ENTOMOPATHOGENIC FUNGI *CORDYCEPS* SENSU LATO: SPECIES DIVERSITY AND HOST ASSOCIATION

LINGSHENG ZHA

DOCTER OF PHILOSOPHY
IN
BIOLOGICAL SCIENCE

SCHOOL OF SCIENCE
MAE FAH LUANG UNIVERSITY
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Lingsheng Zha

Dissertation Title Entomopathogenic Fungi *Cordyceps* Sensu Lato: Species Diversity and Host Association

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ABSTRACT

Cordyceps sensu lato (cordyceps), the most important group of entomopathogenic fungi, have always been highly researched for their species diversity, edible and medicinal values and use for biological control. This group currently has more than 1,000 species, which are distributed in three families (Cordycipitaceae, Ophiocordycipitaceae and partial Clavicipitaceae) in the order Hypocreales. In nature, cordyceps develop mainly on insects, spiders, other cordyceps species and hypogeous fungi *Elaphomyces*. These ascomycetes can reproduce via ascospores, conidia and mycelia that generally inhabit soil, plants, nematodes, other invertebrates, mushrooms and other organisms. Among them, insects account for a major proportion, and 19 Insecta orders have been reported as hosts of cordyceps.

Due to lack of molecular evidence or inconclusive morphology and ecology, more than 100 undetermined cordyceps species need to be confirmed. And due to confusion of sexual and asexual morphs, many cordyceps species still have two or more names. Furthermore, ecology and relationships with host insects have always been poorly studied, which seriously affects the identification, application and exploitation of cordyceps. Based on these problems, related cordyceps researches have been carried out and the findings are included in this dissertation.

This study reviews the medicinal history and distribution of the medicinal mushroom Chanhua and reinvestigates its phylogenetic relationships with allied species. Based on results obtained the study clarifies its taxonomy and reviews its host and biological and ecological aspects. The molecular phylogeny proved Chanhua to be an independent species of the genus *Cordyceps*. Although Chanhua, *I. cicadae* and *I. sinclairii* have generally been accepted as the same species, the latter two lack DNA sequence data from their type localities. *C. cicadae* S.Z. Shing (syn. *C. zhejiangensis*), which has been treated as the sexual morph of Chanhua for over 40 years, has been proved to be *Tolypocladium paradoxum*, and the sexual morph of Chanhua is considered to be *C. kobayasii* (syn. *C. cicadae-sm*). This study proposes to use the scientific name *C. cicadae* (Miq.) Massee to describe or record Chanhua fungi.

Cordyceps qingchengensis sp. nov., found in southwestern China and growing on a cocooned pupa of a silk moth (Lepidoptera: Bombycidae), is described, illustrated and compared with allied taxa. It is morphologically and molecularly related to *C. bifusispora*, Chanhua (*C. cicadae* (Miq.) Massee) and *C. tenuipes*. The taxonomy of *C. tenuipes* and *C. pruinosa* is reviewed and *C. ninchukispora* (≡ *Phytocordyceps ninchukispora*) is considered to be a synonym of *C. pruinosa*. The ecology and life cycles of *C. qingchengensis*, *C. tenuipes*, *C. pruinosa* and *C. ningxiaensis* are recorded and inferred. This study clarifies some taxonomical and biological aspects of related *Cordyceps* species.

Furthermore, the current study discusses the species diversity of Xuefeng Cordyceps and identifies the host insects and the plants on which the insects reside. Xuefeng Cordyceps refers to those fungi that grow on larvae of *Endoclita davidi* living in basal trunks or roots of the medicinal plant *Clerodendrum cyrtophyllum* in the Xuefeng Mountain region, Hunan, China. The fungi deal with *Beauveria bassiana* (sexual and asexual), *Metarhizium guizhouense* (asexual), *Ophiocordyceps macroacicularis*, *O. ramosissimum* and *O. xuefengensis*. The host insects, that were previously mistaken for larvae of *E. nodus*, are corrected as larvae of *E. davidi*. Molecular phylogenetic data confirmed the name of the host plants, and morphological variations of the plant are discussed. The biology and ecology of Xuefeng Cordyceps and the host insects are also outlined. The study provides necessary information for studying the precious traditional Chinese medicine, Xuefeng Cordyceps.

Cicadicolous cordyceps refers to the cordyceps fungi growing on cicadas (Hemiptera: Cicadidae). Currently, there are 35 cicadicolous species distributed worldwide, with many known from southern Asia. In this study, the relationships between cicadicolous species and their relatives are studied based on multigene phylogeny. The taxonomy of 16 known species, 1 new species and 2 new records of cicadicolous species from China are discussed. *O. bannaensis* sp. nov., *Metarhizium owariense* and *Polycephalomyces nipponicus* are newly reported from China. These taxa are detailed with descriptions, photographs, DNA sequence data and taxonomic notes.

A total of 25 cordyceps species have been reported on Orthoptera insects, and this study reviews their hosts and distributions and gives taxonomical notes. Using a combined ITS and TEF1-α sequence dataset, the molecular phylogeny of cordyceps species on Orthoptera insects was constructed, and current species are assigned to three clades: the *Beauveria*, the *Metarhizium* and the *Ophiocordyceps* clade, respectively. The ecology and habits of well-known groups of Orthoptera are explored and cordyceps on them show distinct host specificity. The study also displays the occurrence mechanism and conidiogenous mode of cordyceps. The rhizomorphs of cordyceps, a special root-like structure situated somewhere between fruiting bodies and synnemata, were observed on *Beauveria* spp. and *Metacordyceps taii* (syn. *Metarhizium guizhouense*). Taxonomical revisions of *Beauveria grylli* (Teng) L.S. Zha comb. nov. ≡ *Cordyceps grylli* Teng = *Beauveria loeiensis* Luangsa-ard, Ridkaew

& Tasan., *Beauveria kirkii* (G. Cunn.) L.S. Zha comb. nov. ≡ *Cordyceps kirkii* G. Cunn., are provided. Host corrections were made as follows: *Beauveria grylli* on katydid nymphs (Gryllacrididae) and *O. tettigonia* on cricket adults (Gryllidae).

Elateroidea and Tenebrionoidea are two large groups of Coleoptera, and their larvae are generally called wireworms. Most wireworms live in humid soil, humus layers or decayed wood and are thus easily parasitized by cordyceps fungi. Fifty-five cordyceps species have been reported on wireworms, and this study updates their hosts, summarizes their distributions and provides taxonomic notes. Among them, 52 species are accepted as parasites of wireworms and three are rejected. *O. borealis* sp. nov. and *O. spicatus* sp. nov. are described, photographed and compared with their allies. *Polycephalomyces formosus* is newly reported on wireworms (Elateroidea) and is described from this host. *Metarhizium jiangxiensis* comb. nov. (≡ *Cordyceps jiangxiensis*) is proposed for its typical *Metarhizium*-type conidiogenous structure. The multi-gene phylogeny supports the two new species and the identification of *P. formosus*.

Keywords: Biology, Ecology, Distribution, Morphology, Phylogeny, Taxonomy

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ABBREVIATIONS AND SYMBOLS

cm	centimeter
diam.	diameter
e.g.	for example,
et al.	and others
gen.	genus (singular) and genera (plural)
ibid.	in the same place
mm	millimeter
nov.	novum (Latin for new thing)
s	second
sp.	species
spp.	species (plural)
$\bar{x}$	average
°C	degree centigrade
=	equal (for measurement size)
=	heterotypic (taxonomic, facultative) a synonym
≡	homotypic (nomenclatural, obligate) a synonym
µm	micrometer
µl	microliter
%	percent
≥	greater than or equal to
vs.	versus
viz.	namely

CHAPTER 1

INTRODUCTION

1.1 Insects

Insects (Insecta), the largest group of all organisms, currently comprise nearly 940,000 species which are distributed worldwide (http://www.catalogueoflife.org/). Insects generally have complex life cycles (eggs, larvae/nymphs, naked/cocooned pupae, adults), which can help them go through harsh environments (i.e., lower temperatures, drought, humidity, predators and prey, competition (Wen et al., 2016c). Some insects never live in soil, including dragonflies, most butterflies, some beetles, mosquitoes, and most flies, and some need to live in soil for partial or whole periods of their life cycles, such as moths, cicadas, most beetles and bugs, some bees, ants, and so on (Wen et al., 2016c). The former have seldom, while the latter have frequently been reported as hosts of the fingi *Cordyceps* sensu lato.

1.2 Cordyceps Fungi

Cordyceps sensu lao (*Cordyceps* s.l., also known as cordyceps, Zha et al., 2018), as the most important group of entomopathogenic fungi, has always been highly researched for its species diversity, edible and medicinal values and use for biological control. This group belongs to the order Hypocreales, which includes all species of the families Cordycipitaceae and Ophiocordycipitaceae and some species of the family Clavicipitaceae (Sung et al., 2007; Kepler et al., 2012a). Currently cordyceps fungi have more than 1,000 species with sexual and asexual 47 genera (Sung et al., 2007; Shrestha et al., 2017a; Zha et al., 2018; Table 1.1).

Table 1.1 Current classification system of cordyceps (*Cordyceps* sensu lato)

Partial Clavicipitaceae		**Ophiocordycipitaceae**		**Cordycipitaceae**	
Genus	**Species**	**Genus**	**Species**	**Genus**	**Species**
Aschersonia	55	*Blistum*	1	*Akanthomyces*	13
Conoideocrella	3	*Didymobotryopsis*	3	*Ascopolyporus*	7
Drechmeria	12	*Elaphocordyceps*	1	*Beauveria*	33
Harposporium	36	*Hirsutella*	79	*Beejasamuha*	1
Hypocrella	50	*Hymenostilbe*	14	*Cordyceps*	172
Metacordyceps	5	*Ophiocordyceps*	196	*Coremiopsis*	2
Metarhizium	38	*Paraisaria*	1	*Engyodontium*	4
Moelleriella	39	*Perennicordyceps*	4	*Gibellula*	23
Nomuraea	2	*Polycephalomyces*	14	*Hyperdermium*	2
Orbiocrella	1	*Purpureocillium*	5	*Insecticola*	2
Pochonia	4	*Synnematium*	1	*Isaria*	82
Regiocrella	2	*Tolypocladium*	39	*Lecanicillium*	24
Samuelsia	6			*Leptobacillium*	1
Sphaerocordyceps	2			*Microhilum*	1
Tyrannicordyceps	5			*Parengyodontium*	1
				Phytocordyceps	1
				Pseudogibellula	1
				Rotiferophthora	27
				Simplicillium	9
				Torrubiella	65
Total: 15	260	12	358	20	471
Total: 3 families, 47 genera and 1,089 species					

Source Sung et al. (2007), Shrestha et al. (2017a) and Catalogue of Life: http://www.catalogueoflife.org/, access in July 2023

Cordyceps generally grow in humid soil, humus layers and plant tissues, or on wet ground and plant surfaces, because their growths, as well as those of other fungi, need a sufficiently humid environment. In the ecosystem, cordyceps, like all fungi, always play the role of decomposers, and their decomposing targets are always the organisms who are old, weak, ill or disabled (Wen et al., 2016b). They utilize the nutrition of organisms to continue their lives, enrich their species diversity, and maintain the ecological balance of the ecosystem. In nature, cordyceps develop mainly on insects, spiders, other cordyceps species and hypogeous fungi *Elaphomyces*. These ascomycetes can reproduce via ascospores, conidia and mycelia that generally inhabit soil, plants, nematodes, other invertebrates, mushrooms and other organisms (Sung et al., 2007; Vega et al., 2009; Zha et al., 2019a). Among these targets attacked, insects account for a major proportion, and 19 Insecta orders have been reported as hosts of cordyceps (Araújo and Hughes, 2016; Zha et al., 2019a).

1.3 Problems Needing to Solve in Cordyceps Research

1.3.1 Taxonomic Confusion

Previously, most cordyceps species had been assigned to the old genus *Cordyceps* Fr., so they had been called '*Cordyceps*'. It was not until 2007 that Sung et al. revised the classification system of cordyceps based on molecular and morphological data. In the new classification system, all these entomopathogenic fungi are assigned to three families, and only partial species were retained in the revised *Cordyceps* (Table 1.1). In other words, the concept of cordyceps has been extended from the previous *Cordyceps* genus to *Cordyceps* sensu lato (cordyceps, Zha et al., 2018). Due to a lack of molecular evidence or inconclusive morphology and ecology, more than 100 species are retained in the old genus *Cordyceps* (Sung et al., 2007). Also, due to the confusion of sexual and asexual morphs, many cordyceps species still have two or more names. Clarification of sexual and asexual names and classification of undetermined *Cordyceps* species are still needed for cordyceps research.

1.3.2 Problems Associated with Host Insects (Wen et al., 2016b)

The ecological habits of different insect groups are generally different, which results in the cordyceps species on them being also generally different. Most cordyceps species show distinct host specificity, so host information is specifically important to cordyceps research. Host information mainly includes host name, collecting position, instar stage, geography, and ecology.

For host identification, we should use the scientific name (including genus, family, and even order), and we can’t only use its common name because the latter is difficult to reach consensus in different countries and regions. Some larvae or nymphs are difficult to identify, but we can record, photograph, collect their larvae or nymphs or adults nearby, and resort to other biological and ecological imformations.

Cordyceps may be collected in soil, the humus layer, plant tissue, or on the ground and plant surface. Notably, the collecting position represents the place where the insect died instead of being infected.

The insect stage includes the egg, larva/nymph, pupa (naked and cocooned), and adult. Pupae are present in holometabola insects (Coleoptera, Diptera, Lepidoptera, and Hymenoptera) and absent in paurometabola ones (i.e., Hemiptera, Orthoptera). For paurometabola insects, their nymphs and adults are closely similar, while for holometabola insects, their larvae and adults are morphologically distinctly different. Notably, the insect stage of a cordyceps host refers to the stage when it died, but not when infected.

Geographical data refers to the longitude, latitude, and altitude, which determine the species and distribution of the host insects. Ecology information deals with the surrounding vegetation, the plants where the host insects inhabit, and some ecological factors. All this information is helpful to identify the cordyceps species and to understand its life cycle.

1.4 Thesis Outline

This dissertation consists of eight chapters.

Chapter 1 provides a brief introduction to species diversity and associations with host insects of cordyceps fungi. The problems and objectives of this research are explained.

Chapter 2 systematically introduces the important medicinal mushroom Chanhua (*C. cicadae* (Miq.) Massee). I review the medicinal history and distribution, reconstruct its phylogenetic relationships with allied species, clarify its taxonomy, and investigate its host and its biological and ecological aspects.

Chapter 3 introduces *C. qingchengensis* sp. nov. with a description and multigene phylogeny, revises one species, and clarifies some taxonomical and biological aspects of related *Cordyceps* species.

Chapter 4 investigates the species diversity, hosts, and ecology of the important Chinese medicinal mushrooms, Xuefeng Cordyceps.

Chapter 5 reviews the species diversity and distribution of cordyceps on cicadas (Hemiptera: Cicadidae), constructs the multigene phylogeny of cicadicolous cordyceps, and describes one new species and two new Chinese records.

Chapter 6 reviews the species diversity and distribution of cordyceps on Orthoptera insects, constructs their multigene phylogeny, revises two species, and corrects two host names.

Chapter 7 reviews the species diversity and distribution of cordyceps on wireworms (Elateroidea and Tenebrionoidea, Coleoptera), describes two new species with multigene phylogeny, and updates some host names.

Chapter 8 summarizes the current study and made an outlook on future work.

CHAPTER 2

TAXONOMY AND BIOLOGY OF THE MEDICINAL MUSHROOM CHANHUA (*CORDYCEPS CICADAE* (MIQ.) MASSEE)

2.1 Introduction

Chanhua fungus like other cordyceps (*Cordyceps* sensu lato, Zha et al., 2018) species are used in Traditional Chinese Medicines as they are believed to have special pharmaceutical efficacy and high nutritional value (De Silva et al., 2012, 2013; Chen et al., 2014). As an important member of cordyceps, Chanhua has been extensively studied, second only to *Ophiocordyceps sinensis* (Berk.) G.H. Sung et al. and *Cordyceps militaris* (L.) Fr. Because of its high demand, Chanhua has become scarce in nature, and this demand is being supplemented by artificial culture.

In the pharmaceutical industry and Traditional Chinese Medicine fields, a correct identification for source material is essential, as an incorrect scientific name may lead to legal disputes and ineffective conservation (Chan et al., 2011). Because of historical reasons and taxonomic problems, the use of the scientific name of Chanhua has been controversial (Chen et al., 2014). *Cordyceps cicadae* S.Z. Shing and *Isaria cicadae* Miq. have been the most frequently used, other names viz. *C. cicadae* (Miq.) Massee, *I. sinclairii* (Berk.) Lloyd, *Paecilomyces cicadae* (Miq.) Samson and *O. sobolifera* (Hill ex Watson) G.H. Sung et al. are also used. Besides this, host affiliation, ecological and biological aspects of Chanhua are poorly understood and there is a lack of documentation on these topics.

In this study, I reviewed the medicinal history and distribution of Chanhua, reinvestigated its phylogenetic relationships with related species, clarified its taxonomic placement and provided insights into its host affiliation, ecological and biological aspects. Based on results obtained, existing published information and my practical investigations, I clarified some important aspects of this economically important medicinal mushroom.

2.2 Material and Methods

2.2.1 Specimens

Specimen collections and biological and ecological investigations were made in southern China. A Nikon Coolpix P520 camera, a Nikon Eclipse 80i compound microscope connected with a Cannon EOS 600D camera and an Optec SZ660 stereo dissecting microscope were used for morphologic observing and photographing. Measurements were made using Tarosoft (R) Image Frame Work software. Specimens and strains are deposited in the Herbarium of Guizhou Agricultural College (= Guizhou University, GACP), Institute of Fungus Resources, Guizhou University (GZUIFR) and the Herbarium of Cryptogams of Kunming Institute of Botany, Chinese Academy of Sciences (HKAS).

2.2.2 DNA Extraction, Sequencing and Construction of Phylogenetic Tree

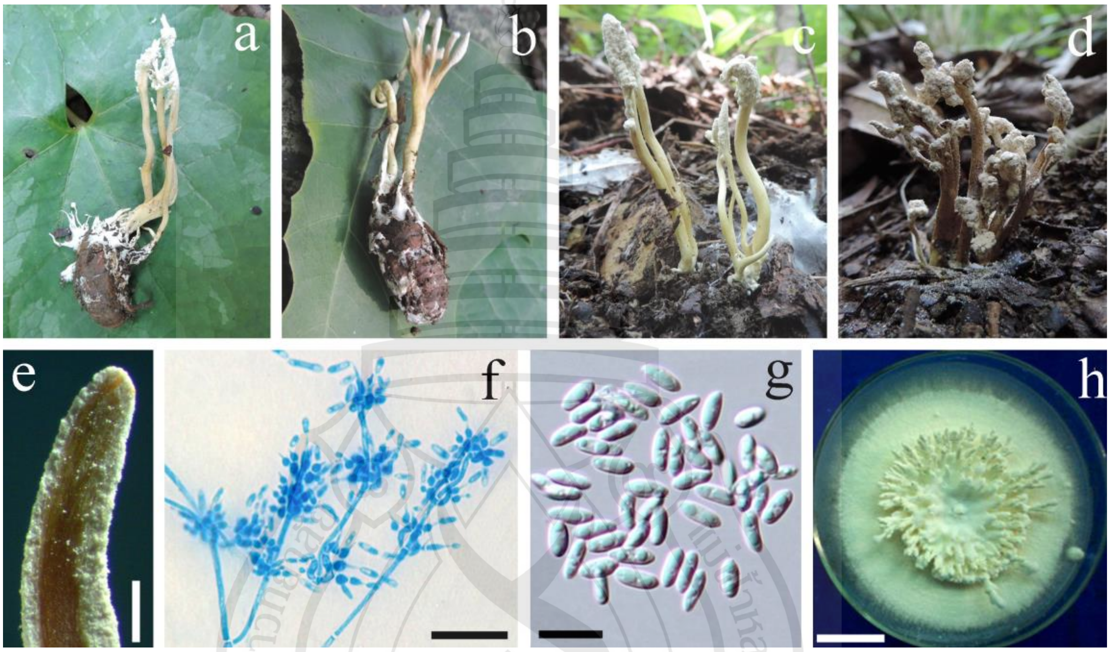
Total genomic DNA were extracted from specimens to confirm fungus identification, using E.Z.N.A.TM Fungal DNA MiniKit (Omega Biotech, CA, USA). Primers ITS4 and ITS5 were used to amplify and sequence the internal transcribed spacer (ITS) gene of ribosomal DNA (rDNA) (White et al., 1990). PCR programs followed those of Agrawal et al. (2014) and amplification reactions were performed in an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR products were purified using the Bioteke’s Purification Kit (Bioteke 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). Blast searches were performed to reveal the closest matches in GenBank database for selection of taxa in the phylogenetic analysis. Clustalx1.81 (Larkin et al., 2007), ContigExpress (Invitrogen, Carlsbad, CA, USA), Chromas230 (http://www.technelysium.com.au/chromas.html) and Mega6.06 (Tamura et al., 2013) were used for sequence assembly and alignment, and alignments were checked visually and improved manually. Molecular phylogeny was constructed using ITS sequence dataset. Maximum likelihood (ML) analysis was performed using RAxML v. 8.2.8 employing a GTRGAMMA model of nucleotide substitution, other details are described in Jeewon et al. (2003) and Hongsanan et al. (2017).

2.3 The Common Name and the Medicinal History

The word Chanhua (Figure 2.1) came from ancient Chinese culture. As a Traditional Chinese Medicine, Chanhua has a long history. The earliest record is in ‘Lei Gong Pao Zhi Lun’ (Lei’s Treatise on Preparing Drugs) written by Xiao Lei (the Southern and Northern Dynasties, 5th century AD) who recorded it as a medicine, that is 300 years earlier than the first record of *O. sinensis* (‘Worm in winter, herb in summer’, ‘Dongchong Xiacao’) (Chen et al., 2014; Zha et al., 2018). Other early records include ‘Yao Xing Lun’ (Theory of Drugs) of Quan Zeng in the Sui and Tang Dynasties (7th century AD); ‘Ben Cao Tu Jing’ (Illustrated Pharmacopoeia) of Song Su, ‘Xi Xi Zong Yu’ (Yao’s Comments on Everything) of Kuan Yao, ‘Yi Bu Fang Wu Lüe Ji’ (Notes on Useful Organisms) of Qi Song, ‘Shen Ji Zong Lu’ (General Records of Holy Universal Relief) of Ji Zhao, ‘Xiao Er Yao Zheng Zhi Jue’ (Craft of Medicinal Treatment for Children) of Xiao-Zhong Yan and ‘Jing Shi Zheng Lei Bei Ji Ben Cao’ (Classic Classified Materia Medica for Emergency) of Shen-Wei Tang in the Song Dynasty (960-1279 AD); ‘Ben Cao Gang Mu’ (Compendium of Materia Medica) of Shi-Zhen Li in the Ming Dynasty (1,596 AD). All these monographs recorded the medicinal value of Chanhua; among them Chanhua was described as ‘flower of cicada’, ‘corolla cicada’, ‘with a horn on the head of a cicada shell, which looks like corollas’. (Ren & Zhu, 2013; Wan et al., 2013; Chen et al., 2014).

Chanhua means ‘flower of cicada’, because it grows on cicada nymphs and resembles flowers. According to the literal meaning and the Chinese ancient records above, Chanhua should only be *Isaria*-like fungi that grow on cicada nymphs. But in Japanese culture, Chanhua (Chen hua, Semihana) is a general name for cicadicolous fungi and occasionally used for *O. sobolifera* (≡ *C. sobolifera* (Hill ex Watson) Berk. & Broome); later, another two names ‘Tsunozemi (horned cicada)’ and ‘Hanazemi (flowering cicada)’ were applied to *O. sobolifera* and *I. sinclairii* (refers to Chanhua) respectively (Kobayasi & Shimizu, 1963).

Obviously, the understandings of Chanhua from Chinese and Japanese cultures are not the same, presumably because of the different interpretations of languages. For example, the 'horn' in the sentence 'with a horn on the head of a cicada shell, which looks like corollas' may easily be misunderstood as a fruiting body.



Note a-d immature and mature synnemata (collected by Yuanpin Xiao and Chuangen Lin in June 2014 from Jiangxi, China; c, GACP 14061604); e sectional view of the apex of a synnema; f conidiophores and conidia cultured on PDA; g conidia; h culture on PDA at 25 °C for one month (Gzuifr-06722). Scale bars: e = 0.5 mm, f = 20 µm, g = 10 µm, h = 2 cm.

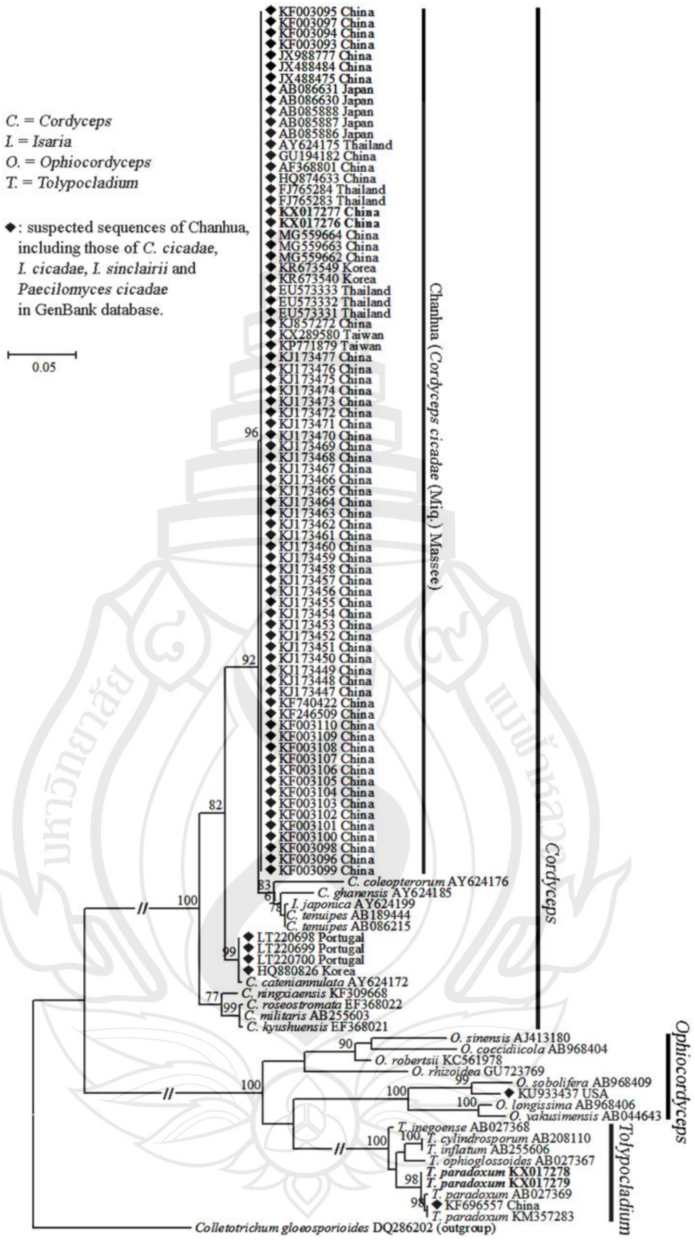
Figure 2.1 Chanhua (*Cordyceps cicadae* (Miq.) Massee)

2.4 Phylogeny

The ITS sequences of suspected species of Chanhua available in GenBank database have been checked and analyzed, which comprises eighty-six sequences that include *I. cicadae*, *I. sinclairii*, *C. cidadae*, *P. cicadae* and my sequencing results for Chanhua (KX017276 (GACP 14061604 from Jiangxi) and KX017277 (GACP 07071701 from Sichuan, China)). Apart from two suspicious sequences (AJ536573 and AJ536574 from China, use names as *C. cidadae* but can not match any known species), the rest eighty-four ones, together with their closest matches, were used for sequence alignment and phylogenetic analysis. The ITS gene dataset comprised 680 characters (including gaps), of which 307 were variable and 230 were parsimony-informative. Using *Colletotrichum gloeosporioides* (Glomerellaceae, Glomerellales) as outgroup taxon, the ML tree was constructed (Figure 2.2).

Among the eighty-four suspected sequences of Chanhua: (1) seventy-eight from Asia (including sixty-three from China, five from Japan, two from Korea, six from Thailand and two from Taiwan) are identical (of them, only five have minor base insertions or gaps, which I think are errors during the process of sequence assembly), and in phylogenetic tree they form an sister group also have the same branch length; (2) HQ880826 from Koera and LT220698, LT220699 and LT220700 from Portugal are identical and represent an *C. cateniannulata*-like species; (3) KU933437 from USA represents an *O. sobolifera*-like species; and (4) KF696557 (*Cordyceps cicadae* S.Z. Shing) from China is actually *Tolypocladium paradoxum* (Figure 2.2).

Undoubtedly, the seventy-eight ITS sequences from southern Asia represent Chanhua, and the molecular phylogenetic analysis strongly supports that Chanhua is one independent species of the genus *Cordyceps*. Chanhua is widely distributed in southern Asia (Figures 2.2, 2.3). *I. cicadae* was originally described from Brazil (Miquel, 1838) and *I. sinclairii* from New Zealand (Berkley, 1855), unfortunately, there are no DNA sequence data from their type localities. Considering the different geographical locations (South America, New Zealand and Asia, Figure 2.3), there is insufficient evidence that Chanhua is *I. cicadae* and *I. sinclairii* is a synonym of *I. cicadae*.



Note Bootstrap support values greater than 60% are indicated above the nodes. The sequences from this study are shown in blod.

Figure 2.2 Maximum likelihood (ML) tree of suspected species of Chanhua (*Cordyceps cicadae* (Miq.) Massee) and their allies generated from ITS dataset

2.5 Distribution

Note Every distributed country is marked with a red '★'.

Source The original map was from: http://m.onegreen.net/maps/HTML/37132.html.

Figure 2.3 Distribution of Chanhua (*Cordyceps cicadae* (Miq.) Massee) including *Isaria cicadae* and *I. sinclairii*

Chanhua, including *I. cicadae* and *I. sinclairii*, are distributed in southern Asia (China including Taiwan, India (Sharma et al., 2015), Indonesia, Japan, Nepal (Shrestha, 2011), South Korea, Sri Lanka, Thailand and Vietnam (Binh et al., 2017)), Oceania (Australia and New Zealand), South America (Brazil, Guatemala and Mexico), North America (Canada) and Africa (Madagascar) (Petch, 1942; Kobayasi & Shimizu, 1963; Samson, 1974; Chen et al., 2014; Figure 2.3).

In China, Chanhua is widely distributed in the Oriental Region of China (the southern regions of the Qinling Mountains and the Huaihe River), including Hainan, Guangdong, Yunnan, Guangxi, Taiwan, Fujian, Guizhou, Jiangxi, Hunan, Sichuan, Chongqing, Zhejiang, Hubei, Anhui (south of the Huaihe River), Shanghai, Shaanxi (south of the Qinling Mountains), Henan (south of the Huaihe River) and Jiangsu (south of the Huaihe River) provinces (from south to north; Chen et al., 2014) .

2.6 Taxonomy

Based on published reports, *C. cicadae* S.Z. Shing, *I. cicadae*, *I. sinclairii*, *O. sobolifera* had routinely been used as the scientific name of Chanhua. Based on a revised ITS based phylogeny done in this study and based on distribution of Chanhua above, all these suspected scientific names are reviewed and taxonomy of Chanhua is summarized as follows.

2.6.1 *Isaria cicadae* Miq.

Isaria cicadae was introduced by Miquel in 1838 with type specimens from Brazil (Miquel, 1838). Massee synonymized it to *C. cicadae* (Miq.) Massee in 1895 (Samson, 1974). Based on morphological evidence, Samson (1974) recircumscribed the genus *Isaria* into the sect. *Isarioidea* of the genus *Paecilomyces* and transferred all *Isaria* species to *Paecilomyces*. *Isaria cicadae*/*C. cicadae* (Miq.) Massee was renamed as *P. cicadae*. Luangsa-ard et al. (2005) analysed DNA sequence of *Paecilomyces* sensu lato and found the genus to be polyphyletic, which lead to the genus *Isaria* including the name *I. cicadae* being revived. Now, *I. cicadae* has generally been accepted as the scientific name of Chanhua, in spite of no DNA sequence data to support such a taxonomic arrangement.

Sexual morph: see *C. kobayasii* below.

2.6.2 *Isaria sinclairii* (Berk.) Lloyd

This species was originally described as *Sphaeria sinclairii* Berk. with types from New Zealand (Berkley, 1855), followed by two revisions from *C. sinclairii* (Berk.) Sacc. to *I. sinclairii* (Kobayasi and Shimizu, 1963; Samson, 1974), and at last Samson (1974) treated it as a synonym of *I. cicadae* based on morphological evidence. Last century in Japan, the name *I. cicadae* had never been used and Chanhua had always been recorded as *I. sinclairii* (Kobayasi & Shimizu, 1963;

Shimizu, 1997). Though *I. sinclairii* is commonly considered as a synonym of *I. cicadae*, they both lack DNA sequence data from their type localities.

Sexual morph: see *C. kobayasii* below.

2.6.3 *Cordyceps kobayasii* Koval and *Cordyceps cicadae-sm* A.Y. Liu & X. Zou, Sexual Morph of Chanhua

Kobayasi (1949) described *C. sinclairii* Kobayasi and regarded it as the sexual morph of *I. sinclairii*, based on one immature specimen from Japan. Considering the same epithet with *C. sinclairii* (Berk.) Sacc., Koval (1984) gave a new scientific name, *C. kobayasii*. Because *I. sinclairii* had already been treated as a synonym of *I. cicadae*, now *C. kobayasii* is generally considered as the sexual morph of *I. cicadae*.

Liu et al. (2011) described the sexual morph of Chanhua, *C. cicadae-sm*, based on one specimen from Leye County, Guangxi, China (Figure 2.4 a, b). Although being a sexual specimen, conidiophores are presented beside the perithecia, and at apices of conidiophores, numerous conidia are clustered together. Depending on microcycle sporulation of ascospores and isolated strains from conidiophores, Liu et al. (2011) confirmed that the compound specimen is Chanhua. Though without molecular evidence, I believe the specimen is the sexual morph of Chanhua, which is also supported by Prof. Zongqi Liang who checked and deposited the specimen in Institute of Fungus Resources, College of Life Sciences, Guizhou University (GZUIFR 08721-6). Unfortunately, I could not extract DNA from the specimen because it has been preserved in solution. Subsequently, Liu (2012) recorded the same collection in her monograph. Prof. Xingliang Wu provided a picture of a similar specimen collected from Dushan, Guizhou, China (Figure 2.4 c, Zha et al., 2018), but the specimen is lost.

Descriptions of *C. kobayasii* (Kobayasi, 1949) and *Cordyceps cicadae-sm* (Liu et al., 2011) are consistent. Since they have been considered as the sexual morphs of *I. cicadae* and Chanhua respectively, together with the molecular phylogeny (Figure 2.2) and the distributions (Figure 2.3) of Chanhua, *C. kobayasii* and *C. cicadae-sm* (= *C. kobayasii*) should be the sexual morph of Chanhua. Unfortunately, *C. kobayasii* and *C. cicadae-sm* lack DNA sequence data.

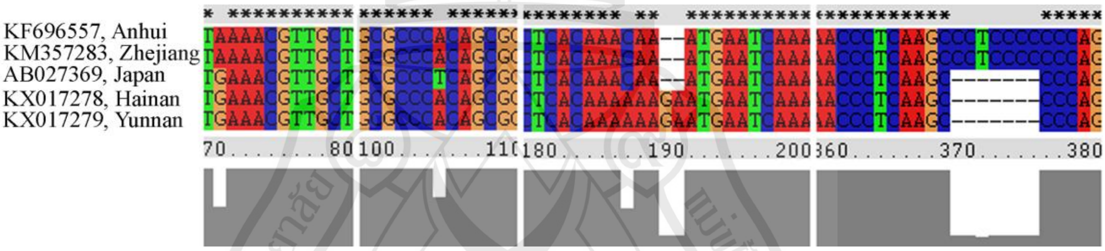
Note a, b sexual morph of Chanhua from Guangxi, China (collected by Xiao Zou on 21 July 2008, GZUIFR 08721-6); c sexual morph of Chanhua from Guizhou, China (phographed by Xingliang Wu in 15 July 2012); d *T. paradoxum* from Zhejiang, China (collected by local people in July 2015 for sale); e, f *O. sobolifera* from Hunan, China (collected by Ping Zhang on 5 July 2011, GACP 110705).

Figure 2.4 Chanhua (*Cordyceps cicadae* (Miq.) Massee), *Tolypocladium paradoxum* and *Ophiocordyceps sobolifera*

2.6.4 *Cordyceps cicadae* S.Z. Shing, a Synonym of *Tolypocladium paradoxum* (Kobayasi) Quandt et al.

Tolypocladium paradoxum (≡ *C. paradoxa* Kobayasi), as well as Chanhua, is also used as a Traditional Chinese Medicine, widely distributed in southern China and has a high yield (Zha et al., 2018). This species produces a single black and hornlike fruiting body, so Chinese people called it 'Du-Jiao-Long' ('One-horned dragon', Figure 2.4 d). Shing (1975) introduced *C. cicadae* S.Z. Shing and treated it as the sexual morph of Chanhua, and in his report the author explicitly stated that its types are 'Du-Jiao-Long' that was bought from a pharmaceutical company in Ningbo, Zhejiang, China. Using morphological evidence and sequence (ITS) data, Chen et al. (2014) clearly explained that 'Du-Jiao-Long' is *T. paradoxum* instead of the sexual morph of Chanhua. I sequenced the ITS gene of 'Du-Jiao-Long' from Yunnan (HKAS 87772, KX017279) and Hainan (GACP 14081002, KX017278), and the sequence alignment result indicates they are similar with that of *T. paradoxum* from Japan (AB027369) and Zhejiang (KM357283) and *C. cicadae* S.Z. Shing from Anhui (KF696557). My molecular phylogeny also supports the view that they are all *T. paradoxum* (Figure 2.2).

As the same epithet between *C. cicadae* (Miq.) Massee and *C. cicadae* S.Z. Shing, Liang (2007) gave a new scientific name *C. zhejiangensis* (Shing) Z.Y. Liu et al. for the latter. So, *C. cicadae* S.Z. Shing, *C. zhejiangensis* (≡ *C. cicadae* S.Z. Shing) are both synonyms of *T. paradoxum*. *T. paradoxum* may include a complex of cryptic species. Although there are no distinct morphological differences (Kobayasi, 1939; Shing, 1975; Figure 2.4 d), the existing five ITS sequences from Japan and China have minor but distinct variations among each other (Figure 2.5).



Note Total length 558 bp alignment.

Figure 2.5 DNA base variations among the existing five ITS sequences of *Tolypocladium paradoxum*

2.6.5 *Ophiocordyceps sobolifera* (Hill ex Watson) G.H. Sung et al.

Ophiocordyceps sobolifera (Figure 2.4 e, f) had previously been regarded as the sexual morph of *I. cicadae/I. sinclairii* (Petch, 1942; Mains, 1951), but its asexual morph was shown to be *Beauveria sobolifera* Z.Y. Liu et al. (Liu et al., 2001; Sung et al., 2007). My molecular phylogeny also proves Chanhua and *O. sobolifera* to be two distinct species (Figure 2.2).

2.6.6 Other Synonyms of Chanhua

Petch (1942), Kobayasi (1941), Kobayasi and Shimizu (1963) and Samson (1974) listed some synonyms of *I. cicadae* and/or *I. sinclairii*, including *C. caespitosa* (Tul. & C. Tul.) Sacc. (≡ *Torrubia caespitosa* Tul. & C. Tul.) from New Zealand, *I.*

arbuscula Har. from Guatemala, *I. basili* (Taylor) Kobayasi (≡ *Sphaeria basili* Taylor) from New Zealand, *I. cosmopsaltriae* Yasuda from Japan, *I. cryptotympanae* Sawada from Taiwan, *I. hariotii* Arnaud from Madagascar and *I. mokanshawii* Lloyd from China. These species lack DNA sequence data. Together with the distributions (Figure 2.3) and the molecular phylogeny (Figure 2.2) of Chanhua, the three Asian species, *I. cosmopsaltriae, I. cryptotympanae* and *I. mokanshawii*, are most likely Chanhua.

