Morphology and molecular study of three new *Cordycipitoid* fungi and its related species collected from Jilin Province, Northeast China

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Research

Keywords: Cordyceps, phylogenetic study, host, new species, relationship

Posted Date: April 26th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-422247/v1

Abstract

Cordyceps are notable medicinal fungi in China, which pathogenic on insects and with high biodiversity in tropical and subtropical regions. Recently, three new *Cordyceps* species, *Cordyceps changchunensis* and *Cordyceps jingyuetanensis* grow on pupae of Lepidoptera, and *Cordyceps changbaiensis* grows on larvae of Lepidoptera, and one new record of Northeast China, *Cordyceps taishanensis*, are found in Jilin Province, China and detailed descriptions were obtained based on morphological and ecological characteristics. These three new species are similar to *Cordyceps militaris* group but distinctly distinguishable from known species. *Cordyceps changchunensis*, characterised by its small and light yellow to orange stromata that forks-like occasionally, covered with white mycelium at base of stipe, globose to ovoid perithecia, is macroscopically similar to *Cordyceps militaris. Cordyceps changbaiensis* clearly discriminated from other *Cordyceps* species by its white to orange and branched stromata, clavate to cylindrical fertile apical portion, immersed and globose to ovoid perithecia. Moreover, unbranched, clavate and orange to light red stromata, almond-shaped to ovoid and immersed perithecia differ *Cordyceps jingyuetanensis* from other *Cordyceps* species. ITS and nrLSU sequences are undertaken, and phylogenetic trees based on Maximum Likelihood analysis and Bayesian inference analysis confirmed the results of our morphological study. The phylogenetic result showed that the three new species gather with *Cordyceps militaris* but formed clades alone. Further analysis shows that the group of *Cordyceps militaris* mainly host on larvae of Lepidoptera.

Introduction

The family Cordycipitaceae belongs to Hypocreales with plant-, animal-, and fungus-based nutrition modes (Sun et al. 2007, Vega et al. 2009). The species of Cordycipitaceae are wide variety infect to invertebrates, and in the tropics and subtropics are known to have the highest species diversity (Kobayasi 1942, 1981). According to statistics, over 900 species of Cordycipitoid fungi are reported worldwide (Zha et al. 2018). In China, more than 146 species are recorded (Yan and Bau 2015).

Cordycipitoid fungi were first described in 1753 when the species was described under the genus *Clavaria* Vaill. ex L. as *Clavaria militaris* L., later be proved to be *Cordyceps militaris* (L.) Fr.. The genus *Cordyceps* Fr. was established by Fries in 1818, encompassing over 450 species (Kobayasi 1982, Luangsa-ard et al. 2007). Compared with a large number of species, subdivisions into infrageneric groups, e.g. subgenera and sections, have been proposed in *Cordyceps* classification, traditionally based on morphological and ecological characters (Stensrud et al. 2005). Modern classification of *Cordyceps* based on the studies of Kobayasi (1941, 1982), three subgenera, *C.* subg. *Cordyceps*, *C.* subg. *Ophiocordyceps* and *C.* subg. *Neocordyceps* were recognised. Subg. *Cordyceps* was characterised by the production of either immersed or superficial perithecia, which approximately right angles to the surface of stroma, and ascospores break into part-spores at maturity. Mains (1958) holds a different viewpoint, the genus *Cordyceps* was divided from a different perspective, and two subgenera, *C.* subg. *Cryptocordyceps* and *C.*subg. *Racemella*, were added. Furthermore, Kobayasi and Mains have conflicting views on the division of *C.* subg. *Ophiocordyceps* and *C.* subg. *Neocordyceps*. Based on the phylogenetic study, the Cordycipitoid fungi belong to six genera (*Cordyceps* Fr., *Metacordyceps* G.H.Sung et al., *Tyrannicordyceps* Kepler & Spatafora, *Elaphocordyceps* G.H.Sung & Spatafora, *Ophiocordyceps* Petch, and *Polycephalomyces* Kobayasi emend. Kepler and Spatafora) across three families, Cordycipitaceae, Clavicipitaceae and Ophiocordycipitaceae (Yan and Bau 2015, Sun et al. 2007).

The host of Cordycipitoid fungi is varied, which always parasitic with larvae of swifts, pupae of Lepidoptera, spider etc. Cordycipitoid fungi have a strong relationship with the environment and its host (Zha et al. 2019).

In this study, three new species and one new record to Northeast China of *Cordyceps* are reported, based on morphology and molecular study. Furthermore, the relationship between the host and *Cordyceps* species is analysed.

Material And Methods

Sampling and morphological studies

Fresh ascocarps were collected in Jilin Province, China. Specimens were photographed in situ. The habitat, altitude, soil characteristics, nearby trees and shrubs were recorded. The size of the stromata was measured when fresh. After examination

and description of the fresh macroscopic characters, the specimens were dried in an electric drier at 40–45°C.

Descriptions of macroscopic characters were based on field notes and photographs. The dried specimens were rehydrated in 94% ethanol for microscopic examination and then mounted in 3% KOH, 1% Congo Red, and Melzer's reagent (Torres et al. 2005), and using a Zeiss Axio Lab. A1 microscope for observation. A minimum of 100 part-spores was measured from 2 different ascocarps collected. The colours correspond to the *Flora of British fungi: colour identification chart* (Royal 1969). The specimens examined are deposited in the Herbarium of Mycology of Jilin Agricultural University (HMJAU).

DNA extraction, PCR amplification and sequencing

Total DNA was extracted from dried specimens and strains using the NuClean Plant Genomic DNA Kit (Kangwei Century Biotechnology Company Limited, Beijing, China). Sequences of the nuclear large ribosomal subunits (LSU) and internal transcribed spacer region (ITS) were used for phylogenetic analysis. The internal transcribed spacer (ITS) was amplified using the primer pair ITS4–ITS5 (White et al. 1990), and nuclear large ribosomal subunits (LSU) was amplified using the primer pair LROR–LR7 (Stensrud et al. 2005).

Reaction programs were the same as those described by Yan and Bau (2015) for ITS. As for LSU, we followed Castillo et al. (2018). PCR products were visualised via UV light after electrophoresis on 1% agarose gels stained with ethidium bromide and purified using Genview High-Efficiency Agarose Gels DNA Purification Kit (Gen-View Scientific Inc., Galveston, TX, USA). The purified PCR products were sent to Sangon Biotech Limited Company (Shanghai, China) for sequencing using the Sanger method. The new sequences were deposited in GenBank.

Data analysis

Based on the results of BLAST and morphological similarities, the sequences obtained are listed in Table 1. A dataset comprising of sequences from this study, 28 representative sequences showing the highest similarity to *Cordyceps* spp. and the outgroup *Metacordyceps* spp., *Nigelia martiale* (Speg.) Luangsa-ard & Thanakitp., *Ophiocordyceps* spp. and *Tolypocladium ophioglossoides* (J.F. Gmel.) C.A. Quandt, Kepler & Spatafora retrieved from GenBank were aligned with using ClustalX (Thompson et al. 1997) and MAFFT (Katoh and Standley 2013). The tree construction procedure was performed in PAUP* version 4.0b10 (Swofford et al. 2002) as described by Jiang et al. (2011). All characters were equally weighted, and gaps were treated as missing data.

