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## Taxonomy and biology of *Cordyceps qingchengensis* sp. nov. and its allies

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### Abstract

*Cordyceps qingchengensis* sp. nov., growing on a cocooned pupa of a silk moth (Lepidoptera: Bombycidae) and collected from southwestern China, is described, illustrated and compared with allied taxa. The species is morphologically similar to *C. bifusispora* and *C. tenuipes*, but can be easily separated from the latter two by the unique host and by branched and thicker stroma. Phylogenetic analyses of single ITS and combined SSU, LSU and TEF1- $\alpha$  datasets indicate that it is closely related to *C. bifusispora*, *C. cicadae* (Miq.) Masee (Chanhua) and *C. tenuipes*, but *C. qingchengensis* has distinct nucleotide differences which support it as new. Taxonomy of *C. tenuipes* and *C. pruinosa* is reviewed and *C. ninchukispora* ( $\equiv$  *Phytocordyceps ninchukispora*) is considered as a synonym of *C. pruinosa*. Ecology and life cycles of *C. qingchengensis*, *C. tenuipes*, *C. pruinosa* and *C. ningxiaensis* are recorded and inferred. We provide important biological information for *C. qingchengensis* and its allies.

**Keywords:** *Cordyceps ningxiaensis*, *Cordyceps pruinosa*, *Cordyceps tenuipes*, life cycle, host, revision

### Introduction

*Cordyceps* (*Cordyceps* sensu lato) fungi have always been highly regarded for their important edible and medicinal values and applications in biological control. *Cordyceps* can grow predominantly on insects, but also occur on spiders, nematodes, other cordyceps, the fungi *Elaphomyces* and even inhabit soil and plant tissues (Sung *et al.* 2007, Vega *et al.* 2009). This group currently has more than 900 species that belong to three families (Cordycipitaceae, Ophiocordycipitaceae and partial Clavicipitaceae) in the order Hypocreales (Zha *et al.* 2018). More than 140 species have been reported in China (Wen *et al.* 2017).

The genus *Cordyceps* Fr. emend. G.H. Sung *et al.* (Cordycipitaceae) comprises 172 accepted species (Roskov *et al.* 2019). Due to a lack of molecular evidence or inconclusive morphology and ecology, more than 100 species are retained from the previous *Cordyceps* Fr. (Sung *et al.* 2007). Also, due to confusion of sexual and asexual morphs, many cordyceps species still have two or more names. Revision of sexual and asexual names and classification of undetermined *Cordyceps* species still need to be worked on.

Occurrence of a cordyceps is closely related with ecological environment and the life cycle of its host. Factors affecting distribution are humidity (air and soil), temperature, light, rainfall, elevation, biogeography and the occurrence season (both the cordyceps and its host). Host information includes host name/group, the instar of death (egg, nymph/larva, pupae/cocoon, adult), the instar that becomes infected, locality of collection (in/on soil, humus layer, rotten wood, tree root or trunk, leaf or twig of plant, etc.) and the plant on which the insects reside (Wen *et al.* 2016). All this biological information is useful for cordyceps research. Unfortunately, biology of cordyceps has always been poorly studied, and this affects its identification, application and exploitation.

We recently collected a new *Cordyceps* species from southwestern China. It is described as *Cordyceps qingchengensis* sp. nov., and is compared to allied species. In addition, we give taxonomic notes and biological information for related *Cordyceps* species.

## Materials and methods

### Specimens

Explorations were made in China. Specimens were observed in the wild and brought to the laboratory for identification. Fungal specimens were examined and photographed using a Nikon Coolpix P520 camera, an Optec SZ660 stereo dissecting microscope and a Nikon Eclipse 80i compound microscope connected with a Cannon EOS 600D camera. Voucher specimens are deposited in Centre of Excellence in Fungal Research, Mae Fah Luang University (MFLU), Chiang Rai, Thailand, and the Herbarium of Guizhou University (GACP), Guiyang, China.

### DNA extraction and sequencing

Total DNA was extracted from specimens dried over silica-gel using a CTAB procedure (Doyle 1987). The ribosomal small and large subunits (SSU and LSU), internal transcribed spacers (ITS) and elongation factor 1 $\alpha$  (TEF1- $\alpha$ ) genes were amplified and sequenced using the primers detailed by White *et al.* (1990) and Ban *et al.* (2015). Amplification reactions were performed in an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA) and PCR programs followed those of Ban *et al.* (2015). PCR products were purified using the Biotek's Purification Kit (Biotek Corporation, Beijing, China), and were sequenced using an ABI 3730 DNA analyzer and an ABI BigDye 3.1 terminator cycle sequencing kit (Sangon Co., Ltd., Shanghai, China). Sequences were aligned and assembled visually and manually using Clustalx1.81 (Larkin *et al.* 2007), Chromas230 and ContigExpress software.