2.6.7 Proposal of the Scientific Name for Chanhua

Recently, the asexual genus *Isaria* has been treated as a synonym of the sexual genus *Cordyceps* (Kepler et al., 2017). So, *I. cicadae* Miq. should be revised to *C. cicadae* (Miq.), that is to say the old name *C. cicadae* (Miq.) Massee should be revived.

With a history of 1,500 years, the Chinese common name Chanhua has been widely used and is far more popular than *C. cicadae* and *I. cicadae*, so it should be protected. *I. cicadae* (Miquel, 1838; Samson, 1974), *I. sinclairii* (Berkley, 1855; Samson, 1974) and Chanhua (Kobayasi & Shimizu, 1963; Liang, 2007) are morphologically nearly identical and have generally been accepted as the same species. Considering these factors, the One Fungus One Name (1F1N; Hawksworth et al., 2011; Taylor, 2011) concept and in order not to cause taxonomic confusion, herein I propose to use the scientific name *C. cicadae* (Miq.) Massee, together with the Chinese common name Chanhua, to describe or record this important medicinal mushroom (Zha et al., 2018).

2.7 Medicinal Values

Chanhua is sweet, innoxious and cold in nature for the body; it is good for the eyes, mainly cure convulsions, palpitations, crying at night for children, and can even cure malaria (Wan et al., 2013; Chen et al., 2014). These medicinal values are closely similar to that of cicada sloughs which were recorded in The Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission, 2015). Recent studies indicate, apart from being safe (Chen et al., 2015c), Chanhua has a high nutritional value and contains many bioactive substances (Chen et al., 2015b; Hsu et al., 2015; Yu et al., 2015), which can enhance immunity significantly (He et al., 2010; Liu and Huang, 2015), improve kidney function (He et al., 2012; Liu et al., 2014; Zhu et al., 2014) and can be used for treating cancer (Chen et al., 2015a; Wang et al., 2014a).

2.8 The Hosts

Chanhua (including *I. cicadae* and *I. sinclairii*) has been reported from insect hosts which are all cicada nymphs (Cicadidae, Hemiptera) (Kobayasi and Shimizu, 1963; Samson, 1974; Liu et al., 2011; Wen et al., 2016b), and shows typical host specificity. Liu (2012) reported that Chanhua had been isolated from a synnema growing on a Scarabaeidae (Coleoptera) larva, however, no description was provided in her report, thus it cannot be confirmed. In Japan, Chanhua is commonly found on the nymphs of *Meimuna opalifera* (Walker & F.) (Kobayasi & Shimizu, 1963); while in southern China, the most common host is the nymphs of *Macrosemia pieli* (Kato).

Known hosts of Chanhua from China include 9 cicada species (Cicadidae), they are *Auritibicen flammata* (Distant), *Cicadatra shaluensis* China, *Cryptotympana atrata* (Fabricius), *Hyalessa ella* (Lei & Chou), *H. maculaticollis* (Motschulsky), *H. ronshana* China, *Macrosemia pieli*, *Mogannia conica* (Germar) and *Platypleura kaempferi* (Fabricius), respectively (updated names).

2.9 Ecology

Chanhua grows in warm, humid and low elevational regions. In China, these areas are covered by bamboo forests, broad-leaved forests or coniferous and broad-leaved mixed forests, at altitudes below 2,500 m, have an average annual temperature of about 15 °C, an annual rainfall between 1,000 and 1,200 mm and about 65% forest coverage. When the temperature rises to 18-24 °C and the soil humidity is above 80% from June to August in these areas, synnemata of Chanhua may be produced on cadavers of cicada nymphs in soil. Chanhua has a conspicuous vertical distribution, and most individuals grow on sunny slopes of 30-40° (summarized from Chen et al. (2014) and my investigations).

In China, sexual morph of Chanhua were only reported from Guangxi (Leye) (Liu et al., 2011) and Guizhou (Dushan) (Zha et al., 2018) where the temperature difference is smaller and the humidity is higher than that of other distributed areas of Chanhua. Apart from more complicated natural and environmental factors, sexual morph of Chanhua might need smaller temperature difference and continuous high humidity than its asexual morph.

2.10 Life Cycle

Based on Chen et al. (2014) and my observations on Chanhua and cicadas, herein I summarize the life cycle of Chanhua.

When temperatures rise to 18-24 °C and soil humidity reaches above 80% (before and after July in southern China, which is just the eclosion period of the cicada nymphs), old-mature cicada nymphs are easily infected by the conidia of the Chanhua fungus. Conidia attach to the body surface of a nymph at first, then germinate and form germ tubes which may penetrate the nymph’s body. After successful infection, the germ tubes will separate into numerous short parts (hyphal bodies). They use blood (hemolymph) and solid organizations of the host as nutrients and reproduce rapidly, and they can occupy the whole-body cavity (haemocoel) of the host within two to three days. These hyphal bodies elongate gradually and form into mycelia, which will fill the whole haemocoel, use up water and nutrients, produce some noxious metabolites, and eventually cause the nymph to die.

After having been infected by the fungus, the nymph will accelerate to mature, which may cause it to molt early and to die easily during molting. Most nymphs are close to the ground with their head upwards when they die (killed by the fungus), only a few can get to the ground. Mycelia within the nymph form a solid and dense sclerotia which can resist harsher environments such as low temperature and drought, and mycelia also produce antibiotics and toxins that can resist attacks of other organisms, and the sclerotium will remain dormant and not rot away.

When temperature rises to 18-24 °C and soil humidity reaches above 80% again (the current or the following year), mycelia within the sclerotium will begin to grow. They gather and entwine (only one mating type) and form one or several synnemata. Gradually the synnemata become larger and form one or several light yellow ‘buds’ which will grow out from the molting line of the dorsal thorax of the nymph. The ‘buds’ grow straightly upwards, break through the soil and grow above the ground. Length and size of a synnema varies according to: (1) depth of the nymph in the soil and thickness of litter layer that covered the hole; (2) temperature, soil humidity and light; (3) the instar stage of the nymph; and (4) number of synnemata growing on the nymph. The apex of a synnema will branch repeatedly and form many conidiophores, on which numerous whorled conidiogeous cells (phialides) will be produced. Numerous conidia will be produced repeatedly from these conidiogeous cells, forming chains of conidia. While forming synnemata, mycelia can also grow out from some thin parts of the body surface (including intersegmental membranes, joints of legs, and so on), cover the cadaver partially or completely.

Dispersed by air or water, the conidia eventually fall to the ground and infiltrate the soil along with water flow. They can attach to the body surface of a nymph if they make contact. Once the nymph is infected successfully, a life cycle of Chanhua is completed.

In southern China, the humidity of shallow soil layer changes continually and this interferes with the life cycle of Chanhua. Together with fungal growth and the habits of host cicada nymphs, I can divide the life cycle of Chanhua into four separate stages (Wen et al., 2016b) in Table 2.1.

Table 2.1 Four stages of life cycle of Chanhua in the wild

Stages	Occurrences	Essential factors
Stage I	In soil, conidia attaching to the body surface of a nymph	Conidia have already infiltrated into the soil
Stage II	Infected–death–a sclerotium formed	Suitable temperature and humid soil environment
Stage III	Sclerotium begins to germinate and an original synnema will be formed	Suitable temperature and humid soil environment
Stage IV	Growth of synnemata	Suitable temperature, humid soil environment and light

Under laboratory conditions, factors such as infection, temperature, humidity, light and even host nymphs can be artificially controlled but these are different from those in the wild. For the needs of both research and industrial production, I compared life cycles of Chanhua in the wild and under artificial culture (Table 2.2).

Table 2.2 A comparison of life cycle of Chanhua in the wild and under artificial culture

Factor	In the wild	Artificial culture
Conidial contact by nymph	By attachment	Most injecting, few spraying
Death of host	Killed by Chanhua fungus, slowly	Killed by injecting or high temperature, quickly
Temperature and humidity	18-24 °C and above generally 80%	Both are controlled steadily; the infection needs a higher temperature
Light	Natural light, not steady	Artificial control, steady
Synnema	One or a few large synnemata produced from dorsal thorax of an undamaged cadaver	Many small synnemata produced from many parts of a rotten cadaver

Table 2.2 (continued)

Factor	In the wild	Artificial culture
Four stages of life cycle	Separate from one another	Connected one by one
Time span of a life cycle	Varies from 2 weeks to one year or more	About 2 weeks
The hosts	Cicada nymphs, 9 species known in China	Cicada nymphs or lepidopteran larvae, alternative

CHAPTER 3

TAXONOMY AND BIOLOGY OF *CORDYCEPS QINGCHENGENSIS* SP. NOV. AND ITS ALLIES

3.1 Introduction

Cordyceps (*Cordyceps* sensu lato) has always been highly researched for its species diversity, edible and medicinal values and applications in biological control. As an important group of invertebrate-pathogenic fungi, cordyceps can grow on insects, spiders, nematodes, other cordyceps, the fungi *Elaphomyces* and even inhaibt soil and plant tissues (Sung et al., 2007; Vega et al., 2009). This group currently has more than 1,000 species (Table 1.1) that belong to three families (Cordycipitaceae, Ophiocordycipitaceae and Clavicipitaceae (in part)) in the order Hypocreales (Sung et al., 2007). More than 140 species are reported from China (Wen et al., 2017).

The genus *Cordyceps* (Cordycipitaceae) comprises 172 accepted species (http://www.catalogueoflife.org/, access in July 2019). Due to lack of molecular evidence or inconclusive morphology and ecology, more than 100 species are retained from the old genus *Cordyceps* (Sung et al., 2007). Also, due to confusion of sexual and asexual morphs, many cordyceps species still have two or more names. Clarification of sexual and asexual names and classification of undetermined *Cordyceps* species are still needed for cordyceps research.

Occurrence of a cordyceps is closely related with its ecological environment and the life cycle of its host insect. Details are often required of natural and ecological factors such as humidity (air and soil), temperature, light, rainfall, elevation, biogeography and the occurrence season (both cordyceps and its host). Important host information is often lacking, including host name/group, the instar of death (egg, nymph/larva, pupae/cocoon, adult), the instar that becomes infected (generally nymph or larva, significantly different from the instar of its death), locality of collection (in soil, on humus layer, on leaf or tree trunk, in root or tree trunk.) and the inhabited plant (Wen et al., 2016b). All of this biological information is useful for the study of cordyceps. Unfortunately, the biology of cordyceps has been often poorly studied, which seriously affects the identification, application and ability to exploit the fungus.

I 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, I clarify the taxonomy of related *Cordyceps* species and record their ecology and life cycles.

3.2 Material and Methods

3.2.1 Specimens

Specimen collections and biological and ecological investigations were made in China in recent years. 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.

3.2.2 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 1α (TEF1-*α*) genes were sequenced using the primers detailed by White et al. (1990) and Ban et al. (2015b). Amplification reactions were performed in an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA) and the PCR programs followed those of Ban et al. (2015b). Sequences were aligned and assembled visually and manually using Clustalx1.81 (Larkin et al., 2007), Chromas230 and ContigExpress software.

3.2.3 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 3.1). Molecular phylogeny was reconstructed using the single ITS, the single TEF1-*α* and combined SSU, LSU and TEF1-*α* sequence datasets. *Ophiocordyceps sinensis* (Berk.) G.H. Sung et al. (EFCC 7287; Sung et al., 2007) was used as the outgroup taxon.

Table 3.1 Voucher information and GenBank numbers for samples appearing in Figures 3.1, 3.5

Species	**Collection**	**ITS**	**SSU**	**LSU**	**TEF1-*α***
Cordyceps bifusispora	spat 08-129	-	MF416576	MF416523	MF416468
C. bifusispora	spat 08-133.1	-	MF416577	MF416524	MF416469
C. chiangdaoensis	BCC 75734	KT261394	-	-	KT261404
***C. cicadae* (Chanhua)**	**GACP 07071701**	**KX017277**	**MK761207**	**MK761212**	**MK770631**
***C. cicadae* (Chanhua)**	**GACP 14061604**	**KX017276**	**MK761208**	**MK761213**	**MK770632**
C. cicadae (Chanhua)	RCEF HP090724-31	-	MF416605	MF416552	MF416496
C. coleopterorum	CBS 110.73	AY624177	JF415965	JF415988	JQ425689
C. exasperata	MCA 2155	-	MF416596	MF416542	MF416486
C. farinosa	CBS 111113	AY624181	AY526474	MF416554	MF416499
C. fumosorosea	CBS 244.31	AY624182	MF416609	MF416557	MF416503
C. kyusyuensis	EFCC 5886	-	EF468960	EF468813	EF468754
C. militaris	OSC 93623	JN049825	AY184977	AY184966	DQ522332
C. morakotii	BCC 55820	KT261389	-	-	KT261399
C. ninchukispora	BCC 2121	FJ765277	FJ765292	FJ765245	FJ765261
C. ninchukispora	BCC 30937	FJ765274	FJ765289	FJ765242	FJ765258
C. ningxiaensis	HMJAU 25074	KF309668	-	KF309671	-
C. oncoperae	AFSEF 4358	-	AF339581	AF339532	EF468785

Table 3.1 (continued)

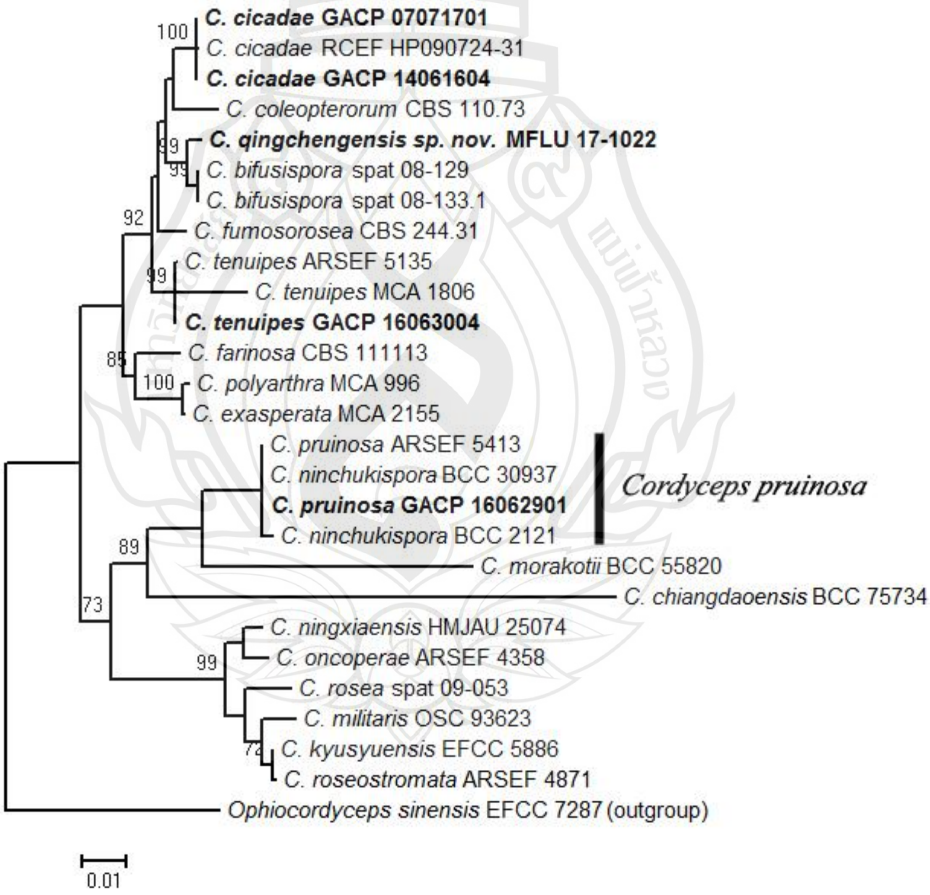
Species	Collection	ITS	SSU	LSU	TEF1-*α*
C. polyarthra	MCA 996	-	MF416597	MF416543	MF416487
C. pruinosa	**GACP 16062901**	**KY423508**	**MK761210**	**MK761215**	**MK770634**
C. pruinosa	ARSEF 5413	JN049826	AY184979	AY184968	DQ522351
C. qingchengensis	**MFLU 17-1022**	**KY423506**	**MK761206**	**MK761211**	**MK770630**
C. rosea	spat 09-053	-	MF416590	MF416536	MF416480
C. roseostromata	ARSEF 4871	-	AF339573	AF339523	-
C. roseostromata	ARSEF 4870	EF368022	-	-	-
C. tenuipes	MCA 1806	-	MF416595	MF416541	MF416485
C. tenuipes	ARSEF 5135	AY624196	MF416612	JF415980	JF416020
C. tenuipes	**GACP 16063004**	**KY423509**	**MK761209**	**MK761214**	**MK770633**
Ophiocordyceps sinensis	EFCC 7287	JN049854	EF468971	EF468827	EF468767

Note Sequencing results in this study are displayed in bold.

The single ITS and TEF1-*α* sequence datasets were analyzed using neighbor-joining (NJ) method (Saitou & Nei, 1987), and the combined SSU, LSU and TEF1-*α* 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, other details are described in Jeewon et al. (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 and also a bootstrap test of 1,000 replicates, other details were outlined by Cai et al. (2006) and Tang et al. (2007).

3.3 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 in 909 characters of the single TEF1-α, and 450 variable and 277 parsimony-informative in 2,679 characters of the combined SSU, LSU and TEF1-α gene dataset. The different analyses resulted in trees with similar topology and support values (results not shown). The ML tree for combined SSU, LSU and TEF1-α sequence dataset is shown in Figure 3.1, and the NJ tree for the single ITS sequence dataset is shown in Figure 3.5.



Note Bootstrap support values greater than 70% are indicated above the nodes.

Figure 3.1 ML tree of *Cordyceps qingchengensis* sp. nov. and its allies inferred from combined SSU, LSU and TEF1-α dataset

3.4 Taxonomy and Biology

Recently in China, I collected and investigated some *Cordyceps* species in the wild. Herein I provide taxonomic notes on three of them and describe a new species. At the same time, I record and infer their ecology and life cycles.

3.4.1 *Cordyceps tenuipes* (Peck) Kepler et al. (Figure 3.2)

≡ *Isaria tenuipes* Peck

≡ *Paecilomyces tenuipes* (Peck) Samson

= *Cordyceps takaomontana* Yakush. & Kumaz.

Cordyceps takaomontana was originally described and illustrated by Kobayasi (1941) with the type specimen from Japan. Kepler et al. (2017) gave a nice 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.

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 meet the characters of *C. takaomontana*, so I 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, I 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 (Figure 3.2). 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 (Figure 3.2). 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. *I. 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.

Figure 3.2 Asexual morph of *Cordyceps tenuipes* in the wild

3.4.2 *Cordyceps qingchengensis* L.S. Zha & T.C. Wen sp. nov. (Figures 3.3, 3.4)

Index Fungorum number IF556460; Facesoffungi number FoF 03405.

Description. Sexual morph: Stromata arising from head of cocooned pupa of a large silk moth (the cocoon is ovate and thick, 21 × 8 mm), fleshy, yellow (fresh specimen), branched, fleshy, 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 ($\overline{X}$=188, n=10) × 2.4-4.0 ($\overline{X}$=3.15, n=30) μm, caps hemispheric, 1.8-2.2 ($\overline{X}$=2.0, n=30) μm high and 2.5-3.2 ($\overline{X}$=2.85, n=30) μm wide, 8-spored. Ascospores filiform, about 180-220 μm long and 0.45-0.65 ($\overline{X}$=0.53, n=30) μm wide, not at all bifusiform and not broken into part-spores. Asexual morph: Unknown.

Note a growing in the wild; b dug out from the soil; c habitat environment.

Figure 3.3 *Cordyceps qingchengensis*

Material examined. CHINA. Sichuan Province: Chengdu, Qingchengshan Mountains, 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).

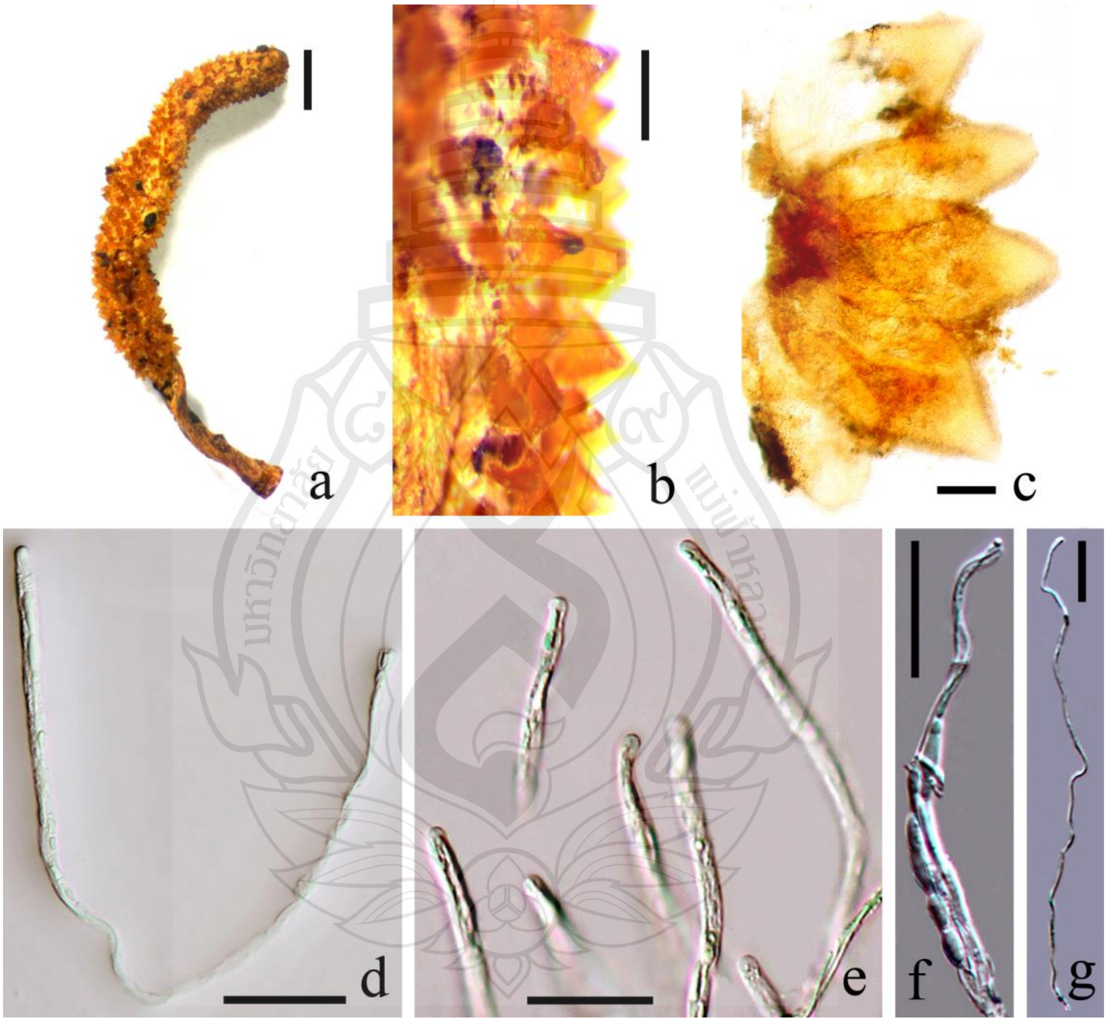
Host. Growing on the ovate cocooned pupa of a large silk moth (Lepidoptera: Bombycidae) in humid soil of a broad-leaved forest.

Etymology. the new species is named after Qingchengshan Mountains, its type locality.

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 (Table 3.2).

ITS sequence of *Cordyceps qingchengensis* is most similar (only 0.9 % bp difference across 569 bp) to that of Chanhua (*C. cicadae* (Miq.) Massee, RCEF HP090724-31 and my collections; Zha et al., 2018), but their TEF1-α sequence are distinctly different (3.1% bp difference across 911 bp). Morphologically, Chanhua has large and irregularly branched stromata, broad ascospores and grows on cicada nymphs, features that are significantly different from *C. qingchengensis* (Table 3.2).

TEF1-*α* sequence of *C. qingchengensis* is most similar (1.7 % bp 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*. Single ITS together with combined SSU, LSU and TEF1-*α* genes phylogenies indicate *C. qingchengensis* is closely related to, but different from *C. bifusispora*, Chanhua and *C. tenuipes* (Figures 3.1, 3.5).

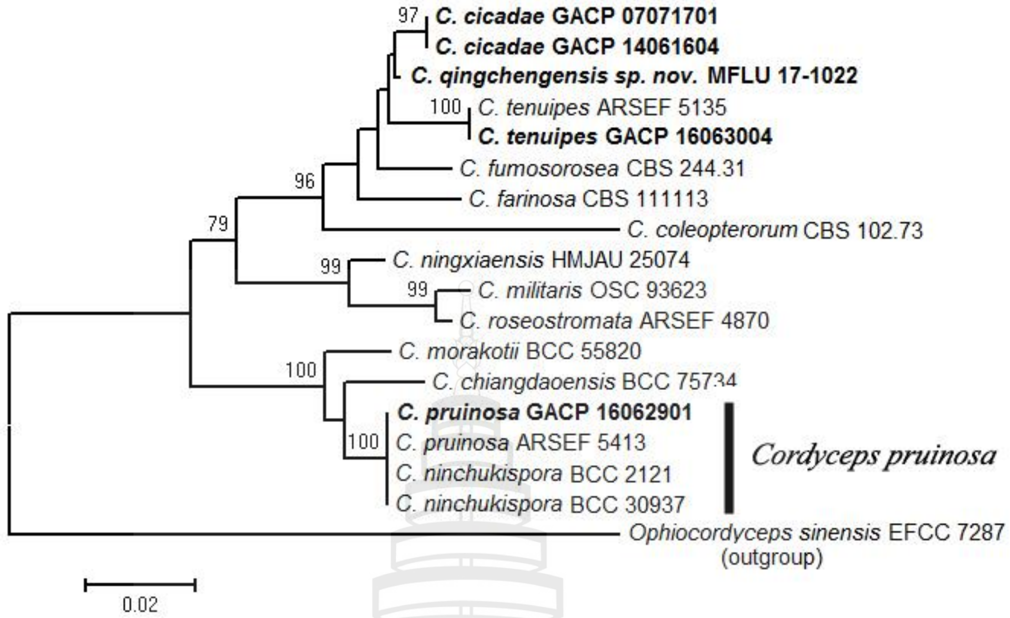


Note a fertile head of dry fruiting body; b surface of fertile head; c perithecia in profile; d-f asci; g ascospore. Scale bars: a = 1 mm, b = 200 µm, c = 100 µm, d-f = 20 µm, g = 10 µm.

Figure 3.4 *Cordyceps qingchengensis* (MFLU 17-1022, holotype)

Table 3.2 Morphological comparison of sexual states of *Cordyceps bifusispora*, *C. qingchengensis*, *C. tenuipes* and Chanhua (*C. cicadae* (Miq.) Massee)

Fungi	Host	Stroma	Fertile head	Ascospores	References
C. bifusispora	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 et al. (2017)
C. qingchengensis	Ovate cocooned pupa of a large silk moth (Lepidoptera: Bombycidae)	Single, branched, basal stipe and upper 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
C. tenuipes	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 et al. (2017)
Chanhua (*C. cicadae*) = *C. sinclairii* from Japan = *C. cicadae-sm* in China (an illegal epithet)	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), Liu et al. (2011), Zha et al. (2018)



Note Bootstrap values greater than 75% are indicated above the nodes.

Figure 3.5 NJ tree of *Cordyceps qingchengensis* and its allies inferred from ITS gene data

Biology. The cocoon of the host is a little rotted (Figure 3.3 b) and the pupa in the cocoon had been seriously degraded by the fungus. I infer the life cycle of the fungus should be as follows.

In the previous summer, an old silk moth larva was preparing 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 advent of the rainy season in the next summer, the sclerotium began to germinate and fruiting body of the fungus formed at last.

3.4.3 *Cordyceps pruinosa* Petch (Figure 3.6)

= *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.

Note 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.

Figure 3.6 Different stages of life cycle of *Cordyceps pruinosa*

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., 2016b), 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 (Figure 3.6).

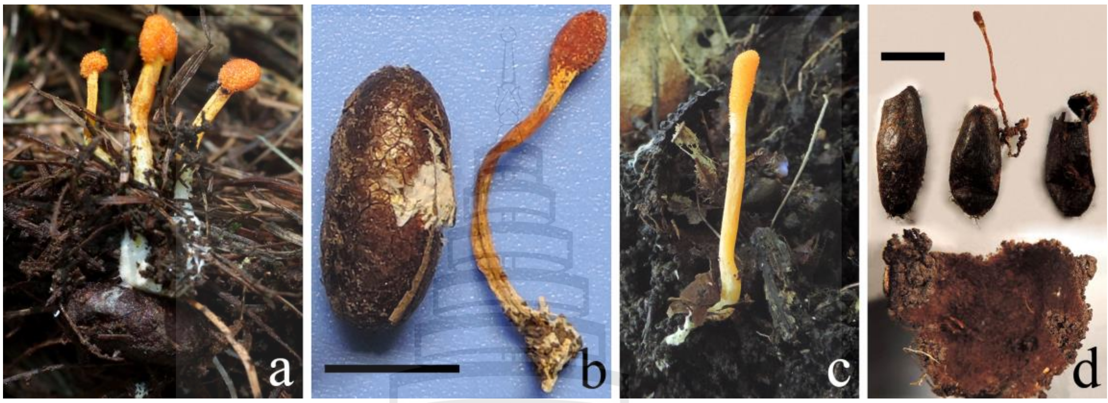
In moist subtropical regions, *C. pruinosa* can occur for most of the year. The reasons may include: (1) many Limacodidae species can reproduce 2-3 generations per year; (2) abundant but non-continuous rainfall all year round; and (3) the infection and the growth of cordyceps are separate (Wen et al.,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

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 creating an effective imitation mechanism (Figure 3.6). *C. ninchukispora* (≡ *Phytocordyceps ninchukispora*), the only exception among all cordyceps species, was reported as a plant pathogen growing on seeds of *Beilschmiedia erythrophloia*, Lauraceae (Su & Wang, 1986). *Beilschmiedia* plants are common hosts of several Limacodidae species, and *Beilschmiedia* seeds are similar to these Limacodidae pupae, so I 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 aligment and Blast search indicate that two of them are not cordyceps and four represent other known cordyceps species (>99.6% similar); the remaining 19 sequences (including one from Taiwan) are >99.5% similar and are probably the same species (the rest <0.5% base difference mainly comes from base insertions or gaps, which I think are errors during the process of sequence assembly). *C. 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). *C. 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 (southern Asia). For these reasons, and following Sung et al. (2007), I synonymize *C. ninchukispora* into *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).

3.4.4 *Cordyceps ningxiaensis* Tolgor Bau & J.Q. Yan (Figure 3.7 a, b)



Note 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 parasitized 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.

Figure 3.7 Two *Cordyceps* species growing on Hymenoptera pupae

Cordyceps ningxiaensis was introduced by Yan and Bau (2015) with the type from Liupanshan Mountains, Longde County, Ningxia Hui Autonomous Region, China. The species is morphological and phylogenetic related to *C. militaris* (L.) Link and its allies (Figures 3.1, 3.5). Hosts of *C. ningxiaensis*, which had originally been identified as fly pupae (Diptera), are actually Scoliidae pupae (Hymenoptera,) that parasitized 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 anesthetize 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* (Figure 3.7 c, d) collected in Changbai Mountains, 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*.

CHAPTER 4

XUEFENG CORDYCEPS: INSIGHTS INTO SPECIES DIVERSITY, LIFE CYCLE AND HOST ASSOCIATION

4.1 Introduction

Xuefeng Mountain, its literal meaning is ‘a mountain with snowy top’, because its top has been covered with snow for most of the year. As an iconic mountain of the Second Step (2,000-3,000 m alt.) of Chinese terrain which inserts between high mountains of the First Step (above 4,000 m alt.) and hills of the Third Step (below 500 m alt.), Xuefeng Mountain is located in west-central Hunan Province, China, and is 350 km long and 80-120 km wide with an altitude which varies between 240 and 1,934 m (Figure 4.1). The annual average temperature in this region is 12.7 °C (varying -10.1-27.3 °C), with a relative humidity of more than 87% and abundant rainfall (annual average rainfall of 1,810 mm). With heavy fog and frost, winter is rather dry and cold while summer is humid and cool (Yuan, 2014).

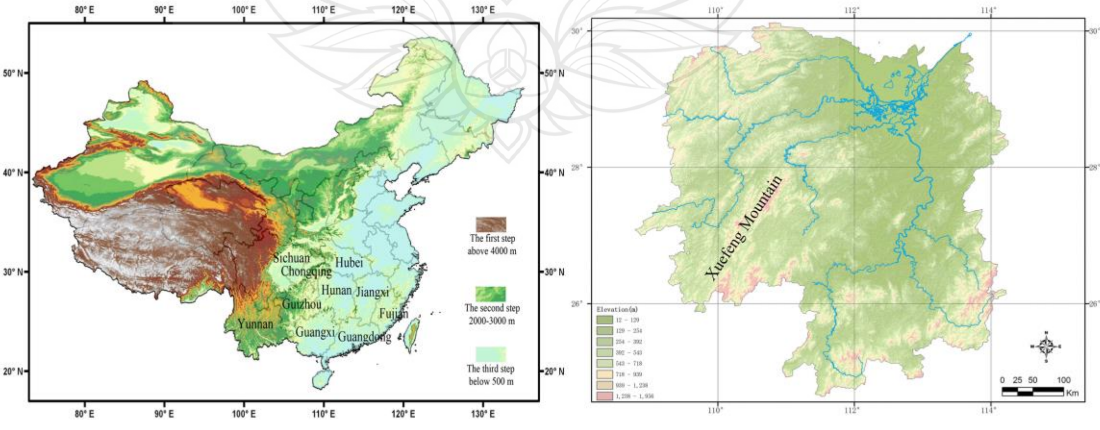


Figure 4.1 Topopraphic maps of China (the left) and Hunan Province (the right)

Cordyceps (*Cordyceps* sensu lato; Zha et al., 2018, 2019b, 2019c) is one of the most important groups of invertebrate pathogens (Hywel-Jones, 2001). Currently it includes more than 1,000 species that belong to three families (Cordycipitaceae, Ophiocordycipitaceae and a part of Clavicipitaceae) in the order Hypocreales (Table 1.1), among which more than 140 species are reported from China (Wen et al., 2015). For a long time, cordyceps researches are mainly focused on species diversity, medicinal values and biological control, however biology, ecology and associations with hosts are rather poorly understood.

The common name 'Xuefeng Cordyceps', previously known as 'Immortal Herb', had originally been recorded in the middle or late Tang Dynasty (about 1,200 years ago), and has been intensively used to cure various cancers and other diseases, improve the physique and extend human longevity (Huang, 2012). Although being a precious traditional Chinese medicine (TCM) by local Yao people, this common name had not been formally reported yet until *Ophiocordyceps xuefengensis* T.C. Wen et al., the first species having been introduced (Wen et al., 2013; Liu et al., 2015). Molecular phylogenetic analysis indicated *O. xuefengensis* is closely related with the important Traditional Chinese Medicine (TCM) *O. sinensis* (Berk.) G.H. Sung et al. (Dong Chong Xia Cao; Winter worm, summer herb) (Wen et al., 2013). Together with similarly growing on Hepialidae larvae (Wang and Yao, 2011), Xuefeng Cordyceps rose to fame swiftly. Also, from then on, the common name Xuefeng Cordyceps came into being, in spite of the second species, *O. ramosissimum* T.C. Wen et al. reported which shares the same host insect that resides on the same host plant as the former (Wen et al., 2014).

Rather than a precious TCM, to a greater extent Xuefeng Cordyceps represent a kind of Chinese triditional culture. This study aims to reveal the species diversity of Xuefeng Cordyceps, and to identify their host insects and the plants on which the insects resided. At the same time, I try to make clear the biology and ecology of Xuefeng Cordyceps and their host insects. To recognize the regional cordyceps correctly, and to inherit the Chinese triditional culture properly, current research provides important taxonomic, biological and ecological information.

4.2 Material and Methods

4.2.1 Explorations and Specimens

Explorations were made in the Xuefeng Mountain region, Hunan Province, China from year to year. Specimens, including the cordyceps fungi, the host insects (larvae and pupae), and the plants on which the insects resided were observed in the wild and brought back to laboratory for identification. A Nikon Coolpix P520 camera and a Nikon Eclipse 80i compound microscope connected with a Cannon EOS 600D camera were used to photograph. Measurements were made using Tarosoft (R) Image Frame Work software. The fungal specimens are deposited in GACP (the Herbarium of Guizhou Agricultural College, Guiyang, Guizhou, China), GZUH (the Herbarium of Guizhou University, Guiyang, = GACP) and MFLU (Centre of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, Thailand).

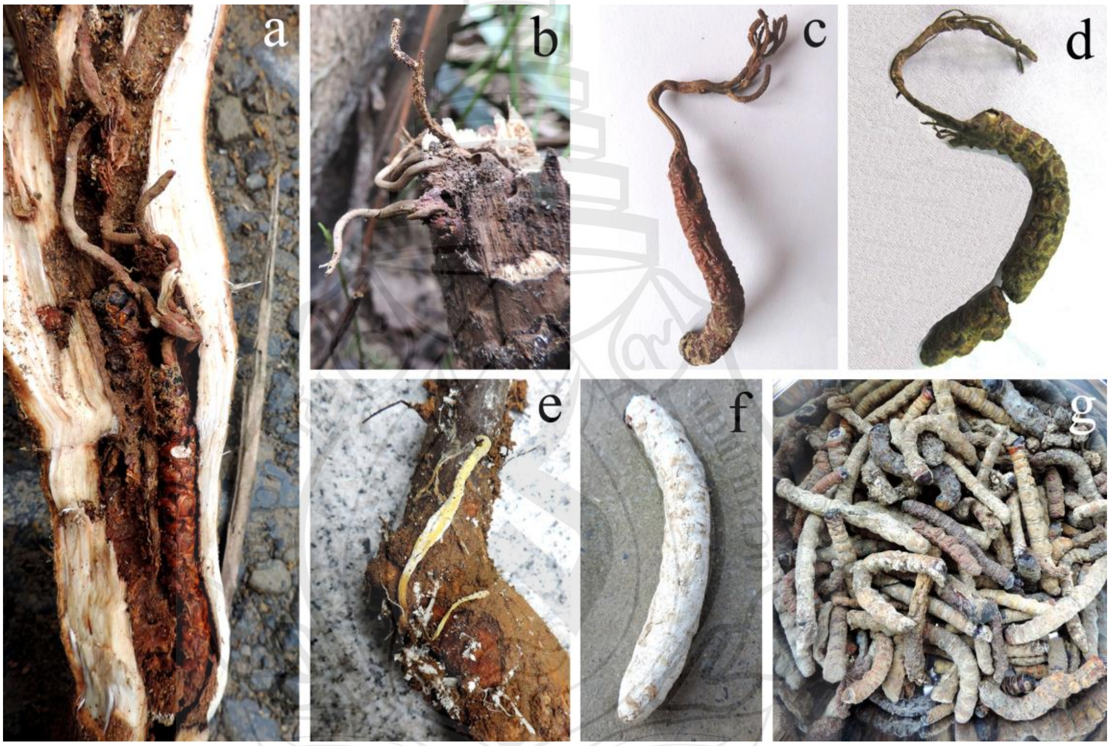
4.2.2 DNA Extraction, PCR, Sequencing, Sequence Alignment and Assembly

Total genomic DNAs were extracted from sclerotia of the cordyceps and leaves of the plants to confirm fungus and plant identification respectively, using E.Z.N.A.TM Fungal DNA MiniKit (Omega Biotech, CA, USA). The primer pairs ITS4/ITS5 were used for PCR amplification and sequencing of ITS gene of the cordyceps and the plants (White et al., 1990). PCR products were sequenced by GenScript Biotechnology Co., Nanjing, China. Alignments were checked visually and improved manually carefully according to sequence diagrams. Clustalx1.81, ContigExpress (Invitrogen, Carlsbad, CA, USA), Chromas230 (http://www.technelysium.com.au/chromas.html) and Mega6.06 biology software was used for sequence assembly and alignment.