MrMODELTEST 2.3 was used to determine the best fitting substitution model for each data set for Bayesian inference, which was calculated with MrBayes 3.2.6 with a general time-reversible DNA substitution model and a gamma distribution rate variation across sites (Ronquist and Huelsenbeck 2003). Four Markov chains were run for two runs from random starting trees for one million generations until the split deviation frequency value was < 0.01, and trees were sampled every 100 generations. RaxMLGUI beta 2.0 (Edler et al. 2021) was used for maximum likelihood (ML) analysis with 1,000 bootstrap replicates using the GTRGAMMA algorithm to perform a tree inference and search for optimal topology (Vizzini et al. 2015)

Table 1
Voucher information and GenBank accession numbers of ITS and LSU rDNA sequences of *Cordyceps changchunensis*, *Cordyceps changbaiensis*, *Cordyceps jingyuetanensis* and related species used in this study

Species name	Specimen/Strain number	Host/Substratum	GenBank accession numbers		References
			ITS	LSU	
A. lecanii	CBS 101247	Scale insects (Homoptera)	JN049836	AF339555	Kepler et al. 2012
A. tuberculatus	NBRC 106949/BCC 16819	Lepidoptera	JN943318	MF416546	Schoch et al. 2012
B. cardinalis	CBS 113414	Lepidoptera	MH862930	MH874497	Vu et al. 2019
B. pseudomilitaris	NBRC 101411	Lepidoptera	JN943308	JN941395	Schoch et al. 2012
C. bassiana	IFO 4848	Lepidoptera	AB027382	AB027382	Nikoh & Fukatsu 2000
C. bifusispora	ARS 5690/EFCC 8260	Lepidoptera	AY245627	EF468807	Kuo et al. 2005, Sung et al. 2007
C. brongniartii	NBRC 101395	Lepidopteran pupae	JN943298	JN941382	Schoch et al. 2012
C. cateniobliqua	CBS 153.83	Lepidoptera	MH861560	——	Vu et al. 2019
C. changbaiensis	**HMJAU 48255**	**Lepidoptera**	**MW893252**	**MW893277**	This study
C. changbaiensis	**CCMJ 48260**	**Lepidoptera**	**MW893270**	**MW893272**	This study
C. changchunensis	**HMJAU 48251**	**Lepidoptera**	**MW893249**	**MW893274**	This study
C. changchunensis	**HMJAU 48252**	**Lepidoptera**	**MW893250**	**MW893275**	This study
C. changchunensis	**HMJAU 48259**	**Lepidoptera**	**MW893251**	**MW893276**	This study
C. chiangdaoensis	BCC 75734/TBRC 7274	Coleopteran larvae	KT261394	MF140732	Tasanathai et al. 2016, Mongkolsamrit et al. 2018
C. coleopterorum	CBS 110.73	Coleoptera	AY624177	JF415988	Luangsa-Ard et al. 2005, Kepler et al. 2012
C. exacerbated	ARSEF 5689	Scarabaeid adult (Coleoptera)	JN049827	AF339524	Kepler et al. 2012, Sung et al. 2007
C. exasperata	MCA 2155	Lepidoptera	——	MF416542	Kepler et al. 2017
C. farinosa	CBS 111113	Lepidoptera	AY624181	MF416554	Luangsa-Ard et al. 2005, Kepler et al. 2017
C. fumosorosea	CBS 244.31	Coleoptera	AY624182	MF416557	Luangsa-Ard et al. 2005, Kepler et al. 2017
C. hepialidicola	——	Lepidoptera	AF315649	——	Unpublished
C. jingyuetanensis	**HMJAU 48253**	**Lepidoptera**	**MW 893253**	**MW893278**	This study
C. jingyuetanensis	**CCMJ 48261**	**Lepidoptera**	**MW 893271**	**MW893273**	This study
C. kyushuensis	HMAS 78115	Lepidoptera	EF368021	EF468813	Wang et al. 2008
C. militaris	OSC 93623	Lepidopteran pupae	JN049825	AY184966	Sung et al. 2007

Species name	Specimen/Strain number	Host/Substratum	GenBank accession numbers		References
			ITS	LSU	
C. militaris	**HMJAU 48256**	**Lepidopteran pupae**	**MW888227**	**MW893279**	This study
C. morakotii	BCC 55820/TBRC 7276	Hymenoptera	KT261389	MF140731	Tasanathai et al. 2016, Mongkolsamrit et al. 2018
C. ninchukispora	BCC 30937	Lepidoptera	FJ765274	FJ765242	Unpublished
C. ningxiaensis	HMJAU 25074	Fly pupae (Diptera)	KF309668	KF309671	Yan et al. 2015
C. polyarthra	——	Lepidoptera	AJ536548	——	Unpublished
C. pruinosa	ARSEF 5413	Lepidoptera	JN049826	MK761215	Kepler et al. 2012, Zha et al. 2019
C. qingchengensis	MFLU 17-1022	Lepidoptera	KY423506	MK761211	Zha et al. 2019
C. rosea	Spat 09–053	Lepidoptera	——	MF416536	Kepler et al. 2017
C. roseostromata	ARSEF 4870	Larva, not specified	AY245637	AF339523	Kuo et al. 2005, Sun et al. 2001
C. spegazzinii	ARSEF 7850	Diptera	DQ196435	DQ196435	Torres et al. 2005
C. taishanensis	——	Lepidoptera	FJ008927	——	Unpublished
C. taishanensis	**HMJAU 48254**	**Lepidoptera**	**MW888228**	**MW893280**	This study
C. tenuipes	TBRC 7266	Lepidoptera	MF140742	——	Mongkolsamrit et al. 2018, Vu et al. 2019
I. cicadae	GACP 07071701	Hemiptera	KX017277	MK761212	Unpublished
I. japonica	BCC 2808	Lepidoptera	AY624199	——	Luangsa-Ard et al. 2005
M. taii	ARSEF 5714	Lepidoptera	JN049829	AF543787	Sung et al. 2007
M. yongmunense	EFCC 2131	Lepidoptera	JN049856	EF468833	Kepler et al. 2012, Sung et al. 2007
N. martiale	HMAS 197472 (S)	Coleoptera	JN049881	JF415975	Kepler et al. 2012
O. acicularis	OSC 12858/OSC 110987	Coleoptera	JN049820	EF468805	Kepler et al. 2012
O. clavata	NBRC 106961	Coleoptera	JN943327	JN941414	Schoch et al. 2012
O. gracilis	EFCC 8572	Lepidoptera	HM142942	EF468811	Zhong et al. 2010, Sung et al. 2007
O. rubiginosoperitheciata	NBRC 106966	Coleoptera	JN943344	JN941437	Schoch et al. 2012
O. sinensis	ARSEF 6282	Lepidopteran pupae	HM595981	HM595885	Chan et al. 2011
T. ophioglossoides	NBRC 106331	*Elaphomyces* sp.	JN943320	JN941408	Schoch et al. 2012

Results

Phylogenetic analysis

The phylogenetic tree based on ITS from Bayesian analysis included sequences from 46 fungal samples representing 42 taxa, and the result showed in Fig. 1. According to the phylogenetic tree, the three new species gather into one branch with *Cordyceps militaris*, *Cordyceps roseostromata* Kobayasi & Shimizu, *Cordyceps taishanensis* B. Liu, P.G. Yuan & J.Z. Cao, *Cordyceps kyushuensis* A. Kawam., and *Cordyceps hepialidicola* Kobayasi & Shimizu, but the species *C. jingyuetanensis* is not gathering in one branch by itself. Meanwhile, the genus *Cordyceps* can be divided into three independent clades. Furthermore, *Cordyceps* and *Akanthomyces* Lebert are a sister clade to *Blackwellomyces* Spatafora & Luangsa-ard.

For these reasons, the combined ITS + LSU dataset included 88 fungal samples representing 42 taxa were used for analysis, and the result showed in Fig. 2. In this time, the three new species are in three independent clades included in *C. militaris* complex, *C. jingyuetanensis* closed to *Cordyceps hepialidicola* Kobayasi & Shimizu, different from Fig. 1. From the phylogenetic tree (Fig. 2), the species of *Cordyceps* are divided into three independent clades. Furthermore, the family Cordycipitaceae gathered into three clades, and the genus *Akanthomyces* formed a sister clade to the genus *Cordyceps*.

Taxonomy

Cordyceps changchunensis J.J. Hu, Bo Zhang & Y. Li **sp. nov.** (Figs. 3, 4)

Mycobank No.: MB 839249

Etymology: *changchunensis*: referring to Changchun, the location of the holotype.