### Construction of phylogenetic tree

Phylogenetic trees were constructed using sequences of *Cordyceps qingchengensis* sp. nov. and voucher sequences of its allies obtained from GenBank (Sung *et al.* 2007, Wang *et al.* 2008, Yan & Tolgor 2015, Kepler *et al.* 2017) (Table 1). Phylogeny was reconstructed using the single gene datasets of ITS, TEF1- $\alpha$  and finally a combined SSU, LSU and TEF1- $\alpha$  sequence dataset. *Ophiocordyceps sinensis* (Berk.) G.H. Sung *et al.* (EFCC 7287, Sung *et al.* 2007) was used as the outgroup taxon.

**TABLE 1.** Voucher information and GenBank accession numbers for samples analysed phylogenetically. New sequences generated in this study are displayed in bold.

Taxa	Specimen/strain number	GenBank accession numbers			
		ITS	SSU	LSU	TEF1- $\alpha$
<i>Cordyceps bifusispora</i>	spat 08-129	–	MF416576	MF416523	MF416468
<i>Cordyceps bifusispora</i>	spat 08-133.1	–	MF416577	MF416524	MF416469
<i>Cordyceps chiangdaoensis</i>	BCC 75734	KT261394	–	–	KT261404
<b><i>Cordyceps cicadae</i> (Chanhua)</b>	<b>GACP 07071701</b>	<b>KX017277</b>	<b>MK761207</b>	<b>MK761212</b>	<b>MK770631</b>
<b><i>Cordyceps cicadae</i> (Chanhua)</b>	<b>GACP 14061604</b>	<b>KX017276</b>	<b>MK761208</b>	<b>MK761213</b>	<b>MK770632</b>
<i>Cordyceps cicadae</i> (Chanhua)	RCEF HP090724-31	–	MF416605	MF416552	MF416496
<i>Cordyceps coleopterorum</i>	CBS 110.73	AY624177	JF415965	JF415988	JQ425689
<i>Cordyceps exasperata</i>	MCA 2155	–	MF416596	MF416542	MF416486
<i>Cordyceps farinosa</i>	CBS 111113	AY624181	AY526474	MF416554	MF416499
<i>Cordyceps fumosorosea</i>	CBS 244.31	AY624182	MF416609	MF416557	MF416503
<i>Cordyceps kyusyuensis</i>	EFCC 5886	–	EF468960	EF468813	EF468754
<i>Cordyceps militaris</i>	OSC 93623	JN049825	AY184977	AY184966	DQ522332
<i>Cordyceps morakotii</i>	BCC 55820	KT261389	–	–	KT261399
<i>Cordyceps ninchukispora</i>	BCC 2121	FJ765277	FJ765292	FJ765245	FJ765261
<i>Cordyceps ninchukispora</i>	BCC 30937	FJ765274	FJ765289	FJ765242	FJ765258
<i>Cordyceps ningxiaensis</i>	HMJAU 25074	KF309668	–	KF309671	–
<i>Cordyceps oncoperae</i>	AFSEF 4358	–	AF339581	AF339532	EF468785
<i>Cordyceps polyarthra</i>	MCA 996	–	MF416597	MF416543	MF416487

.....continued on the next page

**TABLE 1.** (Continued)

Taxa	Specimen/strain number	GenBank accession numbers			
		ITS	SSU	LSU	TEF1- $\alpha$
<i>Cordyceps pruinosa</i>	ARSEF 5413	JN049826	AY184979	AY184968	DQ522351
<b><i>Cordyceps qingchengensis</i></b>	<b>MFLU 17-1022</b>	<b>KY423506</b>	<b>MK761206</b>	<b>MK761211</b>	<b>MK770630</b>
<i>Cordyceps rosea</i>	spat 09-053	–	MF416590	MF416536	MF416480
<i>Cordyceps roseostromata</i>	ARSEF 4871	–	AF339573	AF339523	–
<i>Cordyceps roseostromata</i>	ARSEF 4870	EF368022	–	–	–
<i>Cordyceps tenuipes</i>	MCA 1806	–	MF416595	MF416541	MF416485
<i>Cordyceps tenuipes</i>	ARSEF 5135	AY624196	MF416612	JF415980	JF416020
<b><i>Cordyceps tenuipes</i></b>	<b>GACP 16063004</b>	<b>KY423509</b>	<b>MK761209</b>	<b>MK761214</b>	<b>MK770633</b>
<i>Ophiocordyceps sinensis</i> (outgroup)	EFCC 7287	JN049854	EF468971	EF468827	EF468767

The single ITS and TEF1- $\alpha$  sequence datasets were analyzed using neighbor-joining (NJ) method (Saitou & Nei 1987), and the combined SSU, LSU and TEF1- $\alpha$  sequence dataset using maximum parsimony (MP) and maximum likelihood (ML) methods, respectively. NJ trees were performed with MEGA6 (Tamura *et al.* 2013) using the uncorrected p-distance method (Nei & Kumar 2000) with a bootstrap test of 1,000 replicates. ML tree was generated using RAxML v.8.2.8 employing a GTRGAMMA model of nucleotide substitution with other details described in Jeewon *et al.* (2002, 2003) and Hongsanan *et al.* (2017). MP tree was reconstructed with PAUP\* 4.0b10 (Swofford 2002) and using the heuristic search option with TBR branch swapping, bootstrap of 1,000 replicates, and other details as outlined in Cai *et al.* (2006) and Tang *et al.* (2007).