4.2.3 Construction of Phylogenetic Tree of the Host Plants

A Blast search was performed to reveal the closest matches of the host plants in the GenBank database that would allow the selection of appropriate taxa for phylogenetic analyses. Maximum likelihood (ML) analysis was performed using RAxML v. 8.2.8 employing a GTRGAMMA model of nucleotide substitution, other details are described in Jeewon et al. (2003) and Hongsanan et al. (2017). Maximum parsimony (MP) analysis was performed with PAUP* 4.0b10 (Swofford, 2002) and using the heuristic search option with TBR branch swapping and a bootstrap test of 1,000 replicates, other details were outlined by Cai et al. (2006) and Tang et al. (2007).

4.3 Species Diversity of Xuefeng Cordyceps



Note a, b *Ophiocordyceps xuefengensis*; c *O. ramosissimum*; d *Metacordyceps taii* (syn. of *Metarhizium guizhouense*); e sexual morph of *Beauveria bassiana*; f *B. bassiana*; g ossified insects.

Figure 4.2 Species of Xuefeng Cordyceps

In the Xuefeng Mountain region, Hunan, China, so far, I have found five Xuefeng Cordyceps species. These cordyceps were dug out from basal trunks or roots of the plant *Clerodendrum cyrtophyllum* Turcz. (Lamiales: Lamiaceae), and their hosts are all larvae of *Endoclita davidi* Poujade (Lepidoptera: Hepialidae). The five cordyceps species are reported as follows.

4.3.1 *Beauveria bassiana* (Bals.-Criv.) Vuill.

≡ *Botrytis bassiana* Bals.-Criv.
≡ *Spicaria bassiana* (Bals.-Criv.) Vuill.
≡ *Penicillium bassianum* (Bals.-Criv.) Biourge
Sexual morph
= *Cordyceps bassiana* Z.Z. Li et al.

Material examined. Asexual morph: GACP 15101201, GACP 15101206 (Figure 4.2 f); sexual morph: GACP 14071004, GACP 16080601 (Figure 4.2 e).

Notes. Though the asexual *B. bassiana* and the sexual *C. bassiana* are conspecific (Li et al., 2001), herein I habitually separate them to discuss. *B. bassiana* is very common in the wild, but it has seldom been found in trunks or roots of plants. Notably, in all entomopathogenic fungi, only *B. bassiana* and *O. sinensis* are included in the Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission, 2015). Unlike the common *B. bassiana*, *C. bassiana* is rarely collected in the wild. A third *C. bassiana* specimen (GACP 16041002) in my hands also grew on a Hepialidae larva, but it was dug out from soil (Guizhou, China) instead of in a tree trunk or root. The sequencing results further substantiate that *C. bassiana* is the sexual morph of *B. bassiana*.

4.3.2 *Metarhizium guizhouense* Q.T. Chen & H.L. Guo

= *Metarhizium taii* Z.Q. Liang & A. Y. Liu
= *Metarhizium taii* var. *Chongqingensis* Y.H. Yang et al.
= *Metarhizium taii* var. *taii* Z.Q. Liang & A.Y. Liu
Sexual morph
= *Cordyceps taii* Z. Q. Liang & A. Y. Liu
= *Metacordyceps taii* (Z.Q. Liang & A.Y. Liu) G.H. Sung et al.

Material examined. Sexual morph: GACP 1229 (Figure 4.2 d) and GACP 1230.

Notes. *Metarhizium guizhouense* was originally isolated from a dead Hepialidae larva in Guizhou, China (Guo et al., 1986). Liang et al. (1991) introduced

C. taii and its asexual morph *Metarhizium taii* aslo on Lepidoptera larvae from Guizhou. Sung et al. (2007) introduced the genus *Metacordyceps* and revised *C. taii* to *Metacordyceps taii*. Kepler et al. (2014) revised *Metacordyceps* as a synonym of *Metarhizium*. Huang et al. (2005) treated *Metarhizium taii/C. taii* as synonyms of *Metarhizium anisopliae* (Metschn.) Sorokīn based on ITS sequence data, but this placement was rejected by Bischoff et al. (2009) who treated *Metacordyceps taii*/*Metarhizium taii* as synonyms of *Metarhizium guizhouense* based on a multilocus phylogeny. Evidences from hosts, geographical distributions, morphologies and molecular phylogeny are all supportive of *Metarhizium guizhouense*, *Metacordyceps taii* (≡ *C. taii*) and *Metarhizium taii* to be conspecific.

Metacordyceps taii is widely distributed in southwestern provinces of China and has a high yield, especially in Guizhou (Zha et al., 2018). In Guizhou, this sexual morph can be collected all year around, but its fruiting body stage only occurs between May and August (Zha et al., 2018). Generally, it grows from heads of larvae or pupae of Hepialidae in soil. This is the first report that *Metacordycep taii* can grow in tree trunk or root instead of in soil.

4.3.3 *Ophiocordyceps ramosissimum* T.C. Wen, J.C. Kang & K.D. Hyde

Material examined. MFLU 12-2165 (holotype), GZUH HN8 (Figure 4.2 c), GZUH 2012HN2, GZUH 2012HN10 and GZUH 2012HN12 (paratypes).

Notes. Morphologically, *O. ramosissimum* can be easily differentiated from *O. xuefengensis* and *O. macroacicularis* by its multi-branch stromata, although their ossified insects (Figure 4.2 g) or immature fruiting bodies are very similar and not easy to distinguish.

4.3.4 *Ophiocordyceps xuefengensis* T.C. Wen et al.

Material examined. GZUH 2012HN14 (holotype), GZUH HN13, GZUH 2012HN11, GZUH 2012HN13 and GZUH 2012HN19 (paratypes); GACP 16090901 (Figure 4.2 b), GACP 16092301 and GACP 16092302 (Figure 4.2 a).

Notes. For the special medicinal value, ancient Yao people called these strange ‘herbs’ growing on caterpillars as ‘Immortal Herbs’ (Huang, 2012, 2013). Since the establishment of *O. xuefengensis*, the original name ‘Immortal Herb’ had automatically been replaced by the new name ‘Xuefeng Cordyceps’. In other words, Xuefeng Cordyceps represent a group of cordyceps, and *O. xuefengensis* is just one of them.

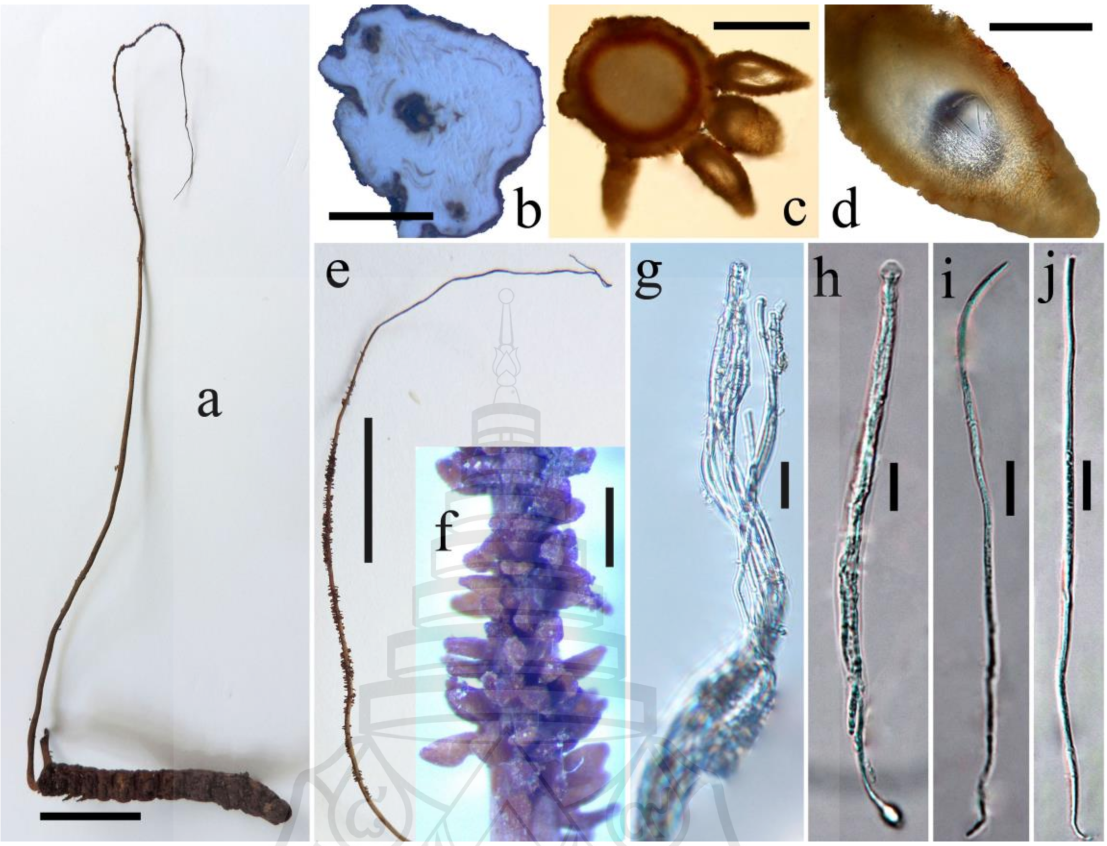
4.3.5 *Ophiocordyceps macroacicularis* S. Ban, T. Sakane & Nakagiri (Figure 4.3)

Material examined. GACP 15092001, GACP 15092002, GACP 15092003, GACP 15092004. These four specimens were dug out within the same plant. Other region's specimens: GACP SG0301 - GACP SG0312 (12 specimens), Match to May, 2016, Guizhou, China, growing from heads of larvae of *Endoclita* sp. living in basal tree trunks or roots, collected by local people.

Redescription. Sexual morph. Stromata: one or two, seldom fasciculate, slender and long, cylindrical at base, then gradually thinning upwards, unbranched, relatively smooth, brown, 80-220 × 0.1-2.5 mm, growing from heads of lepidopteran larva living in basal tree trunks or roots. Stipe: lower part (below middle) sterile, sometimes distal part also sterile due to too slender. Perithecia: superficial, vertically placed to the stipe, ovoid and compressed, apices a little coned, yellowish brown to brown, covering middle to sub-distal part of the stipe, but sparse or absent on the distal part, 390-820 × 250-440 μm (537.4 × 324.7 μm on average). Asci: hyaline, cylindrical, 8-spored, 240-310 μm length (267.6 μm on average), apical cap conspicuous and thick, 5.0-7.2 (5.6 μm on average) in diameter. Ascospores: hyaline, needle-shaped, septate indistinctly, but 10-20 septa barely visible in old-mature specimens, 200-300 × 2.0-3.0 μm (253.8 × 2.5 μm on average); no partspore observed.

Asexual morph. *Hirsutella*-like (Ban et al., 2015b; Zhou et al., 2015).

Hosts. Growing from heads of larvae of *Endoclita* (Hepialidae) or Cossidae living in basal tree trunks or roots, such as *E. davidi* living in *Clerodendrum cyrtophyllum*, or *Cossida* sp. living *Reynoutria japonica* Houtt. (Polygonaceae) (Ban et al., 2015b).



Note a overview of stroma and the host; b section of ossified insect; c section of stroma; d section of ascoma; e distal part of stroma; f brown, superficial ascomata on stroma; g a bunch of asci twisted together; h a mature ascus with ascospores; i, j hyaline and filliform ascospores with indistinct septa; Scale bars: a, e = 20 mm, b = 2 mm, c = 500 μm, d = 200 μm, f = 100 μm, g = 40 μm, h-j = 20 μm.

Figure 4.3 *Ophiocordyceps macroacicularis*

Distribution. China (Hunan (newly recorded), Guizhou), Japan (Kyoto) (Ban et al., 2015b).

My collections were identified as *O. macroacicularis* based on two reasons: (1) morphologically, apart from only 1-2 stromata, my collections are closely similar to *O. macroacicularis* from Japan (Ban et al., 2015b); (2) ITS sequences of my collections (GACP 15092001, GACP 15092002 and GACP SG0301, not uploaded) and *O. macroacicularis* from Japan are highly similar (only one or two DNA bases' differences).

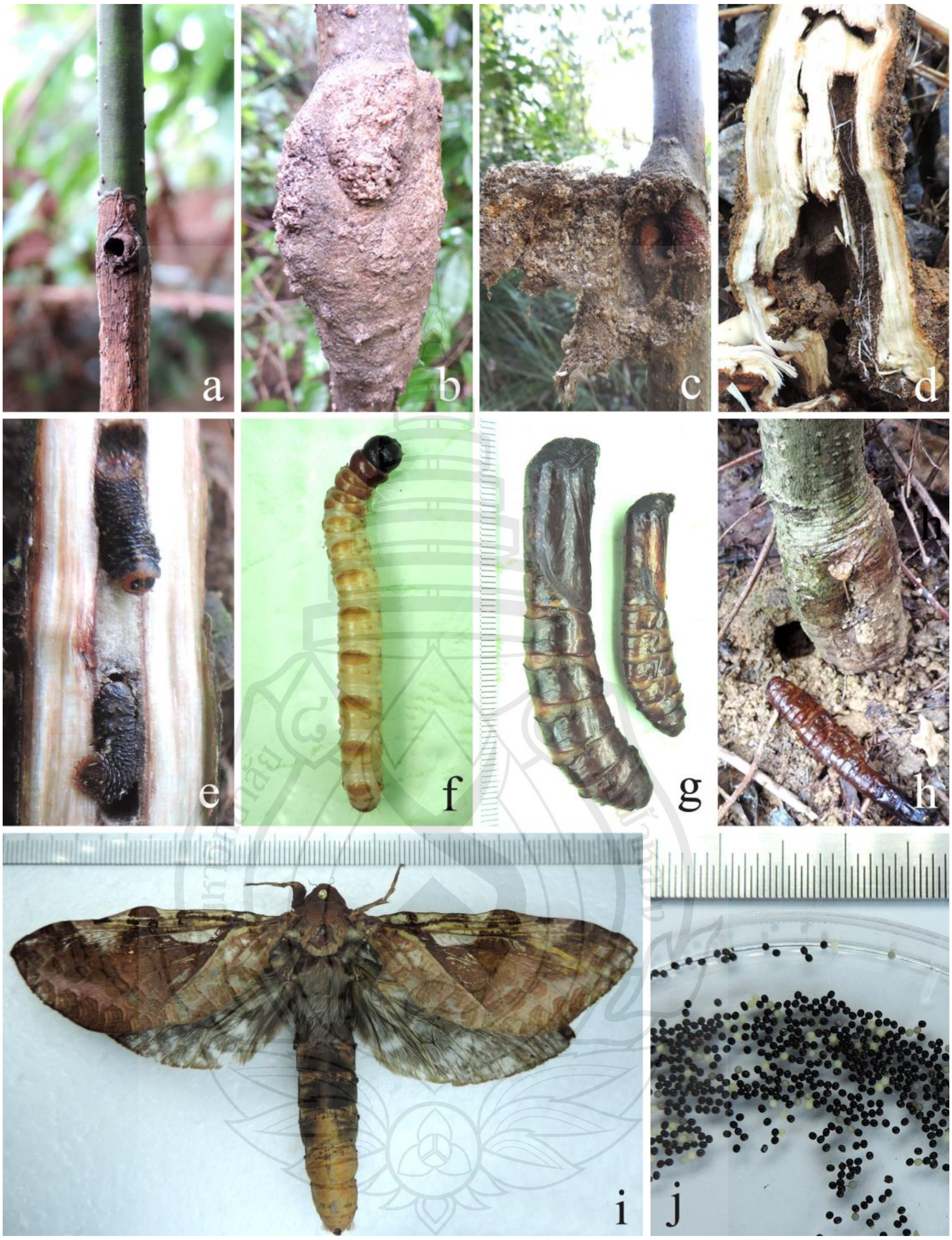
Ban et al. (2015b) named *O. macroacicularis* to distinguish from *O. emeiensis* (A.Y. Liu & Z.Q. Liang) G.H. Sung et al. (Liu et al., 1997), because the latter has only 1-2 stromata and has one distinctly different ITS sequence (AJ309347, uploaded in 2002). Notably, also having 1-2 stromata, my collections are more similar to *O. emeiensis*. So I doubt the unique ITS sequence of *O. emeiensis* (AJ309347) is problematic and *O. macroacicularis* may be a synonym of *O. emeiensis*.

4.4 Host Insects of Xuefeng Cordyceps

Hosts of *O. xuefengensis* and *O. ramosissimum* were originally identified as larvae of *Endoclita nodus* (Chu & Wang) (= *Phassus nodus* Chu & Wang) (Wen et al., 2013, 2014), and in subsequent literature, larvae of *E. nodus* has routinely been treated as the host of Xuefeng Cordyceps.

Recently in the Xuefeng Mountian region, I had dug out several hundred host specimens (including larvae, pupae and ossified insects) from tree trunks or roots. After morphological identification and comparison, the insects had been confirmed as only one *Endoclita* species. Fortunately, some pupae had molted into adults successfully. With its huge size, unique spots presented on fore wing and a distinct protrusion presented at middle of anterior margin of fore wing (Figure 4.4 i), these typical characteristics confirm that the species is *E. davidi* (= *E. giganodus* (Chu & Wang), = *P. giganodus* Chu & Wang, = *E. nankingi* (Daniel), = *P. nankingi* Daniel; Hepialidae, Lepidoptera) (Chu & Wang, 1985; Nielsen et al., 2000; Zhu et al., 2004), instead of *E. nodus* that had formerly been identified and reported.

Biology and ecology. The newborn larvae eat young tree trunks or twigs and dig tunnels within them, and several larvae can live together in one tunnel (Figure 4.4 a, e). In the following 1-2 months they continue to eat in a downward direction and become larger and larger. Entrance of every tunnel is covered by a large frass bag (Figure 4.4 b, c, d, f). These large larvae are aggressive and one will be attacked or be killed by the other when meeting, and as a result of fierce competition, one tunnel can



Note a upper part of a tree trunk being resided by young larvae; b, c frass bags produced by middle-aged larvae; d basal trunk; e newborn larvae; f middle-aged larva; g female (left) and male (right) pupae; h pupa slough; i female adult; j eggs.

Figure 4.4 *Endoclita davidi* and its ecology only be occupied by one larva. During the night with no rainfall, wind and disturbance and under relatively high temperatures, the larvae like to crawl out and this is what makes them more susceptible to be in contact with the ascospores/conidia of Xuefeng Cordyceps. The old-mature larvae pupate and molt in tree roots (Figure 4.4 d, h). Food shortages and disturbances can prompt them to pupate and molt early, while lower temperature will delay their molting. Shortly after molting, the insects can fly and the females begin to lay eggs (no need to mate). They lay eggs continually, especially when vibrating their wings or being disturbed. One female can produce several thousands of eggs, and the eggs are white at first, then become milky yellow and at last black (Figure 4.4 j). The eggs fall on grasses, fallen leaves, humus layers and soil surface, but most of them will be eaten by predatory insects, mice, birds, and so on, and only a few can successfully hatch. The insects overwinter as eggs or pupae and as a result their adults can occur two times (in May and Sep.) every year. A life cycle needs one year and the adults can live for 10-15 days. I presume the insects are capable of parthenogenesis.

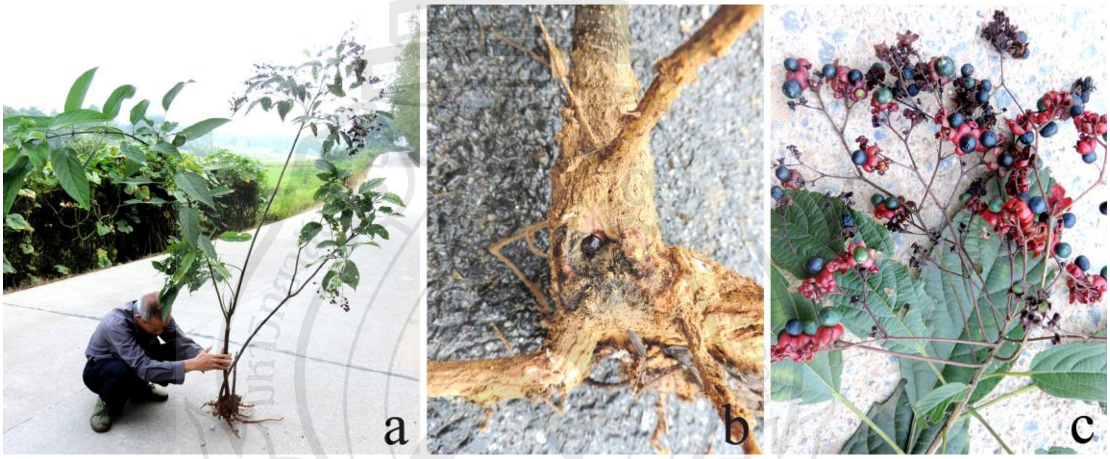
4.5 Infection Mechanism and Life Cycle of Xuefeng Cordyceps

Unlike hosts of most entomopathogenic fungi, larvae of *E. davidi* live in tree trunks or roots instead of in soil, that are rarely influenced by changes of external environment. Hence environmental factors (mainly refer to temperature and humidity; Wen et al., 2016b) are not the direct cause of insects being infected by Xuefeng Cordyceps. Given that the larvae are inherently aggressive, they are easily injured or even killed during fighting. Under these circumstances, this possibly renders them more vulnerable to infections and allow cordyceps species to become opportunistic pathogens. This also explains why the infected hosts are all larvae of irregular instars instead of pupae or adults (Figure 4.2 g), and the reason why several Xuefeng Cordyceps simultaneously grew in neighboring tunnels (Figure 4.2 *a*). Apart from fighting, other injuries can also cause the larvae being infected.

Xuefeng Cordyceps grow slowly due to the limited humidity in the plants, but it can continuously grow because the humidity and temperature are both steady.

Fruiting bodies of three *Ophiocordyceps* spp. can grow for 1-3 years in their plants, which is much longer than that of *B. bassiana* (sexual morph) and *Metarhizium guizhouense* (sexual morph). The fruiting body grows along the tunnel, until it reaches the entrance of the tunnel where it will begin to sporulate. As the largest species in Hepialidae (Zhu et al., 2004), the huge larva of *E. davidi* can provide sufficient nutrition for the fungus to grow and this possibly accounts why Xuefeng Cordyceps are similarly huge. Length of the fruiting body is dependent on the length of the tunnel, and as a result some fruiting bodies can even grow up to more than 40 cm.

4.6 Host Plants of the Host Insects

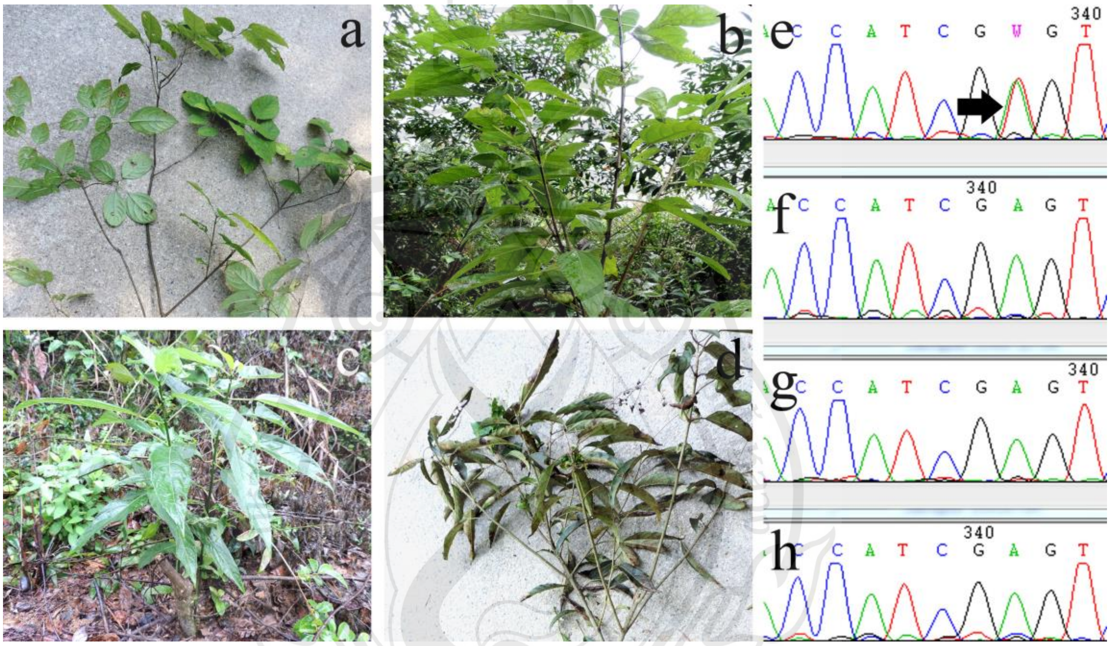


Note a the plant; b roots of the plant being resided by an *E. davidi* larva; c the fruits.

Figure 4.5 *Clerodendrum cyrtophyllum*, the host plant of *Endoclita davidi* in Xuefeng Mountain region, Hunan, China

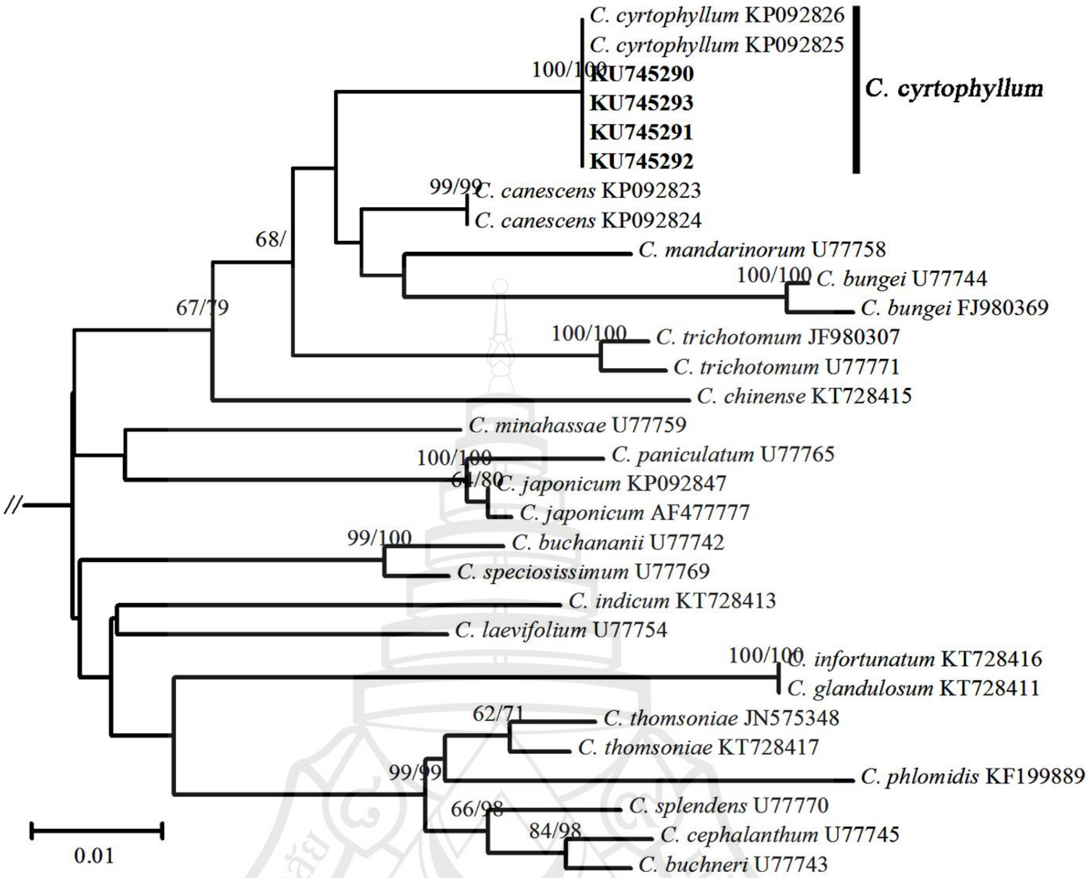
Proper identification of the host plants of *E. davidi* is always controversial. In the Xuefeng Mountain region, Huang (2012, 2013) and local people thought there are three plant species but did not properly identify them. Wen et al. (2013, 2014) reported *Clerodendrum cyrtophyllum* as the host plant, but did not give any further taxonomic and botanical information to properly validate host identity. Given the relative importance of cordyceps in TCM or biocontrol, it is absolutely necessary to properly name host and fungus concerned. In this study, a morphological comparison supplemented by DNA sequence-based data is implemented to better support any taxonomic arrangement proposed.

Depending on tunnels and frass bags produced by *E. davidi*, the host plants were confirmed and collected back for identification. Morphologically, the plants are similar to *Clerodendrum cyrtophyllum*, but the leaf shapes of the plants vary distinctly and can be classified into three types: 1) oval and short (Figure 4.6 a); 2) long and fat (Figure 4.6 b, c); 3) sharp and thin (Figure 4.6 d). In order to make clear what exactly the plants are, molecular phylogenetic analysis have to be introduced.



Note a oval and short leaves; b, c long and fat leaves; d sharp and thin leaves; e-h partial sequencing results of ITS sequences of the corresponding plants a-d with accession numbers in GenBank as KU745290, KU745291, KU745292 and KU745293 respectively.

Figure 4.6 Morphological changes of leaves vs. base variation of ITS sequence of the plants *Clerodendrum cyrtophyllum*



Note Bootstrap support values greater than 60% for ML and MP are indicated above the nodes and separated by '/' (ML/MP). KU745290, KU745291, KU745292 and KU745293 refer to the host plants with different leaf shapes.

Figure 4.7 ML tree of *Clerodendrum cyrtophyllum* and its allies inferred from ITS rDNA gene sequences

DNA sequence analyses reveal that the host plants of different leaf shapes have exactly the same ITS sequence (only one DNA base difference, see arrow in Figure 4.6 e-h). The ITS dataset consisted of 30 samples from 19 *Clerodendrum* species. It comprised 654 characters (including gaps), of which 187 were variable and 123 were parsimony-informative. ML and MP analyses resulted in phylogenetic reconstructions with closely similar topologies, and the ML tree is shown in Figure 4.7. The molecular phylogeny further confirms that the host plants are all *Clerodendrum cyrtophyllum*. It is to be noted that in the Xuefeng Mountain region, other plants such as *Rhus chinensis* Mill., *Loropetalum chinense* Oliv., *Argyreia pierreana* Bois and *Paulownia* sp. can also harbour larvae of *E. davidi*. They may crawl up these plants to eat, but a short time later they will retreat. In trunks or roots of these plants, larvae of *E. davidi* have rarely, and Xuefeng Cordyceps have never been discovered.

4.7 Discussion

According to investigations and analyses above, I can summarize that, Xuefeng Cordyceps refer to those cordyceps fungi that grow on larvae of *E. davidi* living in basal trunks or roots of the plants *Clerodendrum cyrtophyllum* in Xuefeng Mountain, Hunan, China. These cordyceps fungi deal with *B. bassiana* (asexual and sexual), *Metarhizium guizhouense* (sexual), *O. macroacicularis*, *O. ramosissimum* and *O. xuefengensis*, rather than one species, *O. xuefengensis* only that has routinely been regarded by local people and outside world.

With similar chemical compositions (Zhang et al., 2015; Zhu et al., 2015; Qin et al., 2016) and anti-tumor effect (Zheng et al., 2015) to the important TCM *O. sinensis*, Xuefeng Cordyceps have increasingly been accepted by outside world. Its market price, even the ossified insects only (Figure 4.2 g), has already risen up to $30,000 /kg (dry weight) that is just the price of *O. sinensis*. What’s more, they are huge cordyceps, generally a dry specimen has 3-5 g, while *O. sinensis* has only 0.2-0.6 g each. On one occasion, 120 g Xuefeng Cordyceps had at one time been dug out from a big tree (Huang, 2012). Driven by the huge economic benefit, the plants *Clerodendrum cyrtophyllum* have been damaged excessively and yield of Xuefeng Cordyceps decreased drastically in recent years. But prevailing in low altitude unlike *O. sinensis*, artificial culture of Xuefeng Cordyceps will probably be realized in the near future.

CHAPTER 5

CORDYCEPS FUNGI GROWING ON CICADAS IN CHINA

5.1 Introduction

Cicadas (Hemiptera: Cicadidae) are a widely known, diverse group of insects that are considered pests since their nymphs live on the sap of tree roots and the females lay eggs in tree trunks (Li & Wei, 2013). They are also a food source (cicada nymphs) and used in traditional Chinese medicine (cicada sloughs and Chanhua (*Cordyceps cicadae* (Miq.) Massee)) (Chinese Pharmacopoeia Commission, 2015). Cicadas spend most of their lives as nymphs in underground, humid soil for many years, but their adults can only survive for one month or a little more (Gourley & Kuang, 2009). The database ‘Catalogue of Life’ (https://www.catalogueoflife.org/) demonstrates that the family Cicadidae includes 3,145 species worldwide currently, with about 310 species recorded from China.

Cicadicolous cordyceps refers to those cordyceps fungi growing on cicadas. The first species, *Clavaria sobolifera* Hill ex Watson (≡ *Ophiocordyceps sobolifera* (Hill ex Watson) G.H. Sung et al.), was introduced in 1763, and since then many cicadicolous cordyceps have been described. The first systematic report of Cicadicolous cordyceps was from Kobayasi (1939), with records of 5 species and 1 variety. Then, Kobayasi and Shimizu (1963), with 16 species and 2 forms, and Shimizu (1994, 1997), with 22 species, 2 varieties, 12 forms, and 8 unidentified species, subsequently contributed. Shrestha et al. (2017b) listed cordyceps species on Hymenoptera and Hemiptera insects, which deals with 30 taxa (species, subspecies, and synonyms) on cicadas; among them, 21 are accepted species. Systematic reports of Chinese cicadicolous cordyceps were from Liang (2007) with 10 species and Chen et al. (2014), with 14 species and 1 undetermined taxon. Overall, the species diversity of cordyceps on cicadas has not been well studied.

In this study, we review the species diversity, distribution, and host range of Cicadicolous cordyceps. Using DNA-based phylogenetic evidence, we explore the relationships between cicadicolous species and their relatives. The taxonomy of Cicadicolous species from China is discussed. *Ophiocordyceps bannaensis* is introduced as a new species, and *Metarhizium owariense* (Kobayasi) Kepler et al. and *Polycephalomyces nipponicus* (Kobayasi) Kepler & Spatafora are newly recorded for China. The latter three species are described, illustrated, and compared with their allies.

5.2 Material and Methods

5.2.1 Fungal Specimens

Specimens of cicadicolous cordyceps 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. Measurements of microphotographs were made using Tarosoft (R) Image Frame Work software. Voucher specimens were deposited in the Herbarium of the Institute of Microbiology, Chinese Academia of Sciences (HMAS), the Herbarium of Guizhou University (GACP), China, and the Centre of Excellence in Fungal Research, Mae Fah Luang University (MFLU), Thailand.

5.2.2 DNA Extraction, PCR, Sequencing, Sequence Alignment and Assembly

Total DNAs were 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), the second largest subunits of RNA polymerase II (RPB2) and the elongation factor 1α (TEF1-*α*) genes were sequenced using the primers followed by White et al. (1990) and Ban et al. (2015b). Amplification reactions were performed in an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA) and the PCR programs were followed those of Ban et al. (2015b). PCR products were purified using the Bioteke’s Purification Kit (Bioteke 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 have been aligned and assembled visually and manually using Clustalx1.81, Chromas230 and ContigExpress software.

5.2.3 Construction of Molecular Phylogenetic Tree

Molecular phylogeny was reconstructed using the single ITS and combined SSU, ITS, LSU and TEF1-α datasets, respectively. The single ITS sequence dataset was analyzed using neighbor-joining (NJ) method (Saitou & Nei, 1987), and the combined one using maximum likelihood (ML) method. NJ tree was 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, other details are described in Jeewon et al. (2003) and Hongsanan et al. (2017).

5.3 Species and Distribution of Cicadicolous Cordyceps

Currently, cicadicolous cordyceps has a worldwide distribution especially in southern Asia with 34 known species and 1 species which is new to science. Among them, 19 species have been recorded in China. Following the new classification system of cordyceps (Sung et al., 2007) and its latest taxonomic updates (Kepler et al., 2013, 2014; Quandt et al., 2014; Ban et al., 2015a; Kepler et al., 2017), we list the names and distribution of all cicadicolous species in Table 5.1.

35 cicadicolous species are included in seven genera of three families of the order Hypocreales. They are respectively 12 *Ophiocordyceps*, 1 *Perennicordyceps*, 3 *Polycephalomyces*, 1 *Purpureocillium* and 3 *Tolypocladium* species in the family Ophiocordycipitaceae, 4 *Metarhizium* species in the family Clavicipitaceae and 11 *Cordyceps* species in the family Cordycipitaceae. Among them, 1 *Hirsutella* (= *Ophiocordyceps*), 2 *Isaria*/*Paecilomyces* (= *Cordyceps*) and some *Cordyceps* species which came from the old genus *Cordyceps* (Sung et al., 2007), have not been revised.

Table 5.1 Species diversity and distributions of cicadicolous cordyceps (35 species)

Species	Distribution	Main references
Cordyceps cicadae (Miq.) Massee (Chanhua)	The tropics and subtropics, widely distributed	Zha et al. (2018)
C. ctenocephala P. Syd	New Guinea	Kobayasi and Shimizu (1963)
C. hesleri Mains	USA (Tennessee)	Mains (1939)
C. imagamiana Kobayasi & Shimizu	China (Guangdong), Japan	Liang (2007), Kobayasi and Shimizu (1983)
C. ishikariensis Kobayasi & Shimizu	Japan, Nepal	Shimizu (1994), Shrestha (2011)
**C. minuta* Kobayasi	Japan	Kobayasi and Shimizu (1963)
C. pleuricapitata Kobayasi & Shimizu	Japan	Kobayasi and Shimizu (1982a)
C. polycephala Kobayasi & Shimizu	Japan	Kobayasi and Shimizu (1983)
C. coxii Olliff	Australia	Kobayasi and Shimizu (1963)
Hirsutella graptopsaltriae (Kobayasi) H.C. Evans & Samson	Japan	Kobayasi (1939)
Isaria oncotympanae Kobayasi	Japan	Kobayasi (1939)
Metarhizium cylindrosporum Q.T. Chen & H.L. Guo	China (Anhui, Guizhou, Taiwan), India, Japan, Thailand	Luangsa-ard et al. (2008), Li et al. (2010), Balakrishnan et al. (2012)
M. guniujiangense (C.R. Li et al.) Kepler et al.	China (Anhui)	Li et al. (2010)
M. owariense (Kobayasi) Kepler et al.	Cameroon, China (Hunan), Jamaica?, Japan, Slovenia	Kobayasi (1939), Kobayasi and Shimizu (1963), this study
M. viridulum (Tzean et al.) B. Huang & Z.Z. Li	China (Anhui, Taiwan)	Tzean et al. (1992), Huang et al. (2004)
Ophiocordyceps araracuarensis T. Sanjuan & J.W. Spatafora	Colombia (Amazon region)	Sanjuan et al. (2015)
O. bannaensis sp. nov.	China (Yunnan)	This study
**O. cicadicola* (Teng) G.H. Sung	China (Guangdong, Hainan)	Liang (2007)

Table 5.1 (continued)

Species	**Distribution**	**Main references**
**O. evdogeorgiae* (Koval) G.H. Sung et al.	Slovenia (Primorska)	Kobayasi and Shimizu (1963)
O. heteropoda (Kobayasi) G.H. Sung et al.	China (Anhui, Fujian), Japan, Korea, the Congo	Kobayasi (1939), Li et al. (2006) Liang, (2007), Sung et al. (2011)
O. longissima (Kobayasi) G.H. Sung et al.	China (Anhui), Japan, Korea, Thailand	Kobayasi and Shimizu, 1963, Li et al. (1999), Sung et al. (2007), Aung et al. (2008)
O. pseudolongissima (Kobayasi & Shimizu) G.H. Sung et al.	Japan	Kobayasi and Shimizu (1982b)
O. sobolifera (Hill ex Watson) G.H. Sung et al.	The tropics and subtropics, widely distributed	Chen et al. (2014)
O. wuyishanensis (Z.Q. Liang et al.) G.H. Sung et al.	China (Fujian)	Liang et al. (2002)
O. voeltzkowii (Henn.) G.H. Sung et al.	Madagaskar	Hennings (1908)
O. yakusimensis (Kobayasi) G.H. Sung et al.	China (Anhui, Zhejiang, Taiwan), Japan, Korea	Kobayasi and Shimizu, (1963), Sung et al. (2007), Chen et al. (2014)
Paecilomyces xylariiformis (Lloyd) Samson	China (Yunnan), Brazil	Samson (1974), Liu et al. (2010)
Perennicordyceps prolifica (Kobayasi) Matočec & I. Kušan	Japan	Kobayasi and Shimizu, (1963)
Polycephalomyces kanzashianus (Kobayasi & Shimizu) Kepler & Spatafora	China (Anhui), Japan	Kobayasi and Shimizu (1982a), Zhang (2013)
P. nipponicus (Kobayasi) Kepler & Spatafora	China (Yunnan), Japan, Thailand	Kobayasi (1939), Luangsa-ard et al. (2008), this study
P. ramosopulvinatus (Kobayasi & Shimizu) Kepler & Spatafora	China (Yunnan), Japan, Korea	Kobayasi and Shimizu (1983), Sung et al. (2010), Chen et al. (2014)

Table 5.1 (continued)

Species	Distribution	Main references
**Purpureocillium takamizusanense* (Kobayasi) S. Ban et al.	Japan	Kobayasi and Shimizu (1963), Ban et al. (2015a)
Tolypocladium inegoense (Kobayasi) Quandt et al.	China (Fujian Taiwan), Japan, Korea	Kobayasi and Shimizu (1963), Liang (2007), Sung et al. (2007)
T. paradoxum (Kobayasi) Quandt et al.	China, Japan, Korea	Chen et al. (2014)
T. toriharamontanum (Kobayasi) Quandt et al.	Japan	Kobayasi and Shimizu (1963)

Note Species on adult cicadas are marked with ‘*’, the rests are all on cicada nymphs.