Diagnosis: *Cordyceps changchunensis* can be easily differentiated from closely related species *C. militaris* by its unique host, smaller stromata, immersed perithecia and bigger part-spores (2.6–6×1.0–1.4 μm).

Type: CHINA: Jilin Province: Changchun City, Jingyuetan national forest park, 27 August 2018, Jia-Jun Hu, Bo Zhang and Gui-Ping Zhao (HMJAU 48251, holotype; GenBank Acc. nos.: ITS = MW 893249, LSU = MW893274),

Description: Sexual Morph: Stromata 2.4–4.5 cm long, single or multiple, solitary to gregarious, arising from pupa; branched, sometimes single at base, then branched into two forks. Fertile apical portion, orange, clavate to globose, sometimes irregular, 2.0–3.5 cm long and 0.4–0.6 cm wide, distinctly distinguishable from the stipe. Sterile stipe fleshy, light yellow to orange, cylindrical, 1.3–3.3 cm long, and ca. 0.4 cm wide, usually with white mycelium at the base. Perithecia immersed at right angle to the surface of the fruiting body, globose to ovoid, 180–600×180–520 μm, with a thick wall about 10–15 μm. Asci cylindrical, 80–300×2.5–5 μm, 8–spored, apex of ascus hemispheric, 3.0–4.0×2.0–3.0 μm. Ascospores oblong, 2.6–6×1.0–1.4 μm, smooth, hyaline in 3% KOH, thin-walled, inamyloid.

Asexual Morph: Unknown.

Other specimen examined: CHINA. Jilin Province: Changchun City, Jingyuetan national forest park, 20 August 2015, Bo Zhang (HMJAU 48259, GenBank Acc. nos.: ITS = MW893251; LSU = MW893276); Changchun City, Jingyuetan national forest park, 18 August 2018, Bo Zhang (HMJAU 48252, GenBank Acc. nos.: ITS = MW893250; LSU = MW893275).

Distribution: China (Jilin Province).

Host: Growing on pupae of Lepidoptera.

Note

Species of *Cordyceps changchunensis* sp. nov. has a significant difference between the common species of *Cordyceps*, *C. militaris*, morphologically. The stromata of *C. changchunensis* is thinner than *C. militaris*, besides stromata single to multiple.

Cordyceps changbaiensis J.J. Hu, Bo Zhang & Y. Li **sp. nov.** (Figs. 5, 6)

Mycobank No.: MB 839250

Etymology: *changbaiensis*: referring to Mt. changbai, the location of the holotype.

Diagnosis: The species is characterised by stromata and perithecia. It can be easily distinguished from related species by orange to white and branched stromata, globose to ovoid perithecia and bigger part-spores (3.0–7.0×1.0–1.4 μm).

Type: CHINA. Jilin Province: Yanbian Korean Autonomous Prefecture, Antu County, Changbai Mountain, 6 September 2019, Jia-Jun Hu and Bo Zhang (HMJAU 48255, holotype, GenBank Acc. nos.: ITS = MW893252; LSU = MW893277).

Description: Sexual Morph: Stromata 2.4–5.2 cm long, single or multiple, solitary, arising from the head of the host insect covered with white mycelia. Fertile apical portion, orange, clavate to cylindrical, 0.6–1.5 cm long and 0.2–0.6 cm wide, obviously distinguishable from the stipe. Sterile stipe fleshy, white to light yellow, cylindrical, 1.8–3.7 cm long and 0.2–0.5 cm wide. Perithecia immersed to the surface of the fruiting body, globose to ovoid, 120–230×90–170 μm, with a thick wall about 15 μm. Asci cylindrical, 225–625×4–5 μm, 8–spored, apex of ascus hemispheric, 3.0–4.0×2.2–3.2 μm. Ascospores oblong, 3.0–7.0×1.0–1.4 μm, smooth, hyaline in 3% KOH, thin-walled, inamyloid.

Asexual Morph: Unknown.

Distribution: China (Jilin Province).

Host: Growing on larvae of Lepidoptera.

Note

The most significant difference between *C. changbaiensis* and other *C. militaris* complex species is that the stromata of *C. changbaiensis* are orange, white at the base, perithecia globose to ovoid and the bigger part-spores.

Cordyceps **jinyuetanensis** J.J. Hu, Bo Zhang & Y. Li **sp. nov.** (Figs. 7, 8)

Mycobank No.: MB 839251

Etymology: *jinyuetanensis*: referring to Jingyuetan national forest park, the location of the holotype.

Diagnosis: *C. jingyuetanensis* different from other species by grows on pupae, orange to light red stromata, immersed and almond-shaped to ovoid perithecia.

Type: CHINA. Jilin Province: Changchun City, Jingyuetan national forest park, 27 August 2018, Jia-Jun Hu, Bo Zhang and Gui-Ping Zhano (HMJAU 48253, holotype, GenBank Acc. nos.: ITS = MW893253; LSU = 893278).

Description: Sexual Morph: Stromata 4–4.5 cm long, multiple, solitary, arising from pupae of Lepidoptera. Fertile apical portion, orange to light red, clavate, 0.8–1.3 cm long and 0.1–0.2 cm wide, obviously distinguishable from the stipe. Sterile stipe fleshy, light yellow to orange, cylindrical, 2.7–3.7 cm long and 0.1–0.2 cm wide, usually with white mycelium at the base. Perithecia immersed to the surface of the fruiting body, almond-shaped to ovoid, 220–340×110–220 μm, with a thick wall about 15–20 μm. Asci cylindrical, 225–475×3–5 μm, 8-spored, apex of ascus hemispheric to irregular, 3.0–4.0×1.4–2.8 μm. Ascospores oblong, 2.8–5.0×1.0–1.4 μm, smooth, hyaline in 3% KOH, thin-walled, inamyloid.

Asexual Morph: Unknown.

Distribution: China (Jilin Province).

Host: Growing on pupae of Lepidoptera.

Note

There are about 20 species of Cordycipitiod fungi hosts on pupae, like *Cordyceps morakotii* Tasan., Thanakitp. & Luangsa-ard reported from Thailand in 2016, which is host on ant pupae. *C. jingyuetanensis* is different from these species in host, stromata, perithecia or the size of part-spores.

Table 2
Morphological comparisons of sexual states of *Cordyceps changchunensis*, *Cordyceps changbaiensis* and *Cordyceps jingyuetanensis* with its closed species

Species	Host	Stromata	Fertile part	Perithecia	Asci	Ascospores	Reference
Beauveria bassiana	Larvae of Lepidoptera	Single or several, unbranched, slender and cylindrical, brownish yellow to yellowish	18.7–33.3×2.8–8.0 mm	Ovalia, 610–720×230–320 μm, immersed to surface	Cylindrical, 230–590×3.5–4.0 μm with ascus cap 3.6-4.0 μm in diameter	Filamentous, 300–570×1.0 μm, not broken into part-spores	Li et al. 2001
Blackwellomyces pseudomilitaris	Larvae of Lepidoptera	Single or cluster, simple or branched, cylindrical, white to white-orange	15–30×0.9–3 mm	Elongate-ellipsoid or elongate-ovoid, 290–570×120–245 μm, superficial	Filiformis, 290–410×5–6 μm,	Filiformis, 280–390×1 μm, not broken into part-spores	Hywel-Jones 1994
Cordyceps bifusispora	Larvae of Lepidoptera	Simple, cylindrical clavate, whitish	6×1.3 mm	pyriform, with protruding apices, yellowish, 300×150–170 μm, immersed	Cylindrical, 200–220×3–4.5 μm	Bifusiform, 145–220 μm in length, central part filiform about 0.4 μm wide, terminal parts narrowly fusiform, about 30×1.6 μm and 3 septate	Eriksson 1982
Cordyceps kyushuensis	Larvae of Lepidoptera	Cluster, cylindrical, Light yellow to orange red	20–30×5–8 mm	Ovalia, 300–500×200–300 μm, half-buried	Cylindrical, 3–4.5 μm wide	Short cylindrical, part-spores 5–7×0.7–1 μm	Li et al. 2015, Guo & Li 2000
Cordyceps militaris	Lepidopteran pupae	Single or several, clavate, orange	10–20×3–5 mm	Conical, half-buried	Clavate, 300–400×4–5 μm	Filiformis, part spores 2–3×1 μm	Li et al. 2015
Cordyceps ningxiaensis	Fly pupae (Diptera)	One to two in a group, clavate, orange	1.2–3×1.2–2.8 mm	Ellipsoid to ovoid, 288–400×103–240 μm, with a wall about 10 μm thick, loosely embedded at right angles to the surface	Cylindrical, 168–205×(3.7–)4.1–5.5(–6.6) μm, with oblate spheroid or hemispherical refractive cap 3.4–3.8×2.9–3.4 μm at apex	Filiform, irregularly multiseptate, part-spores 3.6–7.8×1.0–1.4 μm	Yan & Bau 2015