## Results

### Phylogeny

The single ITS gene dataset comprised 545 characters (including gaps), of which 151 were variable and 74 were parsimony-informative. There were 293 variable and 205 parsimony-informative characters within the 909 characters of the single TEF1- $\alpha$ , and 450 variable and 277 parsimony-informative within the 2,679 characters of the combined SSU, LSU and TEF1- $\alpha$  gene dataset. The different analyses resulted in trees with similar topologies and support values (results not shown). The ML tree for combined SSU, LSU and TEF1- $\alpha$  sequence dataset is shown in Fig. 1, and the NJ tree for the single ITS sequence dataset is shown in Fig. 5.

The phylogenetic analyses show that *C. qingchengensis* (MFLU 17-1022) is closely related to, but separated from *C. bifusispora*, *C. cicadae* (Miq.) Masee (Chanhua) and *C. tenuipes* while *C. pruinosa* and *C. ninchukispora* cluster together (Figs 1, 5).

## Taxonomy

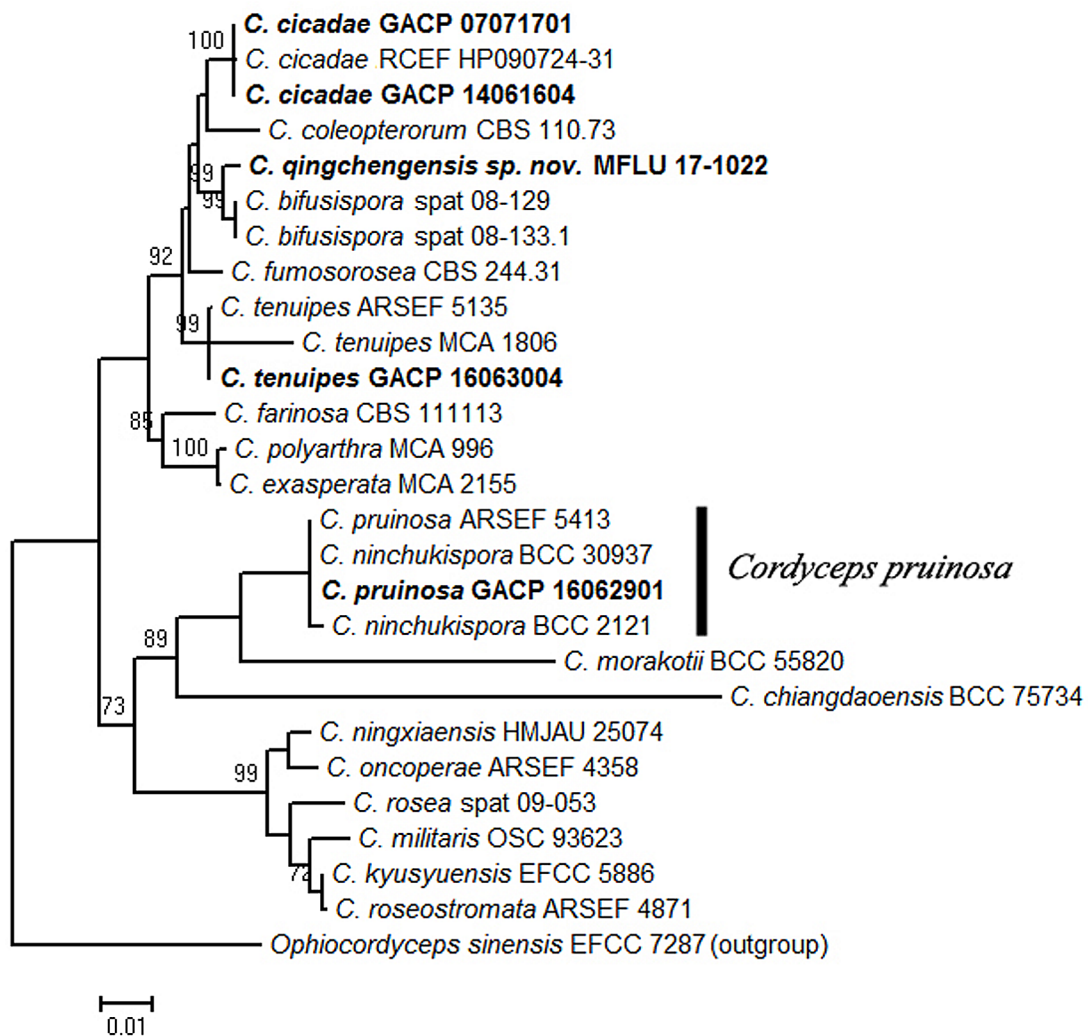
***Cordyceps tenuipes*** (Peck) Kepler *et al.* (Fig. 2)

≡ *Isaria tenuipes* Peck

≡ *Paecilomyces tenuipes* (Peck) Samson

= *Cordyceps takaomontana* Yakush. & Kumaz.