5.4 Molecular Phylogenetic Analysis

Among the 35 cicadicolous species, 21 have nucleotide sequences in GenBank. Blast search were performed to reveal the closest matches in NCBI database that would allow the selection of appropriate taxa for phylogenetic analyses. Information of voucher sequences for this study were obtained from GenBank and are listed in Table 5.2. The single ITS and combined SSU, ITS, LSU and TEF1-α datasets comprised 67 taxa (60 species) respectively, 25 of which come from cicadicolous cordyceps (21 species) and 42 from their relatives. Using *Colletotrichum gloeosporioides* (FAU553, Glomerellales) as outgroup, NJ and ML trees were reconstructed respectively. The single ITS dataset comprised 790 characters (include the gaps), of which 462 characters were variable and 397 were parsimony-informative. The combined SSU, ITS, LSU and TEF1-α dataset comprised 3,245 characters, of which 895 were variable and 635 were parsimony-informative. Trees generated under NJ and ML analyses resulted in trees with similar topology and support values (results not shown), and the ML tree is shown in Figure 5.1.

Table 5.2 Sequence information of samples appearing in Figure 5.1

Fungal Name	Specimen/strain	Host	ITS	SSU	LSU	TEF1-*α*	Literature cited
Cordyceps cateniobliqua	CBS 153.83	Tortricidae	AY624173	AY526466	-	JQ425688	Luangsa-ard et al. (2005)
C. cicadae (Chanhua)	GACP 07071701	Cicadidae	KX017277	MK761207	MK761212	MK770631	Zha et al. (2019b)
C. cicadae (Chanhua)	GACP 14061604	Cicadidae	KX017276	MK761208	MK761213	MK770632	Zha et al. (2019b)
C. coleopterorum	CBS 110.73	Lampyridae	AY624177	JF415965	JF415988	JQ425689	Luangsa-ard et al. (2005)
C. fumosorosea	CBS 244.31	Butter	AY624182	MF416609	MF416557	MF416503	Luangsa-ard et al. (2005)
C. ghanensis	CBS 105.73	Lepidoptera	AY624185	-	-	-	Luangsa-ard et al. (2005)
C. imagamiana	10605	Cicadidae	AJ536547	-	-	-	NCBI
C. kyushuensis	HMAS 78115	Sphingidae	EF368021	-	-	-	Wang et al. (2008)
C. militaris	OSC 93623	Lepidoptera	JN049825	AY184977	AY184966	DQ522332	Kepler et al. (2013)
C. ningxiaensis	HMJAU 25074	Scoliidae	KF309668	-	NG 058862	-	Yan and Tolgor (2015)
C. pleuricapitata	NBRC 100746	Cicadidae	JN943306	KF049607	KF049625	KF049680	Kepler et al. (2013)
C. qingchengensis	MFLU 17-1022	Lepidoptera	KY423506	MK761206	MK761211	MK770630	Zha et al. (2019a)
C.roseostromata	ARSEF 4870	Coleoptera	EF368022	-	-	-	Wang et al. (2008)
C. tenuipes	GACP 16063004	Lepidoptera	KY423509	MK761209	MK761214	MK770633	Zha et al. (2019a)
C. tenuipes	ARSEF 5135	Lepidoptera	AY624196	MF416612	JF415980	JF416020	Kepler et al. (2013)
Isaria xylariiformis	GZUIFR-4606	Cicadidae	FJ479746	-	-	-	Liu et al. (2010)
Metacordyceps chlamydosporia	CBS 101244	Nematode /Rotifera	JN049821	DQ522544	DQ518758	DQ522327	Kepler et al. (2012a)
M. shibinensis	GZUH SB13050311	Lepidoptera	KR153585	KR153588	-	KR153589	Wen et al. (2015)

Table 5.2 (continued)

Fungal Name	Specimen/strain	Host	ITS	SSU	LSU	TEF1-*α*	Literature cited
M. taii	ARSEF 5714	Lepidoptera	JN049829	AF543763	AF543787	AF543775	Kepler et al. (2012a)
Metarhizium anisopliae	ARSEF 3145	Coleoptera	JN049834	AF339579	AF339530	AF543774	Kepler et al. (2012a)
M. brittlebankisoides	GZAAS5.1002	Coleoptera	KC561835	-	-	KC561899	NCBI
M. cylindrosporum	RCEF 3632	Cicadidae	JN049872	JF415959	JF415982	JF416022	Kepler et al. (2012a)
M. guizhouense	**GACP CY0703**	**Hepialidae**	**MG029525**	**-**	**-**	**-**	**This study**
M. guniujiangense	RCEF 2001	Cicadidae	AY913758	-	-	-	Li et al. (2010)
M. indigoticum	TNS F18553	Lepidoptera	JN049874	JF415953	JF415968	JF416010	Kepler et al. (2012a)
M. kusanagiense	TNS F18494	Coleoptera	JN049873	JF415954	JF415972	JF416014	Kepler et al. (2012a)
M. owariense	NBRC 33258	Cicadidae	JN049883	-	JF415976	JF416017	Kepler et al. (2012a)
M. owariense	**GACP ZP756**	**Cicadidae**	**MG029522**	**-**	**-**	**-**	**This study**
M. viridulum	WH000702-01	Cicadidae	AF368500	-	-	-	Li et al. (2010)
M. yongmunense	EFCC 2131	Lepidoptera	JN049856	EF468977	EF468833	EF468770	Kepler et al. (2012a)
Ophiocordyceps araracuarensis	HUA 186135	Cicadidae	KP200891	KC610788	KC610769	KC610738	Sanjuan et al. (2015)
***O. bannaensis* sp. nov.**	**HKAS 100059**	**Cicadidae**	**MG029521**	**MG725822**	**MG725824**	**MG729401**	**This study**
O. coccidiicola	NBRC 100682	Hemiptera	AB968404	AB968391	AB968419	AB968583	Ban et al. (2015b)
O. coenomyiae	NBRC 108993	*Coenomyia*	AB968396	AB968384	AB968412	AB968570	Ban et al. (2015b)
O. entomorrhiza	KEW 53484	Lepidoptera	JN049850	EF468954	EF468809	EF468749	Quandt et al. (2014)
O. gracilis	EFCC 8572	Lepidoptera	JN049851	EF468956	EF468811	EF468751	Kepler et al. (2012a)

Table 5.2 (continued)

Fungal Name	Specimen/strain	Host	ITS	SSU	LSU	TEF1-*α*	Literature cited
O. heteropoda	EFCC 10125	Cicadidae	JN049852	EF468957	EF468812	EF468752	Sanjuan et al. (2015)
O. longissima	NBRC 106965	Cicadidae	AB968406	AB968392	AB968420	AB968584	Ban et al. (2015b)
O. macroacicularis	NBRC 100685	*Cossida*	AB968400	AB968388	AB968416	AB968574	Ban et al. (2015b)
O. rhizoidea	NHJ11941.01	Isoptera	GU723769	-	-	GU797113	Luangsa-Ard et al. (2011)
O. robertsii	KEW 27083	Hepialidae	AJ309335	-	EF468826	EF468766	Sung et al. (2007)
O. sinensis	EFCC 7287	Lepidoptera	JN049854	EF468971	EF468827	EF468767	Sung et al. (2007)
O. sobolifera	NBRC 106967	Cicadidae	AB968409	AB968395	AB968422	AB968590	Ban et al. (2015b)
O. yakusimensis	–	Cicadidae	AB044643	AB044632	-	-	Nikoh and Fukatsu (2001)
Perennicordyceps cuboidea	NBRC 103835	Coleoptera	JN943333	JN941722	JN941419	-	Schoch et al. (2012)
P. paracuboidea	NBRC 105892	Coleoptera	AB925954	-	AB925988	-	NCBI
P. prolifica	NBRC H-12581	Cicadidae	AB378676	-	AB378658	-	Ban et al. (2009)
P. ryogamiensis	NBRC 103837	Coleoptera	JN943346	JN941702	JN941439	-	Schoch et al. (2012)
Polycephalomyces formosus	ARSEF 1424	Coleoptera	KF049661	KF049615	AY259544	DQ118754	Kepler et al. (2013)
P. kanzashianus	–	Cicadidae	AB027371	AB027325	AB044639	-	Nikoh and Fukatsu (2000)
P. nipponicus	**HKAS 100058**	**Cicadidae**	**MG029523**	**MG725823**	**MG725825**	**-**	**This study**
P. nipponicus	NBRC 101406	Cicadidae	JN943301	JN941753	JN941388	-	Schoch et al. (2012)
P. nipponicus	BCC 1682	Neuroptera	KF049664	KF049620	KF049638	KF049694	Kepler et al. (2013)

Table 5.2 (continued)

Fungal Name	**Specimen/strain**	**Host**	**ITS**	**SSU**	**LSU**	**TEF1-*α***	**Literature cited**
P. ramosopulvinatus	EFCC 5566	Cicadidae	KF049658	-	KF049627	KF049682	Kepler et al. (2013)
P. tomentosus	BL4	Trichiales	KF049666	KF049623	AY259545	KF049697	Kepler et al. (2013)
Purpureocillium lavendulum	FMR 10376	Soil	FR734106	-	FR775489	FR775516	Perdomo et al. (2013)
P. lilacinum	CBS 284.36	Soil	FR734101	AY526475	FR775484	FR734156	Perdomo et al. (2013)
P. takamizusanense	NBRC 100742	Cicadidae	LC008197	-	-	LC008333	Ban et al. (2015a)
Tolypocladium album	GB5502	Soil	AF389192	-	AF245297	-	Bills et al. (2002)
T. cylindrosporum	NBRC 100548	Peat	AB208110	-	-	-	Yokoyama et al. (2006)
T. inegoense	–	Cicadidae	AB027368	AB027322	-	-	Nikoh and Fukatsu (2000)
T. inflatum	OSC 71235	?	JN049844	EF469124	EF469077	EF469061	Ban et al. (2015b)
T. ophioglossoides	NBRC 8992	*Elaphomyces*	JN943316	JN941736	JN941405	AB968601	Ban et al. (2015b)
T. paradoxum	**HKAS 87772**	**Cicadidae**	**KX017279**	**-**	**-**	**-**	**This study**
T. paradoxum	-	Cicadidae	AB027369	-	-	-	Nikoh and Fukatsu (2000)
T. pustulatum	GB6597	Soil	AF389189	-	AF389190	-	Bills et al. (2002)
Tyrannicordyceps fratricida	TNS 19011	Fungus	JQ349068	JQ257022	JQ257023	JQ257028	Kepler et al. (2012b)

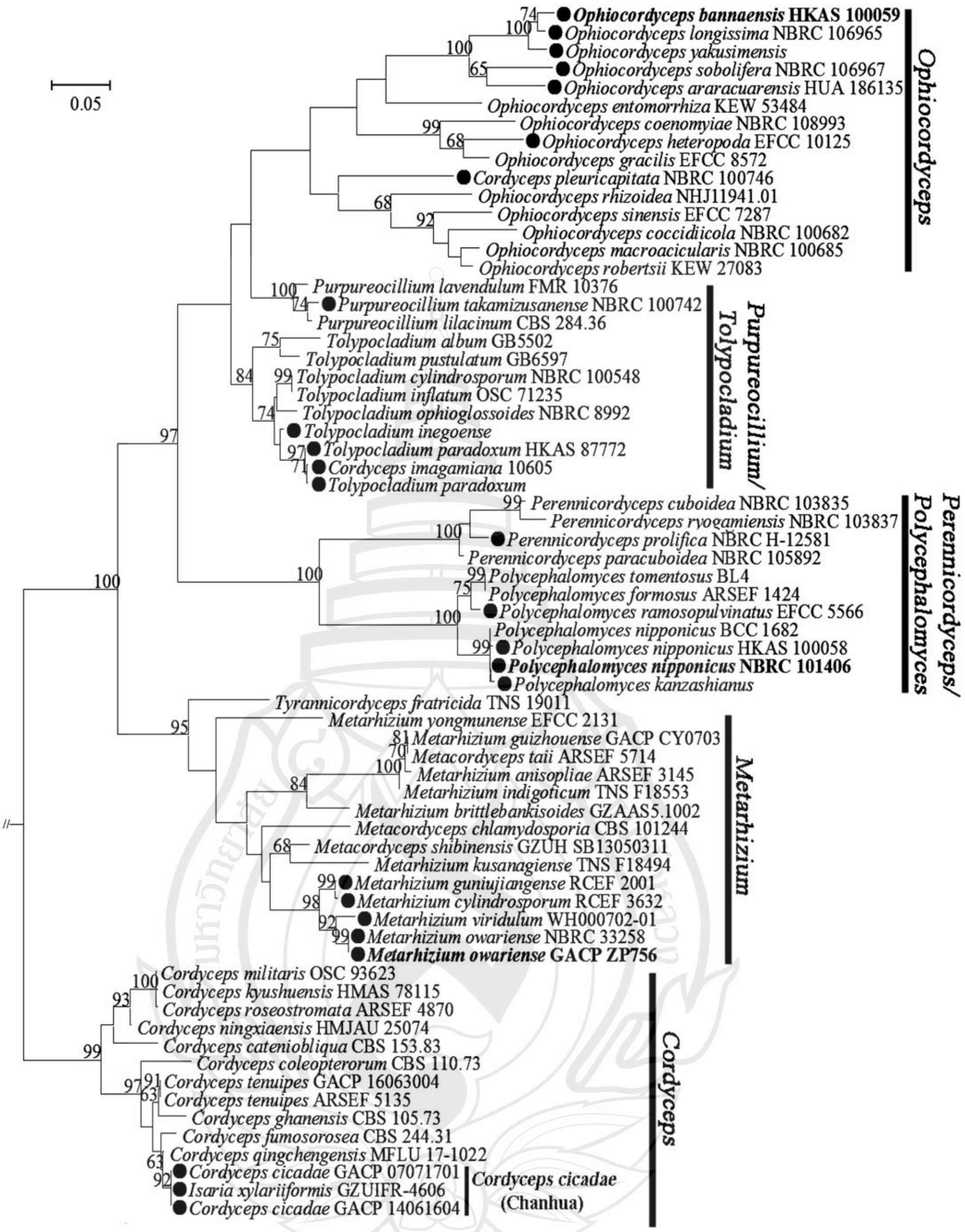
Note Sequencing results in this study are displayed in bold.

On the phylogenetic tree, the 21 cicadicolous species are assigned to five clades: the *Cordyceps* clade, the *Metarhizium* clade, the *Polycephalomyces*/*Perennicordyceps* clade, the *Purpureocillium*/*Tolypocladium* clade and the *Ophiocordyceps* clade.

DNA sequences of Chanhua (*Cordyceps cicadae* (Miq.) Massee) and Chinese *Paecilomyces xylariiformis* (only ITS, Liu et al., 2010) are 100% identical and clustered together in the *Cordyceps* clade. ITS data of *Pa. xylariiformis* come from China instead of Brazil, the type locality (see notes of Chanhua below). Chanhua is nearest to *C. qingchengensis* L.S. Zha et al. on a Lepidoptera cocoon (Zha et al., 2019a).

The *Metarhizium* clade includes 4 cicadicolous species which are closely related: *M. guniujiangense* and *M. cylindrosporum* are closely related (ML = 99), while *M. owariense* and *M. viridulum* constitute independent lineages. The taxon nearest to the above 4 species is *Metacordyceps shibinensis* T.C. Wen et al. on Lepidoptera larvae (Wen et al., 2015).

Based on morphological evidence, Matočec et al. (2014) introduced the genus *Perennicordyceps* to distinguish *Polycephalomyces*. Current molecular phylogeny strongly supports the monophyly of *Perennicordyceps*, and *Perennicordyceps* is closely related to *Polycephalomyces.* These two genera constitute a strongly supported monophyletic clade (*Polycephalomyces/Perennicordyceps* clade). 3 cicadicolous species (*Po. kanzashianus*, *Po. nipponicus* and *Po. ramosopulvinatus*) are included in the former and 1 (*Pe. prolifica*) in the latter. *Cordyceps pleuricapitata*, the other cicadicolous species, was synonymized as *Po. pleuricapitata* by Wang (2016), however in our study, this species is phylogenetically assigned to the *Ophiocordyceps* clade. Hence, taxonomic status of *C. pleuricapitata* needs further investigations.



Note Bootstrap support values greater than 60% are indicated above the nodes. Cicadicolous species (25 samples including 21 species) are marked with ‘●’.

Figure 5.1 ML tree of cicadicolous cordyceps species and their allies inferred from combined SSU, ITS, LSU and TEF1-α dataset

The *Purpureocillium*/*Tolypocladium* clade also includes two sister genera: *Tolypocladium* and *Purpureocillium*. 3 allied cicadicolous species (*T. paradoxum*, *Cordyceps imagamiana* and *T. inegoense*) are included in the former and 1 (*Pu. takamizusanense*) in the latter. *T. paradoxum* and *C. imagamiana* are closely related (ML = 97), also they share similar morphologies (Kobayasi, 1939; Kobayasi & Shimizu, 1983). Following the new classification system of cordyceps (Sung et al., 2007) and its update (Kepler et al., 2013), *C. imagamiana* should be combined into *T. imagamiana*. In *Purpureocillium*, *Pu. takamizusanense* is related to *Pu. lilacinum* (Thom) Luangsa-ard et al. which was isolated from soil (Perdomo et al., 2013).

The *Ophiocordyceps* clade includes 7 cicadicolous species. *Ophiocordyceps bannaensis* sp. nov. is closely related to *O. longissima* and *O. yakusimensis* (ML = 100), *O. sobolifera* and *O. araracuarensis* are also closely related. These 5 species are grouped well (ML = 100). *O. heteropoda* is related to *O. coenomyia* Ban et al. and *O. gracilis* (Grev.) G.H. Sung et al. that grow on Diptera (Ban et al., 2015b) and Lepidoptera larvae (Stensrud et al., 2005) respectively. *Cordyceps pleuricapitata* is still undetermined, and the current molecular phylogeny can also not resolve its lineages.

5.5 The Hosts

Cicadicolous cordyceps generally grow on cicada nymphs (Hemiptera: Cicadidae) and show typical host specificity. Though *Cordyceps minuta*, *Ophiocordyceps cicadicola* (≡ *C. cicadicola* Teng), *O. evdogeorgiae* (≡ *C. evdogeorgiae* Koval) and *Purpureocillium takamizusanense* (= *C. ryogamimontana* Kobayasi) grew on cicada adults, the infections of their hosts should occur before eclosion, because there is a time span between the infection and the death of a host. Due to the lack of sufficient moisture in the air, cicada adults are almost impossible to be infected by cordyceps; but if an ill or injured cicada drops to the ground, under humid environment and not being eaten by animals or predatory insects, it will easily be infected and quickly form asexual cordyceps of having a short life cycle (Wen et al., 2016b). Considering the similar biological habits of cicadas and the difficulty in identifying cicada nymphs, we do not list the host names of these cicadicolous cordyceps (Table 5.1).

5.6 Species Diversity of Cicadicolous Cordyceps in China

The 19 cicadicolous species in China deal with five cordyceps genera, including 3 *Cordyceps* (including 1 unrevised *Paecilomyces*), 4 *Metarhizium*, 7 *Ophiocordyceps*, 3 *Polycephalomyces* and 2 *Tolypocladium* species. Herein we give their names and distributions and discuss their taxonomies.

5.6.1 Chanhua (*Cordyceps cicadae* (Miq.) Massee)

≡? *Isaria cicadae* Miq.
=? *Sphaeria sinclairii* Berk.
=? *Sphaeria basili* Taylor
=? *Torrubia caespitosa* Tul. & C. Tul.
=? *Cordyceps caespitosa* (Tul. & C. Tul.) Sacc.
=? *Cordyceps sinclairii* (Berk.) Sacc.
=? *Isaria arbuscula* Har.
=? *Isaria hariotii* Arnaud
= *Isaria cosmopsaltriae* Yasuda
= *Isaria mokanshawii* Lloyd
=? *Isaria sinclairii* (Berk.) Lloyd
=? *Isaria xylariiformis* Lloyd
=? *Isaria nipponica* Kobayasi
=? *Isaria basili* (Taylor) Kobayasi
= *Cordyceps sinclairii* Kobayasi
= *Isaria cryptotympanae* Sawada
≡? *Paecilomyces cicadae* (Miq.) Samson
=? *Paecilomyces xylariiformis* (Lloyd) Samson
= *Cordyceps kobayasii* Koval
= *Cordyceps cicadae-sm* A.Y. Liu & X. Zou

Distribution. Distributed in southern Asia (China, India, Indonesia, Japan, Nepal, South Korea, Sri Lanka, Thailand and Vietnam) and suspected in Oceania (Australia and New Zealand), South America (Brazil, Guatemala and Mexico), North America (Canada) and Africa (Madagascar) (Zha et al., 2019b).

In China, Chanhua is widely distributed in the Oriental Region of China (the southern regions of the Qinling Mountain and the Huaihe River), including Anhui, Chongqing, Fujian, Guangdong, Guangxi, Guizhou, Hainan, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Shaanxi, Shanghai, Sichuan, Taiwan, Yunnan and Zhejiang Provinces (Zha et al., 2019b).

Notes. Based on molecular evidence, recently we confirmed Chanhua to be one species of the genus *Cordyceps* (Zha et al., 2019b). Currently, Chanhua from Asia, *Isaria cicadae* from Brazil and *I. sinclairii* from New Zealand are generally accepted as the same species, but the latter two species lack DNA sequence data from their type localities. For these reasons and together with the One Fungus One Name (1F1N) concept, we proposed to use *C. cicadae* (Miq.) Massee (≡ *I. cicadae*) as the temporary scientific name for Chanhua (Zha et al., 2018). Apart from the synonyms formerly reported (Kobayasi and Shimizu, 1963; Samson, 1974), *Paecilomyces xylariiformis* (≡ *I. xylariiformis*) and *I. nipponica* (see *Polycephalomyces nipponicus* below) might also be synonyms of Chanhua.

Paecilomyces xylariiformis (≡ *I. xylariiformis*) was originally reported from Brazil and is morphologically similar to Chanhua (Lloyd, 1923; Samson, 1974). Liu et al. (2010) reported *I. xylariiformis* from China based on specimens from Yunnan (Mojiang and Fengyang). With broad-rounded, globose or occasionally cylindric conidia, the specimens are morphologically very similar to *I. xylariiformis* from Brazil (Lloyd, 1923). Liu et al. (2010) identified them as *I. xylariiformis* using morphological and ITS sequence data, but the ITS sequence (FJ479746) used is 100% identical to that of Chanhua. Considering its morphologically similar to Chanhua, we can conclude the specimens from Yunnan are Chanhua instead of *I. xylariiformis* (Figire 1). In addition, considering the fact that *I. xylariiformis* and Chanhua can produce broad-rounded or globose conidia, we infer *I. xylariiformis* might be a synonym of *C. cicadae* (Miq.) Massee, after all, they are both originally reported from Brazil.

5.6.2 *Cordyceps imagamiana* Kobayasi & Shimizu

Distribution. China (Guangdong) (Liang, 2007), Japan (Kobayasi & Shimizu, 1983).

Note. In GenBank, this species has only one ITS sequence data (AJ536547) obtained from China instead of Japan, its type locality. Our phylogeny indicates that it is closely related to *Tolypocladium paradoxum* (Figure 1).

5.6.3 *Metarhizium cylindrosporum* Q.T. Chen & H.L. Guo [as '*cylindrosporae*']

≡ *Nomuraea cylindrosporae* (Q.T. Chen & H.L. Guo) Tzean et al. [as '*cylindrospora*']

Distribution. China (Anhui, Guizhou, Taiwan), India, Japan, Thailand

Notes. *Metarhizium cylindrosporum* was originally isolated from an unknown larva in soil in a tea garden of Guiyang, Guizhou, China (Guo et al., 1986). This species was frequently reported growing on cicada nymphs and adults and has a wide distribution (Luangsa-ard et al., 2008; Li et al., 2010; Balakrishnan et al., 2012; Kepler et al., 2012a), but its sexual morph remains unknown. Molecular phylogeny indicates that it is related to *M. guniujiangense*, *M. viridulum* and *M. owariense* (Figure 5.1).

5.6.4 *Metarhizium guniujiangense* (C.R. Li et al.) Kepler et al.

≡ *Metacordyceps guniujiangensis* C.R. Li, B. Huang, M.Z. Fan & Z.Z. Li

Distribution. China (Anhui).

Notes. Morphologically, *Metarhizium guniujiangense* is very similar to *M. owariense*, but their ITS sequence data are distinctly different (4.5% differences across 605 bp) (Li et al., 2010; Kepler et al., 2014).

5.6.5 *Metarhizium owariense* (Kobayasi) Kepler et al. (Figure 5.2)

≡ *Cordyceps owariensis* Kobayasi

= *Cordyceps owariensis* f. *owariensis* Kobayasi

= *Cordyceps owariensis* f. *viridescens* Uchiy. & Udagawa

≡ *Ophiocordyceps owariensis* (Kobayasi) G.H. Sung et al.

= *Ophiocordyceps owariensis* f. *owariensis* (Kobayasi) G.H. Sung et al.

= *Ophiocordyceps owariensis* f. *viridescens* (Uchiy. & Udagawa) G.H. Sung et al.

≡ *Metacordyceps owariensis* (Kobayasi) Kepler et al.

= *Metacordyceps owariensis* f. *owariensis* (Kobayasi) Kepler et al.

= *Metacordyceps owariensis* f. *viridescens* (Uchiy. & Udagawa) Kepler et al.

= *Metarhizium owariense* f. *owariense* (Kobayasi) Kepler et al.

= *Metarhizium owariense* f. *viridescens* (Uchiy. & Udagawa) Kepler et al.

= *Metarhizium owariense* var. *owariense* (Kobayasi) Kepler et al.

MycoBank: MB806094

Description. Sexual morph: Stroma arising from the vertex of a cicada nymph in soil, solitary at base, then branched for 3-4 times upward, fleshy, 8.5 cm long in total. Stalk nearly cylindric, 2.4-6 mm thick, milky yellow and with glabrous surface. Fertile part located at end of every branch, not clearly defined from stalk, dark green, surface very rough, 0.8-3.6 cm long and 2.5-5 mm thick; sterile tips 3.5-4 mm, conical, glabrous, yellowish green when fresh but pale after dried, apexes acute. Perithecia obliquely immersed, asymmetrical, ampullaceous, 360-460 × 170-240 µm, with somewhat long neck, walls pale ochraceous, 22-35 µm thick. Asci 130-180 × 3.3-5.0 µm, caps depressed globose, 2-2.8 µm wide. Ascospores and part-spores immature. Asexual morph: Undetermined.

Specimen examined. CHINA. Hunan Province, Dongkou, 23 June 2014, collected by Ping Zhang (GACP ZP756).

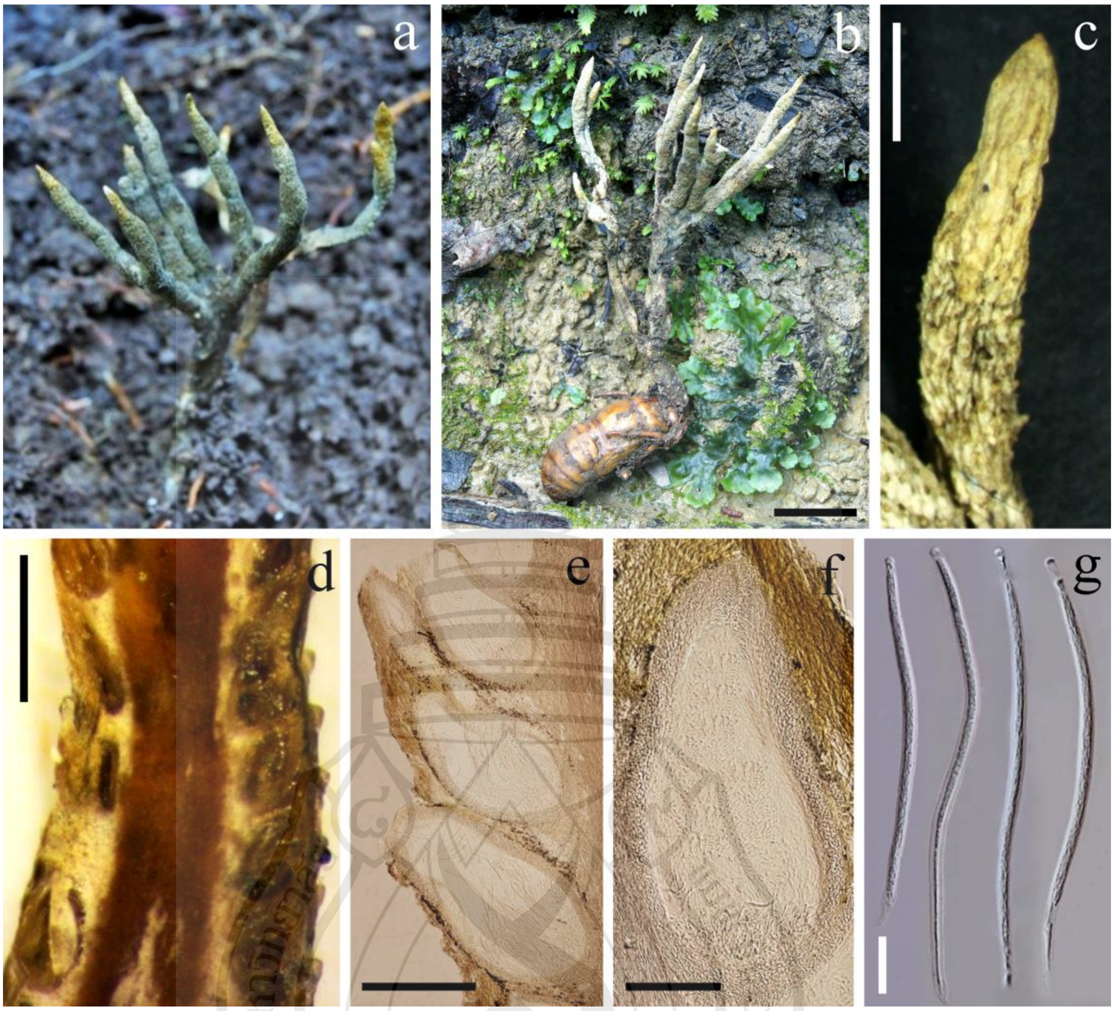
Sequence information. ITS (MG029522).

Habitat. Found in shallow soil layer of a broad-leaved forest of a humid subtropical rainforest.

Host. Cicada nymphs (Hemiptera: Cicadidae).

Distribution. Cameroon, China (Hunan, this study), Jamaica, Japan, Slovenia (Kobayasi, 1939; Kobayasi & Shimizu, 1963).

Notes. *Metarhizium owariense* was originally described by Kobayasi (1939) with type material from Japan. We identify our specimen as *M. owariense* based on their similar morphological characters and 100% identical ITS sequences (603 bp). *M. owariense* is newly recorded species for China.



Note a growing in the wild; b dug it out; c fertile part; d fertile part in profile; e, f perithecia in profile; g immature asci. Scale bars: b = 2 cm, c = 5 mm, d = 2 mm, e = 200 µm, f = 100 µm, g = 20 µm.

Figure 5.2 *Metarhizium owariense* (GACP ZP756)

5.6.6 *Metarhizium viridulum* (Tzean et al.) B. Huang & Z.Z. Li

≡ *Nomuraea viridula* Tzean et al. [as ‘*viridulus*’]

Distribution. China (Anhui, Taiwan) (Tzean et al., 1992; Huang et al., 2004).

Notes. The type locality is Taiwan (Taibei; Tzean et al., 1992), but the ITS sequence (AF368500) has been obtained by a specimen from Anhui (Wuhe County; Huang et al., 2004). Its sexual morph is also unknown.

5.6.7 *Ophiocordyceps bannaensis* L.S. Zha sp. nov. (Figure 5.3)

Index Fungorum number IF556563; Facesoffungi number: FoF 03919

Description. Sexual morph: Stromata arising from the vertex of a cicada nymph in shallow soil layer, single. Stalk unbranched, glabrous, fleshy and cylindric when fresh while flexible and a little compressed after dried, 12 cm long and 1.6-3.0 mm thick (dry specimen), yellowish brown but distal part darker than basal part. Fertile part located at the end of stalk, 4 cm long, barely incrassate and with obtuse apex, its surface densely dotted with black ostioles. Perithecia wholly immersed at right angles, densely arranged, long-spindle-like and with neck, 360-500 × 80-130 µm, walls dark brown and 15-20 µm thick, ostioles slightly thickened and slightly protruding. Asci cylindric, 6.3-8.6 µm in diameter, caps 7.7-8.3 µm wide and 4.3-4.8 µm high. Ascospores extremely easy to break; part-spores cylindric, obtuse at both ends, 12-18 × 1.3-2.2 µm. Asexual morph: Unknown.

Material examined. CHINA. Yunnan Province: Xishuangbanna Autonomous Prefecture, Jinghong (Nabanhe), 27 July 2016, collected by Lei Ye (HKAS 100059, holotype; MFLU 18-0041, isotype).

Sequence information. ITS: MG029521; SSU: MG725822; LSU: MG725824; RPB2: MG729402; TEF1-α: MG729401.

Habitat. Found in shallow soil layer of a broad-leaved forest in a humid tropical rainforest.

Host. A cicada nymph (Hemiptera: Cicadidae).

Distribution. China (Yunnan).

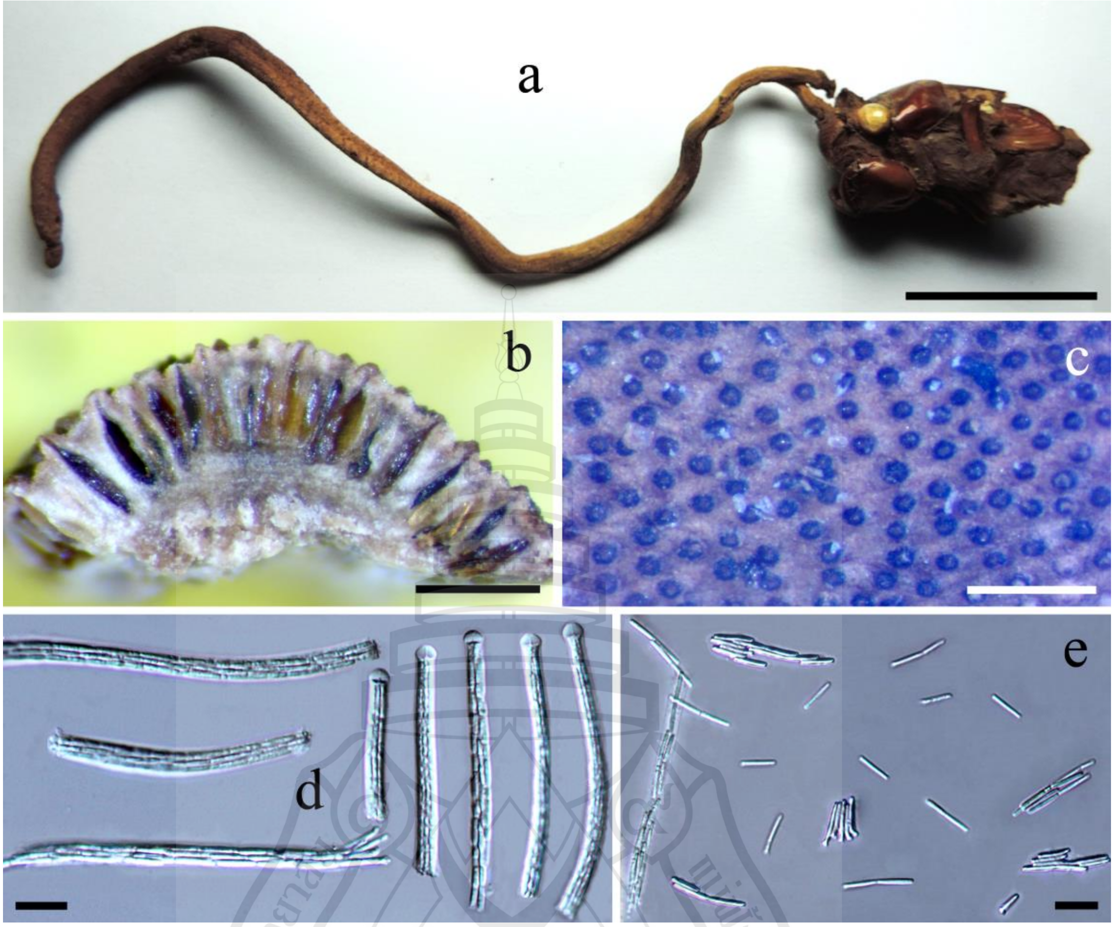
Etymology. The epithet is derived from the type locality: Xishuangbanna Autonomous Prefecture.

Note. Morphologically, *Ophiocordyceps bannaensis* sp. nov. is similar to *O. longissima* from Japan (Kobayasi & Shimizu, 1963) and China (Li et al., 1999), but differs by a barely incrassate fertile head, long spindle perithecia and larger asci and part-spores (Table 5.3). The new species can be easily distinguished from *O. pseudolongissima* by elongate stromata, barely incrassate fertile head, long spindle perithecia and distinctly larger asci and part-spores; from *O. yakusimensis* by the incrassate fertile head and without protruding ostioles of the latter (Kobayasi & Shimizu, 1963); and from *O. araracuarensis* by the dark brown fruiting body, the incrassate fertile head and pyriform perithecia of the latter (Sanjuan et al., 2015) (Table 5.3).

Table 5.3 Main differences among *Ophiocordyceps araracuarensis*, *O. longissima*, *O. pseudolongissima*, *O. yakusimensis* and *O. bannaensis* sp. nov.