Species	Host	Stromata	Fertile part	Perithecia	Asci	Ascospores	Reference
Cordyceps polyarthra	Larvae of Lepidoptera	Cespitose, narrowly clavate, light yellow to reddish brown	—	Ovoid, 250–450×125–250 μm, brown, with a definite wall 25 μm thick, embedded at right angles to the surface	Cylindrical, 150–260×3–4 μm, with a 1.5–2 μm thick cap	Filiform, part-spores 4–6×0.75–1 μm	Mains 1958
Cordyceps pruinosa	Larvae of Lepidoptera	Solitary or several, clavate, orange to red	2–8×1–3 mm	Ovoid to fusiform, 360–400×130–200 μm, crowded, red, ordinal in orientation, immersed	Cylindrical, 100–200×2.5–4 μm	Filiformis, part-spores 4–6×1 μm	Li et al. 2015
Cordyceps qingcengensis	Lepidopteran pupae	Branched (single at base, then branched into several (often 3) forks, basal stipe and upper branches slightly cylindrical, moderate width), yellow	7–9×2.0–2.5 mm	Ovoid but apex sharply pointed, 335–490×145–240 μm, partially immersed at right angle to the surface	Cylindrical, 180–200×2.4–4.0 μm wide, caps hemispheric, 1.8–2.2×2.5–3.2 μm	Filiform, 180–220×0.45–0.65 μm, not at all bifusiform and not broken into part-spores	Zha et al. 2019
Cordyceps roseostromata	Larva, not specified	Single or branched (single at base, then branched into several (2–4) forks	1.2–5×1.5–2.2 mm	Pyriform, 280–300×140–160 μm, Superficial	3–3.5×2.5–3 μm	4–5×1 μm	Kobayasi & Shimizu 1983
Cordyceps changchunensis	Lepidopteran pupae	Single or multiple, clavate, orange	2.0–3.5×0.4–0.6 mm	Globose to ovoid, 180–600×180–520 μm, with a thick wall about 10–15 μm, partially immersed at right angles to the surface	Cylindrical, 80–300×2.5–5 μm, caps hemispheric, 3.0–4.0×2.0–3.0 at apex	Oblong, 2.6–6×1.0–1.4 μm	This study

Species	Host	Stromata	Fertile part	Perithecia	Asci	Ascospores	Reference
Cordyceps changbaiensis	Larvae of Lepidoptera	Single or multiple, clavate, white to orange	0.6–1.5×0.2–0.6 mm	Globose to ovoid, 120–230×90–170 µm, with a thick wall about 15 µm, immersed to surface	Cylindrical, 225–625×4–5 µm, caps hemispheric, 3.0–4.0×2.2–3.2 µm at apex	Oblong, 3.0–7.0×1.0–1.4 µm	This study
Cordyceps jingyuetanensis	Lepidopteran pupae	Single or multiple, clavate, orange to light red	0.8–1.3×0.1–0.2 mm	Almond-shaped to ovoid, 220–340×110–220 µm, with a thick wall about 15–20 µm, immersed to surface	Cylindrical, 225–475×3–5 µm, caps hemispheric to irregular, 3.0–4.0×1.4–2.8 µm at apex	Oblong, 2.8–5.0×1.0–1.4 µm	This study

Cordyceps taishanensis B. Liu, P.G. Yuan & J.Z. Cao, Acta Mycol. Sin. 3(4): 192 (1984)

Description: Sexual Morph: Stromata 3–5.5 cm long, single or multiple, solitary to gregarious, arising from the head and abdomen of the host insect covered with white mycelia. Fertile apical portion, orange, clavate to cylindrical, 1.0–3.0 cm long and 0.1–0.3 cm wide, obviously distinguishable from the stipe. Sterile stipe fleshy, light yellow to orange, cylindrical, 2–2.5 cm long and 0.1–0.3 cm wide, usually with white mycelium at the base. Perithecia immersed at right angles to the surface of the fruiting body, ovoid, 240–370×80–190 µm, with a thick wall about 10 µm. Asci cylindrical, 200–400×3–5 µm, 8–spored, apex of ascus hemispheric, 3.0–4.1×2.2–3.0 µm. Ascospores oblong, 3.1–5.4×1.0–1.4 µm, smooth, hyaline in 3% KOH, thin-walled, inamyloid.

Asexual Morph: Unknown.

Specimen examined: CHINA. Jilin Province: Changchun City, Jingyuetan national forest park, 27 August 2018, Jia-Jun Hu, Bo Zhang and Gui-Ping Zhao (HMJAU 48254, GenBank Acc. nos.: ITS = MW888228; LSU = MW893280).

Distribution: China (Jilin and Shandong Province).

Host: Growing on larvae of Lepidoptera.

Note

Cordyceps taishanensis was first described by Liu et al. in 1984. It was found in Mount Taishan, Taian City, Shandong Province. Moreover, at this time, it was the first time found in Northeast China-Changchun City, Jilin Province.

Cordyceps militaris (L.) Fr., Observ. mycol. (Havniae) 2: 317 (cancellans) (1818)

Specimen examined: CHINA. Yunnan Province: Qujin City, Huize County, 30 July 2019, Jia-Jun Hu, Bo Zhang and Di-zhe Guo (HMJAU 48256, GenBank Acc. nos.: ITS = MW888227; LSU = MW893279); Jilin Province: Changchun City, Jingyuetan national forest park, 25 August 2018, Jia-Jun Hu and Yong-Lan Tuo (HMJAU 48257); Changchun City, Jingyuetan national forest park, 25 August 2018, Jia-Jun Hu, Bo Zhang and Gui-Ping Zhano (HMJAU 48258).

Note

Cordyceps militaris is a widely distributed species and also a well-known medicinal fungus in China. At this time, we collected samples from many different places. The morphological and molecular evidence shows no apparent differences between each

other. However, the habitat is markedly different.

Discussion

In this study, three new species and one new record of Northeast China in the *Cordyceps militaris* group are described. *C. changchunensis* was found in the broad-leaved forest around the hillside in Jingyuetan national forest park in Jilin Province. The place where we found *C. changchunensis* is also present *C. militaris*, but both can be clearly separated by morphology. The stromata of *C. militaris* are bigger, single or gregarious, perithecia half-buried, thick clavate, brown, 500–1089×132–264 µm, asci 142–574×4–6 µm, part-spores 2–4×1 µm, besides the host and the base of sterile stipe covered with white mycelium. While the stromata of *C. changchunensis* are single or branched, perithecia immersed, ovoid, 180–600×180–520 µm, part-spores 2.6–6×1.0–1.4 µm and white at the base of sterile stipe.