*Notes:*—*Cordyceps takaomontana* was originally described and illustrated by Kobayasi (1941) with the type specimen from Japan. Kepler *et al.* (2017) provided a photograph of *C. takaomontana* co-occurring with *Isaria tenuipes*. According to the information of the two reports above, *C. takaomontana* should grow on slender cocooned pupae of small moths (may represent several groups of Lepidoptera), its stromata are single to several, abbreviated, clavate, unbranched and yellow, and its fertile heads are distinctly expanded.



**FIGURE 1.** ML tree of *Cordyceps qingchengensis* sp. nov. and its allies inferred from a combined SSU, LSU and TEF1- $\alpha$  dataset. Bootstrap support values greater than 70% are indicated above the nodes.



**FIGURE 2.** Asexual morph of *Cordyceps tenuipes* in the wild.

In China, Liang *et al.* (2003) described a specimen from Guizhou as *C. takaomontana*. The specimen grew on a larva (Lepidoptera), its stromata are fasciculate, orange and slender, and its fertile heads are only slightly expanded. These characters do not match the characters of *C. takaomontana*, so we think the specimen is not *C. takaomontana*. Unfortunately, the specimen has no DNA sequence data.

Another description of *C. takaomontana* from China was provided by Li *et al.* (2007) who collected a specimen from Anhui. The specimen grew on a large and nearly rounded (slightly ovate) cocooned pupae (Lepidoptera), its stromata were fasciculate, slender and light yellow, and its fertile heads were also only slightly expanded. Due to these different characters, we think this specimen is also not *C. takaomontana*. The specimen also lacks DNA sequence data.

Though *C. takaomontana* is scarce, its asexual morph, *I. tenuipes* is quite common (Fig. 2). In the wild, *I. tenuipes* can readily be found on leaf litter or humus layer in humid environments. It generally grows on slender and cocooned pupae of numerous small moths (Lepidoptera), such as Arctiidae (Fig. 2). Old Arctiidae larvae move into shallow soil layer, or to soil surface and hide in dead leaves to pupate. During the process of pupation, probably due to continuous rainfall or very humid environment, these old larvae are easily infected by conidia of *I. tenuipes* that attach to their body surface. *Isaria tenuipes* grows rapidly and under suitable humidity, temperature and light, synnemata will soon be produced on the slender and cocooned pupae instead of the previous larvae. The insects are infected as old larvae and then die as cocooned pupae.

***Cordyceps qingchengensis*** L.S. Zha & T.C. Wen *sp. nov.* (Figs 3, 4)

Index Fungorum number IF556460; Facesoffungi number FoF 03405.

Etymology:—the new species is named after Qingchengshan Mountain, its type locality.

Holotype:—MFLU 17-1022 (isotype: GACP 16070401).

**Description:**—**Sexual morph:** *Stromata* arising from head of cocooned pupa of a large silk moth, fleshy, yellow (fresh specimen), branched, total length 25 mm; single at base, then branched into several (often 3) forks, basal stipe and upper branches slightly cylindrical, moderate width, covered by light yellow mycelia, apices of branches obtuse. *Fertile part* located at the terminal part of every branch, 7–9 × 2.0–2.5 mm, slightly wider than basal stipe and upper branches. *Perithecia* partially immersed at right angle to surface of fruiting body, ovoid but apex sharply pointed, 335–490 × 145–240 μm. *Asci* cylindrical, 180–200 ( $\bar{X}$  = 188, n = 10) long and 2.4–4.0 ( $\bar{X}$  = 3.15, n = 30) μm wide, caps hemispheric, 1.8–2.2 ( $\bar{X}$  = 2.0, n = 30) μm high and 2.5–3.2 ( $\bar{X}$  = 2.85, n = 30) μm wide, 8-spored. *Ascospores* filiform, 180–220 μm long and 0.45–0.65 ( $\bar{X}$  = 0.53, n = 30) μm wide, not at all bifusiform and not broken into part-spores. **Asexual morph:** Unknown.

**Material examined:**—CHINA, Sichuan Province: Chengdu, Qingchengshan Mountain, 30°55'30"N, 103°29'44"E, about 1000 m altitude, 4 July 2016, Ling-Sheng Zha (MFLU 17-1022, **holotype**; GACP 16070401, isotype).

**Distribution:**—China (Sichuan).

**Host:**—Growing on the ovate cocooned pupa of a large silk moth (Lepidoptera: Bombycidae) in humid soil of a broad-leaved forest. The cocoon is ovate and thick, 21 × 8 mm.

**Notes:**—Morphologically, *Cordyceps qingchengensis* (sexual morph) is similar to *C. bifusispora* O.E. Erikss. and *C. tenuipes* (= *C. takaomontana*), but can easily be identified by its unique host (ovate cocooned pupa of a large silk moth) and branched stroma. Morphological differences of *Cordyceps qingchengensis* and its allies are outlined in Table 2.