Species	**Fertile head**	**Perithecia**	**Asci**	Part-spores
Ophiocordyceps araracuarensis	Clearly incrassate	Pyriform with a basal long neck, 550-650 × 150-240 µm	5-6 µm in diameter, caps 5-6 µm wide	9-12 (-16) × 1µm
O. bannaensis sp. nov.	Barely incrassate, unconspicuous	Long spindle, 360-500 × 80-130 µm	6.3-8.6 µm in diameter, caps 7.7-8.3 µm wide	12-18 × 1.3-2.2 µm
O. longissima from Japan	Clearly incrassate	Ovoid, 600-650 × 280-310 µm	5-6 µm in diameter, caps 4 µm wide	9-11 × 1-1.2 µm
O. longissima from China	Clearly incrassate	Long ovoid, 550-630 × 130-230 µm	4.5-5.5 µm in diameter, caps 4.8-5.0 µm wide	7.8-13 × 1.0-1.4 µm
O. pseudolongissima	Clearly incrassate	Long ovoid, 470-500 × 150-180 µm	4 µm in diameter, caps 3 µm wide	8-11 × 1 µm
O. yakusimensis	Clearly incrassate	Long spindle, 740-800 × 170-230 µm	5 µm in diameter	10-15 × 1 µm

Nucleotide sequences of the new species are similar to but different from those of *O. longissima* (NBRC 106965; Ban et al., 2015b): 32.2% bp difference across 1086 bp in SSU, 3.3% bp difference across 609 bp in ITS, 1.4% bp difference across 971 bp in TEF1-α and minor differences (<0.8%) in LSU (887 bp) and RBP2 (889 bp) gene sequences. When the SSU gene was compared to *O. longissima* (NBRC 106965; Ban et al., 2015b) and other allies, there is a fragment of a total 333 bp missing in our new species.



Note a stroma; b perithecia in profile; c surface of fertile part of fruiting body; d asci; e part-spores. Scale bars: a = 2 cm, b-c = 0.5 mm, d-e = 20 µm.

Figure 5.3 *Ophiocordyceps bannaensis* (HKAS 100059, holotype)

5.6.8 *Ophiocordyceps cicadicola* (Teng) G.H. Sung et al.

≡ *Cordyceps cicadicola* Teng

Distribution. China (Guangdong, Hainan) (Liang, 2007).

Notes. Descriptions of *Cordyceps cicadicola* and *C. ryogamimontana* are almost identical (Kobayasi & Shimizu, 1963; Liang, 2007), also their hosts are both adult cicadas. So *Purpureocillium takamizusanense* (≡ *Isaria takamizusanensis* Kobayasi, = *C. ryogamimontana*; Ban et al., 2015a) is most likely a synonym of *Ophiocordyceps cicadicola*.

5.6.9 *Ophiocordyceps heteropoda* (Kobayasi) G.H. Sung et al.

≡ *Cordyceps heteropoda* Kobayasi

= *Cordyceps heteropoda* var. *heteropoda* Kobayasi

= *Cordyceps heteropoda* var. *langyashanensis* C.R. Li et al.

= *Hirsutella heteropoda* C.R. Li et al.

Distribution. China (Anhui, Fujian) (Li et al., 2006; Liang, 2007), Japan (Kobayasi, 1939), Korea, the Congo (Sung et al., 2011).

5.6.10 *Ophiocordyceps longissima* (Kobayasi) G.H. Sung et al.

≡ *Cordyceps longissima* Kobayasi

= *Hirsutella longissima* C.R. Li et al.

Distribution. China (Anhui) (Li et al., 1999), Japan (Kobayasi & Shimizu, 1963), Korea (Sung et al., 2007), Thailand (Aung et al., 2008).

5.6.11 *Ophiocordyceps sobolifera* (Hill ex Watson) G.H. Sung et al.

≡ *Clavaria sobolifera* Hill ex Watson

≡ *Cordyceps sobolifera* (Hill ex Watson) Berk. & Broome [as '*Cordiceps*']

≡ *Sphaeria sobolifera* (Hill ex Watson) Berk.

≡ *Torrubia sobolifera* (Hill ex Watson) Tul. & C. Tul.

= *Cordyceps sobolifera* var. *sobolifera* (Hill ex Watson) Berk. & Broome

= *Cordyceps sobolifera* var. *takaoensis* Kobayasi

= *Cordyceps takaoensis* (Kobayasi) Kobayasi

= *Ophiocordyceps takaoensis* (Kobayasi) G.H. Sung et al. [as '*takaoënsis*']

Distribution. Widely distributed in the tropics and subtropics. In China, it widely distributed in southern provinces.

Notes. In China, *Ophiocordyceps sobolifera* has been called 'Xiao-Chan-Cao' ('small cicada herb'), to separate from 'Da-Chan-Cao' ('large cicada herb', refers to *Cordyceps cicadae* S.Z. Shing, see *Tolypocladium paradoxum* below). And as a medicinal mushroom, this species is often mistakenly called Chanhua (*Cordyceps cicadae* (Miq.) Massee) (Zha et al., 2018).

5.6.12 *Ophiocordyceps wuyishanensis* (Z.Q. Liang et al.) G.H. Sung et al.

≡ *Cordyceps wuyishanensis* Z.Q. Liang, A.Y. Liu & J.Z. Huang

Distribution. China (Fujian) (Liang et al., 2002).

5.6.13 *Ophiocordyceps yakusimensis* (Kobayasi) G.H. Sung et al.

≡ *Cordyceps yakusimensis* Kobayasi

Distribution. China (Anhui, Zhejiang, Taiwan), Japan, Korea (Kobayasi & Shimizu, 1963; Sung et al., 2007; Chen et al., 2014).

5.6.14 *Paecilomyces xylariiformis* (Lloyd) Samson

≡ *Isaria xylariiformis* Lloyd [as '*xylariaformis*']

Distribution. Brazil, China (Yunnan) (Samson, 1974; Liu et al., 2010).

Note. *Paecilomyces xylariiformis* from China is actually Chanhua, and *Pa. xylariiformis* from Brazil might be a synonym of *Cordyceps cicadae* (Miq.) Massee (see notes of Chanhua above).

5.6.15 *Polycephalomyces kanzashianus* (Kobayasi & Shimizu) Kepler & Spatafora

≡ *Cordyceps kanzashiana* Kobayasi & Shimizu

Distribution. China (Anhui) (Zhang, 2013), Japan (Kobayasi & Shimizu, 1982a).

Notes. Zhang (2013) introduced two immature specimens which seem to be *Polycephalomyces kanzashianus*, but their ITS information provided is possibly inaccurate (unassembled sequences, only 93% similar to that of *Po. kanzashianus* from Japan). The specimens were collected from Anhui.

5.6.16 *Polycephalomyces nipponicus* (Kobayasi) Kepler & Spatafora (Figure 5.4)

≡ *Cordyceps nipponica* Kobayasi

MycoBank: MB804389

Description. Sexual morph: Stromata arising from the vertex of a cicada nymph in soil, solitary but polycephalous, 5.3 cm long and 1.0-2.5 mm thick. Stalk cylindric, simple, flexible; basal and middle parts (4 cm) buried in the soil, not branched, surface cinnamomeous but covered by muds; terminal part exposed in the air, brown, branched, apex obtuse. Fertile parts laterally attach the terminal stalk and its branches, conglomerated or tuberiform, milky yellow, 0.1-0.4 mm high and 0.5-1.5 mm wide, surface dotted with ostioles, mammillate. Perithecia immersed, ampullaceous or ovoid, 240-290 × 120-160 µm, walls about 10 µm thick, almost hyaline; ostioles protruding, milky yellow to dark brown, l0-20 µm high and 60-90 µm in diameter, apex obtuse. Asci 3-4 µm thick, with very rough surface, easy to break, caps 3-3.4 × 1.7-2 µm; part-spores minute, 2.9-4.5 × 0.7-1.1 µm, truncated at both ends. Asexual morph: Undetermined.

Specimen examined. CHINA. Yunnan Province: Xishuangbanna Autonomous Prefecture, Jinghong (Nabanhe), 27 July 2016, collected by Lei Ye (HKAS 100058).

Sequence information. SSU: MG725823; ITS: MG029523; LSU: MG725825.

Habitat. Found in the soil of a broad-leaved forest of a humid tropical rainforest.

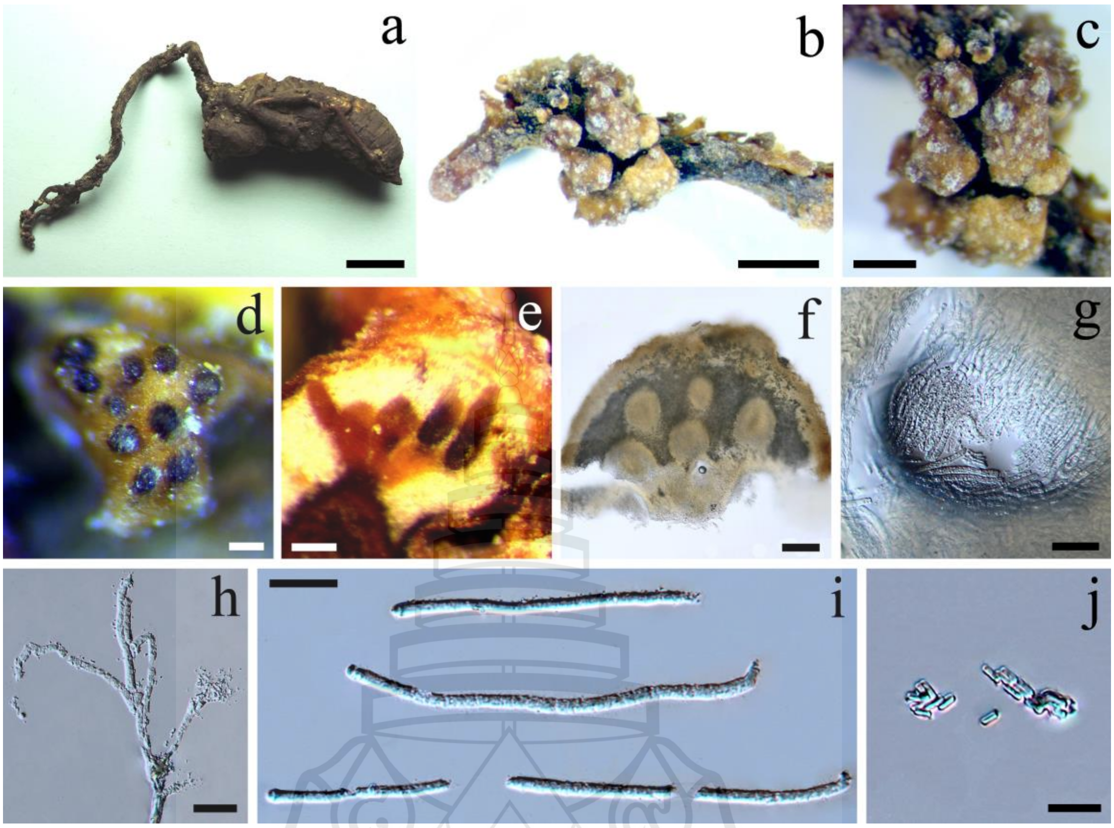
Host. Cicada nymph (Hemiptera: Cicadidae).

Distribution. China (Yunnan), Japan (Kobayasi, 1939), Thailand (Luangsa-ard et al., 2008).

Notes. Apart from smaller perithecia, our specimen is closely similar to *Polycephalomyces nipponicus* from Japan (perithecia 800-950 × 300-370 µm; Kobayasi, 1939). Its ITS sequence is 99.7% similar (across 661 bp) to those of *Po. nipponicus* from Thailand (Schoch et al., 2012; Kepler et al., 2013). For these reasons, we identify our specimen as *Po. nipponicus* (Jeewon & Hyde, 2016). *Po. nipponicus* is newly recorded species for China.

Po. nipponicus is similar to *Po. kanzashianus*. In *Po. kanzashianus*, apex of stroma expanded, either globose or ellipsoidal, and fertile parts also globose or ellipsoidal (Kobayasi & Shimizu, 1982a); while in *Po. nipponicus*, apex of stroma is not at all expanded, and fertile parts conglomerated or tuberiform. Both species are closely related in the phylogenetic tree (Figure 5.1).

Asexual morph of *Po. nipponicus* should be *Polycephalomyces*-like of instead *Isaria*-like; and *Isaria nipponica*, a *Isaria cicadae*-like species and originally reported as the asexual morph of *Po. nipponicus* (Kobayasi, 1939), is most likely Chanhua (*Cordyceps cicadae* (Miq.) Massee).



Note a stromata; b, c fertile part of fruiting body; d surface of fertile part; e-g perithecia in profile; h, i asci; j part-spores. Scale bars: a = 1 cm, b = 1 mm, c = 0.5 mm, d-f = 0.1 mm, g-i = 20 µm, j = 10 µm.

Figure 5.4 *Polycephalomyces nipponicus* (HKAS 100058)

5.6.17 *Polycephalomyces ramosopulvinatus* (Kobayasi & Shimizu) Kepler & Spatafora

≡ *Cordyceps ramosopulvinata* Kobayasi & Shimizu

Distribution. China (Yunnan) (Chen et al., 2014), Japan (Kobayasi & Shimizu, 1983), Korea (Sung et al., 2010).

5.6.18 *Tolypocladium inegoense* (Kobayasi) Quandt et al.

≡ *Cordyceps inegoensis* Kobayasi

≡ *Elaphocordyceps inegoensis* (Kobayasi) G.H. Sung et al. [as ‘*inegoënsis*’]

Distribution. China (Fujian, Taiwan) (Liang, 2007), Japan (Kobayasi & Shimizu, 1963), Korea (Sung et al., 2007).

5.6.19 *Tolypocladium paradoxum* (Kobayasi) Quandt et al.

≡ *Cordyceps paradoxa* Kobayasi

= *Cordyceps cicadae* S.Z. Shing

= *Cordyceps zhejiangensis* (S.Z. Shing) Z. Y. Liu et al.

≡ *Elaphocordyceps paradoxa* (Kobayasi) G.H. Sung et al.

Distribution. China, Japan, Koera (Chen et al., 2014). In China, it is widely distributed in southern provinces.

Notes. This is the well-known *Cordyceps cicadae* S.Z. Shing and in the last 40 years it had been regarded as the sexual morph of Chanhua (Chen et al., 2014; Zha et al., 2018). As a medicinal mushroom and with a black and hornlike fruiting body, Chinese people call it 'Du–Jiao–Long' ('one–horned dragon'; Zha et al., 2018); and in order to separate from 'Xiao-Chan-Cao' ('small cicada herb', refer to *Ophiocordyceps sobolifera*), it has also been called 'Da-Chan-Cao' ('large cicada herb'; Shing, 1975).

5.6.20 Other cicadicolous species

Chen et al. (2014) recorded an unknown species of cicadicolous cordyceps based on a specimen from Zhejiang, China. This species was described as: sexual and asexual fruiting bodies simultaneously presented at the end of stroma; the asexual sporulation structures immersed in the out layer of the asexual fruiting body, flask-shaped; conidia polyhedral or nearly globose, 2.6-3.6 × 2-3 μm (Chen et al., 2014).

Besides, Wang (2016) introduced three new cicadicolous species from China: *Polycephalomyces cicadae* H. Yu & Y.B. Wang from Jiangsu, *Po. daweiensis* H. Yu & Y.B. Wang and *Po. roseoalbus* H. Yu & Y.B. Wang from Yunnan, but they have not been formally published.

CHAPTER 6

CORDYCEPS FUNGI GROWING ON ORTHOPTERA INSECTS

6.1 Introduction

Orthoptera are the sixth largest order of Insecta, they mainly consist of six well known groups: locusts, katydids, crickets, mole crickets, pygmy locusts and pygmy mole crickets (Table 6.1). Among them, locusts are the largest group while mole crickets have only 208 species. Orthoptera insects are mostly pests, because they mainly feed on green plants. Many locust species, i.e., *Locusta migratoria* (Linnaeus) and *Oxya* spp., can swarm and form huge populations that cause immense damage to agricultural crops (Lomer et al., 2001). But as edible insects, some locust species have been bred and exploited for food or animal feed (Paul et al., 2016). In traditional Chinese culture, some katydids and crickets have been collected or bred for folk entertainments.

Cordyceps (= *Cordyceps* sensu lato, Zha et al., 2018), as the most popular topic of entomopathogenic fungi, has always been highly researched for its species diversity, edible and medicinal values and use for biological control. This group currently has more than 1,000 species (Table 6.1) that grow on insects, spiders, nematodes, other cordyceps, the fungi *Elaphomyces* and even inhabit soil and plant tissues (Sung et al., 2007; Vega et al., 2009). Due to lack of molecular evidence or inconclusive morphology and ecology, more than 100 species are retained in the old genus *Cordyceps* (Sung et al., 2007). Also, due to confusion of sexual and asexual morphs, many cordyceps species still have two or more names. Clarification of sexual and asexual names and classification of undetermined *Cordyceps* species are still needed for cordyceps research.

How many cordyceps species grow on Orthoptera insects? The early systematic reports came from Petch (1934) who reported five species and Kobayasi

(1941) with six species. Later, Shimizu (1997) recorded four species and Liang (2007) with three which distributed in China. Recently Sanjuan et al. (2014) gave a report for species diversity of Orthoptera cordyceps which deals with seven known species and a new one. However, Orthoptera cordyceps has far more than eight species, and several names are undetermined and several hosts had been mistakenly identified. In a word, Orthoptera cordyceps has been poorly studied since Petch (1934) and Kobayasi (1941).

In this report, we review the species diversity of cordyceps on Orthoptera insects, including their hosts, distributions and taxonomical notes. Molecular phylogeny of Orthoptera cordyceps was constructed and ecology and habits of the six groups of Orthoptera are explored. We also exploit the occurrence mechanism and conidiogenous mode of cordyceps funiculi. Among these Orthoptera cordyceps, two undetermined names are revised, one name is synonymized, and hosts of two species are corrected. We aim to provide more taxonomical and ecological information for cordyceps on Orthoptera insects.

6.2 Material and Methods

6.2.1 Specimens

Specimen collections and ecological observations were made in southern China and Thailand. Fungal specimens were examined and photographed using an Optec SZ660 stereo dissecting microscope, a Nikon Coolpix P520 camera and a Nikon Eclipse 80i compound microscope connected with a Cannon EOS 600D camera. Measurements of microphotographs were made using Tarosoft (R) Image Frame Work software. Voucher specimens are deposited in the Centre of Excellence in Fungal Research, Mae Fah Luang University (MFLU), Chiang Rai, Thailand, the Herbarium of the Institute of Microbiology, Chinese Academia of Sciences (HMAS), and the Herbarium of Guizhou University (GACP), Guiyang, China.

6.2.2 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 1α (TEF1-*α*) genes were amplified and sequenced using the primers detailed by White et al. (1990) and Ban et al. (2015b). PCR programs followed those of Ban et al. (2015b) and amplification reactions were performed in an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR products were purified using the Bioteke's Purification Kit (Bioteke 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, Chromas230, ContigExpress and Mega6.06 software.

6.2.3 Construction of Phylogenetic Tree

ITS and TEF1-*α* sequence data of *Cordyceps grylli* and its allies were acquired from GenBank and our sequencing results, which consists of 44 samples of 36 species that deal with *Beauveria*, *Cordyceps* and species on Orthoptera insects (Table 6.2). Molecular phylogeny was constructed using the single ITS, the single TEF1-*α* and combined ITS and TEF1-*α* sequence datasets, respectively. The single ITS and TEF1-*α* sequence datasets were analyzed using neighbor-joining (NJ) method (Saitou & Nei, 1987), and the combined ITS and TEF1-*α* one using maximum likelihood (ML) method. NJ trees were performed with MEGA6.06 (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, other details are described in Jeewon et al. (2003) and Hongsanan et al. (2017).

6.3 Phylogeney

The different analyses resulted in trees with similar topology and support values (results not shown), and the ML tree for combined ITS and TEF1-*α* sequence dataset is shown in Figure 6.1.

Table 6.1 Species totals of Insecta, Orthoptera, Fungi and cordyceps

Insecta	Species total	Orthoptera	Species total	Fungi	Species total	Cordyceps	Species total
Coleoptera (beetles)	331,855	Locusts (the superfamily group Acridomorpha)	10,070	Ascomycota	83,837	Cordycipitaceae	471
Diptera (flies and mosquitoes)	153,953	Katydids (Tettigoniidea)	8,951	Basidiomycota	48,405	Ophiocordycipitaceae	358
Lepidoptera (moths and butterflies)	149,845	Crickets (Grylloidea)	5,662	Zygomycota	1,366	Clavicipitaceae (partially)	260
Hymenoptera (bees and ants)	118,110	Pygmy locusts (Tetrigoidea)	2,043	Chytridiomycota	1,218	-	-
Hemiptera (stinkbugs, cicadas)	85,032	Pygmy mole crickets (Tridactylidea)	245	Glomeromycota	284	-	-
Orthoptera	28,111	Mole crickets (Gryllotalpoidea)	208	-	-	-	-
Other 38 orders	70,614	Others	932	-	-	-	-
Total: 44 orders	937,520	-	28,111	-	135,110	-	1089

Note Counted from Catalogue of Life (http://www.catalogueoflife.org/) and Orthoptera Species File (Cigliano et al., 2019), retrieval date: 5 April 2019. 'Species total' refers to numbers of accepted species and doubtful species, all infraspecific names were not included.

Table 6.2 Sequence information of samples appearing in Figure 6.1

Fungal species	Collection	Host	GenBank numbers		Reference
			ITS	**TEF1-α**	
Beauveria acridophila	AV1815	Orthoptera: Acrididae: *Ommatolampis* (locust)	JQ958600	JQ958614	Sanjuan et al. (2014)
B. acridophila	AV1845	Orthoptera: Acrididae: *Ommatolampis* (locust)	JQ958601	JQ958615	Sanjuan et al. (2014)
B. amorpha	ARSEF 2641	Hymenoptera: Formicidae	HQ880808	AY531917	Rehner et al. (2011)
B. araneola	GZU0317bea	Spider	KT961700	KT961699	Chen et al. (2017)
B. asiatica	ARSEF 4384	Coleoptera: Scarabaeidae	AY532026	AY531935	Rehner et al. (2011)
B. asiatica	ARSEF 4474	Coleoptera: Scarabaeidae	AY532027	AY531936	Rehner et al. (2011)
B. australis	ARSEF 4598	Soil	HQ880789	HQ880995	Rehner et al. (2011)
B. bassiana	ARSEF 1564	Lepidoptera: Arctiidae (locust)	HQ880761	HQ880974	Rehner et al. (2011)
B. bassiana	**GACP 16041002**	**Lepidoptera: Hepialidae (larva)**	**MF179621**	**MK860196**	**This study**
B. bassiana	**GACP 14071004**	**Lepidoptera: Hepialidae: *Endoclita davidi* (larva)**	**MF179620**	**MK860195**	**This study**
B. blattidicola	MCA 1727	Blattaria (cockroach adult)	-	MF416483	Kepler et al. (2017)
B. blattidicola	MCA 1814	Blattaria (cockroach adult)	-	MF416484	Kepler et al. (2017)
B. brongniartii	ARSEF 617	Coleoptera: Scarabaeidae	HQ880782	HQ880991	Rehner et al. (2011)
B. caledonica	ARSEF 2567	Soil	HQ880817	AY531915	Rehner et al. (2011)
B. diapheromeriphila	MCA1557	Phasmatodea: Diapheromeridae	JQ958608	JQ958612	Kepler et al. (2017)
B. gryllotalpidicola	BCC26300	Orthoptera: Gryllotalpidae (mole cricket)	FJ459787	FJ459795	Ariyawansa et al. (2015)
B. hoplocheli	B507	Coleoptera: Melolonthidae	KC339680	KC339707	Robene et al. (2015)
B. kipukae	ARSEF 7032	Homoptera: Delphacidae	HQ880803	HQ881005	Rehner et al. (2011)

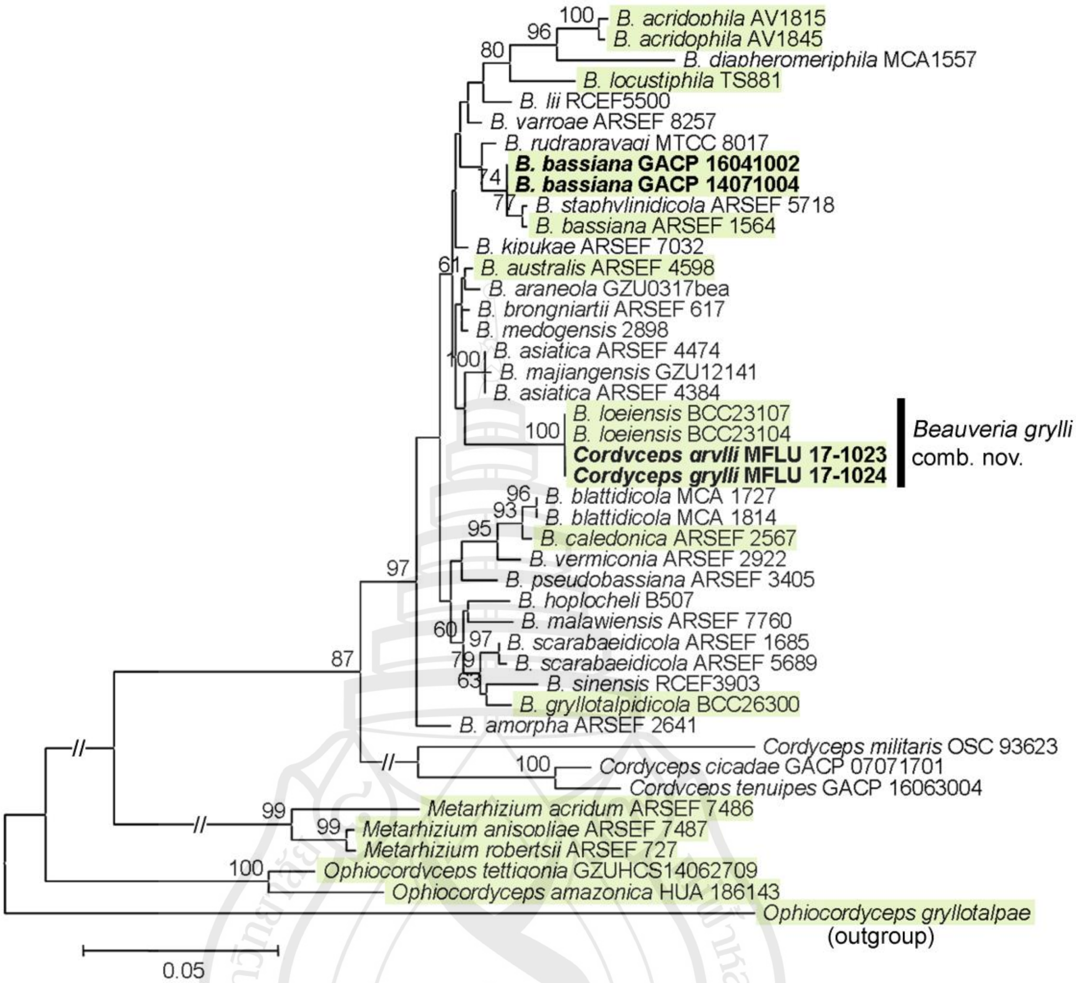
Table 6.2 (continued)

Fungal species	**Collection**	**Host**	**GenBank numbers**		**Reference**
			ITS	**TEF1-*α***	
B. lii	RCEF5500	Coleoptera: Coccinellidae	JN689372	JN689371	Zhang et al. (2013)
B. locustiphila	TS881	Orthoptera: Romaleidae: *Colpolopha sinuata* (locust)	JQ958606	JQ958619	Sanjuan et al. (2014)
B. loeiensis	BCC23104	Orthoptera: Gryllacrididae (katydid)	FJ459784	FJ459792	Ariyawansa et al. (2015)
B. loeiensis	BCC23107	Orthoptera: Gryllacrididae (katydid)	FJ459786	FJ459794	Ariyawansa et al. (2015)
B. majiangensis	GZU12141	Coleoptera: Scarabaeoidea	MG052642	MG052640	Chen et al. (2018)
B. malawiensis	ARSEF 7760	Coleoptera: Cerambycidae	HQ880825	DQ376246	Rehner et al. (2011)
B. medogensis	2898	Soil	KU994837	KU994833	Imoulan et al. (2016)
B. pseudobassiana	ARSEF 3405	Lepidoptera: Tortricidae	HQ880792	AY531931	Rehner et al. (2011)
B. rudraprayagi	MTCC 8017	Lepidoptera (silkworm)	JQ266173	JQ990914	Agrawal et al. (2014)
B. scarabaeidicola	ARSEF 1685	Coleoptera: Scarabaeidae	HQ880809	AY531899	Rehner et al. (2011)
B. scarabaeidicola	ARSEF 5689	Coleoptera: Scarabaeidae (adult)	HQ880810	DQ522335	Rehner et al. (2011)
B. sinensis	RCEF3903	Lepidoptera: Geometridae (larva)	HQ270152	HQ270151	Chen et al. (2013b)
B. staphylinidicola	ARSEF 5718	Coleoptera: Staphylinidae (pupa)	-	EF468776	Sung et al. (2007)
B. varroae	ARSEF 8257	Acari: Varroidae	HQ880800	HQ881002	Rehner et al. (2011)
B. vermiconia	ARSEF 2922	Soil	HQ880822	AY531920	Rehner et al. (2011)
Cordyceps cicadae (Chanhua)	GACP 07071701	Hemiptera: Cicadidae (nymph)	KX017277	MK770631	Zha et al. (2019b)

Table 6.2 (continued)

Fungal species	**Collection**	**Host**	**GenBank numbers**		**Reference**
			ITS	**TEF1-α**	
C. grylli	**MFLU 17-1023**	**Orthoptera: Gryllacrididae (katydid nymph)**	**MF179618**	**MK860193**	**This study**
C. grylli	**MFLU 17-1024**	**Orthoptera: Gryllacrididae (katydid nymph)**	**MF179619**	**MK860194**	**This study**
C. militaris	OSC 93623	Lepidoptera (pupa)	JN049825	DQ522332	Sung et al. (2007)
C. tenuipes	GACP 16063004	Lepidoptera (cocooned pupae of small moth)	KY423509	MK770633	Zha et al. (2019a)
Metarhizium acridum	ARSEF 7486	Orthoptera	HQ331458	EU248845	Bischoff et al. (2009)
M. anisopliae	ARSEF 7487	Orthoptera	HQ331446	DQ463996	Bischoff et al. (2009)
M. robertsii	ARSEF 727	Orthoptera	HQ331453	DQ463994	Bischoff et al. (2009)
Ophiocordyceps amazonica	HUA 186143	Orthoptera: Acrididae (locust adult)	-	KM411989	Sanjuan et al. (2015)
O. gryllotalpae	-	Orthoptera: Gryllotalpidae (mole cricket)	AJ536569	-	NCBI
O. tettigonia	GZUH CS14062709	Orthoptera: Gryllidae (cricket adult)	KT345954	KT375440	Wen et al. (2016a)

Note Sequencing results in this study are displayed in bold.



Note Species maked in green are pathogens of Orthoptera insects. Bootstrap support values greater than 60% are indicated above the nodes.

Figure 6.1 Maximum likelihood (ML) tree of *Beauveria grylli* and its allies inferred from combined ITS and TEF1-*α* dataset

The combined ITS and TEF1-*α* dataset comprises 1,511 characters (including gaps), of which 535 are variable and 317 are parsimony- informative. The ML tree consists of 36 species (44 samples) of *Cordyceps grylli* and its allies. Among them, species of *Beauveria* (26 species) and Orthoptera cordyceps (14 species), that their ITS and/or TEF1-*α* sequence data can be acquired in GenBank, are all included.

In the ML tree, *Cordyceps grylli* is assigned in the *Beauveria* clade and grouped together with *Beauveria loeiensis* (support values of ML and NJ trees are all

100%). Together with evidences of morphology, hosts and geography, the two species will be combined at below (see *Beauveria grylli*).

Also in the tree, Orthoptera cordyceps are assigned to three groups: the *Beauveria*, the *Metarhizium* and the *Ophiocordyceps* clade, respectively. No one is included in the *Cordyceps* clade, so those undetermined Orthoptera cordyceps (8 *Cordyceps* spp. and 1 *Syngliocladium* sp.) may will be assigned to these three clades in future research.

6.4 Species Diversity of Cordyceps on Orthoptera Insects

6.4.1 *Beauveria acridophila* (T. Sanjuan & Franco-Mol.) T. Sanjuan et al.

≡ *Cordyceps acridophila* T. Sanjuan & Franco-Mol.

Host. Many locust nymphs (the superfamily group Acridomorpha) (Sanjuan et al., 2014).

Known distribution. Colombia, Ecuador and Guyana (Sanjuan et al., 2014).

6.4.2 *Beauveria australis* S.A. Rehner & Humber

Host. Isolated from soil and locusts (Acrididae) (Rehner et al., 2011).

Known distribution. Australia (Rehner et al., 2011).

6.4.3 *Beauveria bassiana* (Bals.-Criv.) Vuill.

≡ *Botrytis bassiana* Bals.-Criv.

≡ *Spicaria bassiana* (Bals.-Criv.) Vuill.

≡ *Penicillium bassianum* (Bals.-Criv.) Biourge

= *Cordyceps bassiana* Z.Z. Li et al.

Host. Many insect groups including Orthoptera; inhabit soil, plant surfaces and plant internal tissues (Meyling et al., 2009; Agrawal et al., 2014; Bamisile et al., 2018).

Distribution. Widely distributed.

6.4.4 *Beauveria caledonica* Bissett & Widden

Host. Many insect orders including Orthoptera (eg. katydid (Gryllacrididae)); inhabit soil (Bissett & Widden, 1988; Rehner et al., 2011).

Distribution. Widely distributed (Rehner et al., 2011).

6.4.5 *Beauveria grylli* (Teng) L.S. Zha comb. nov. (Figures 6.2. 6.3)

MycoBank:

≡ *Cordyceps grylli* Teng

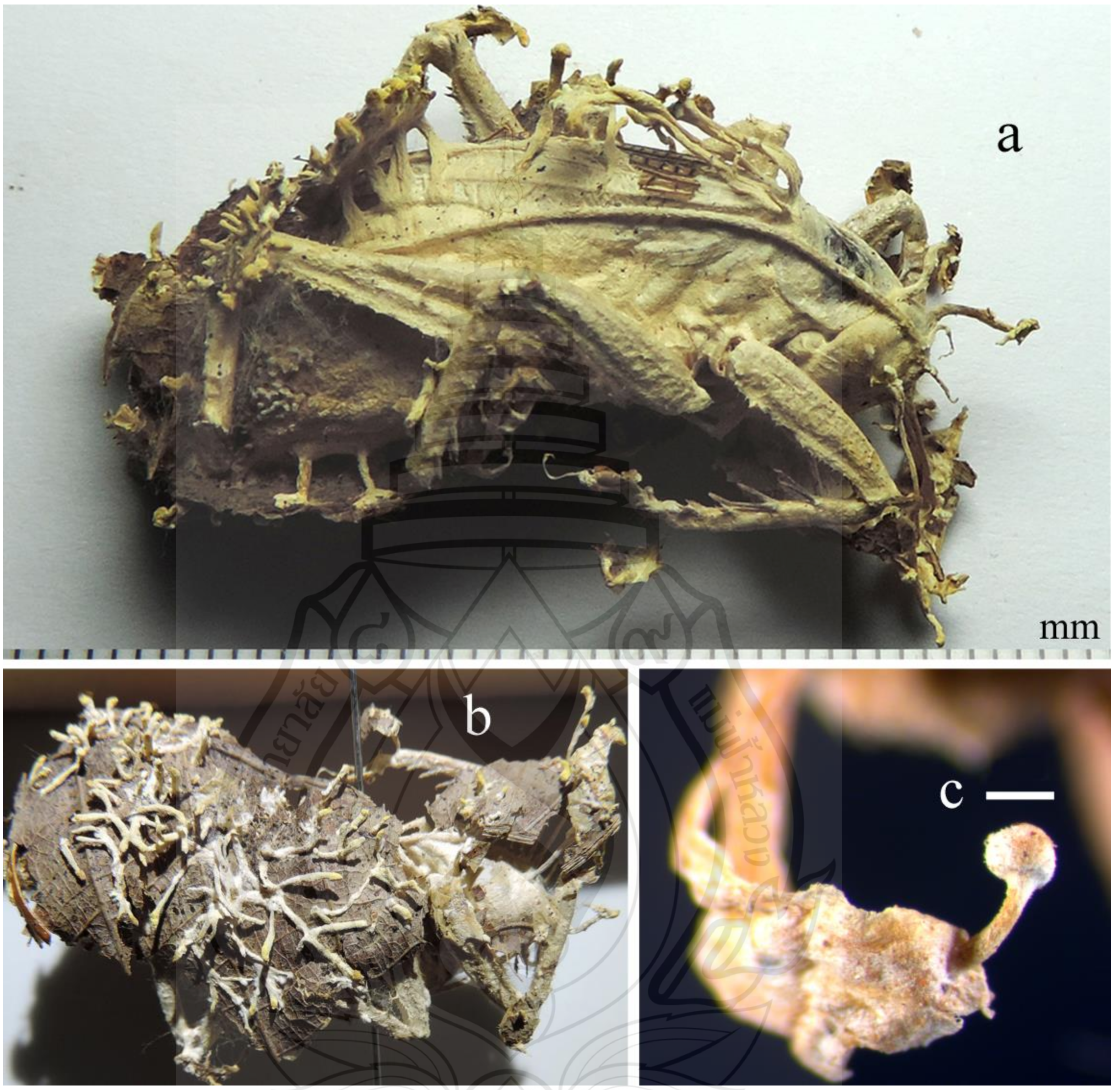
= *Beauveria loeiensis* Luangsa-ard, Ridkaew & Tasan.

Notes. *Cordyceps grylli* was originally described by Teng (1936) with specimens from China (Fujian, Guangdong and Hainan). Huang (1998) recorded this species with the original description, the distribution and a colour photograph. Luo and Zhang (2017) reported it with a specimen from Guizhou, also gave a similar photograph. According to the two photographs, the hosts of *C. grylli*, that were identified as adult crickets (Gryllidae), are actually katydid nymphs (Gryllacrididae). Gryllacrididae insects look like crickets (Grylloidea), but their extremely enlongate antennae and their nymphs wrapped in leaves can easily separate them from other insect groups. Our collections exactly match the description, the photograph and the hosts, so we identify them as *C. grylli*. Following the new revision of the genus *Beauveria* (Kepler et al., 2017) and the molecular phylogeny (Figure 6.1), we combine *C. grylli* to *B. grylli*. Notably, the epithet '*grylli*' came from the family Gryllidae of crickets instead of katydids.

Ariyawansa et al. (2015) introduced *B. loeiensis* with specimens from Thailand. Its description and phtographs are consistent with those of Teng (1936), Huang (1998), Luo and Zhang (2017) and our collections. Also DNA sequences of *B. loeiensis* (ITS, SSU and TEF1-α) and our collections are all identical. For these reasons and following the priority of *C. grylli*, we synonymize *B. loeiensis* as *B. grylli*.

Asexual synnemata of *B. grylli* are commonly accompanied with its sexual fruiting bodies, and in nature, the solitary sexual morph has not been reported yet. Recently in northern Thailand, we first discovered the solitary asexual morph of *B. grylli* in the wild. It grew on a young Gryllacrididae nymph that wrapped in a fallen leaf (Figure 6.3), and morphological and molecular evidences also prove it to be *B. grylli*.

Beauveria grylli is morphologically closely similar to *B. kirkii* comb. nov. (≡ *C. kirkii*, see notes of *B. kirkii* below).