C. changbaiensis was founded on a broad-leaved forest covered with ferns from Changbai Mountain in Jilin Province. *C. changbaiensis*, *Cordyceps roseostromata* and *Cordyceps kyushuensis* are closed species. The stromata of *Cordyceps kyushuensis* are 4–8 cm long and 5–8 mm wide, gregarious or fascicled, and growth from the head or abdomen of the host, perithecia half-buried, ovoid, 300–500×200–300 µm, pale brown, part-spores cylindrical, 5–7×0.7–1 µm (Li et al. 2015). As for *Cordyceps roseostromata*, stromata single or branched, perithecia not immersed, pyriform, 280–300×140–160 µm, part-spores 4–5×1 µm, and host on larva of Coleoptera (Kobayasi & Shimizu 1983). While the stromata of *C. changbaiensis* are single or branched, perithecia immersed, ovoid, globose to ovoid, 120–230×90–170 µm, asci cylindrical, 225–625×4–5 µm, ascospores oblong, 3.0–7.0×1.0–1.4 µm, host on larva of Lepidoptera, and growth from the tail or abdomen of the host.

Cordyceps hepialidicola reported from Japan close to *C. jingyuetanensis* in phylogenetic, but there are distinct morphological differences. The stromata of *Cordyceps hepialidicola* multiple, branched on the top sometimes, grow from the head of the host, perithecia immersed, 300–350×500 µm, part-spores 3–4×1 µm. While the stromata of *C. jingyuetanensis* is single or multiple, orange to light red the most apparent difference. Perithecia are smaller than *Cordyceps hepialidicola*, 220–340×110–220µm, part-spores a little bigger than *C. hepialidicola*. A review of the literature reveals that we found a few Cordycipitiod fungi hosts on the pupae, like the unusual medicinal fungi *Ophiocordyceps sinensis*, *C. militaris*, *Isaria cicadae*, and also like the two new species, *C. ningxiaensis* and *C. qingchengensis*, reported from China in 2015 and 2019. Nevertheless, we can easily distinguish between these species and the two newly species reported in this study, *C. ningxiaensis* growth on the pupae of Diptera, *I. cicadae* growth on the pupae of Hemiptera, and the stromata of *C. qingchengensis* are yellow, single or branched on the top.

According to Yan and Bau (2015) and other literature, about 38 species belong to *C. militaris* group. All the ITS and LSU sequence were downloaded from NCBI, and phylogenetic trees were built with the three new species. The result shows that the phylogenetic tree is mainly divided into two branches, one is Cordycipitaceae, the other is Clavicipitaceae and Ophiocordycipitaceae. Moreover, the Cordycipitaceae branch is divided into three clades, the *Akanthomyces* clade near the *Cordyceps* clade. The new species included in the *Cordyceps militaris*, *Cordyceps kyushuensis, Cordyceps hepialidicola*, *Cordyceps rosea*, and *Cordyceps taishanensis* clade, and formed clades alone.

In China, a Cordycipitiod fungus grows on *Platylomia pieli* Kato was treated as *I. cicadae*, named based on a Brazilian specimen for a long time. In recent days, the teleomorph was discovered in Mt. Jinggang, Jiangxi Province, China. The result shows that there was a misunderstanding about *I. cicadae* in China. The species called *I. cicadae* in China is a new species, *Cordyceps chanhua* Z.Z. Li, F.G. Luan, Hywel-Jones, C.R. Li & S.L. Zhang (Li et al. 2021). In our phylogenetic study, *I. cicadae*, based on Chinese sequence, gathers into one branch with *Cordyceps* species, showing a similar result. Furthermore, *Isaria japonica* Yasuda reported from Japan, shows exceptionally high affinity with the genus *Cordyceps*, however, the teleomorph was still a mystery, and need a more intensive study.

The host information is collected (Table 1). The result shows that the *C. militaris* complex fungi mainly host on the larva of Lepidoptera and possibly infect the larva of Coleoptera, Hemiptera, Hymenoptera or Diptera. Moreover, most fungi in *C. militaris* complex infect the pupae of Lepidoptera, only *C. morakotii* growth on the pupae of Hymenoptera. From the result, research on Cordycipitoid fungi, the host is the most crucial feature.

Declarations

Acknowledgement

This study is funded by the Development and Utilisation of Economic Fungi Resources in Changbai Mountain (2018YFGH000047), the National Natural Science Foundation of China (No. 31770012), the China Agriculture Research System (CARS20), and the Jilin Province Science and Technology Development Plan Project (20190201256JC). The authors are very thankful to Dr. Xue-Fei Li, Hui-Ze Hu, Fang-Fang Zhang from Engineering Research Centre of Edible and Medicinal Fungi, Ministry of Education, for help during this study.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Authors' contributions

HJJ, ZGP, TYL, RG, QZX and ZZH collected the specimen, DD helped modify the English expression, ZB and LY designed this experiment.

References

Castillo LP, Osorio A, Vargas N, Sanjuan T, Grajales A, Restrepo S: Genetic diversity of the entomopathogenic fungus *Cordyceps tenuipes* in forests and butterfly gardens in Quindío, Colombia. Fungal biology 2018, 122:891-899.

Chan WH, Ling KH, Chiu SW, Shaw PC, But PPH: Molecular Analyses of *Cordyceps gunnii* in China. Journal of Food & Drug Analysis 2011, 19:18-25.

Eriksson O: *Cordyceps bifusispora* spec. nov. Mycotaxon 1982, 15:185-188.

Edler D, Klein J, Antonelli A, Silvestro D: raxmlGUI 2.0 beta: a graphical interface and toolkit for phylogenetic analyses using RAxML. Methods in Ecology and Evolution 2021, 12:373-377.

Guo Y, Li C: *Cordyceps kyushuensis* new to China. Mycosystema 2000, 19:296.

Hywel-Jones NL: *Cordyceps khaoyaiensis* and *C. pseudomilitaris*, two new pathogens of lepidopteran larvae from Thailand. Mycological Research 1994, 98: 939-942.

Jiang X, Yu H, Xiang M, Liu X, Liu X: *Echinochlamydosporium variabile*, a new genus and species of Zygomycota from soil nematodes. Fungal Diversity 2011, 46:43-51.

Kobayasi Y: The genus *Cordyceps* and its allies. Science reports of the Tokyo Bunrika Daigaku 1941, 84:53–260.

Kobayasi Y: Keys to the taxa of the genera *Cordyceps* and *Torrubiella*. Transactions of the Mycological Society of Japan 1982, 23:329-54.

Kobayasi Y, Shimizu D: *Cordyceps* species from Japan 6. Bulletin of the National Science Museum, Tokyo 1983, 9:1-21.

Katoh K, Standley DM: MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular biology and evolution 2013, 30:772-780.

Kepler RM, Sung GH, Ban S, Nakagiri A, Chen MJ, Huang B, Li Z, Spatafora JW: New teleomorph combinations in the entomopathogenic genus *Metacordyceps*. Mycologia 2012, 104:182-197.

Kepler RM, Luangsa-Ard JJ, Hywel-Jones NL, Quandt CA, Sung GH, Rehner SA, Aime MC, Henkel TW, Sanjuan T, Zare R, Chen M, Li Z, Rossman AY, Spatafora JW, Shrestha B: A phylogenetically-based nomenclature for Cordycipitaceae (Hypocreales). IMA Fungus 2017, 8:335-353.

Kuo HC, Su YL, Yang HL, Chen TY: Identification of Chinese medicinal fungus *Cordyceps sinensis* by PCR-single-stranded conformation polymorphism and phylogenetic relationship. Journal of agricultural and food chemistry 2005, 53:3963-3968.

Li Y, Li TH, Yang ZL, Bau T, Dai YC: Atlas of Chinese macrofungal resources. Central China Farmers Publishing House, China 2015.

Li Z, Li C, Huang B, Fan M: Discovery and demonstration of the teleomorph of *Beauveria bassiana* (Bals.) Vuill., an important entomogenous fungus. Chinese Science Bulletin 2001, 46:751-753.

Li ZZ, Luan FG, HYWEL-JONES Nigel L, Zhang SL, Chen MJ, Huang B, Sun CS, Chen ZA, Li CR: Biodiversity of cordycipitoid fungi associated with *Isaria cicadae* Miquel II: Teleomorph discovery and nomenclature of chanhua, an important medicinal fungus in China. Mycosystema 2021, 40(1):95-107.