ITS sequence of *Cordyceps qingchengensis* is most similar (only 0.9 % bp difference across 569 bp) to that of *C. cicadae* (Miq.) Masee (Chanhua, RCEF HP090724-31 and our collections, Zha *et al.* 2018), but their TEF1- $\alpha$  sequence are sufficiently different (3.1% bp difference across 911 bp). Morphologically, *C. cicadae* (Miq.) Masee has large and irregularly branched stromata, broad ascospores and grows on cicada nymphs, features that are significantly different from *C. qingchengensis* (Table 2). TEF1- $\alpha$  sequence of *C. qingchengensis* is most similar (1.7 % difference across 866 bp) to that of *C. bifusispora* (EFCC 8260, spat 08-129 and spat 08-133.1). Unfortunately, there is no ITS sequence for *C. bifusispora*. The phylogenetic analyses also support *C. qingchengensis* as a new taxon despite being closely related to *C. bifusispora*, *C. cicadae* (Miq.) Masee and *C. tenuipes* (Figs 1, 5).

**Biology:**—The cocoon of the host is a little rotted (Fig. 3b) and the pupa in the cocoon had been seriously degraded by the fungus. We infer the life cycle of the fungus should be as follows.

In the previous summer, an old silk moth larva prepared for pupation in the soil when there was heavy rain, and due to extremely humid soil environment, the insect was infected by the fungus. Several days later a pupa had formed in its thick silk cocoon and the insect was killed by the fungus in the cocoon, and as a result a sclerotium (ossified insect) came into being. Due to either dry soil environment or low temperatures in the next year, the sclerotium did not germinate, but with the advent of the rainy season in the next summer, the sclerotium began to germinate and fruiting body of the fungus formed at last.

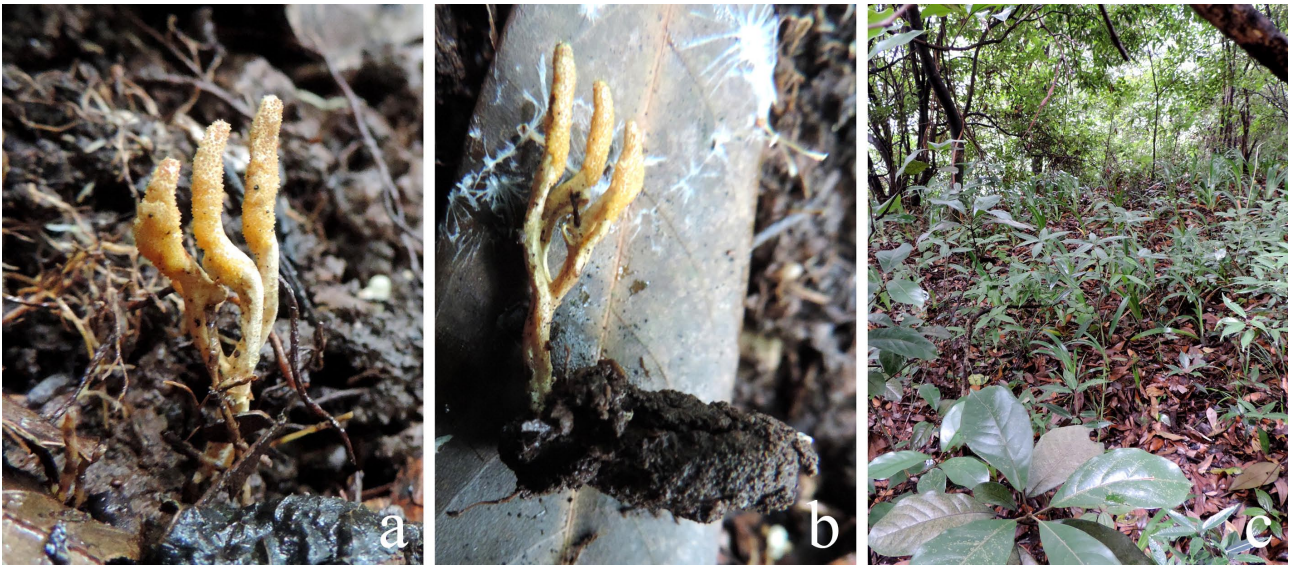


FIGURE 3. *Cordyceps qingchengensis* (MFLU 17-1022, holotype). a-b. Growing in the wild and dug out. c. Habitat environment.

