06311 and YJ06312 are members of the Cordycipitaceae and belong to the genus *Cordyceps*. Ant-pathogenic species are mostly found in the Ophiocordycipitaceae, rarely in the Cordycipitaceae. Tasanathai *et al*. (2016) reported an ant-pathogenic species, *Cordyceps morakotii* Tasanathai, Thanakitpipattana & Luangsa-ard. Compared with the typical characteristics, strain YJ06221 has a close relationship with *C. morakotii* by having ant host, and conidia formed in an imbricate chain. But strain YJ06221 can be distinguished from *C. morakotii* which has longer phialides (16–20 × 2–3 µm), and bigger aseptate conidia (4–12 × 1–2 µm). Thus, morphological characters suggest that strain YJ06221 is a new species in the genus *Cordyceps*, and it is described here as *C. yinjiangensis*.

Phylogenetic analyses of *Cordyceps* have been previously based on *LSU*, *SSU*, *RPB1*, *RPB2* and *TEF* (Sung *et al*. 2007). Tasanathai *et al*. (2016) reported two new species by *ITS* and *TEF* loci. In the present study, concatenated analyses of (*LSU+RPB2+TEF*) and (*ITS+TEF*) produced ML and Bayesian trees that were largely congruent. The majority of branches were strongly supported in both analyses. The six strains of *C. yinjiangensis* clustered together, and were distinct from *C. morakotii*. Thus, molecular phylogenetic results supported the morphologically based conclusion that *C. yinjiangensis* is a new species.

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