TAN You- Jiu DONG Jian -Feil

Liu B, Yuan TG, Cao JZ: A NEW SPECIES OF *CORDYCEPS* FROM CHINA. Mycosystema 1984, 3:192-195.

Luangsa-Ard JJ, Hywel-Jones NL, Manoch L, Samson RA: On the relationships of *Paecilomyces* sect. *Isarioidea* species. Mycological research 2005, 109:581-589.

Luangsa-ard JJ, Tasanathai K, Mongkolsamrit S, Hywel-Jones N: Atlats of Invertebrate-Pathogenic Fungi of Thailand. National Center of Genetic Engineering and Biotechnology, Thailand 2007.

Mains EB: North American entomogenous species of *Cordyceps*. Mycologia 1958, 50:169-222.

Mongkolsamrit S, Noisripoom W, Thanakitpipattana D, Wutikhun T, Spatafora JW, Luangsa-ard J: Disentangling cryptic species with isaria-like morphs in Cordycipitaceae. Mycologia 2018, 110:230-257.

Nikoh N, Fukatsu T: Interkingdom host jumping underground: phylogenetic analysis of entomoparasitic fungi of the genus *Cordyceps*. Molecular Biology and Evolution 2000, 17:629-638.

Ronquist F, Huelsenbeck JP: MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 2003, 19 :1572-1574.

Royal Botanic Garden E: Flora of British fungi: colour identification chart. HM Stationery Office 1969.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium: Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences 2012, 109:6241-6246.

Stensrud Ø, Hywel-Jones NL, Schumacher T: Towards a phylogenetic classification of *Cordyceps*: ITS nrDNA sequence data confirm divergent lineages and paraphyly. Mycological research 2005, 109:41-56.

Sung GH, Spatafora JW, Zare R, Hodge KT, Gams W: A revision of *Verticillium* sect. *Prostrata*. II. Phylogenetic analyses of SSU and LSU nuclear rDNA sequences from anamorphs and teleomorphs of the Clavicipitaceae. Nova Hedwigia 2001, 72:311-328.

Sung GH, Hywel-Jones NL, Sung JM, Luangsa-ard JJ, Shrestha B, Spatafora JW: Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. Studies in mycology 2007, 57:5-59.

Swofford DL: Phylogenetic analysis using parsimony (* and other methods) 2002.

Tasanathai K, Thanakitpipattana D, Noisripoom W, Khonsanit A, Kumsao J, Luangsa-ard JJ: Two new *Cordyceps* species from a community forest in Thailand. Mycological progress 2016, 15:28.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG: The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic acids research 1997, 25:4876-4882.

Torres MS, White J, Bischoff JF: *Cordyceps spegazzinii* sp. nov., a new species of the *C. militaris* group. Mycotaxon 2005, 94:253-263.

Vega FE, Goettel MS, Blackwell M, Chandler D, Jackson MA, Keller S, Koike M, Maniania NK, Monzon A, Ownley BH, Pell JK, Rangel DEN, Roy HE: Fungal entomopathogens: new insights on their ecology. Fungal Ecol 2009, 2:149–159.

Vizzini A, Vladimír Antonín, Sesli E, Contu M: *Gymnopus trabzonensis* sp. nov. (Omphalotaceae) and *Tricholoma virgatum* var. *fulvoumbonatum* var. nov. (Tricholomataceae), two new white-spored Agarics from turkey. Phytotaxa 2021, 226(2):119-130.

Vu D, Groenewald M, De Vries M, Gehrmann T, Stielow B, Eberhardt U, Al-Hatmi A, Groenewald JZ, Cardinali G, Houbraken J, Boekhout T, Crous PW, Robert V, Verkley GJM: Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Studies in mycology 2019, 92:135-154.

Wang L, Zhang WM, Hu B, Chen YQ, Qu LH: Genetic variation of *Cordyceps militaris* and its allies based on phylogenetic analysis of rDNA ITS sequence data. Fungal Divers 2008, 31:147-155.

White TJ, Bruns T, Lee SJWT, Taylor J: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 1990, 18:315-322.

Yan JQ, Bau T: *Cordyceps ningxiaensis* sp. nov., a new species from dipteran pupaee in Ningxia Hui Autonomous Region of China. Nova hedwigia 2015, 100:251-258.

Zha L, Huang SK, Xiao YP, Boonmee S, Eungwanichayapant P, McKenzie E, Kryukov V, Wu XL, Hyde KD, Wen TC: An Evaluation of Common *Cordyceps* (Ascomycetes) Species Found in Chinese Markets. International journal of medicinal mushrooms 2018, 20:1149-1162.

Zha LS, Wen TC, Huang SK, Boonmee S, Eungwanichayapant PD: Taxonomy and biology of *Cordyceps qingchengensis* sp. nov. and its allies. Phytotaxa 2019, 416:14-24.

Zhong X, Peng Q, Qi L, Lei W, Liu X: rDNA-targeted PCR primers and FISH probe in the detection of *Ophiocordyceps sinensis* hyphae and conidia. Journal of microbiological methods 2010, 83:188-193.