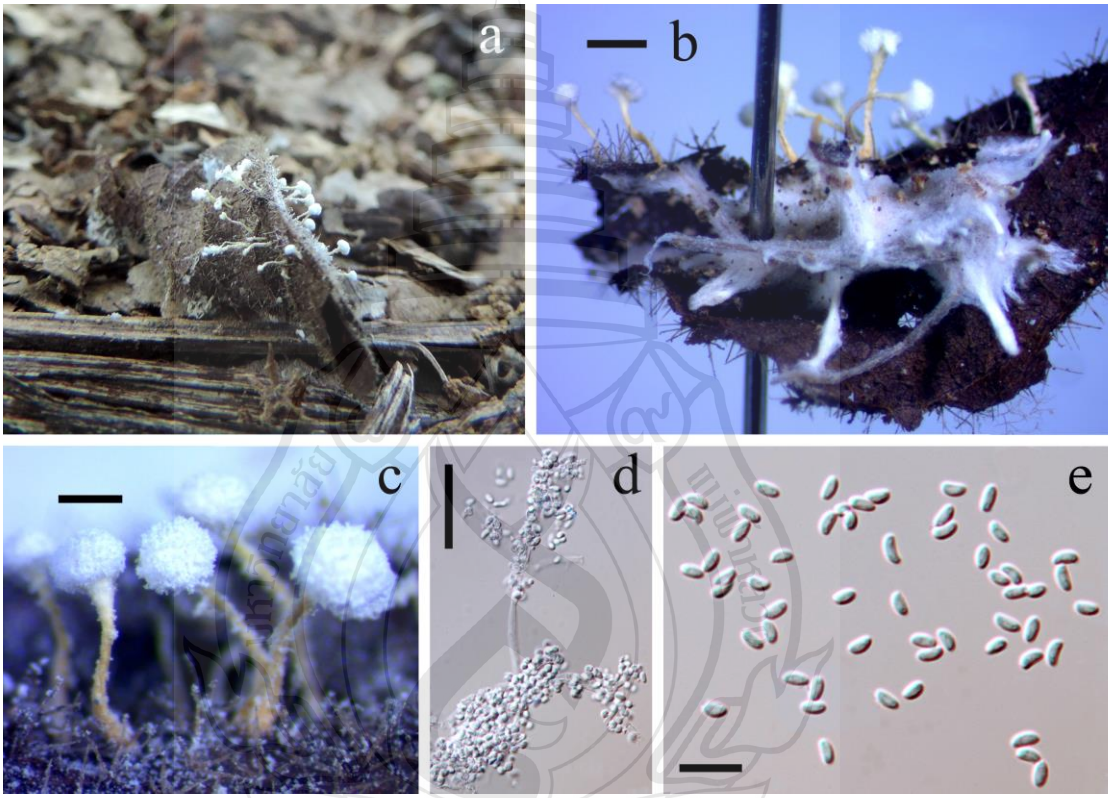


Note **a** lateral view; **b** turned upside down; **c** funiculus and conidiogenous structure. Scale bar: **c** = 0.5 mm.

Figure 6.2 *Beauveria grylli* on an old Gryllacrididae nymph, sexual morph (immature) co-occurred with asexual morph (MFLU 17-1023)

Specimens examined. MFLU 17-1023, HKAS100041, HKAS100042, HKAS100043 and HKAS100044; Jinghong (Nabanhe), Yunnan, China; 29-31 July 2016, collected by Lei Ye, Samantha C. Karunarathna and Hui-Li Li; MFLU 17-1024, Mae Fah Luang University, Chiang Rai, Thailand, N20°3'21.83", E99°55'6.26", 406 m, 20 November 2016, collected by Lingsheng Zha.

Hosts. Katydid nymphs (Orthoptera: Gryllacrididae).



Note **a** the host was enveloped in a fallen leaf; **b** many synnemata arising from outer side of the fallen leaf (the host is pinned upside down); **c** synnemata; **d**, **e** conidiophores and conidia. Scale bars: **b** = 1 mm, **c** = 0.5 mm, **d** = 30 µm, **e** = 10 um.

Figure 6.3 *Beauveria grylli* on a young Gryllacrididae nymph, asexual morph (MFLU 17-1024)

Sequence information. MFLU 17-1023: ITS, MF179618; SSU, MK863048; LSU, MK863055; TEF1-*α*, MK860193. MFLU 17-1024: ITS, MF179619; SSU, MK863049; LSU, MK863056; TEF1-*α*, MK860194.

Known distribution. China (Fujian, Guangdong, Guizhou, Hainan, Yunnan), Thailand.

Ecology. Gryllacrididae adults lay eggs on leaves or grasses at first, then fold the plant tissues to wrap their eggs. The eggs will hatch out and the young nymphs will live in the folded plant tissues. When the folded plant tissues had been destroyed (by winds, animals or naturally fell off) and dropped onto the humid ground, the nymphs within them might easily be infected by *B. grylli*. Occurrence of sexual or asexual morph of the fungus mainly depends on the environmental humidity and the nymph’s instar, and the sexual life cycle is much longer than its asexual ones.

6.4.6 *Beauveria gryllotalpidicola* Luangsa-ard, Ridkaew & Tasan.

Host. Mole crickets (Gryllotalpidae) (Ariyawansa et al., 2015)

Known distribution. Thailand.

6.4.7 *Beauveria kirkii* (G. Cunn.) L.S. Zha comb. nov.

MycoBank:

≡ *Cordyceps kirkii* G. Cunn.

Host. Katydid nymphs (Anostostomatidae: *Deinacrida rugosa*) (Cunningham, 1922; Kobayasi, 1941).

Known distribution. New Zealand.

Notes. *Cordyceps kirkii* was originally described by Cunningham (1922) with types from New Zealand (Stephen Island), unfortunately it has no molecular data so far. Just like *Beauveria grylli* (≡ *C. grylli*, = *B. loeiensis*), it also grew on katydid nymphs (Anostostomatidae: *Deinacrida rugosa*), and its conidial phase accompanied its sexual fruiting bodies as well. According to the original description and photographs, *C. kirkii* is closely similar to *B. grylli* (Teng, 1936; Huang, 1998; Ariyawansa et al., 2015; Luo & Zhang, 2017; Figures 6.2, 6.3), but the former has narrower, denser and apically obtuse perithecia, also their hosts are different. Though both are katydids, biological habits of Anostostomatidae and Gryllacrididae are distinctly different. Considering the similarity of the two species and together with the phylogeny of Orthoptera cordyceps (Figure 6.1), *C. kirkii* is undoubtedly a *Beauveria* species. Herein we transfer *C. kirkii* to the genus *Beauveria* and combine it to *B. kirkii.*

6.4.8 *Beauveria locustiphila* (Henn.) B. Shrestha, Kepler & Spatafora

≡ *Cordyceps locustiphila* Henn.

Hosts. Locust nymphs (Romaleidae: *Colpolopha*) (Sanjuan et al., 2014).

Known distribution. The Amazon: Brazil, Colombia, Ecuador, Peru (Petch, 1934; Kobayasi, 1941; Sanjuan et al., 2014)

6.4.9 *Cordyceps albida* Berk. & M.A. Curtis ex Cooke

Hosts. Crickets (adults?) (Cooke, 1884).

Known distribution. Cuba.

Note. Undetermined species which came from the old genus *Cordyceps*.

6.4.10 *Cordyceps ctenocephala* P. Syd.

Host. Locust.

Known distribution. New Guinea (Petch, 1934).

Note. Undetermined species which came from the old genus *Cordyceps*. According to the original description and figure, Petch (1934) treated it as a synonym of *Cordyceps uleana*.

6.4.11 *Cordyceps lilacina* Moureau

Host. Locust (Moureau, 1949).

Known distribution. Congo.

Note. Undetermined species which came from the old genus *Cordyceps*.

6.4.12 *Cordyceps neogryllotalpae* Kobayasi

Host. Mole crickets (Gryllotalpidae: *Gryllotalpa*) (Kobayasi & Shimizu, 1976; Shimizu, 1997).

Known distribution. New Guinea.

Note. Undetermined species which came from the old genus *Cordyceps*.

6.4.13 *Cordyceps parvula* Mains

Host. An Orthoptera insect (Mains, 1959), katydid?

Known distribution. Venezuela (Guarico).

Note. Undetermined species which came from the old genus *Cordyceps*. According to the photograph from Mains (1959), the host of the species appears to be a katydid.

6.4.14 *Cordyceps stiphrodes* P. Syd.

Host. Adult locusts (Petch, 1934; Kobayasi, 1941).

Known distribution. New Guinea.

Note. Undetermined species which came from the old genus *Cordyceps*. According to the original description and figure, Petch (1934) treated it as a synonym of *Beauveria locustiphila* (≡ *C. locustiphila*).

6.4.15 *Cordyceps trinidadensis* Mains

Host. Cricket (Mains, 1959).

Known distribution. Venezuela (Trinidad).

Note. Undetermined species which came from the old genus *Cordyceps*.

6.4.16 *Cordyceps uleana* Henn.

Host. Locusts (Orthoptera), Diapheromeridae (Phasmida), oothecae (Mantodea) (Hennings, 1904; Petch, 1933a; Moureau, 1949; Sanjuan et al., 2014).

Known distribution. Peru (Hennings, 1904), Madagascar (Petch, 1933a), Congo (Moureau, 1949).

Note. Undetermined species which came from the old genus *Cordyceps*.

6.4.17 *Metarhizium acridum* (Driver & Milner) J.F. Bisch., S.A. Rehner & Humber

≡ *Metarhizium anisopliae* var. acridum Driver & Milner

Host. Locust adults (Bischoff et al., 2009; Hu et al., 2014).

Distribution. Widely distributed.

Note. *Metarhizium acridum* has been most widely used for controlling locusts.

6.4.18 *Metarhizium anisopliae* (Metschn.) Sorokīn

≡ *Entomophthora anisopliae* Metschn.

≡ *Isaria anisopliae* (Metschn.) R.H. Pettit

≡ *Penicillium anisopliae* (Metschn.) Vuill.

= *Isaria destructor* Metschn.

= *Oospora destructor* (Metschn.) Delacr.

= *Isaria anisoplia*e var. *americana* R.H. Pettit

= *Penicillium cicadinum* Höhn.

= *Metarhizium cicadinum* (Höhn.) Petch

= *Sporotrichum paranaense* Marchion.

Host. More than seven insect orders including Orthoptera; inhabit soil as saprobes, rhizosphere inhabitants and endophytes (Hu et al., 2014; Brunner-Mendoza et al., 2019).

Distribution. Widely distributed (Bischoff et al., 2009).

6.4.19 ***Metarhizium robertsii*** J.F. Bisch., S.A. Rehner & Humber

Host. More than seven insect orders including Orthoptera; inhabit soil as saprobes, rhizosphere inhabitants and endophytes (Hu et al., 2014; Bamisile et al., 2018; Brunner-Mendoza et al., 2019).

Distribution. Widely distributed (Bischoff et al., 2009).

6.4.20 *Ophiocordyceps acridiorum* (H.C. Evans & P.A. Shah) B. Shrestha, G.H. Sung & Spatafora [as 'acridiora']

≡ *Syngliocladium acridiorum* H.C. Evans & P.A. Shah

= *Ophiocordyceps acridiorum* var. *madagascariensis* (H.C. Evans & P.A. Shah) B. Shrestha, G.H. Sung & Spatafora

= *Syngliocladium acridiorum* var. *madagascariense* H.C. Evans & P.A. Shah [as 'madagascariensis']

Host. Locust nymphs and adults (Acrididae) (Evans and Shah, 2002).

Known distribution. Africa: Benin, Chad, Madagascar, Mali, Niger.

6.4.21 *Ophiocordyceps amazonica* (Henn.) G.H. Sung et al.

≡ *Cordyceps amazonica* Henn.

= *Cordyceps amazonica* var. *neoamazonica* Kobayasi & Hara

= *Ophiocordyceps amazonic*a var. *Neoamazonica* (Kobayasi & Hara) G.H. Sung et al.

Host. Nymphs and adults of locusts (Acrididae and Romaleidae) (Kobayasi, 1941; Sanjuan et al., 2015).

Known distribution. Argentina, Bolivia, Brazil, Colombia, Ecuador, Guyana, Peru (Sanjuan et al., 2015).

6.4.22 *Ophiocordyceps gryllotalpae* (Kobayasi) Petch

≡ *Cordyceps gryllotalpae* (non Ellis & Seaver) Kobayasi

≡ *Cordyceps koreana* Kobayasi

=? *Cordyceps gryllotalpae* Lloyd

Host. Nymphs and adults of mole crickets (Gryllotalpidae: *Gryllotalpa*).

Known distribution. China (Guangdong, Taiwan), Japan, New Guinea, North America, North Korea, Russia (Kobayasi, 1941; Kobayasi & Shimizu, 1976, 1981; Shimizu, 1997; Liang 2007)

Notes. Lloyd (1920) introduded *Cordyceps gryllotalpae* Lloyd for a specimen on a mole cricket from North America, it consists of many immature dark gray stromata. Kobayasi (1941) treated a mature cordyceps on a mole cricket (*Gryllotalpa*) in Japan to be the same, and redescribed it as *C. gryllotalpae* Ellis & Seaver. Because of the uncertainty whether the two specimens represent the same species, Kobayasi (1981) give a new name *C. koreana* Kobayasi to replace the latter. Petch (1942) confirmed the Japanese specimen to be *Ophiocordyceps* and combined *C. gryllotalpae* Ellis & Seaver to *O. gryllotalpae* (Kobayasi) Petch. For those immature specimens on *Gryllotalpa* from Korea and Russia, Kobayasi (1981) still identified them as *C. gryllotalpae* Lloyd. Considering these taxonomic confusions, we temporarily treat *C. gryllotalpae* Lloyd as a synonym of *O. gryllotalpae*.

6.4.23 *Ophiocordyceps monticola* (Mains) G.H. Sung et al.

≡ *Cordyceps monticola* Mains

Host. Mole cricket (Gryllotalpidae: *Neocurtilla hexadactyla*) (Mains, 1940).

Known distribution. U.S.A. (Tennessee).

6.4.24 *Ophiocordyceps tettigonia* T.C. Wen, Y.P. Xiao & K.D. Hyde

Host. Cricket adult (Gryllidae) (newly corrected!).

Known distribution. China (Guizhou) (Wen et al., 2016a).

Note. Host of *Ophiocordyceps tettigonia,* that had originally been identified as a katydid adult (Tettigoniidae: *Tettigonia*; Wen et al., 2016a), is herein corrected as a cricket adult (Gryllidae). Notably, the epithet '*tettigonia*' came from the genus *Tettigonia* of katydids insetad of crickets.

6.4.25 *Syngliocladium* sp.

Host. Mole crickets (Gryllotalpidae: *Neoscapteriscus vicinus*).

Known distribution. USA (Florida) (Pendland & Boudas, 1987).

Note. An *Ophiocordyceps* sp..

6.5 Discussion

6.5.1 Ecological Habits of Orthoptera Insects vs. Species Diversity of Orthoptera Cordyceps

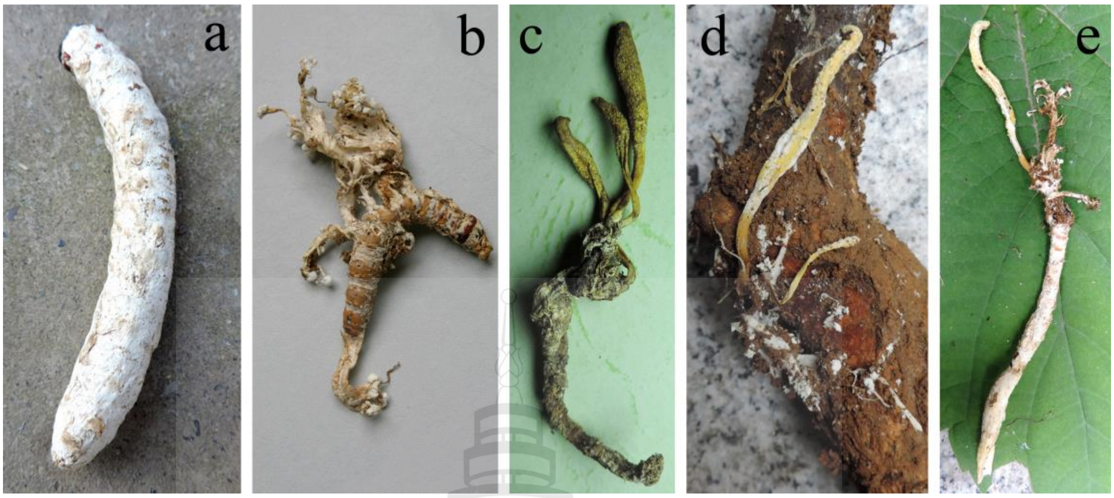
Orthoptera insects are all paurometabola, meaning their nymphs and adults have similar morphologies. Therefore, during identifying the hosts of entomopathogenic fungi, Orthoptera nymphs are easily be mistaken for adults. Ecological habits of different Orthoptera groups are distinctly different, which determine their levels of being attack by cordyceps. Locusts, pygmy mole crickets and katydids generally live on green plants, and crickets are usually found in dry caves or on dry ground; living away from humid soil and ground, these four groups are not easily infected by cordyceps. Mole crickets dig caves in soil to live in and crawl up to the ground surfaces for foods, and are thus easily infected by cordyceps. These five groups are terrestrial and can not endure extreme humidity, and in extremely humid environment, their old, weak, ill and disabled individuals will easily be infected by cordyceps, especially those who have dropped onto the ground (Wen et al., 2016b). Pygmy locusts are generally semiaquatic, they live in humid environment, also they mainly feed on humus instead of green plants; having already adapted for humid environment, pygmy locusts are almost impossible to be parasitized by cordyceps (Zha et al., 2015).

Among the 25 Orthoptera cordycpes species, *Beauveria bassiana*, *B. caledonica*, *Metarhizium anisopliae* and *M. robertsii* are generalists, they can parasitize a broad range of insect orders and even inhabit soil and plant tissues. The rest species show typical host specificity, including: *B. acridophila*, *B. australis*, *B. locustiphila*, *Cordyceps ctenocephala*, *C. lilacina*, *C. stiphrodes*, *C. uleana*, *M. acridum*, *Ophiocordyceps acridiorum* and *O. amazonica* on locusts (10,070 species with a huge population); *B. gryllotalpidicola*, *C. neogryllotalpae*, *O. gryllotalpae*, *O. monticola* and *Syngliocladium* sp. on mole crickets (only 208 species); *B. grylli*, *B. kirkii* and *C. parvula* on katydids (8,951 species); and *C. albida*, *C. trinidadensis* and *O. tettigonia* on crickets (5,662 species). No cordyceps has been found on pygmy mole crickets (only 245 species) and pygmy locusts (2,043 species), maybe the former are too small and too few, while the latter have already adapted for humid environment. These statistical results indicate that ecological habits play important roles for insects to be parasitized by cordyceps.

6.5.2 Funiculi of Cordyceps Fungi

Funiculi of cordyceps are a special structure between sexual fruiting bodies and asexual synnemata. Previously, we reported the funiculi of *Metacordyceps taii* (Z.Q. Liang & A.Y. Liu) G.H. Sung et al. (syn. of *Metarhizium guizhouense* Q.T. Chen & H.L. Guo) from Guizhou. In rainy and low-temperature (10-20 °C) October in tea gardens in Guizhou, a large number of Hepialidae larvae had been infected by *M. taii*; and with the humidity decreasing after the rain, the ossified insects of *M. taii* could only produce funiculi instead of fruiting bodies (Zha et al., 2018). Under suitable conditions, funiculi of cordyceps can produce synnemata of conidial phase on their terminal ends, which was observed on *Beauveria grylli* (Figure 6.2 c) and *B. bassiana* (Figure 6.4 b).

The essence of life is to reproduce, and cordyceps is no exception. Under the great pressure of natural selection, cordyceps fungi had evolved many different reproductive modes. Sometimes, one or several modes can simultaneously occur on the same host (Figures 6.2, 6.4). These complex and variable reproductive modes are more favorable in intraspecies and interspecific competitions, and ensure the cordyceps fungi to continue their lives on any occasions.



Note **a** mycelia; **b** funiculi co-occurred with mycelia; **c** fruiting bodies co-occurred with mycelia; **d**, **e** fruiting bodies co-occurred with funiculi and mycelia. Specimen ID: **a** GACP 15101201; **b** GACP 15101206; **c** GACP 16041002; **d**, **e** GACP 14071004.

Figure 6.4 A wide range of reproductive modes of *Beauveria bassiana*

CHAPTER 7

CORDYCEPS FUNGI ON WIREWORMS (ELATEROIDEA AND TENEBRIONOIDEA, COLEOPTERA)

7.1 Introduction

The superfamilies Elateroidea and Tenebrionoidea are two large groups of Coleoptera. They are phytophagous, xylophagous, saprophagous or omnivorous, and most of them are important agricultural pests (Gullan & Cranston, 2010). Elateroidea larvae are the well-known wireworms, which closely resemble Tenebrionoidea larvae that are known as mealworms or pseudo wireworms. As a result, in practice, the larvae of both Elateroidea or Tenebrionoidea are generally called wireworms. Most wireworms live in humid soil, humus layer or decayed wood, and they are thus exposed to and easily infected by entomopathogenic fungi (Kabaluk et al., 2017).

As the most important group of entomopathogenic fungi, cordyceps (*Cordyceps* sensu lato) has always been of interest for its species diversity, edible and medicinal values and applications in biological control. As a large group and with special morphology and biological habits, wireworms are distinctly different from other groups of Coleoptera, which determines the species diversity of cordyceps on them, and many cordyceps species on wireworms have been reported and described. Shimizu (1997) recorded and illustrated species diversity of cordyceps from Japan, which included many species on wireworms and wireworm-like insects. A recent statistical report for cordyceps on wireworms involved only 20 species (Shrestha et al., 2016), which is fewer than the number reported from Japan. Overall, cordyceps on wireworms has not been systematically studied. In this study, species diversity of cordyceps on wireworms (Elateroidea and Tenebrionoidea) are systematically reviewed. We update their hosts, summarize their distribution and provide taxonomic notes. In addition, we describe two new memberof this group, *Ophiocordyceps borealis* sp. nov. and *O. spicatus* sp. nov. *Polycephalomyces formosus* Kobayasi is described and illustrated and is reported on wireworms (Elateroidea) for the first time. *Cordyceps jiangxiensis* is transferred to the genus *Metarhizium*. We give multi-gene phylogeny for the two new species and to confirm the identification of *P. formosus*.

7.2 Material and Methods

7.2.1 Specimens

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 Canon EOS 600D camera. Measurements were made using Tarosoft (R) Image Frame Work software. Voucher specimens were deposited in the Fungarium of the Centre of Excellence in Fungal Research, Mae Fah Luang University (MFLU), Chiang Rai, Thailand, and the Herbarium of Guizhou University (GACP), Guiyang, China.

7.2.2 DNA Extraction, PCR, Sequencing, Alignment and Assembly

Total DNA was extracted from specimens dried over silica-gel using a CTAB procedure (Doyle, 1987). The ribosomal internal transcribed spacers (ITS), small and large subunits (SSU and LSU) and elongation factor 1α (TEF1-*α*) genes were sequenced using the primers detailed by White et al. (1990) and Ban et al. (2015b). PCR programs followed those of Ban et al. (2015b) and amplification reactions were performed in an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR products were purified using Bioteke's Purification Kit (Bioteke Corporation, Beijing, China), and were sequenced using an ABI 3730 DNA analyzer and an ABI BigDye 3.1 terminator cycle sequencing kit (Sangon Co., Shanghai, China). Sequences were aligned and assembled visually and manually using Clustalx1.81, Chromas230, ContigExpress and MEGA6 software.

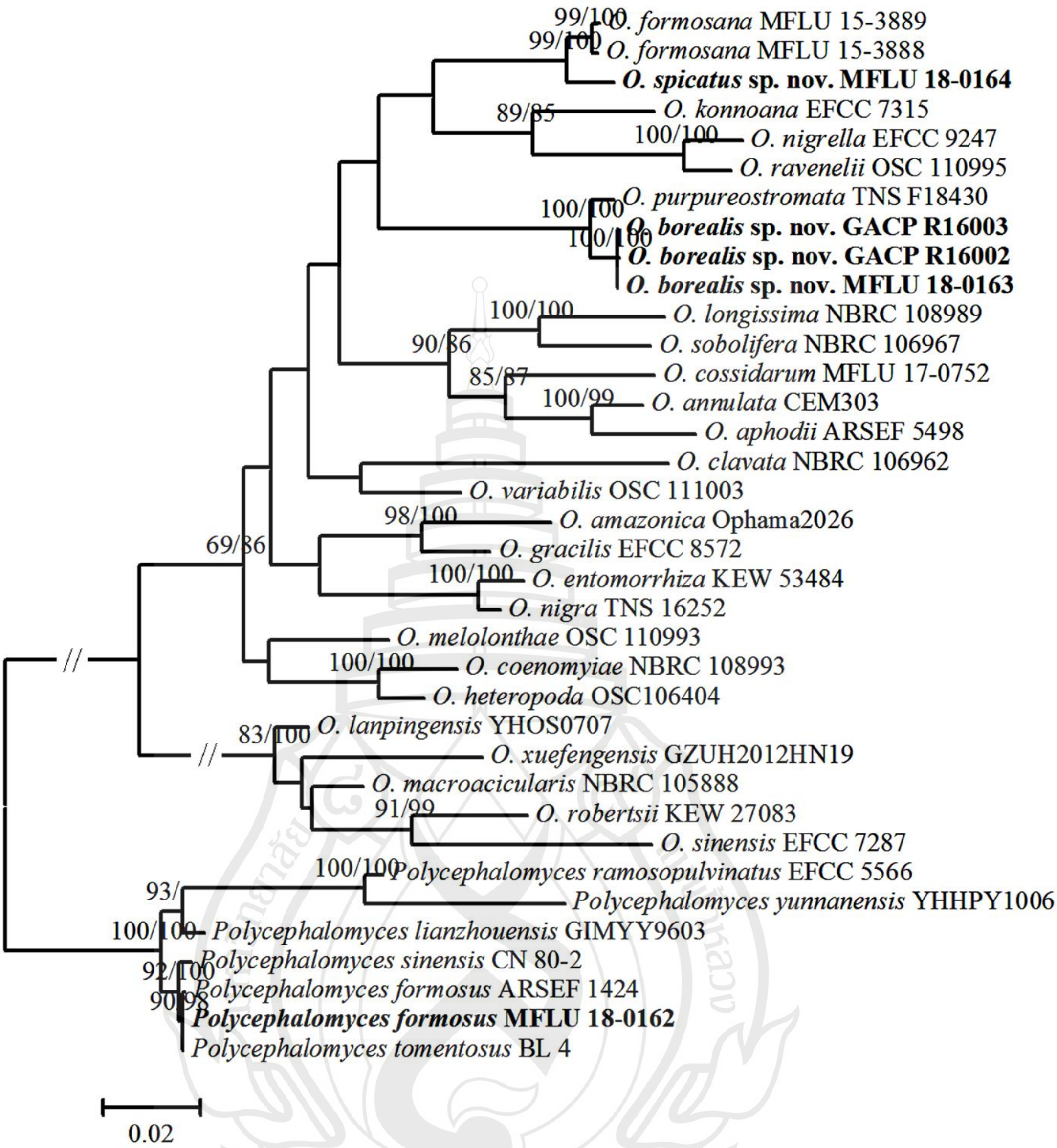
7.2.3 Construction of Phylogenetic Tree

A Blast search was performed to reveal the closest matches in the NCBI database that would allow the selection of appropriate taxa for phylogenetic analyses. Information of voucher sequences obtained from GenBank is listed in Table 7.1. Molecular phylogeny was reconstructed using combined SSU, ITS, LSU and TEF1-*α* sequence dataset. Maximum likelihood (ML) analysis was performed using RAxML v. 8.2.8 employing a GTRGAMMA model of nucleotide substitution, other details are described in Jeewon et al. (2003) and Hongsanan et al. (2017). Maximum parsimony (MP) analysis was performed using PAUP* 4.0b10 (Swofford, 2002) and the heuristic search option with TBR branch swapping and a bootstrap test of 1,000 replicates, other details were outlined by Cai et al. (2006) and Tang et al. (2007).

7.3 Molecular Phylogeny

The combined SSU, ITS, LSU and TEF1-*α* gene dataset consisted of 36 samples from 32 Ophiocordycipitaceae species (*Ophiocordyceps* and *Polycephalomyces*). The dataset comprised 3,535 characters (including gaps), of which 1,271 were variable and 829 were parsimony-informative. ML and MP analyses of the combined dataset resulted in phylogenetic reconstructions with closely similar topologies, and the ML tree is shown in Figure 7.1.

According to the phylogenetic tree, *Ophiocordyceps borealis* sp. nov. (MFLU 18-0163, GACP R16002 and GACP R1600) are grouped together (100% ML and 100% MP) and are related to, but separate from *O. purpureostromata*. *O. spicatus* sp. nov. (MFLU 18-0164) is related to, but separate from *O. formosana*. The two *Polycephalomyces formosus* samples (MFLU 18-0162, ARSEF 1424) grouped together and cannot be separated (Figure 7.1).



Note Bootstrap support values greater than 60% for ML and maximum parsimony (MP) are indicated above the nodes and separated by '/' (ML/MP).

Figure 7.1 Maximum likelihood (ML) tree of *Ophiocordyceps borealis* sp. nov., *O. spicatus* sp. nov. and their allies inferred from combined SSU, ITS, LSU and TEF1-*α* dataset

7.4 Annotated List of Cordyceps Species on Wireworms (Elateroidea and Tenebrionoidea)

The Order Hypocreales Lindau

I. The Family Cordycipitaceae Kreisel ex G.H. Sung et al.

7.4.1 *Akanthomyces lecanii* (Zimm.) Spatafora et al.

≡ *Cephalosporium lecanii* Zimm.

≡ *Verticillium lecanii* (Zimm.) Viégas

≡ *Lecanicillium lecanii* (Zimm.) Zare & W. Gams

= *Hirsutella confragosa* Mains

= *Torrubiella confragosa* Mains

= *Cordyceps confragosa* (Mains) G.H. Sung et al.

Hosts. Spiders, insects from various orders (eg. Tenebrionidae: *Alphitobius diaperinus*), phytopathogenic fungi and plant-parasitic nematodes (Humber & Hansen, 2005; Shinya et al., 2008).

Known distribution. Widely distributed (Shinya et al., 2008).

7.4.2 *Beauveria bassiana* sensu lato

Hosts. Many insect orders including Coleoptera (eg. Elateroidea and Tenebrionoidea spp.; Humber & Hansen, 2005; Reddy et al., 2014; Sufyan et al., 2017); inhabit soil, plant surfaces and plant internal tissues (Bamisile et al., 2018).

Distribution. Widely distributed.

Note. *Beauveria bassiana* sensu lato includes a large complex of cryptic species with wide host ranges including many families of Coleoptera (Rehner et al., 2011; Imoulan et al., 2017).

7.4.3 *Cordyceps aurantiaca* Lohwag

Hosts. Elateroidea or Tenebrionoidea larvae (Keissler & Lohwag, 1937).

Known distribution. China (Keissler & Lohwag, 1937).

Note. Undetermined species which was described from the old genus *Cordyceps*.

7.4.4 *Cordyceps chishuiensis* Z.Q. Liang & A.Y. Liu

Host. Elateroidea larva (Liang, 2007).

Known distribution. China (Guizhou) (Liang, 2007).

Note. Undetermined species from the old genus *Cordyceps*. According to the original description (Liang, 2007), *Cordyceps chishuiensis* (sexual morph) might be a *Metarhizium* species.

7.4.5 *Cordyceps farinosa* (Holmsk.) Kepler et al.

≡ *Ramaria farinosa* Holmsk.

≡ *Clavaria farinosa* (Holmsk.) Dicks.

≡ *Corynoides farinosa* (Holmsk.) Gray

≡ *Isaria farinosa* (Holmsk.) Fr.

≡ *Spicaria farinosa* (Holmsk.) Vuill.

≡ *Penicillium farinosum* (Holmsk.) Biourge

≡ *Paecilomyces farinosus* (Holmsk.) A.H.S. Br. & G. Sm.

Hosts. Insects from various orders (eg. Tenebrionoidea spp.) (Humber & Hansen, 2005; Zimmermann, 2008).

Distribution. Widely distributed.

7.4.6 *Cordyceps fumosorosea* (Wize) Kepler et al.

≡ *Isaria fumosorosea* Wize

≡ *Spicaria fumosorosea* (Wize) Vassiljevsky

≡ *Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm.

Hosts. Insects from various orders (eg. Tenebrionoidea spp.) (Humber & Hansen, 2005; Zimmermann, 2008).

Distribution. Widely distributed.

7.4.7 *Cordyceps huntii* Giard [as 'hunti', 'lunti']

Host. Elateroidea larva (Massee, 1899).

Known distribution. Gaul (Massee, 1899).

Note. Undetermined species from the old genus *Cordyceps*. Sung et al. (2007) treated it as a synonym of *Nigelia martiale* (≡ *Cordyceps martialis*).

7.4.8 *Cordyceps militaris* (L.) Fr.

≡ *Clavaria militaris* L.
= *Clavaria granulosa* Bull.
≡ *Sphaeria militaris* (L.) J.F. Gmel.
= *Sphaeria militaris* var. *sphaerocephala* J.C. Schmidt
≡ *Hypoxylon militare* (L.) Mérat
≡ *Xylaria militaris* (L.) Gray
≡ *Corynesphaera militaris* (L.) Dumort.
≡ *Torrubia militaris* (L.) Tul. & C. Tul.
= *Cordyceps militaris* f. *sphaerocephala* (J.C. Schmidt) Sacc.
= *Cordyceps militaris* f. *alba* Kobayasi & Shimizu ex Y.J. Yao [as 'albina']

Hosts. Commonly on Lepidoptera larvae and pupae, infrequently on Hymenoptera (Kryukov et al., 2011; Shrestha et al., 2012); species of Coleoptera (Tenebrionidae: *Tenebrio molitor*) that was noted by De Bary (1867) and cited by Shrestha et al. (2012) and others.

Distribution. Widely distributed.

Notes. Under laboratory conditions and injection of hyphal bodies into the hemocoel of insects, the species can infect pupae of *Tenebrio molitor* (Sato and Shimazu, 2002). So the conclusion that wireworms (eg. *Tenebrio molitor*) are the hosts of *Cordyceps militaris*, is probably untenable.

7.4.9 *Cordyceps nanatakiensis* Kobayasi & Shimizu

Host. Tenebrionoidea larva (Kobayasi & Shimizu, 1983; Shimizu 1997).

Known distribution. Japan (Kobayasi & Shimizu, 1983).

Note. Undetermined species from the old genus *Cordyceps*.

7.4.10 *Cordyceps nirtolii* Negi et al.

Host. Larva of Elateridae (*Melanotus*) (Negi et al., 2012).

Known distribution. India (Himalaya) (Negi et al., 2012).

Note. *Melanotus communis*, that was reported as the host of *Cordyceps nirtolii* (Negi et al., 2012), is in fact a mushroom (Agaricales: Strophariaceae). *Melanotus* is also a genus in Elateridae.

7.4.11 *Cordyceps roseostromata* Kobayasi & Shimizu

Host. Tenebrionoidea larva (Kobayasi & Shimizu, 1983; Shimizu, 1997).

Known distribution. Japan (Kobayasi & Shimizu, 1983).

7.4.12 *Cordyceps rubiginosistipitata* Kobayasi & Shimizu [as 'rubiginosostipitata']

Host. Tenebrionoidea or Elateroidea larva.

Known distribution. Japan (Kobayasi & Shimizu, 1983).

Note. Undetermined species from the old genus *Cordyceps*. According to the illustration by Shimizu (1997), the host is a Tenebrionoidea or Elateroidea larva.

7.4.13 *Cordyceps rubra* Möller

Host. Elateroidea larva (Möller, 1901).

Known distribution. Brazil (Möller, 1901).

Note. Undetermined species from the old genus *Cordyceps*.

7.4.14 *Cordyceps shanxiensis* B. Liu, Rong & H.S. Jin

Hosts. Elateridae larvae (Liu et al., 1985).

Known distribution. China (Shanxi) (Liu et al., 1985).

Note. Undetermined species from the old genus *Cordyceps*. According to the original description, *Cordyceps shanxiensis* is morphologically similar to *Ophiocordyceps gracilis* (Grev.) G.H. Sung that grows on Lepidoptera larvae. Notably, the host names, *Melanotus caudex* and *Pleonomus canaliculatus* (Liu et al., 1985), are both unaccepted.

7.4.15 *Cordyceps submilitaris* Henn.

Hosts. Elateroidea or Tenebrionoidea larvae (Petch, 1933b).

Known distribution. South America (Petch, 1933b).

Note. Undetermined species from the old genus *Cordyceps*. Petch (1933b) considered the species as a synonym of *Nigelia martiale* (≡ *Cordyceps martialis*).

7.4.16 *Cordyceps velutipes* Massee

Hosts. Larvae of Elateroidea and Scarabaeidae (*Melolontha* sp.) (Massee, 1895; Moureau, 1949).

Known distribution. Africa (Massee, 1895).

Note. Undetermined species from the old genus *Cordyceps*.

II. The family Clavicipitaceae O.E. Erikss.

7.4.17 *Metarhizium anisopliae* sensu lato

Host. More than seven insect orders including Coleoptera (eg. Elateroidea and Tenebrionoide spp.; Kabaluk et al., 2005; Humber & Hansen, 2005); inhabit soil as saprobes, rhizosphere inhabitants and endophytes (Hu et al., 2014; Brunner-Mendoza et al., 2019).

Distribution. Widely distributed.

Note. *Metarhizium anisopliae* sensu lato, as well as *Beauveria bassiana* sensu lato, includes a complex of cryptic species with wide host ranges (Bischoff et al., 2009; Kepler et al., 2014).

7.4.18 *Metarhizium atrovirens* (Kobayasi & Shimizu) Kepler et al.

≡ *Cordyceps atrovirens* Kobayasi & Shimizu

≡ *Metacordyceps atrovirens* (Kobayasi & Shimizu) Kepler et al.

Hosts. Tenebrionoidea larvae (Shimizu, 1997).

Known distribution. Japan (Kobayasi & Shimizu, 1978; Shimizu, 1997).

7.4.19 *Metarhizium brunneum* Petch

Hosts. More than seven insect orders (eg. Elateroidea spp.; Reddy et al., 2014; Kabaluk et al., 2017); inhabit soil as saprobes, rhizosphere inhabitants and endophytes (Hu et al., 2014; Bamisile et al., 2018; Brunner-Mendoza et al., 2019).

Distribution. Widely distributed.

7.4.20 *Metarhizium jiangxiensis* (Z.Q. Liang, A.Y. Liu & Yong C. Jiang) L.S. Zha comb. nov.

MycoBank:

≡ *Ophiocordyceps jiangxiensis* (Z.Q. Liang, A.Y. Liu & Yong C. Jiang) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Stud. Mycol. 57: 43 (2007), MycoBank MB504285

≡ *Cordyceps jiangxiensis* Z.Q. Liang, A.Y. Liu & Yong C. Jiang, Mycosystema 20(3): 306 (2001), MycoBank MB484871

Hosts. Elateridae larvae (*Campsosternus* spp.) (Liang et al., 2001; Zha et al., 2018).

Known distribution. China (Jiangxi, Fujian, Yunnan) (Zha et al., 2018).

Notes. This is a large and beautiful cordyceps species, its yellow and broom-like stromata commonly accompanied by dense and dark green conidiogenous structure, and its hosts are large Elateridae larvae (Liang et al., 2001; Zha et al., 2018). Due to its typical *Metarhizium*-type conidiogenous structure, herein we combine the species to *Metarhizium*.

7.4.21 *Metarhizium robertsii* J.F. Bisch., S.A. Rehner & Humber

Host. More than seven insect orders (eg. Elateroidea; Reddy et al., 2014); inhabit soil as saprobes, rhizosphere inhabitants and endophytes (Hu et al., 2014; Bamisile et al., 2018; Brunner-Mendoza et al., 2019).

Distribution. Widely distributed.

7.4.22 *Metarhizium pseudoatrovirens* (Kobayasi & Shimizu) Kepler et al.

≡ *Cordyceps pseudoatrovirens* Kobayasi & Shimizu

≡ *Metacordyceps pseudoatrovirens* (Kobayasi & Shimizu) Kepler et al.

Hosts. Larvae of Tenebrionoidea (Shimizu, 1997) and Elateroidea (Liang, 2007).

Known distribution. China (Guizhou); Japan (Kobayasi & Shimizu, 1982b; Liang, 2007).

7.4.23 *Metarhizium campsosterni* (W.M. Zhang & T.H. Li) Kepler et al.

≡ *Cordyceps campsosterni* W.M. Zhang & T.H. Li [as 'campsosterna']

≡ *Metacordyceps campsosterni* (W.M. Zhang & T.H. Li) G.H. Sung et al.

Hosts. Larva and adult of *Campsosternus auratus* (Elateridae) (Zhang et al., 2004).

Known distribution. China (Guangdong) (Zhang et al., 2004).