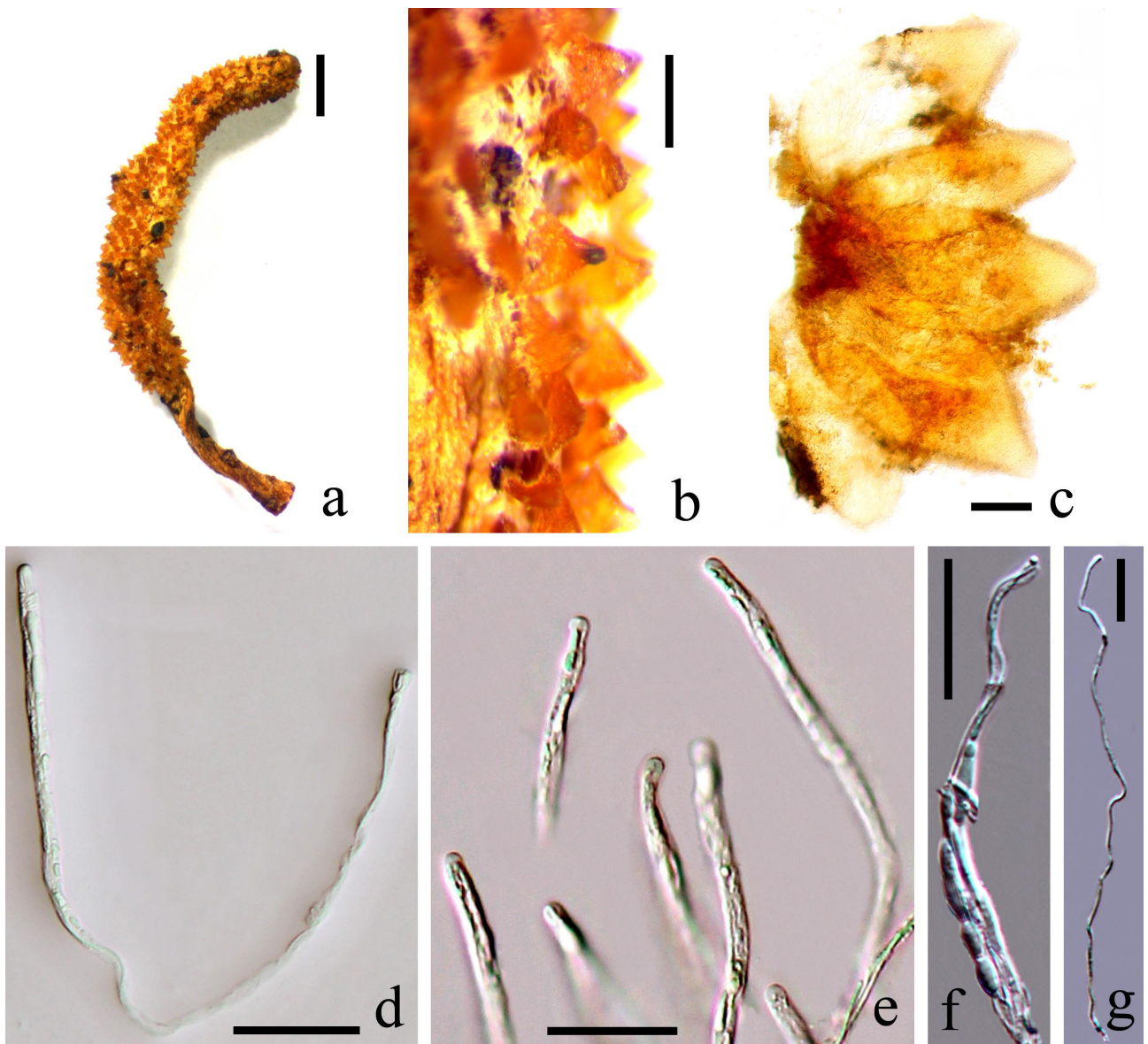
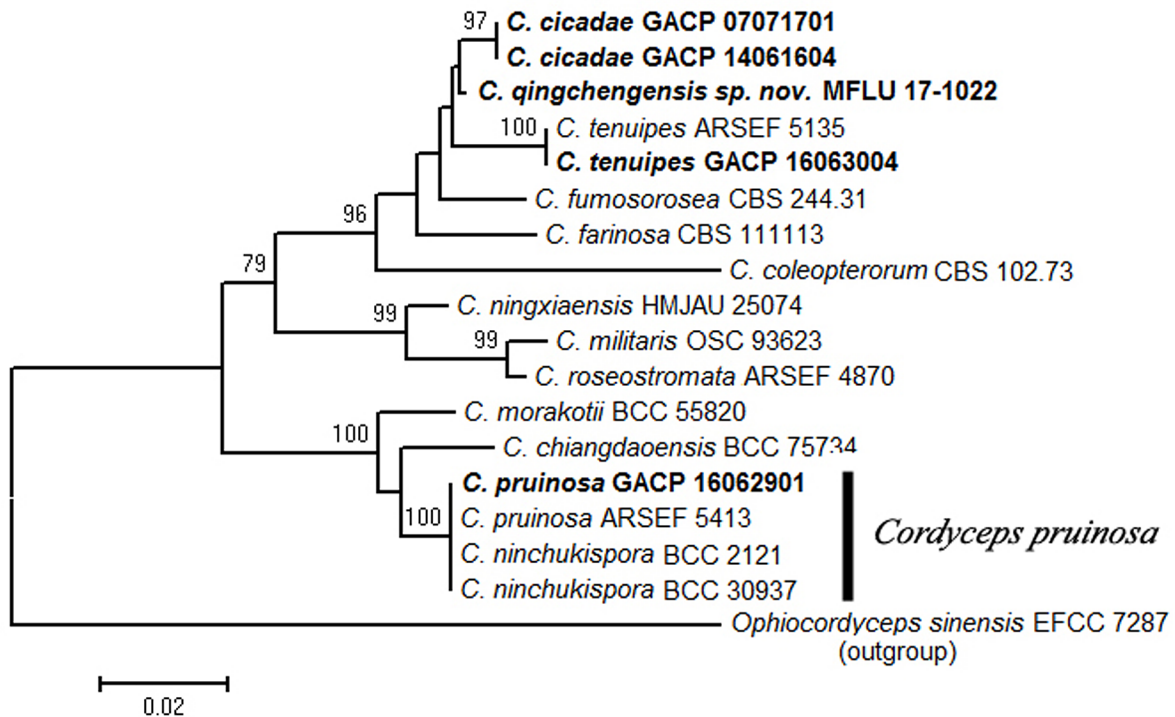