Figures

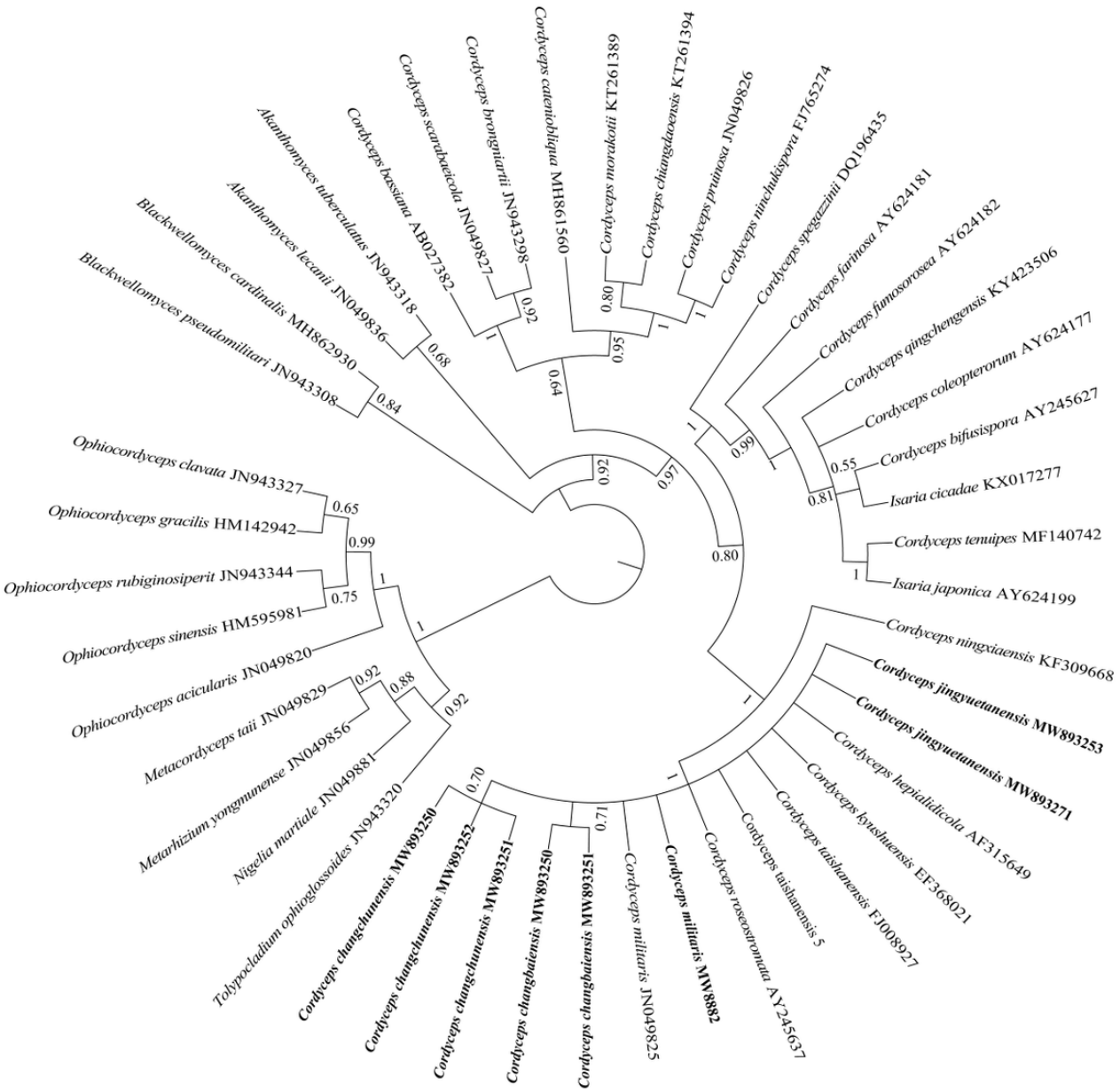

Figure 1

Phylogenetic tree of Cordycepitiod fungi based on ITS from Bayesian analysis

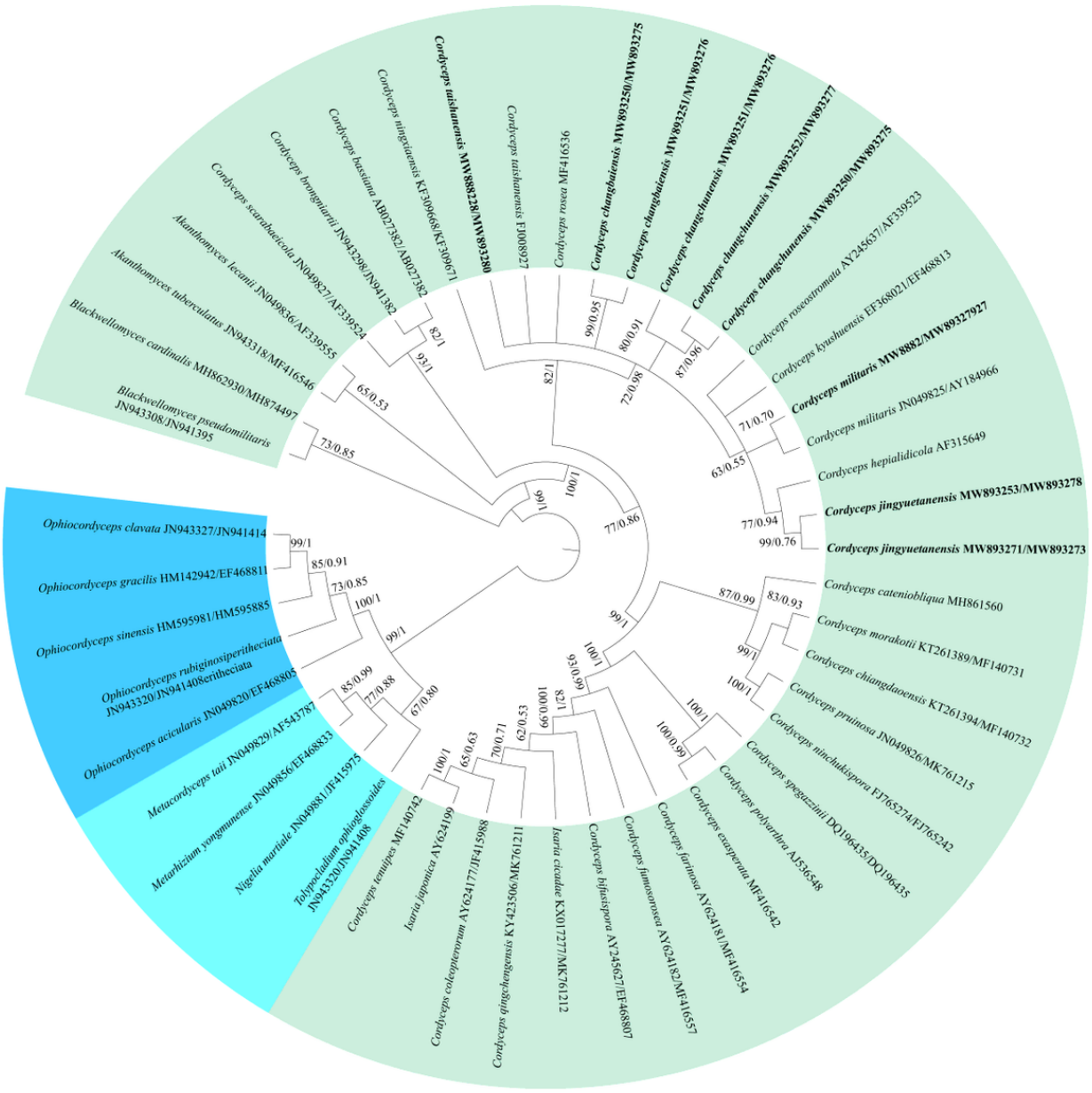

Figure 2

Phylogenetic tree of Cordycepitiod fungi based on ITS and LSU from Bayesian analysis and Maximum Likelihood analysis; self-examined sequences are shown in bold.

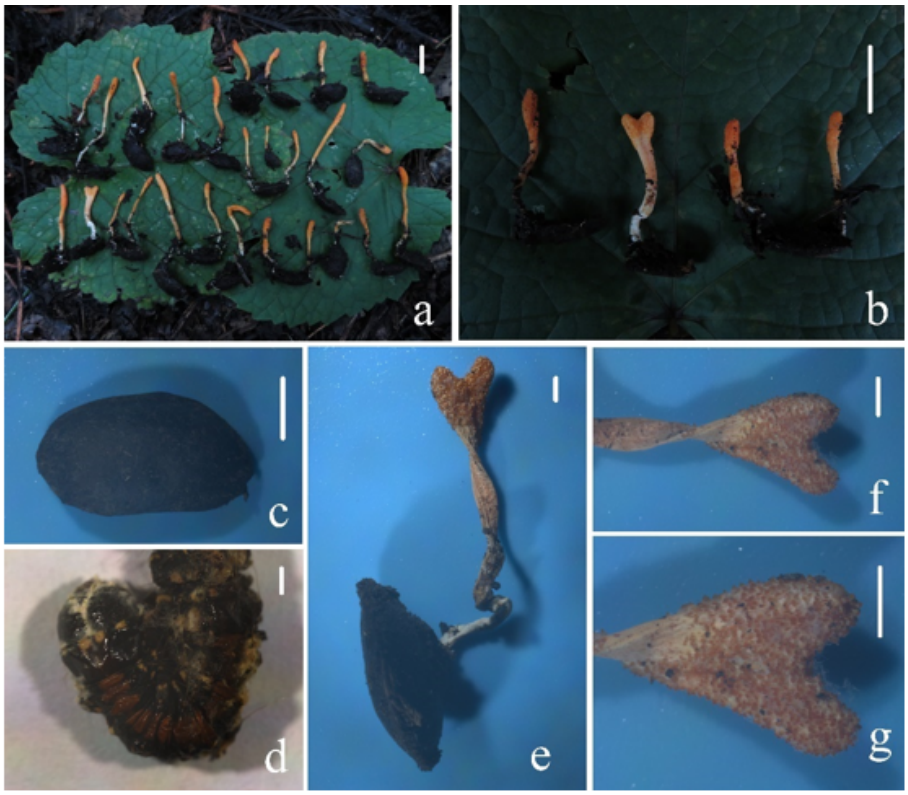

Figure 3

Macrocharacter of Cordyceps changchunensis (HMJAU 48251, holotype). a, b, e: Stromata and host of Cordyceps changchunensis. c, d: Host of Cordyceps changchunensis; f, g: Surface of fertile apex of ascostroma. Bars: a, b= 1 cm; c= 5 mm; d=1 mm; e, f, g= 2 mm.