7.4.24 *Nigelia martiale* (Speg.) Luangsa-ard & Thanakitp.

≡ *Cordyceps martialis* Speg.

≡ *Metacordyceps martialis* (Speg.) Kepler et al.

≡ *Metarhizium martiale* (Speg.) Kepler et al.

Hosts. Larvae of Elateroidea (eg. *Hemirhipus* sp.) and Lepidoptera (Kobayasi, 1941; Liang, 2007; Shrestha et al., 2016).

Known distribution. Brazil; China (Guangdong, Zhejiang, Taiwan); West Indies (Kobayasi, 1941; Liang, 2007; Shrestha et al., 2016).

III. The family Ophiocordycipitaceae G.H. Sung et al.

7.4.25 *Ophiocordyceps acicularis* (Ravenel) Petch

≡ *Cordyceps acicularis* Ravenel

Hosts. Larvae of Tenebrionoidea and Elateroidea (Massee, 1895; Kobayasi & Shimizu, 1980a; Shimizu, 1997; Liang, 2007).

Known distribution. China (Jiangsu, Guangdong, Guizhou, Hainan); Japan; Russia (Far East); U.S.A. (Carolina) (Massee, 1895; Kobayasi & Shimizu, 1980a; Koval, 1984; Liang, 2007).

7.4.26 *Ophiocordyceps agriotidis* (Kawam.) G.H. Sung et al.

≡ *Cordyceps agriotidis* Kawam. [as 'agriota']

Hosts. Elateroidea and Tenebrionoidea larvae.

Known distribution. China (Guizhou, Jilin); Japan (Kobayasi and Shimizu, 1980a; Yang, 2004; Liang, 2007).

Notes. Shimizu (1997) recorded the species on Elateroidea larvae. Yang (2004) and Liang (2007) recorded its hosts also as Elateroidea larvae, but their attached photographs appear to be Tenebrionoidea larvae.

7.4.27 *Ophiocordyceps annullata* (Kobayasi & Shimizu) Spatafora et al.

≡ *Cordyceps annullata* Kobayasi & Shimizu

Host. Tenebrionoidea or Elateroidea larva.

Known distribution. Japan (Kobayasi & Shimizu, 1982a).

Note. According to the illustration by Shimizu (1997), the host of the species is a Tenebrionoidea or Elateroidea larva.

7.4.28 *Ophiocordyceps appendiculata* (Kobayasi & Shimizu) G.H. Sung et al.

≡ *Cordyceps appendiculata* Kobayasi & Shimizu

Host. Tenebrionoidea larva (Shimizu, 1997).

Known distribution. Japan (Kobayasi & Shimizu, 1983).

7.4.29 *Ophiocordyceps asyuensis* (Kobayasi & Shimizu) G.H. Sung et al. [as 'asyuënsis']

≡ *Cordyceps asyuensis* Kobayasi & Shimizu

Hosts. Elateroidea larvae.

Known distribution. Japan (Kobayasi & Shimizu, 1980b).

Note. According to the illustration by Shimizu (1997), the hosts of the species appear to be Elateroidea larvae.

7.4.30 *Ophiocordyceps borealis* L.S. Zha & P. Chomnunti sp. nov. (Figure 7.2)

Index Fungorum number: IF 558114; Facesoffungi number: FOF 04101

Description. Parasitized Elateroidea larvae (Coleoptera) living in humid humus layer. The larvae are cylindrical, 11 mm long and 1.1-1.3 mm thick, yellowish brown; their body cavity stuffed with milky yellow mycelia, and their intersegmental membranes covered with many milky yellow and flocculent funiculi. Sexual morph: Stromata arising from any part of larval body, single or paired, unbranched. Stipe grey, slender and cylindrical, fibrous and flexible, curved more or less, 10-13 mm long and 0.25-0.6 mm thick, surface relatively smooth but with many longitudinal wrinkles, apex pointed. Fertile part irregularly attached on one side of the surface of distal part of stipe, which resembles a mass of insect eggs that are clustered together or separated into several lumps; substrate layer milky white, surface milky yellow accompanied with lavender and dotted with numerous black ostioles. Perithecia immersed, densely arranged, obliquely or at right angles to the surface of stipe, pyriform, neck unconspicuous, 220-290 × 120-150 µm and their tops obtuse; walls dark brown and 25-32 µm thick; ostioles slightly thickened and slightly protruding over the surface of fertile part. Asci cylindrical, 6-8 µm in diameter; caps hemispherical, 5-6 µm wide and 3.5-5 µm high. Ascospores filiform and elongate, multi-septate (far more than 3), not easy to break into part-spores; part-spores cylindrical, truncated at both ends, 10-15 µm × 2 µm. Asexual morph: Undetermined.

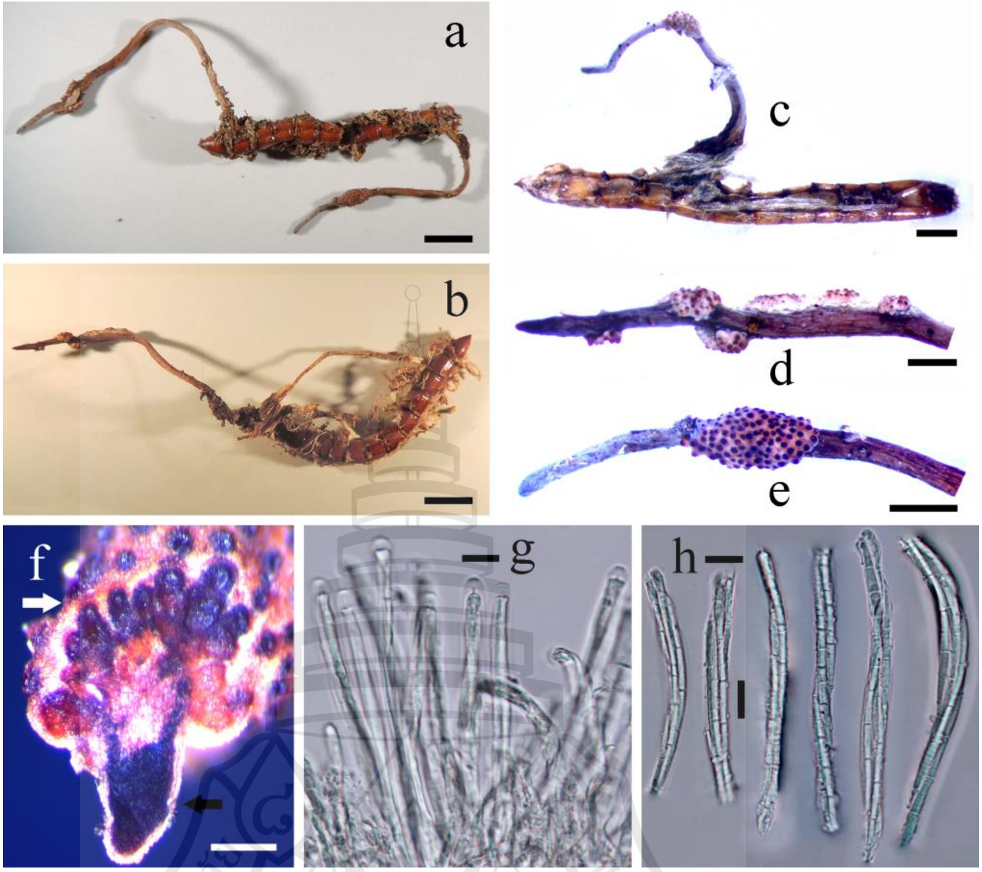
Material examined. RUSSIA. Russian Far East, Primorye, National Park Kedrovaya Pad, 10 August 2016, 43°6′00″N, 131°32′24″E, col. Olga N Yaroslavtseva and Vadim Kryukov, MFLU 18-0163, holotype; ibid, GACP R16002 and GACP R16003, paratypes.

Distribution. Russia (Primorye).

Host. Growing on Elateroidea larvae (Coleoptera) living in humid humus layer in a broad-leaved forest.

Etymology. The epithet *borealis* (northern) refers to the cold northern region where the species was collected.

Notes. The new species is similar to *Ophiocordyceps purpureostromata* (≡ *Cordyceps purpureostromata*), but ascospores of the two species are distinctly different. Those of *O. purpureostromata* are only 65-75 × 10 µm long and 3-septate and the part-spores are 13-23 µm long. The stipe of *O. borealis* is thin and hairless, while that of *O. purpureostromata* is thick (0.6-1 mm in diameter) and has hairs (Kobayasi, 1980b).



Note **a-c** stromata; **d**, **e** apical ends of stromata; **f** fertile part in oblique profile: perithecia in profile (white arrow) and end of stroma in oblique profile (black arrow); **g** asci; **h** ascospores. Scale bars: **a**, **b** = 2 mm, **c-e** = 1 mm, **f**, **g** = 0.2 mm, **h**, **i** = 10 µm.

Figure 7.2 *Ophiocordyceps borealis*

Nucleotide sequences of *O. borealis* are similar to those of *O. purpureostromata* (TNS F18430, Quandt et al, 2014), but there are 2.3% bp difference across 804 bp in TEF1-α, 0.5% bp difference across 845 bp in LSU, and 0.1% bp difference across 1,061 bp in SSU (ITS absent in the latter). ITS of *O. borealis* is >14.1% different to all ITS available in GeneBank. In the phylogenetic tree, the new species is related to, but separate from *O. purpureostromata* (Figure 7.1).

In Russia, this northern species has been routinely identified as *O. purpureostromata*, a species that may be only distributed in Southeast Asia.

7.4.31 *Ophiocordyceps brunneipunctata* (Hywel-Jones) G.H. Sung et al.

≡ *Cordyceps brunneipunctata* Hywel-Jones [as 'brunneapunctata']

Hosts. Elateroidea larvae (Hywel-Jones, 1995).

Known distribution. Thailand (Hywel-Jones, 1995).

7.4.32 *Ophiocordyceps clavata* (Kobayasi & Shimizu) G.H. Sung et al.

≡ *Cordyceps clavata* Kobayasi & Shimizu

Hosts. Tenebrionoidea larvae (Shimizu, 1997).

Known distribution. Japan (Kobayasi & Shimizu, 1980b).

7.4.33 *Ophiocordyceps elateridicola* (Kobayasi & Shimizu) G.H. Sung et al.

≡ *Cordyceps elateridicola* Kobayasi & Shimizu

Host. Elateroidea larva (Kobayasi & Shimizu, 1983).

Known distribution. Japan (Kobayasi & Shimizu, 1983).

7.4.34 *Ophiocordyceps entomorrhiza* (Dicks.) G.H. Sung et al.

≡ *Sphaeria entomorrhiza* Dicks.

= *Isaria eleutheratorum* Nees

≡ *Xylaria entomorrhiza* (Dicks.) Gray

≡ *Cordyceps entomorrhiza* (Dicks.) Fr.

= *Torrubia cinerea* Tul. & C. Tul.

= *Cordyceps cinerea* (Tul. & C. Tul.) Sacc.

= *Cordyceps meneristitis* F. Muell. & Berk. [as 'menesteridis']

= *Cordyceps entomorrhiza* var. *meneristitis* (F. Muell. & Berk.) Cooke [as 'mesenteridis']

= *Cordyceps carabi* Quél.

= *Tilachlidiopsis nigra* Yakush. & Kumaz.

= *Hirsutella eleutheratorum* (Nees) Petch

Hosts. Many Coleoptera larvae and adults, eg. Tenebrionoidea larva (Shrestha et al., 2016), Lampyridae larvae (Shimizu, 1997).

Distribution. Widely distributed.

Note. According to the illustrations by Shimizu (1997), we identify the hosts of the specimens from Japan as Lampyridae larvae (Elateroidea).

7.4.35 *Ophiocordyceps falcatoides* (Kobayasi & Shimizu) G.H. Sung et al.

≡ *Cordyceps falcatoides* Kobayasi & Shimizu

Host. Tenebrionoidea or Elateroidea larva.

Known distribution. Japan (Kobayasi & Shimizu, 1980a).

Note. According to the illustration by Shimizu (1997), host of the species is a Tenebrionoidea or Elateroidea larva.

7.4.36 *Ophiocordyceps ferruginosa* (Kobayasi & Shimizu) G.H. Sung et al.

≡ *Cordyceps ferruginosa* Kobayasi & Shimizu

Hosts. Diptera (Xylophagidae) larvae.

Known distribution. Japan (Kobayasi & Shimizu, 1980b).

Note. In the original description, the hosts were identified as Coleoptera larvae living in decayed wood (Kobayasi and Shimizu, 1980b; Shimizu, 1997), but according to the illustrations by Shimizu (1997), the hosts are actually Diptera (Xylophagidae) larvae. Considering the very similar morphology and the same hosts for *Ophiocordyceps ferruginosa* and *O. variabilis*, the former might be a synonym of the latter (see notes for *O. variabilis* below).

7.4.37 ***Ophiocordyceps formosana*** (Kobayasi & Shimizu) Yen W. Wang et al.

≡ *Cordyceps formosana* Kobayasi & Shimizu

Hosts. Tenebrionoidea larvae (Li et al., 2002, 2016).

Known distribution. China (Anhui, Fujian, Hunan, Taiwan); Japan (Kobayasi & Shimizu, 1981; Li et al., 2002, 2016).

7.4.38 *Ophiocordyceps gracilioides* (Kobayasi) G.H. Sung et al.

≡ *Cordyceps gracilioides* Kobayasi

Hosts. Larvae of Coleoptera (Cossidae, Elateroidea) and Lepidoptera (Kobayasi, 1941; Yahagi, 2008; Liang, 2007).

Known distribution. China (Fujian, Anhui), Japan, Russia (Far East) (Koval, 1984; Kobayasi, 1941; Liang, 2007).

7.4.39 ***Ophiocordyceps larvicola*** (Quél.) Van Vooren

≡ *Cordyceps larvicola* Quél.

Hosts. Larvae of Cerambycidae, Scarabaeidae and Tenebrionoidea (eg. *Cylindronotus* sp., *Helops* spp.) (Kobayasi, 1941; Shrestha et al., 2016).

Known distribution. France (Kobayasi, 1941).

7.4.40 ***Ophiocordyceps melolonthae*** **(Tul. & C. Tul.) G.H. Sung et al.**

≡ *Torrubia melolonthae* Tul. & C. Tul.

≡ *Cordyceps melolonthae* (Tul. & C. Tul.) Sacc.

= *Cordyceps rickii* Lloyd

= *Cordyceps melolonthae* var. *rickii* (Lloyd) Mains,

= *Ophiocordyceps melolonthae* var. *rickii* (Lloyd) G.H. Sung et al.

Hosts. Many Scarabaeidae larvae (Shrestha et al., 2016); Elateroidea larva (Shimizu, 1997).

Known distribution. Widely distributed (Mains, 1958).

7.4.41 ***Ophiocordyceps nigripoda*** **(Kobayasi & Shimizu) G.H. Sung et al. [as 'nigripes']**

≡ *Cordyceps nigripoda* Kobayasi & Shimizu

Host. Elateroidea larva.

Known distribution. Japan (Kobayasi & Shimizu, 1982b).

Note. According to the illustration by Shimizu (1997), the host of the species is an Elateroidea larva.

7.4.42 ***Ophiocordyceps purpureostromata*** **(Kobayasi) G.H. Sung et al.**

≡ *Cordyceps purpureostromata* Kobayasi

= *Cordyceps purpureostromata* f. *recurvata* Kobayasi

= *Ophiocordyceps purpureostromata* f. *recurvata* (Kobayasi) G.H. Sung et al.

Hosts. Elateroidea larvae (Shimizu, 1997).

Known distribution. Japan (Kobayasi & Shimizu, 1980b).

7.4.43 *Ophiocordyceps rubiginosiperitheciata* (Kobayasi & Shimizu) G.H. Sung et al.

≡ *Cordyceps rubiginosiperitheciata* Kobayasi & Shimizu [as 'rubiginosoperitheciata']

Host. Elateroidea or Tenebrionoidea larva.

Known distribution. Japan (Kobayasi & Shimizu, 1983).

Note. According to the illustration by Shimizu (1997), host of the species is an Elateroidea or Tenebrionoidea larva.

7.4.44 *Ophiocordyceps rubripunctata* (Moreau) G.H. Sung et al.

≡ *Cordyceps rubripunctata* Moreau

Hosts. Elateroidea larvae (Samson et al., 1982).

Known distribution. Congo; Ghana (Samson et al., 1982).

7.4.45 *Ophiocordyceps salebrosa* (Mains) G.H. Sung et al.

≡ *Cordyceps salebrosa* Mains

Host. Elateroidea larva (Mains, 1947).

Known distribution. Panama Canal Zone (Barro Colorado Island) (Mains, 1947).

7.4.46 *Ophiocordyceps spicatus* L.S. Zha & P. Chomnunti sp. nov. (Figure 7.3)

Index Fungorum number: IF 558115; Facesoffungi number: FOF 04102

Description. Parasitized a Tenebrionoidea larva (Coleoptera) living in humid and decayed wood. The larva is cylindrical, 7.5 mm long and 1.0-1.1 mm thick, yellowish brown. White mycelia stuff the body cavity, also partially cover the intersegmental membranes of body surface. Sexual morph: Stroma arising from the first quarter of the larval body, single, fleshy, 5 mm in length. Stipe yellow, cylindrical, 3.5 mm long and 0.35-0.4 mm thick, surface rough and pubescent. Fertile head spicate, unbranched, orange, 1.5 mm long and 0.5-0.7 mm thick, obviously differentiated from stipe; its surface rugged and consisted of many humps (outer portions of perithecia), tops of the humps obtuse and with opening ostioles, darker in color. Perithecia partially immersed and obliquely or at right angles to the surface of stipe, broadly pyriform, 200-250 × 170-200 μm; walls 25-35 μm thick. Asci cylindrical, 5-9 μm thick, middle part wider than two terminal parts; caps hemispheric, 4.6-5.3 μm wide and 4.0-4.6 μm high. Ascospores filiform; part-spores cylindric, truncated at both ends, 3.5-6.5 ($\overline{x}$ = 4.7) μm long and 1.7-2.0 μm thick. Asexual morph: Undetermined.

Material examined. CHINA. Guizhou Province, Leishan County, Leigongshan Mountains, 26°22′18″N, 108°11′28″E, 1430 m alt, 2 August 2016, col. Lingsheng Zha, MFLU 18-0164, holotype.

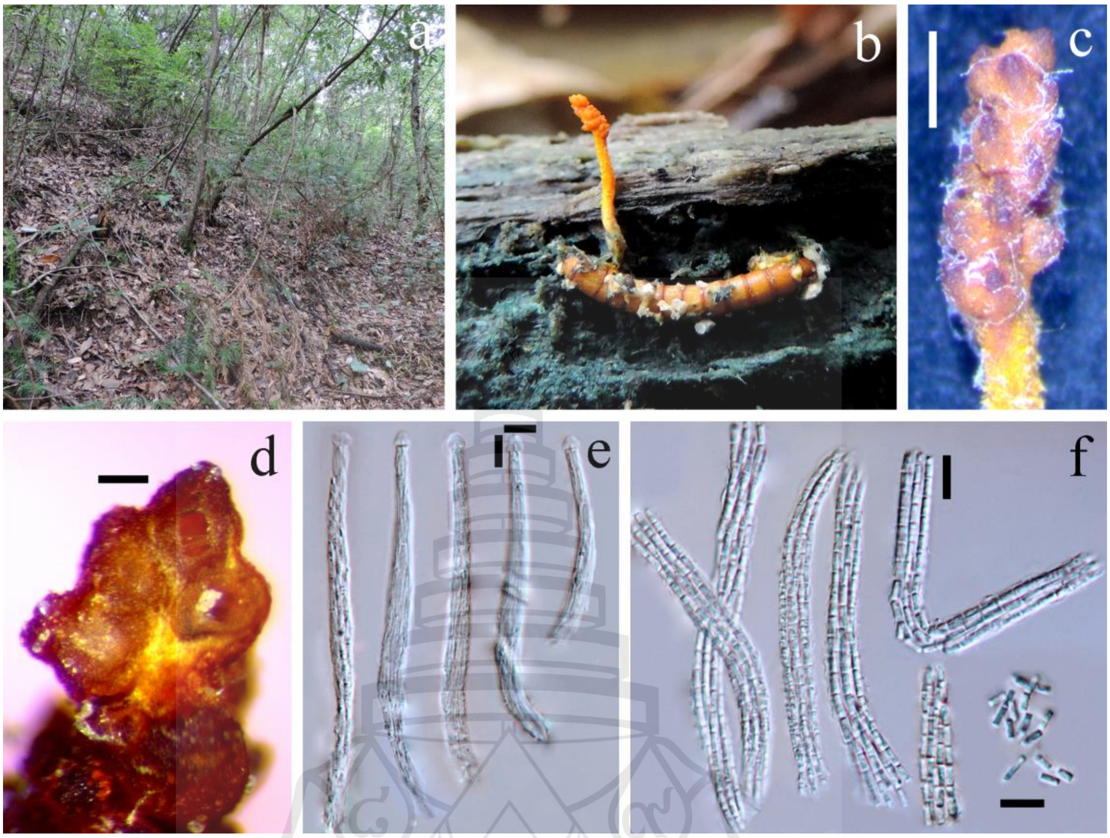
Distribution. China (Guizhou).

Host. Growing on a Tenebrionoidea larva (Coleoptera) living in humid and decayed wood in a broad-leaved forest.

Etymology. The epithet refers to the spicate fertile head.

Notes. *Ophiocordyceps spicatus* is morphologically similar to *O. formosana* (Kobayashi & Shimizu, 1981; Li et al., 2016), but it has a much smaller stroma, a spicate and rugged fertile head, and partially immersed perithecia.

Nucleotide sequences of *O. spicatus* are similar to those of *O. formosana*, but there are >5.2% bp difference in ITS, >2.0% bp difference in TEF1-*α* and >0.1% bp difference in SSU (LSU absent in *O. formosana*). LSU of *O. spicatus* is >5.6% bp different to all ITS available in GeneBank. Also in the phylogenetic tree, *O. spicatus* is related to, but separated from, *O. formosana* (Figure 7.1).



Note **a** habitat environment; **b** infected larva in decayed wood; **c** fertile head of stroma; **d** fertile head in profile; **e** asci; **f** ascospores and part-spores. Scale bars: **c** = 0.5 mm, **d** = 0.1 mm, **e**-**f** = 10 µm.

Figure 7.3 *Ophiocordyceps spicatus* (MFLU 18-0164)

7.4.47 *Ophiocordyceps sporangifera* Y.P. Xiao, T.C. Wen & K.D. Hyde

Host. Elateroidea or Tenebrionoidea larva.

Known distribution. Thailand (Xiao et al., 2019).

Note. The host was originally recorded as an Elateridae larva (Xiao et al., 2019).

7.4.48 *Ophiocordyceps stylophora* (Berk. & Broome) G.H. Sung et al.

≡ *Cordyceps stylophora* Berk. & Broome

Hosts. Larvae of Coleoptera (Cerambycidae, Elateroidea (eg. *Denticollis linearis*), Scarabaeidae) and Lepidoptera (Shrestha et al., 2016).

Known distribution. Canada (Nova Scotia); China (Guangxi, Jilin, Zhejiang); Japan; Russia (Far East); U.S.A. (Carolina) (Kobayasi, 1941; Mains, 1941; Koval, 1984; Liang, 2007).

7.4.49 *Ophiocordyceps subflavida* (Mains) G.H. Sung et al.

≡ *Cordyceps albida* Pat. & Gaillard

≡ *Cordyceps subflavida* Mains

Hosts. Elateroidea larvae (Shimizu, 1997).

Known distribution. Japan (Shimizu, 1997); Venezuela (Mains, 1959).

7.4.50 *Ophiocordyceps variabilis* (Petch) G.H. Sung et al.

≡ *Cordyceps variabilis* Petch

= *Cordyceps viperina* Mains

Hosts. Xylophagidae larvae (Diptera) (Hodge et al., 1998).

Known distribution. China (Shaanxi); Europe; North America (Petch, 1937; Liang, 2007; Hodge et al., 1998).

Notes. In early literature, *Ophiocordyceps variabilis* were recorded on Coleoptera (eg. Elateridae) and Diptera larvae in rotten wood (Petch, 1937; Mains, 1958; Liang, 2007). Hodge et al. (1998) checked many samples and confirmed the hosts to be Xylophagidae larvae (Diptera). More than 40 samples of the species were collected in Russian Far East and all of them were on Xylophagidae larvae (Kryukov et al., unpublished). Ecological habits and morphology of Xylophagidae and Elateridae larvae are closely similar, but their last abdominal segments are distinctly different.

7.4.51 *Perennicordyceps cuboidea* (Kobayasi & Shimizu) Matočec & I. Kušan

≡ *Cordyceps cuboidea* Kobayasi & Shimizu

≡ *Ophiocordyceps cuboidea* (Kobayasi & Shimizu) S. Ban, Sakane & Nakagiri

≡ *Polycephalomyces cuboideus* (Kobayasi & Shimizu) Kepler & Spatafora

= *Cordyceps alboperitheciata* Kobayasi & Shimizu

Hosts. Tenebrionoidea and Elateroidea larvae (Shimizu, 1997; Ban et al., 2009); stroma of *Ophiocordyceps stylophora* (Ban et al., 2009).

Known distribution. Japan (Kobayasi and Shimizu, 1980b).

Note. According to the illustrations by Shimizu (1997) and Ban et al. (2009), the hosts are Tenebrionoidea and Elateroidea larvae.

7.4.52 *Perennicordyceps ryogamiensis* (Kobayasi & Shimizu) Matočec & I. Kušan

≡ *Cordyceps ryogamiensis* Kobayasi & Shimizu

≡ *Ophiocordyceps ryogamiensis* (Kobayasi & Shimizu) G.H. Sung et al.

≡ *Polycephalomyces ryogamiensis* (Kobayasi & Shimizu) Kepler & Spatafora

Host. Tenebrionoidea larva.

Known distribution. Japan (Kobayasi & Shimizu, 1983).

Note. According to the illustration by Shimizu (1997), the host is a Tenebrionoidea larva.

7.4.53 *Polycephalomyces formosus* Kobayasi (Figure 7.4)

MycoBank: MB 289806; Facesoffungi number: FOF 04100

Notes. *Polycephalomyces formosus* was reported on Coleoptera larvae, stromata of *Ophiocordyceps barnesii* (Thwaites) G.H. Sung et al., *O. falcata* (Berk.) G.H. Sung et al. and *O. cantharelloides* (Samson & H.C. Evans) G.H. Sung et al., and distributed in Ecuador, Japan and Sri Lanka (Kobayasi, 1941; Samson & Evans, 1985; Wang, 2016).

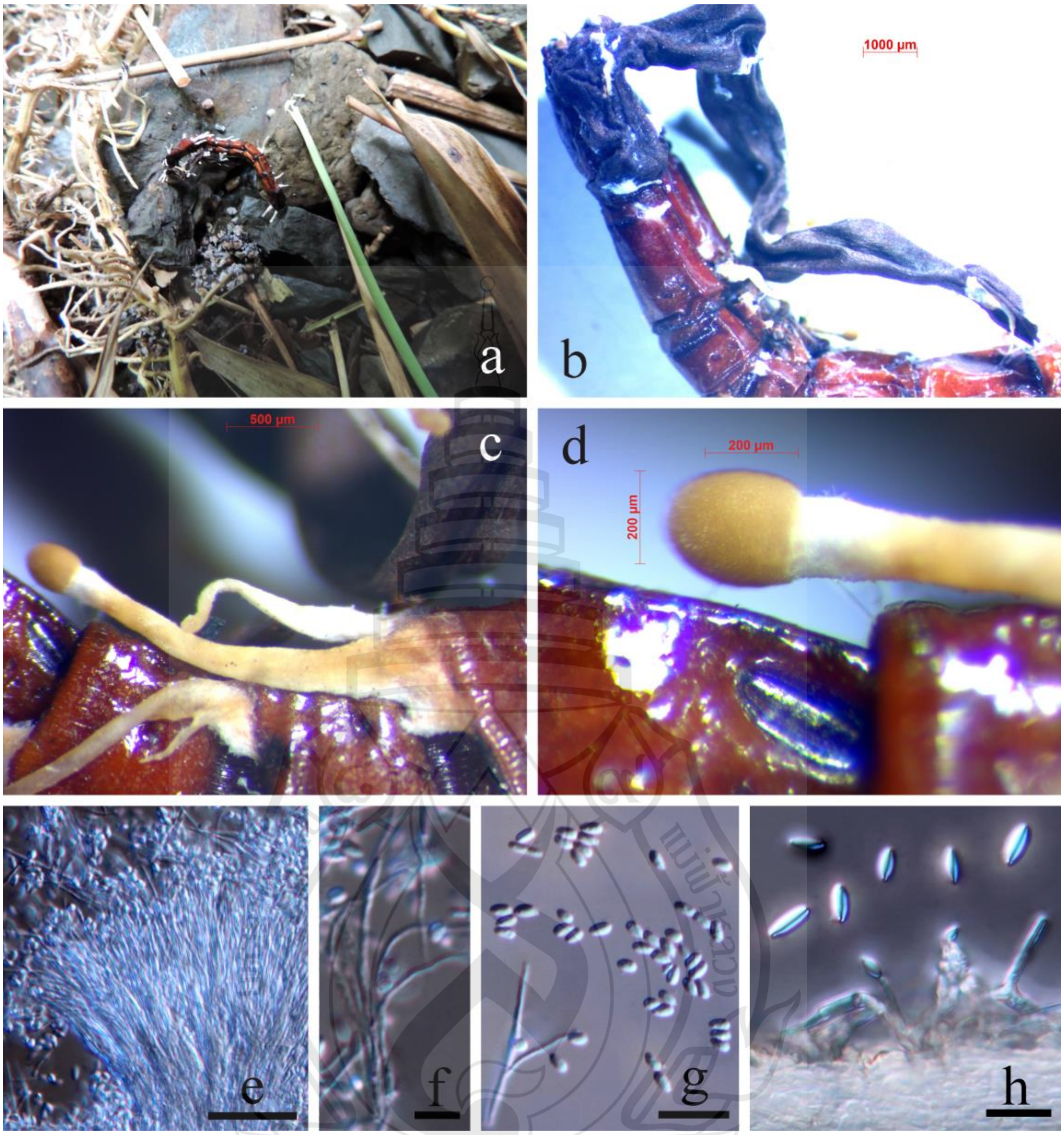
We collected a *P. formosus*-like specimen on the stroma of *Ophiocordyceps* sp. on an Elateroidea larva from Guizhou, China. Morphological and phylogenetic evidence proves it to be *P. formosus*. This is the first report that *P. formosus* can grow on wireworms.

Description. Growing on the stroma of *Ophiocordyceps* sp. on an Elateroidea larva. Stroma single, arising from the body end of the host larva, unbranched. The larva reddish brown, cylindrical, 21 × 1.3-1.6 mm, intersegmental membranes conspicuous. Stipe of the stroma shiny black, stiff, band-like but twisted and deeply wrinkled (dry specimen), more than 20 mm long and 1.0-1.3 mm thick, surface smooth (the fertile head was missing). Asexual morph: Synnemata solitary or caespitose, arising from the intersegmental membranes of the larva and the surface of the stroma, mostly unbranched, generally straight, capitate, 1-3.5 mm long and 50-600 µm thick. Stipe basally broad and compressed, then gradually cylindrical upward, white, greyish white to yellowish brown, surface smooth. Fertile head (including spore mass) abruptly expanded, ellipsoidal, 100-300 × 80-250 µm, located at the top of every synnema and distinctly separated from the stipe. Spore mass covers the surface of every fertile head, 15-25 µm thick, yellowish brown and composed by hymenia. Phialides of two types, A-phialides produced on fertile heads, B-phialides arising laterally along the entire stipe. A-phialides 3-5 in terminal whorl on basal conidiophores, cylindrical to narrowly conical, straight or curved, non-uniform, 10-20 × 1.5-2 µm, basally and terminally narrow, neck narrow to 0.5 µm, collarettes and periclinal thickening not visible; A-conidia obovate to obpyriform, smooth-walled, hyaline, 2.1-3.2 × 1.5-2.2 µm. B-phialides single or in terminal whorls of 2-3 on basal conidiophores, straight, symmetrical or asymmetrical, hyaline, generally cylindrical, 10-25 µm long, 2-3.5 µm thick at the base, 0.5-0.8 µm thick at the end, collarettes and periclinal thickening not visible; B-conidia fusiform, hyaline, smooth-walled, 3.2-6.0 × 1-1.8 µm. Sexual morph: Undetermined.

Material examined. CHINA. Guizhou, Tongzi County, Baiqing Natural Reserve, N28°52'31", E107°9'10", about 1300 m alt., 13 July 2016, col. Lingsheng Zha, MFLU 18-0162, holotype.

Diagnosis. Our specimen is morphologically consistent with *P. formosus* from Japan (Kobayasi 1941). Sequences of SSU, ITS, LSU and TEF1-α are all identical to those of *P. formosus* (ARSEF 1424), and they also grouped together and cannot be separated in the phylogenetic tree (Figure 7.1). For these reasons, we identify it as *P. formosus*.

Host and ecology. On the stroma of *Ophiocordyceps* sp. on an Elateroidea larva on the ground in a humid bamboo (*Chimonobambusa quadrangularis*) forest in Guizhou karst regions. The larva might live in soil or decayed wood at first, but was then infected by *Ophiocordyceps* sp. and produced a sexual stroma. Following heavy rains, the host together with the stroma of *Ophiocordyceps* sp. was washed away, and exposed on the ground, and at last was parasitized by *Polycephalomyces formosus*. The fertile head of the stroma might have been lost during the floods.



Note **a** collected on the ground in a bamboo forest; **b** produced on the stroma of *Ophiocordyceps* sp. (the fertile head was missing) on an Elateroidea larva; **c-d** synnemata; **e-g** A-type phialides and A-type conidia; **h** B-type phialides and B-type conidia. Scale bars: **e** = 20 µm, **f** = 5 µm, **g-h** = 10 µm.

Figure 7.4 *Polycephalomyces formosus* (MFLU 18-0162)

7.4.54 *Tolypocladium cylindrosporum* W. Gams

≡ *Beauveria cylindrospora* (W. Gams) Arx

Hosts. Coleoptera (eg. Elateroidea sp.), Diptera, Hymenoptera and Lepidoptera (Humber and Hansen, 2005); inhabit soil (Scorsetti et al., 2012).

Known distribution. Widely distributed.

7.4.55 *Tolypocladium inflatum* W. Gams

= *Cordyceps subsessilis* Petch

= *Cordyceps facis* Kobayasi & Shimizu [as 'Codyceps']

= *Elaphocordyceps subsessilis* (Petch) G.H. Sung et al.

= *Tolypocladium niveum* (O. Rostr.) Bissett

Hosts. Elateroidea larvae (Shimizu, 1997).

Known distribution. Widely distributed (Petch, 1937; Kobayasi, 1982; Sung et al., 2007).

7.5 Discussion

The superfamilies Elateroidea and Tenebrionoidea are a very large group and contain more than 50 coleopterous families (http://www.catalogueoflife.org/), including Lampyridae (fireflies), Elateridae (click beetles), Phengodidae (glowworm beetles), Cantharidae (soldier beetles) and their relatives in Elateroidea, and Meloidae (blister beetles), Anthicidae (ant-like flower beetles), Mordellidae (tumbling flower beetles), Tenebrionidae (darkling beetle), Ciidae (the minute tree-fungus beetles), Zopheridae (ironclad beetles) and their relatives in Tenebrionoidea. Considering the difficulties in identifying their larvae (wireworms), we suggest using the superfamily names (Elateroidea or Tenebrionoidea) to record the hosts of related entomopathogenic fungi, unless we can definitely know their taxa. In this report, the taxa of the wireworm hosts are all updated.

To date, total 55 cordyceps species have been reported on wireworms, including 16 species (3 genera) in Cordycipitaceae, 8 species (2 genera) in Clavicipitaceae and 31 species (4 genera) in Ophiocordycipitaceae in the order

Hypocreales. Among them, *Cordyceps militaris*, *Ophiocordyceps ferruginosa* and *O. variabilis* are rejected and the remaining 52 species are accepted as parasites of wireworms. A third can parasitize other arthropods, nematodes, plants, other cordyceps fungi or inhabit soil, while the remaining two-thirds show host specificity. These cordyceps fungi were found in humid soil, humus layer or decayed wood where wireworms inhabit. They grow in warm and humid environments, and most of them were reported from Southeast Asia (Japan, Southwest China and Thailand) and the Amazon of South America.

Our work provides basic information for future research of species diversity, biological control, and edible and medicinal insects and fungi. Limited by a lack of information and taxonomic knowledge of larvae, some wireworms cannot be assigned to a family and some wireworms may be incorrectly identified.

Table 7.1 Sequence information of samples appearing in Figure 7.1

Fungal species	Specimen/ strain No.	Host/substratum	SSU	ITS	LUS	TEF1-α	References
Ophiocordyceps amazonica	Ophama2026	Orthoptera: Acrididae (nymph)	KJ917562	-	KJ917571	KM411989	Sanjuan et al. (2015)
O. annulata	CEM303	Coleoptera	KJ878915	-	KJ878881	KJ878962	Quandt et al. (2014)
O. aphodii	ARSEF 5498	Coleoptera	DQ522541	-	DQ518755	DQ522323	Spatafora et al. (2007)
O. borealis	**MFLU 18-0163**	**Coleoptera: Elateroidea (larva)**	**MK863044**	**MK863251**	**MK863051**	**MK860189**	**This study**
O. borealis	**GACP R16002**	**Coleoptera: Elateroidea (larva)**	**MK863045**	**MK863252**	**MK863052**	**MK860190**	**This study**
O. borealis	**GACP R16003**	**Coleoptera: Elateroidea (larva)**	**MK863046**	**MK863253**	**MK863053**	**MK860191**	**This study**
O. clavata	NBRC 106962	Coleoptera (larva)	JN941726	JN943328	JN941415	AB968587	Schoch et al. (2012)
O. coenomyiae	NBRC 108993	Diptera: *Coenomyia* (larva)	AB968384	AB968396	AB968412	AB968570	Ban et al. (2015b)
O. cossidarum	MFLU 17-0752	Lepidoptera (larva)	MF398186	-	MF398187	MF928403	Hyde et al. (2018)
O. entomorrhiza	KEW 53484	Lepidoptera	EF468954	JN049850	EF468809	EF468749	Quandt et al. (2014)
O. formosana	MFLU 15-3889	Tenebrionoidea (larva)	-	-	-	KU854950	Li et al. (2016)
O. formosana	MFLU 15-3888	Tenebrionoidea (larva)	KU854951	-	-	KU854949	Li et al. (2016)
O. gracilis	EFCC 8572	Lepidoptera (larva)	EF468956	JN049851	EF468811	EF468751	Kepler et al. (2012a)
O. heteropoda	OSC106404	Hemiptera (cicada nymph)	AY489690	-	AY489722	AY489617	Castlebury et al. (2004)
O. konnoana	EFCC 7315	Coleoptera (larva)	EF468959	-	-	EF468753	Sung et al. (2007)

Table 7.1 (continued)

Fungal species	**Specimen/ strain No.**	**Host/substratum**	**SSU**	**ITS**	**LUS**	**TEF1-*α***	**References**
O. lanpingensis	YHOS0707	Lepidoptera: Hepialidae (larva)	KC417459	-	KC417461	KC417463	Chen et al. (2013a)
O. longissima	NBRC 108989	Hemiptera (cicada nymph)	AB968394	AB968407	AB968421	AB968585	Sanjuan et al. (2015)
O. macroacicularis	NBRC 105888	Lepidoptera (larva)	AB968389	AB968401	AB968417	AB968575	Ban et al. (2015b)
O. melolonthae	OSC 110993	Coleoptera: Scarabeidae (larva)	DQ522548	-	DQ518762	DQ522331	Spatafora et al. (2007)
O. nigra	TNS 16252	Hemiptera	KJ878941	-	KJ878906	KJ878986	Quandt et al. (2014)
O. nigrella	EFCC 9247	Lepidoptera (larva)	EF468963	JN049853	EF468818	EF468758	Sung et al. (2007)
O. purpureostromata	TNS F18430	Coleoptera	KJ878931	-	KJ878897	KJ878977	Quandt et al. (2014)
O. ravenelii	OSC 110995	Coleoptera (larva)	DQ522550	-	DQ518764	DQ522334	Spatafora et al. (2007)
O. robertsii	KEW 27083	Lepidoptera: Hepialidae (larva)	-	AJ309335	EF468826	EF468766	Sung et al. (2007)
O. sinensis	EFCC 7287	Lepidoptera (pupa)	EF468971	JN049854	EF468827	EF468767	Sung et al. (2007)
O. sobolifera	NBRC 106967	Hemiptera (cicada nymph)	AB968395	AB968409	AB968422	AB968590	Ban et al. (2015b)
***O. spicatus* sp. nov.**	**MFLU 18-0164**	**Coleoptera: Tenebrionoidea (larva)**	**MK863047**	**MK863254**	**MK863054**	**MK860192**	**This study**
O. variabilis	OSC 111003	Diptera (larva)	EF468985	-	EF468839.	EF468779	Sung et al. (2007)

Table 7.1 (continued)

Fungal species	Specimen/ strain No.	Host/substratum	SSU	ITS	LUS	TEF1-α	References
O. xuefengensis	GZUH2012HN19	Lepidoptera: *Endoclita nodus* (larva)	KC631788	KC631803	-	KC631794	Wen et al. (2013)
P. formosus	**MFLU 18-0162**	***Ophiocordyceps* sp. (stroma) on an Elateroidea larva**	**MK863043**	**MK863250**	**MK863050**	**MK860188**	**This study**
P. formosus	ARSEF 1424	Coleoptera	KF049615	KF049661	KF049634	DQ118754	Chaverri et al. (2005)
P. lianzhouensis	GIMYY9603	Lepidoptera	KF226249	EU149922	KF226250	KF226252	Wang et al. (2014b)
P. ramosopulvinatus	EFCC 5566	Hemiptera	-	KF049658	KF049627	KF049682	Kepler et al. (2013)
P. sinensis	CN 80-2	*O. sinensis* (stroma)	HQ832887	HQ832884	HQ832886	HQ832890	Wang et al. (2012)
P. tomentosus	BL 4	Trichiales	KF049623	KF049666	KF049641	KF049697	Kepler et al. (2013)
P. yunnanensis	YHHPY1006	*O. nutans* (stroma)	-	KF977849	-	KF977851	Wang et al. (2015)

Note Sequencing results in this study are displayed in bold.