FIGURE 4. *Cordyceps qingchengensis* (MFLU 17-1022, holotype). a. Fertile head of dry fruiting body. b. Surface of fertile head. c. Perithecia in profile. d-e. Asci. f-h. Ascospores. Scale bars: a = 1 mm, b = 200 µm, c = 100 µm, d-e = 20 µm, f-h = 10 µm.



**FIGURE 5.** NJ tree of *Cordyceps qingchengensis* and its allies inferred from ITS gene data. Bootstrap values greater than 75% are indicated above the nodes.

**TABLE 2.** Morphological comparison of sexual states of *Cordyceps bifusispora*, *C. qingchengensis*, *C. tenuipes* and *C. cicadae* (Miq.) Massee (Chanhua).

	Host	Stroma	Fertile head	Ascospores	References
<i>C. bifusispora</i>	Cocooned pupae of noctuids (Lepidoptera: Noctuidae)	Single or several, unbranched, slender and cylindrical, whitish to light yellow	1.3 mm thick, slightly thicker than stalk	Bifusiform, 0.4–1.6 µm thick; not broken into part-spores	Eriksson 1982, Liang 2007, Kepler <i>et al.</i> 2017
<i>C. qingchengensis</i>	Ovate cocooned pupa of a large silk moth (Lepidoptera: Bombycidae)	Single, branched, stipe and branches moderate width, yellow	2.0–2.5 mm thick, slightly thicker than basal stalk and upper branches	Filiform, 0.45–0.65 µm thick, not broken into part-spores	This study
<i>C. tenuipes</i>	Slender and cocooned pupae of small moths (Lepidoptera)	Single to several, unbranched, abbreviated and clavate, yellow	1.5–3.5 mm thick, distinctly expanded	Filiform, 0.8–1 µm thick, easily broken into part-spores	Kobayasi 1941, Kepler <i>et al.</i> 2017
<i>C. cicadae</i> (Miq.) Massee	Cicada nymphs (Hemiptera: Cicadidae)	Single or several, large, irregularly branched, yellow to yellowish brown	1–12 mm thick, decidedly thicker than stalk; synnemata presented at the top	Filiform, 2–2.2 µm thick; easily broken into part-spores	Kobayasi 1949, Zha <i>et al.</i> 2018

***Cordyceps pruinosa*** Petch (Fig. 6)

= *Mariannaea pruinosa* Z.Q. Liang

= *Phytocordyceps ninchukispora* C.H. Su & H.H. Wang

= *Cordyceps ninchukispora* (C.H. Su & H.H. Wang) G.H. Sung *et al.*

*Notes:*—Larvae of Limacodidae (Lepidoptera) live on leaves of many plants at first, then some species crawl into soil to pupate (species pupating on plants cannot be infected by cordyceps). During the process of pupation, once the soil is sufficiently humid, an old larva may easily be infected by ascospores or conidia of *C. pruinosa* that attach to its body surface. As the pupation proceeds, pupa shell of the insect will gradually be formed, and the insect will at last be killed by the fungus in its pupa shell. When the temperature, humidity and light are suitable for the fungus to grow again (separate from the stage of the previous infection, Wen *et al.* 2016), fruiting bodies of the fungus will break through the pupa shell. This sexual morph *Cordyceps* grows slowly; its young fruiting bodies are light yellow, then become pink, followed by reddish orange and finally rust red (Fig. 6).



**FIGURE 6.** Different stages of life cycle of *Cordyceps pruinosa*. **a.** A larva of *Phocoderma betis* (Lepidoptera: Limacodidae) killed by *C. pruinosa* in its pupa shell. **b, c.** Young fruiting bodies. **d.** Immature fruiting bodies. **e.** Mature fruiting bodies. **f.** Fruiting bodies before dying. Photographs **b, c** and **d** represent the same specimen. Specimens were collected in a tea garden (Guizhou, China), and the *Phocoderma betis* pupae are similar to the seeds of the tea trees.