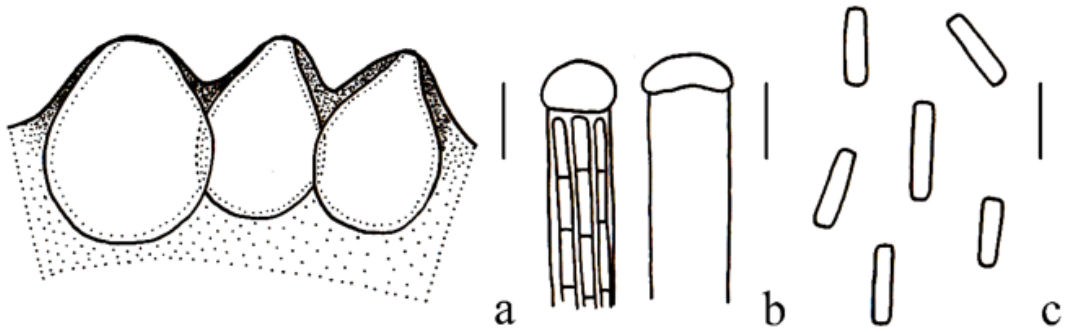

Figure 4

Microscopical characters of Cordyceps changchunensis (HMJAU 48251, holotype). a. Perithecia. b. Apex of ascus. c. Part-spores. Bars: a=100 μm; b, e: 5 μm.

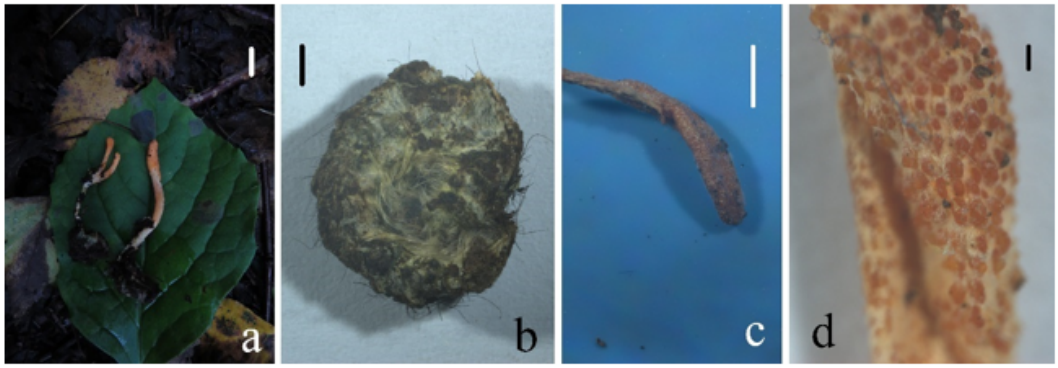

Figure 5

Macrocharacter of Cordyceps changbaiensis (HMJAU 48255, holotype). a: Stromata and host of Cordyceps changbaiensis. b: Host of Cordyceps changbaiensis; c, d: Surface of fertile apex of ascostroma. Bars: a= 1 cm; b-c=5 mm d= 200 μm.

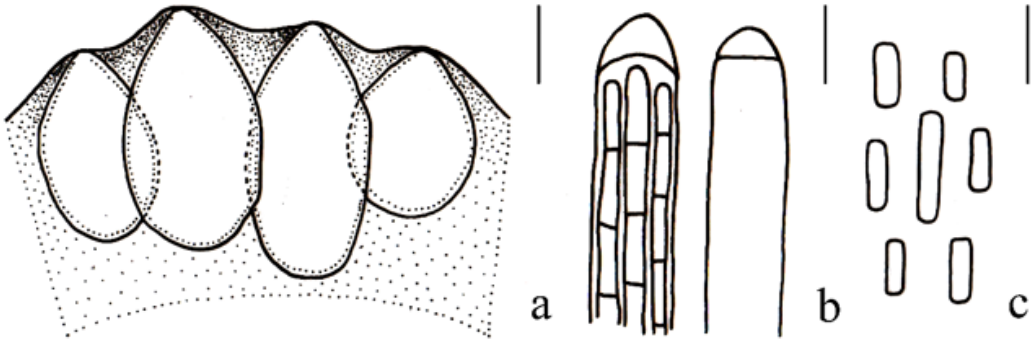

Figure 6

Microscopical characters of Cordyceps changbaiensis (HMJAU 48255, holotype). a. Perithecia. b. Apex of ascus. c. Part-spores. Bars: a=100 μm; b, c=5 μm.

Figure 7

Macrocharacter of Cordyceps jingyuetanensis (HMJAU 48253, holotype). a: Stromata and host of Cordyceps jingyuetanensis. b: Host of Cordyceps jingyuetanensis; c, d: Surface of fertile apex of ascostroma. Bars: a= 1 cm; b, c= 2 mm; c= 5 mm; d= 500 μm.

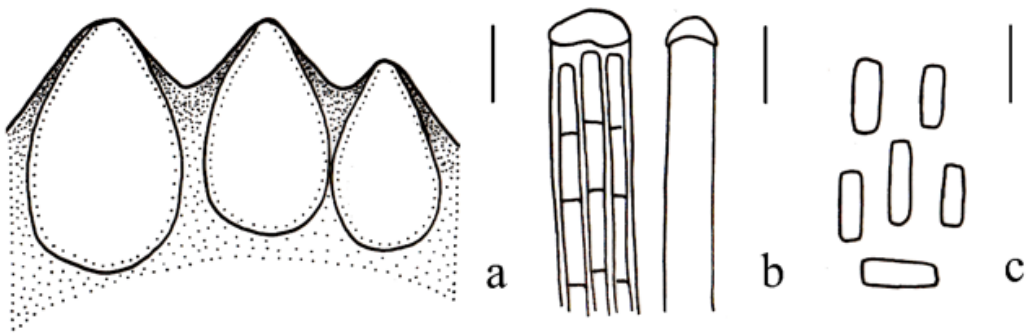

Figure 8

Microscopical characters of Cordyceps jingyuetanensis (HMJAU 48253, holotype). a. Perithecia. b. Apex of ascus. c. Part-spores. Bars: a=100 μm; b, c=5 μm.

Figure 9

Macrocharacter of Cordyceps taishanensis (HMJAU 48254). a: Stromata and host of Cordyceps taishanensis. b: Host of Cordyceps taishanensis; c, d: Surface of fertile apex of Ascostroma. Bars: b-c=5 mm; d=1 mm.

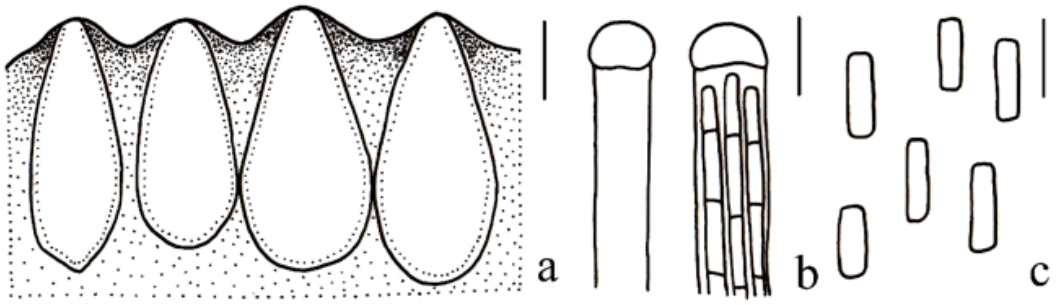

Figure 10

Microscopical characters of Cordyceps taishanensis (HMJAU 48254). a. Perithecia. b. Apex of ascus. c. Part-spores. Bars: a=100 μm; b, c=5 μm.

Figure 11

Macrocharacter of Cordyceps militaris. a-e: Stromata and host of Cordyceps militaris (a collected from Great Xingan Mountains, Heilongjiang Province; b collected from Ji'an County, Tonghua City, Jilin Province; c, e collected from Changchun City, Jilin Province; d collected from Qujin City, Huize County, Yunnan Province). Bars: a-e= 1 cm.