In moist subtropical regions, *C. pruinosa* can occur for most of the year. The reasons may include: 1) many Limacodidae species can reproduce 2–3 generations per year; 2) abundant but non-continuous rainfall all year round; and 3) the infection and the growth of cordyceps occur at distinct phases (Wen *et al.* 2016).

The pupa shells of Limacodidae are 4–15 mm long, rounded or nearly rounded, dark brown, brown to light yellow, generally their surface is relatively smooth and their texture is relatively hard. Interestingly, many of these pupae are similar to the seeds of the plants on which the insects feed, thus creating an effective imitation mechanism (Fig. 6). *C. ninchukispora* ( $\equiv$  *Phytocordyceps ninchukispora*), the only exception among all cordyceps species, was reported as a plant pathogen growing on seeds of *Beilschmiedia erythrophloia* (Su & Wang 1986). *Beilschmiedia* plants are common hosts of several Limacodidae species, and *Beilschmiedia* seeds are similar to these Limacodidae pupae, so we strongly believe the host of *C. ninchukispora* is not *Beilschmiedia* seeds, but Limacodidae pupae.

There are 25 ITS sequences of *C. pruinosa* and *C. ninchukispora* in GenBank. Sequence alignment and Blast search indicate that two of them are not cordyceps and four represent other known cordyceps species (>99.6% similarity); the



remaining 19 sequences (including one from Taiwan) are >99.5% similar and are probably the same species (the <0.5% base difference mainly comes from base insertions or gaps, which we think are errors during the process of sequence assembly). *Cordyceps pruinosa* was originally described from Sri Lanka (Petch 1924) and subsequently reported from Japan (Kobayasi 1941), China (Liang 2007), Korea and Thailand (Sung *et al.* 2007). *Cordyceps ninchukispora* was originally reported from Taiwan (Su & Wang 1986) and this epithet has been commonly used in Thailand. All these collections have the same macroscopic characters, the same hosts (Limacodidae pupae) and the same geographical distribution (south Asia). The phylogenetic analyses also support that the two species are conspecific (Figs 1, 5). For these reasons and following the suggestion of Sung *et al.* (2007), we herein synonymize *C. ninchukispora* to *C. pruinosa*. Their apparently different ascospores may be due to observations made at different stages of ascospore development — disarticulated (Petch 1924, Kobayasi 1941), bifusiform (Sung *et al.* 2007) and filiform at first then broken into disarticulated and bifusiform (Liang 2007).

***Cordyceps ningxiaensis*** Tolgor Bau & J.Q. Yan (Fig. 7a, b)

*Notes*:—*Cordyceps ningxiaensis* was introduced by Yan & Bau (2015) with the type from Liupanshan Mountain, Longde County, Ningxia Hui Autonomous Region, China. The species is morphologically similar and phylogenetically related to *C. militaris* (L.) Link and its allies (Figs 1, 5). Hosts of *C. ningxiaensis*, which had originally been identified as fly pupae (Diptera), are actually Scoliidae pupae (Hymenoptera,) that parasitize the bodies of Scarabaeidae larvae (Coleoptera).

In nature, scoliid wasps (Scoliidae) are the natural enemy of Scarabaeidae larvae. The female scoliid wasps can bury themselves into soil freely to look for Scarabaeidae larvae. They sting and anaesthetize the larvae, then lay eggs into their bodies which later hatch and become larvae. Using tissues of the Scarabaeidae larvae as nutrition, the wasp larvae will grow and the Scarabaeidae larvae will be consumed, until all tissues have been eaten and only exocuticle left, the wasp larvae will become pupae and finally adults (Fabre 1886).

During the process of pupation, once the soil layer cover is shallow and sufficiently humid, the old larvae of scoliid wasps may easily be infected by the ascospores or conidia of *C. ningxiaensis* that attach to their body surface. After being successfully infected, the insects will be killed in their pupal stage. When environmental conditions are suitable for the fungus, its fruiting bodies will break through the pupae, and a piece of ragged exocuticle of a Scarabaeidae larva is left beside the host.

Recently, a similar hyperparasite was discovered in a specimen of *C. cf. militaris* (Fig. 7c, d) collected in Changbai Mountain, Jilin, China. The host, which seems to be a Lepidoptera cocooned pupa, is in fact Braconidae naked pupae (Hymenoptera) that parasitized the cocoon. Apart from the different host species, its biology is similar to that of *C. ningxiaensis*.



**FIGURE 7.** Two *Cordyceps* species growing on Hymenoptera pupae. **a, b.** *C. ningxiaensis* (type specimen) growing on Scoliidae pupae that parasitized a Scarabaeidae larva (the Scarabaeidae larva had already been eaten by the Scoliidae insects). **c, d.** *C. cf. militaris* growing on Braconidae naked pupae that parasitize a Lepidoptera cocooned pupa (the ovate cocoon was cut open, three infected pupae are hidden in it, and the Lepidoptera pupa had already been eaten by the Braconidae larvae). Scale bars = 5 mm.

## Conflict of interest

The authors declare no conflicts of interest to disclose.

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