CHAPTER 8

OVERALL CONCLUSIONS

8.1 Taxonomy and Biology of the Medicinal Mushroom Chanhua (*Cordyceps cicadae* (Miq.) Massee)

Chanhua fungus is an important traditional Chinese medicine and has had a history of use for about 1,500 years. Chanhua has commonly been referred to as *Cordyceps cicadae* S.Z. Shing, *Isaria cicadae*, *I. sinclairii*, *Ophiocordyceps sobolifera*, and by other names. This has resulted in taxonomic confusion and nomenclatural problems. The biology, ecology, and host affiliation of Chanhua are also poorly understood, and these restrict further development and application in various areas, especially in traditional medicine. In this dissertation, we reviewed the medicinal history and distribution of Chanhua and reinvestigated its phylogenetic relationships with allied species. Based on the results obtained, we clarified its taxonomy and reviewed its host and its biological and ecological aspects. The phylogeny based on ITS sequence data indicates that Chanhua is an independent species of the genus *Cordyceps*. Although Chanhua, *I. cicadae*, and *I. sinclairii* have generally been accepted as the same species, the latter two lack DNA sequence data from their type localities. *C. cicadae* S.Z. Shing (syn. C. zhejiangensis), which has been treated as the sexual morph of Chanhua for over 40 years, has been reported to be *Tolypocladium paradoxum*, and the sexual morph of Chanhua is considered to be *C. kobayasii* (syn. *C. cicadae-sm*). I propose to use the scientific name *C. cicadae* (Miq.) Massee, together with the Chinese common name Chanhua, to describe or record this important medicinal mushroom.

8.2 Taxonomy and Biology of *Cordyceps qingchengensis* sp. nov. and Its Allies

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-*α* datasets indicate that it is closely related to *C. bifusispora*, *C. cicadae* (Miq.) Massee (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* (≡ *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. I provide important biological information for *C. qingchengensis* and its allies.

8.3 Xuefeng Cordyceps: Insights into Species Diversity, Life Cycle and Host Association

Cordyceps fungi are rather well known as entomopathogens and also for their medicinal importance in curing many diseases. Many cordyceps species with widespread distribution throughout China has been recorded earlier. However, their taxonomy, hosts, life cycles and the plants on which the host insects reside in are poorly understood, and have sometimes led to confusion in the proper identification of the species. In this dissertation, Xuefeng Cordyceps collected from Xuefeng Mountain, Hunan, China are identified and an assessment of their life cycles, proper hosts and plants they are associated with is made through morphological and DNA sequence-based analyses. Morphological characterization reveals that *Ophiocordyceps xuefengensis* from this region as commonly accepted by mycologists and local people. Species collected also include *Beauveria bassiana* (sexual and asexual), *Metacordyceps tai*i (syn. of *Metarhizium guizhouense*), *Ophiocordyceps macroacicularis* and *Ophiocordyceps ramosissimum*.

Current result challenges previous taxonomic arrangement with regard to host identity. The hosts, previously mistaken for larvae of *Endoclita nodus,* are corrected herein as larvae of *Endoclita davidi* (Lepidoptera: Hepialidae). Ribosomal DNA sequence analyses based on the ITS regions also confirm that *Clerodendrum cyrtophyllum* (Lamiaceae) is the plant on which the host insects reside. Morphological illustrations, where appropriate, are given for the cordyceps species, hosts and plants. The infection mechanism and life cycle of the cordyceps are also outlined.

8.4 Cordyceps Fungi Growing on Cicadas in China

Cicadicolous cordyceps refer to the cordyceps fungi growing on cicadas (Hemiptera: Cicadidae). Currently, there are 35 known species distributed worldwide, with many known from south Asia. In this dissertation, the relationships of cicadicolous species and their relatives are studied based on DNA sequence phylogeny. Taxonomy of 16 known species, 1 new species and 2 new records of cicadicolous species from China are discussed. *Ophiocordyceps bannaensis* sp. nov., *Metarhizium owariense* and *Polycephalomyces nipponicus* are newly reported from China. These taxa are detailed with descriptions, photographs, DNA sequence data and taxonomic notes.

8.5 Cordyceps Fungi Growing on Orthoptera Insects

Totally 25 cordyceps species have been reported on Orthoptera insects. Their hosts and distributions are reviewed and taxonomical notes for species are given. Using combined ITS and TEF1-α sequence dataset, molecular phylogeny of cordyceps species on Orthoptera insects was constructed. Current species were assigned to three clades: the *Beauveria*, the *Metarhizium* and the *Ophiocordyceps* clades. Based on ecology and habits of the six well known groups of Orthoptera, the relation between species diversity and host of Orthoptera cordyceps is discussed.

The rhizomorphs of cordyceps, a special root-like structure situated somewhere between fruiting bodies and synnemata, were observed on *Beauveria* spp. and *Metacordyceps* taii (syn. *Metarhizium guizhouense*). *Beauveria grylli* comb. nov. ≡ *Cordyceps grylli* = *Beauveria loeiensis* and *Beauveria kirkii* comb. nov. ≡ *Cordyceps kirkii* are proposed. The hosts of *Beauveria grylli* and *Ophiocordyceps tettigonia* are corrected.

8.6 Cordyceps Fungi on Wireworms (Elateroidea and Tenebrionoidea, Coleoptera)

Species of *Cordyceps* sensu lato (Hypocreales, Sordariomycetes) have always attracted much scientific attention for their abundant species diversity, important medicinal values and biological control applications. The insect superfamilies Elateroidea and Tenebrionoidea are two large groups of Coleoptera and their larvae are generally called wireworms. Most wireworms inhabit humid soil or fallen wood and are often infected with *Cordyceps* s.l.

However, the species diversity of *Cordyceps* s.l. on Elateroidea and Tenebrionoidea is poorly known. In this dissertation, I summarise taxonomic information of 63 *Cordyceps* s.l. species that have been reported as pathogens of wireworms. I review their hosts and geographic distributions and provide taxonomic notes for species. Of those, 60 fungal species are accepted as natural pathogens of wireworms and three species (*Cordyceps militaris*, *Ophiocordyceps ferruginosa* and *O. variabilis*) are excluded. Two new species, *O. borealis* from Russia (Primorsky Krai) and *O. spicatus* from China (Guizhou), are described and compared with their closest allies. *Polycephalomyces formosus* is also described because it is reported as a pathogen of wireworms for the first time. Phylogeny was reconstructed from a combined dataset, comprising SSU, LSU and TEF1-α gene sequences. The results, presented in this study, support the establishment of the new species and confirm the identification of *P. formosus*.

8.7 Future Works

On the basis of curret research, future work of cordycpes fungi will be carried out mainly from aspects as follows:

1. continue to reveal species diversity of cordyceps fungi, especially those micro ascomycetes;
2. continue to clarify taxonomic confusions of sexual and asexual names;
3. continue to reveal host association and life cycle of cordycpes fungi;
4. to explore the application and development of cordyceps fungi, and to serve biological control and human health.

REFERENCES

REFERENCES

Araújo, J. P. M., & Hughes, D. P. (2016). Diversity of entomopathogenic fungi: Which groups conquered the insect body? *Advances in Genetics*, *94*, 1–39.

Agrawal, Y., Mual, P., & Shenoy, B. D. (2014). Multi-gene genealogies reveal cryptic species *Beauveria rudraprayagi* sp. nov. from India. *Mycosphere*, *5*, 719–736.

Ariyawansa, H. A., Hyde, K. D., Jayasiri, S. C., Buyck, B., Chethana, K. W. T., Dai, D. Q., Dai, Y. C., Daranagama, D. A., Jayawardena, R. S., Lücking, R., Ghobad-Nejhad, M., Niskanen, T., Thambugala, K. M., Voigt, K., Zhao, R. L., Li, G. J., Doilom, M., Boonmee, S., Yang, Z. L., Cai, Q., . . .Chen, X. H. (2015). Fungal diversity notes 111-252—taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity*, *75*, 27–274.

Aung, O. M., Soytong, K., & Hyde, K. D. (2008). Diversity of entomopathogenic fungi in rainforests of Chiang Mai Province, Thailand. *Fungal Diversity*, *30*, 15–22.

Balakrishnan, M. M., Reddy, G. V. M., & Kumar, P. K. V. (2012). Record of *Metarhizium cylindrosporum* (Hypocreales: Clavicipitaceae) on *Terpnosia psecas* from India. *Insect Environment*, *18*, 6–7.

Bamisile, B. S., Dash, C. K., Akutse, K. S., Keppanan, R., Afolabi, O. G., Hussain, M., . . .Wang, L. (2018). Prospects of endophytic fungal entomopathogens as biocontrol and plant growth promoting agents: an insight on how artificial inoculation methods affect endophytic colonization of host plants. *Microbiological Research*, *217*, 34–50.

Ban, S., Sakane, T., Toyama, K., & Nakagiri, A. (2009). Teleomorph-anamorph relationships and reclassification of *Cordyceps cuboidea* and its allied species. *Mycoscience*, *50*, 261–272.

Ban, S., Azuma, Y., Sato, H., Suzuki, K., & Nakagiri, A. (2015a). *Isaria takamizusanensis* is the anamorph of *Cordyceps ryogamimontana*, warranting a new combination, *Purpureocillium takamizusanense* comb. nov. *International Journal of Systematic and Evolutionary Microbiology, 65*, 2459–2465.

Ban, S., Sakane, T., & Nakagiri, A. (2015b). Three new species of *Ophiocordyceps* and overview of anamorph types in the genus and the family Ophiocordyceptaceae. *Mycological Progress*, *14*(1), 1017–1028.

Berkley, M. J. (1855). Supplementum. In J. D. Hooker (Ed.), *Flora novae-zelandiae part II, flowerless plants*. Published under the Authority of the Lords of the Admiralty.

Bills, G. F., Polishook, J. D., Goetz, M. A., Sullivan, R. F., & White, J. J. F. (2002). *Chaunopycnis pustulata* sp. nov., a new clavicipitalean anamorph producing metabolites that modulate potassium ion channels. *Mycological Progress*, *1*, 3–17.

Binh, D. N., Giang, N. T., Ly, L. T., Tuyen, D. T., Tao, V. X., & Diep, L. H. (2017). Isolation and successful cultivation of *Cordyceps cicadae* in Viet Nam. *VNU Journal of Science, Natural Sciences and Technology, 33*. https://doi.org/10.25073/2588-1140/vnunst.4571

Bischoff, J. F., Rehner, S. A., & Humber, R. A. (2009). A multilocus phylogeny of the *Metarhizium anisopliae* lineage. *Mycologia*, *101*(4), 512–530.

Bissett, J. & Widden, P. 1988. A new species of *Beauveria* isolated from Scottish moorland soil. *Canadian Journal of Botany*, *66*, 361–362.

Brunner-Mendoza, C., Reyes-Montes, M. D. R., Moonjely, S., Bidochka, M. J., & Toriello, C. (2019). A review on the genus *Metarhizium* as an entomopathogenic microbial biocontrol agent with emphasis on its use and utility in Mexico. *Biocontrol Science and Technology*, *29*(1), 83–102.

Cai, L., Jeewon, R., & Hyde, K. D. (2006). Molecular systematics of *Zopfiella* and allied genera: Evidence from multigene sequence analyses. *Mycological Research*, *110*, 359–368.

Chan, W. H., Ling, K. H., Chiu, S. W., Shaw, P. C., & But, P. P. H. (2011). Molecular analyses of *Cordyceps gunnii* in China. *Journal of Food and Drug Analysis*, *19*, 18–25.

Chaverri, P., Bischoff, J. F., Evans, H. C., & Hodge, K. T. (2005). *Regiocrella*, a new entomopathogenic genus with a pycnidial anamorph and its phylogenetic placement in the Clavicipitaceae. *Mycologia*, *97*(6), 1225–1237.

Chen, Z. H., Dai, Y. D., Yu, H., Yang, K., Yang, Z. L., Yuan, F., & Zeng, W. B. (2013a). Systematic analyses of *Ophiocordyceps lanpingensis* sp. nov., a new species of *Ophiocordyceps* in China. *Microbiological Research*, *168*(8), 525–532.

Chen, M. J., Huang, B., Li, Z. Z., & Spatafora, J. W. (2013b). Morphological and genetic characterization of *Beauveria sinensis* sp. nov. from China. *Mycotaxon*, *124*, 301–308.

Chen, Z. A., Li, Z. Z., & Chen, Y. P. (2014). *Jin-Chanhua (A precious Cordyceps: Isaria cicadae Miq.)*. Publishing House of Ancient Chinese Medical Books.

Chen, A. H., Shao, Y., Li, J. W., Jiang, Z. Z., & Chen, H. W. (2015a). Antitumor activity of cultured fruiting bodies of *Cordyceps cicadae*. *Food Science*, *36*, 194–197.

Chen, C., Xu, J. W., Sun, X. J., Jia, J. Q., & Gui, Z. Z. (2015b). Strain isolation and polysaccharide biological activity of *Isaria cicadae* in Maoshan region. *Jiangsu Agricultural Sciences*, *43*, 347–352.

Chen, Y. L., Yeh, S. H., Lin, T. W., Chen, C. C., Chen, C. S., & Kuo, C. F. (2015c). A 90-day subchronic toxicity study of submerged mycelial culture of *Cordyceps cicadae* (Ascomycetes) in rats. *International Journal of Medicinal Mushrooms*, *17*, 771–781.

Chen, W. H., Han, Y. F., Liang, Z. Q., & Jin, D. C. (2017). A new araneogenous fungus in the genus *Beauveria* from Guizhou, China. *Phytotaxa*, *302*, 57–64.

Chen, W. H., Liu, M., Huang, Z. X., Yang, G. M., Han, Y. F., Liang, J. D, . . .Liang, Z. Q. (2018). *Beauveria majiangensis*, a new entomopathogenic fungus from Guizhou, China. *Phytotaxa*, *333*(2), 243–250.

Chinese Pharmacopoeia Commission (2015). *Chinese pharmacopoeia* (10th ed., Vol. 1). China Medical Science Press.

Chu, H. F., & Wang, L. Y. (1985). On the stem-borers of Chinese hepialids (Lepidoptera: Hepialidae). *Acta Entomologica Sinica*, *28*, 293–301.

Cigliano, M. M., Braun, H., Eades, D. C., & Otte, D. (2019). *Orthoptera species file, version 5.0/5.0.* http://Orthoptera.SpeciesFile.org

Cooke, M. C. (1884). New British fungi. *Grevillea*, *12*(63), 65–70.

Cunningham, G. H. (1922). A singular *Cordyceps* from Stephen Island, New Zealand. *Transactions of the British Mycological Society*, *8*, 72–75.

Currie, C. R., Wong, B., Stuart, A. E., Schultz, T. R., Rehner, S. A., Mueller, U. G., . . .Straus, N. A. (2003). Ancient tripartite coevolution in the attine ant-microbe symbiosis. *Science*, *299*(5605), 386–388.

De Bary, A. (1867). Zur Kenntniss insectentoedtender Pilze. *Bot. Ztg.*, *25*, 17–21.

De Silva, D. D., Rapior, S., Hyde, K. D., & Bahkali, A. H. (2012). Medicinal mushrooms in prevention and control of diabetes mellitus. *Fungal Diversity*, *56*, 1–29.

De Silva, D. D., Rapior, S., Sudarman, E., Stadler, M., Xu, J. C., Alias, S. A., . . .Hyde, K. D. (2013). Bioactive metabolites from macrofungi: Ethnopharmacology, biological activities and chemistry. *Fungal Diversity, 62*, 1–40.

Doyle, J. J. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, *19*(1), 11–15.

Eriksson, O. (1982). *Cordyceps bifusispora* spec. nov.. *Mycotaxon*, *15*, 185–188.

Evans, H. C., & Shah, P. A. (2002). Taxonomic status of the genera *Sorosporella* and *Synglíocladium* associated with grasshoppers and locusts (Orthoptera: Acridoidea) in Africa. *Mycological Research*, *106*, 737–744.

Fabre, J. H. (1886). *Souvenirs Entomologiques, Livre III*. Étude sur l’instinct et les mœurs des insectes.

Gourley, S. A., & Kuang, Y. (2009). Dynamics of a neutral delay equation for an insect population with long larval and short adult phases. *Journal of Differential Equations*, *246*, 4653–4669.

Gullan, P. J., & Cranston, P. S. (2010). *The insects: An outline of entomology* (4th ed.). Wiley-Blackwell.

Guo, H. L., Ye, B. L., Yue, Y. Y., Chen, Q. T., & Fu, C. S. (1986). Three new species of *Metarhizium. Acta Mycologica Sinica*, *5*, 177–184.

Hawksworth, D. L., Crous, P. W., Redhead, S. A., Reynolds, D. R., Samson, R. A., Seifert, K. A., Taylor, J. W., Wingfield, M. J., Abaci, Ö., Aime, C., Asan, A., Bai, F. Y., de Beer, Z. W., Begerow, D., Berikten, D., Boekhout, T., Buchanan, P. K., Burgess, T., Buzina, W., Cai, L., . . .Zhang, N. (2011). The Amsterdam Declaration on fungal nomenclature. *IMA Fungus*, *2*(1), 105–112.

He, Y. H., He, X. Q., Yang, Y., Ma, M. Z., & Lou, K. M. (2012). Reseach process on treatment of diabetic nephropathy using Chanhua. *Heilongjiang Medicine Journal*, *25*, 868–869.

Hennings, P. (1904). Fungi Amazonici a cl. Ernesto Ule collecti II. *Hedwigia*, *43*, 242–273.

Hennings, P. (1908). Fungi von Madagaskar, den Comoren und Ostafrika. *Reise in Ostafrika in den Jahren 1903–1905*, *3*(1), 15–33.

Hodge, K. T., Humber, R. A., & Wozniak, A. C. (1998). *Cordyceps variabilis* and the genus *Synglioc ladium*. *Mycologia*, *90*(5), 743–753.

Hölldobler, B., & Wilson, E. O. (2009). *The superorganism: The beauty, elegance, and strangeness of insect societies*. WW Norton & Company.

Hongsanan, S., Maharachchikumbura, S. S. N., Hyde, K. D., Samarakoon, M. C., Jeewon, R., Zhao, Q., . . .Bahkali, A. H. (2017). An updated phylogeny of Sordariomycetes based on phylogenetic and molecular clock evidence. *Fungal Diversity*, 84, 25–41.

Hsu, J. H., Jhou, B. Y., Yeh, S. H., Chen, Y. L., & Chen, C. C. (2015). Healthcare functions of *Cordyceps cicadae*. *Journal of Nutrition & Food Sciences*, *5*, 432.

Hu, X., Xiao, G., Zheng, P., Shang, Y., Su, Y., Zhang, X., . . .Wang, C. (2014). Trajectory and genomic determinants of fungal-pathogen speciation and host adaptation. *Proceedings of the National Academy of Sciences*, *111*, 16796–16801.

Huang, N. L. (1998). *The primary illustrations of macrofungi in China*. China Agricultural Press.

Huang, B., Li, S. G., Li, C. R., Fan, M. Z., & Li, Z. Z. (2004). Studies on the taxonomic status of *Metarhizium cylindrospora* and *Nomuraea viridula*. *Mycosystema*, *23*, 33–37.

Huang, B., Li, C., Humber, R. A., Hodge, K. T., Fan, M., & Li, Z. (2005). Molecular evidence for the taxonomic status of *Metarhizium taii* and its teleomorph, *Cordyceps taii* (Hypocreales, Clavicipitaceae). *Mycotaxon*, *94*, 137–147.

Huang, M. H. (2012). *A book for exploring Xuefeng Cordyceps (Xuefeng Chongcao Tansuo Duben).* Guangming Daily Publishing House.

Huang, M. H. (2013). *An introduction to ecological sociology of Xuefeng Cordyceps (Xuefeng Chongcao Shengtai Shehuixue Daolun)*. Guangming Daily Publishing House.

Humber, R. A., & Hansen, K. S. (2005). *USDA-ARS collection of entomopathogenic fungal cultures (ARSEF), ARSEF-index: Host by fungus*. http://arsef.fpsnl.cornell.edu

Hyde, K. D., Chaiwan, N., Norphanphoun, C., Boonmee, S., Camporesi, E., Chethana, K. W. T., Dayarathne, M. C., de Silva, N. I., Dissanayake, A. J., Ekanayaka, A. H., Hongsanan, S., Huang, S, K., Jayasiri, S. C., Jayawardena, R. S., Jiang, H. B., Karunarathna, A., Lin, C. G., Liu, J. K., Liu, N. G., Lu, Y. Z., . . .Zhao, Q. (2018). Mycosphere notes 169-224. *Mycosphere, 9*(2), 271–430.

Hywel-Jones, N. L. (1995). *Cordyceps brunneapunctata* sp. nov. infecting beetle larvae in Thailand. *Mycological Research, 99*(10), 1195–1198.

Hywel-Jones, N. L. (2001). The biological diversity of invertebrate pathogenic fungi. In K. D. Hyde (Ed.), *Biodiversity of tropical microfungi*. Hong Kong University Press.

Imoulan, A., Wu, H. J., Lu, W. L., Li, Y., Li, B. B., Yang, R. H., . . .Yao, Y. J. (2016). *Beauveria medogensis* sp. nov., a new fungus of the entomopathogenic genus from China. *Journal of Invertebrate Pathology*, *139*, 74–81.

Imoulan, A., Hussain, M., Kirk, P. M., Meziane, A. E., & Yao, Y. J. (2017). Entomopathogenic fungus *Beauveria*: Host specificity, ecology and significance of morpho-molecular characterization in accurate taxonomic classification. *Journal of Asia-Pacific Entomology*, *20*(4), 1204–1212.

Jeewon, R., Liew, E. C. Y., Simpson, J. A., Hodgkiss, I. J., & Hyde, K. D. (2003). Phylogenetic significance of morphological characters in the taxonomy of *Pestalotiopsis* species. *Molecular Phylogenetics and Evolution*, *27*, 372–383.

Kabaluk, T., Goettel, M., Erlandson, M., Ericsson, J., Duke, G., & Vernon, B. (2005). *Metarhizium anisopliae* as a biological control for wireworms and a report of some other naturally-occurring parasites. *IOBC/WPRS Bulletin*, *28*, 109–115.

Kabaluk, T., Li-Leger, E., & Nam, S. (2017). *Metarhizium brunneum*, an enzootic wireworm disease and evidence for its suppression by bacterial symbionts. *Journal of Invertebrate Pathology*, *150*, 82–87.

Keissler, K., & Lohwag, H. (1937). Part II fungi. In H. Handel-Mazzetti (Ed.), *Symbolae sinicae*. Springer.

Kepler, R. M., Sung, G. H., Ban, S., Nakagiri, A. Chen, M. J., Huang, B., . . .Spatafora, J. W. (2012a). New teleomorph combinations in the entomopathogenic genus *Metacordyceps*. *Mycologia*, *104*, 182–197.

Kepler, R. M., Sung, G. H., Harada, Y., Tanaka, K., Tanaka, E., Hosoya, T., . . .Spatafora, J. W. (2012b). Host jumping onto close relatives and across kingdoms by *Tyrannicordyceps* (Clavicipitaceae) gen. nov. and *Ustilaginoidea* (Clavicipitaceae). *American Journal of Botany*, *99*, 552–561.

Kepler, R., Ban, S., Nakagiri, A., Bischoff, J., Hywel-Jones, N., Owensby, C. A., . . .Spatafora, J. W. (2013). The phylogenetic placement of hypocrealean insect pathogens in the genus *Polycephalomyces*: An application of One Fungus One Name. *Fungal Biology*, *117*, 611–622.

Kepler, R. M., Humber, R. A., Bischoff, J. F., & Rehner, S. A. (2014). Clarification of generic and species boundaries for *Metarhizium* and related fungi through multigene phylogenetics. *Mycologia*, *106*(4), 811–829.

Kepler, R. M., Luangsa-ard, J. J., Hywel-Jones, N. L., Quandt, C. A., Sung, G. H., Rehner, S. A., . . .Shrestha, B. (2017). A phylogenetically-based nomenclature for Cordycipitaceae (Hypocreales). *IMA Fungus, 8,* 335–353.

Kobayasi, Y. (1939). On the genus *Cordyceps* and its allies on Cicadidae from Japan. *Bulletin of the Biogeographical Society of Japan, 9*, 145–176.

Kobayasi, Y. (1941). The genus *Cordyceps* and its allies. *Science Reports of the Tokyo Bunrika Daigaku, Section B, 5,* 53–260.

Kobayasi, Y. (1949). Several species of the genus *Cordyceps* and their conidial forms. *Journal of Japanese Botany*, *24,* 176–180.

Kobayasi, Y. (1981). Revision of the genus *Cordyceps* and its allies, I. *Bulletin of the National Science Museum Tokyo, Series B, Botany*, *7,* 1–13.

Kobayasi, Y. (1982). Key to the taxa of the genera *Cordyceps* and *Torrubiella*. *Transactions of the Mycological Society of Japan*, *23*, 329–364.

Kobayasi, Y., & Shimizu, D. (1963). Monographic studies of *Cordyceps* 2, group parasitic on cicadae. *Bulletin of the National Science Museum Tokyo*, *6,* 286–314.

Kobayasi, Y., & Shimizu, D. (1976). The genus *Cordyceps* and its allies from New Guinea. *Bulletin of the National Science Museum Tokyo, Series B, Botany*, *2,* 133–152.

Kobayasi, Y., & Shimizu, D. (1978). *Cordyceps* species from Japan. *Bulletin of the National Science Museum, Tokyo, Series B, Botany*, *4*(2), 43–63.

Kobayasi, Y., & Shimizu, D. (1980a). *Cordyceps* species from Japan 2. *Bulletin of the National Science Museum, Tokyo, Series B, Botany*, *6*(3), 77–96.

Kobayasi, Y., & Shimizu, D. (1980b). *Cordyceps* species from Japan 3. *Bulletin of the National Science Museum, Tokyo, Series B, Botany*, *6*(4), 125–145.

Kobayasi, Y., & Shimizu, D. (1981). The genus *Cordyceps* and its allies from Taiwan (Formosa). *Bulletin of the National Science Museum Tokyo, Series B, Botany*, *7*, 113–122.

Kobayasi, Y., & Shimizu, D. (1982a). *Cordyceps* species from Japan 4. *Bulletin of the National Science Museum, Tokyo, Series B, Botany*, *8*(3), 79–91.

Kobayasi, Y., & Shimizu, D. (1982b). *Cordyceps* species from Japan 5. *Bulletin of the National Science Museum, Tokyo, Series B, Botany*, *8*(4), 111–123.

Kobayasi, Y., & Shimizu, D. (1983). *Cordyceps* species from Japan 6. *Bulletin of the National Science Museum, Tokyo B, Botany*, *9*(1), 1–21.

Koval, E. Z. (1984). *Clavicipitalean fungi of the USSR*. Kiev: Naukova Dumka.

Kryukov, V. Y., Yaroslavtseva, O. N., Lednev, G. R., & Borisov, B. A. (2011). Local epizootics caused by teleomorphic cordycipitoid fungi (Ascomycota: Hypocreales) in populations of forest lepidopterans and sawflies of the summer-autumn complex in Siberia. *Microbiology*, *80*(2), 286–295.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., . . .Valentin, F. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, *23*, 2947–2948.

Li, Q. L., & Wei, C. (2013). Antennal morphology of the cicada *Meimuna mongolica* (Distant) (Hemiptera: Cicadidae), with a deduction of its nymphal instars and discussion of the antennal morphological variations in relation to niche changes. *Entomotaxonomia*, *35*, 81–94.

Li, C. R., Huang, B., Fan, M. Z., & Li, Z. Z. (1999) *Cordyceps longissima* and its *Hirsutella* anamorph. *Journal of Anhui Agricultural University*, *26*, 374–377.

Li, Z. Z., Li, C. R., Huang, B., & Fan, M. Z. (2001). Discovery and demonstration of the teleomorph of *Beauveria bassiana* (Bals.) Vuill., an important entomogenous fungus. *Chinese Science Bulletin*, *46*(9), 751–753.

Li, C. R., Fan, M. Z., Huang, B., Wang, S. B., & Li, Z. Z. (2002). The genus *Cordyceps* and its allies from Anhui I. *Mycosystema*, *21*(2), 167–171.

Li, C. R., Chen, M. J., Wang, M., Lin, Y. R., Fan, M. Z., & Li, Z. Z. (2006). *Hirsutella heteropoda* sp. nov. and its teleomorph, new variety of *Cordyceps heteropoda*. *Mycosystema*, *25*, 163–168.

Li, C. R., Zuo, D. P., Nam, S. H., Pu, S. C., Fan, M. Z., & Li, Z. Z. (2007). *Cordyceps takaomontana* and its anamorph *Paecilomyces tenuipes*. *Mycosystema*, *26*(2), 217–220.

Li, C. R., Huang, B., Fan, M. Z., Lin, Y. R., & Li, Z. Z. (2010) *Metacordyceps guniujiangensis* and its *Metarhizium* anamorph: A new pathogen on cicada nymphs. *Mycotaxon*, *111*, 221–231.

Li, G. J., Hyde, K. D., Zhao, R. L., Sinang, H., Abdel-Aziz, F. A., Abdel-Wahab, M. A., Alvarado, P., Alves-Silva, G., Ammirati, J., Ariyawansa, H. A., Baghela, A., Bahkali, A. H., Beug, M., Bhat, D. J., Bojantchev, D., Boonpratuang, T., Timur Bulgakov, T., Camporesi, E., Boro, M. C., Ceska, O., . . .Zhou, L. W. (2016). Fungal diversity notes 253-366: Taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity*, *78*(1), 1–237.

Liang, Z. Q. (2007). *Flora fungorum sinicorum* (Vol. 32), *Cordyceps*. Science Press.

Liang, Z. Q., Liu, A. Y., & Liu, J. L. (1991). A new species of the genus *Cordyceps* and its *Metarhizium* anamorph. *Acta Mycologica Sinica*, *10*, 257–262.

Liang, Z. Q., Liu, A. Y., & Jiang, Y. C. (2001). Two new species of *Cordyceps* from Jinggang Mountains. *Mycosystema*, *20*(3), 306–309.

Liang, Z. Q., Liu, A. Y., & Huang, J. Z. (2002). Some entomogenous fungi from Wuyishan and Zhangjiajie Nature Reserves I, *Cordyceps*. *Mycosystema*, *21*, 162–166.

Liang, Z. Q., Liu, A. Y., Liu, M. H., & Kang, J. C. (2003). The genus *Cordyceps* and its allies from the Kuankuoshui Reserve in Guizhou III. *Fungal Diversity*, *14*, 95–101.

Liu, B., Rong, F., & Jin, H. (1985). A new species of the genus *Cordyceps*. *Journal of Wuhan Botanical Research*, *3*(1), 23–24.

Liu, A. Y. (2012). *Research and application of resource of Isaria cicadae Miq. in China*. Guiyang: Guizhou Science and Technology Press.

Liu, Y., & Huang, K. (2015). Anti-fatigue activity of *Paecilomyce cicadae*. *Journal of Tongren University*, *17*, 15–18.

Liu, A. Y., Liang, Z. Q., & Liu, Z. Y. (1997). *Cordyceps* spp. and some other entomopathogenic fungi from the Emei Mountain Preserve in China. *Mycosystema*, *16*, 139–143.

Liu, Z. Y., Liang, Z. Q., Whalley, A. J. S. , Liu, A. Y., & Yao, Y. J. (2001). A new species of *Beauveria*, the anamorph of *Cordyceps sobolifera*. *Fungal Diversity*, *7*, 61–70.

Liu, A. Y., Hu, H. Y., Liang, Z. Q., & Zou, X. (2010). A new record of *Isaria* in China. *Mycosystema*, *29*, 128–131.

Liu, A. Y., Zou, X., Luo, L., & Chen, B. (2011). A new species of *Cordyceps*, the telemorph of *Isaria cicadae* Miq. *Agriculture of China*, *4*(11), 10–14.

Liu, W. J., Tang, Y. M., Xie, S. H., Liu, Y. N., Pan, Q. J., Wu, H. L., . . .Liu, H. F. (2014). *Cordyceps cicadae* prevents the development of diabetic nephropathy through up-regulating the expression of tubular SIRT1 in diabetic rats. *Chinese Journal of Integrated Traditional and Western Nephrology*, *15*, 665–668.

Liu, H., Zhen L. P., Zhu, R. C., Zhang, S. H., & Huang, H. Y. (2015). Studies on macroscopic and microscopic characteristics of *Ophiocordyceps xuefengensis*. *China Journal of Chinese Materia Medica*, *40*, 2820–2824.

Lloyd, C. G. (1920). Mycological notes 62. *Mycological Writings, 6*(62), 904–944.

Lloyd, C. G. (1923) Mycological notes 69. *Mycological Writings*, *7*, 1185–1218.

Lomer, C. J., Bateman, R. P., Johnson, D. L., Langewald, J., & Thomas, M. (2001). Biological control of locusts and grasshoppers. *Annual Review of Entomology, 46*, 667–702.

Luangsa-ard, J. J., Hywel-Jones, N. L., Manoch, L., & Samson, R. A. (2005). On the relationships of *Paecilomyces* sect. *Isarioidea* species. *Mycological Research, 109*, 581–589.

Luangsa-ard, J. J., Tasanathai, K., Mongkolsamrit, S., & Hywel-Jones, N. (2008). *Atlas of invertebrate-pathogenic fungi of Thailand* (vol. 2). National Center for Genetic Engineering and Biotechnology.

Luangsa-Ard, J. J., Ridkaew, R., Tasanathai, K., Thanakitpipattana, D., & Hywel-Jones, N. (2011). *Ophiocordyceps halabalaensis*: A new species of phiocordyceps pathogenic to *Camponotus gigas* in Hala Bala Wildlife Sanctuary, Southern Thailand. *Fungal Biology*, *115*, 608–614.

Luo, G. T., & Zhang, W. Q. (2017). Report on macrofungi resources in Qiandongnan, Guizhou, II. *Guizhou Science*, *35*(1), 19–21.

Mains, E. B. (1939). *Cordyceps* from the mountains of North Carolina and Tennessee. *Journal of the Elisha Mitchell Scientific Society*, *55*, 117–129.

Mains, E. B. (1940). Species of *Cordyceps*. *Mycologia*, *32*(3), 310–320.

Mains, E. B. (1941). *Cordyceps stylophora* and *Cordyceps ravenelii*. *Mycologia*, *33*, 611–617.

Mains, E. B. (1947). New and interesting species of *Cordyceps*. *Mycologia, 39*(5), 535–545.

Mains, E. B. (1951). Notes concerning entomogenous fungi. *Bulletin of the Torrey Botanical Club*, *78*, 122–133.

Mains, E. B. (1958). North American entomogenous species of *Cordyceps*. *Mycologia*, *50*, 169–222.

Mains, E. B. (1959). *Cordyceps* species. *Bulletin of the Torrey Botanical Club*, *86*(1), 46–58

Massee, G. (1895). A revision of the genus *Cordyceps*. *Annals of Botany*, *9*(33), 1–44.

Massee, G. (1899). Revision du genre *Cordyceps*. *Revue Mycologique*, *21*, 1–16.

Matočec, N., Kušan, I., & Ozimec, R. (2014). The genus *Polycephalomyces* (Hypocreales) in the frame of monitoring Veternica cave (Croatia) with a new segregate genus *Perennicordyceps*. *Ascomycete.org*, *6*, 125–133.

Meyling, N. V., Lubeck, M., Buckley, E. P., Eilenberg, J., & Rehner, S. A. (2009). Community composition, host range and genetic structure of the fungal entomopathogen *Beauveria* in adjoining agricultural and seminatural habitats. *Molecular Ecology*, *18*, 1282–1293.

Miquel, F. A. W. (1838). Observations sur les Cycad^es de I'Herbier royal a Leyde. *Bulletin des Sciences Physiques et Naturelles en Néerlande*, 82–85.

Möller, A. (1901). Phycomyceten und Ascomyceten, Untersuchungen aus Brasilien. *Botanische Mittheilungen aus den Tropen*, *9*, 1–319.

Moureau, J. (1949). *Cordyceps* du Congo Belge. *Mémoires de l'Institut Royal Colonial Belge*, *7*, 1–58.

Negi, P. S., Singh, R., Koranga, P. S., & Ahmed, Z. (2012). Two new for science species of genus *Cordyceps* Fr. (Ascomycetes) from Indian Himalaya. *International Journal of Medicinal Mushrooms*, *14*(5), 501–506.

Nei, M., & Kumar, S. (2000). *Molecular evolution and phylogenetics*. Oxford University Press.

Nielsen, E. S., Robinson, G. S., & Wagner, D. L. (2000). Ghost-moths of the world: A global inventory and bibliography of the Exoporia (Mnesarchaeoidea and Hepialoidea) (Lepidoptera). *Journal of Natural History*, *34*, 823–878.

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

Nikoh, N., & Fukatsu, T. (2001). Evolutionary dynamics of multiple group I introns in nuclear ribosomal RNA genes of endoparasitic fungi of the genus *Cordyceps*. *Molecular Biology and Evolution*, *18*, 1631–1642.

Paul, A., Frederich, M., Uyttenbroeck, R., Hatt, S., Malik, P., Lebecque, S., . . .Danthine, S. (2016). Grasshoppers as a food source? A review. *Biotechnology, Agronomy, Society and Environment*, *20*, 337–352.

Pendland, J. C., & Boudas, D. G. (1987). The hyphomycete *Sorosporetta-Syngliocladium* from mole cricket, *Scapteriscus vicinus*. *Mycopathologia*, *99*, 25–30.

Perdomo, H., Cano, J., Gené, J., García, D., Hernández, M., & Guarro, J. (2013). Polyphasic analysis of *Purpureocillium lilacinum* isolates from different origins and proposal of the new species *Purpureocillium lavendulum*. *Mycologia*, *105*, 151–161.

Petch, T. (1924). Studies in entomogenous fungi, IV, some Ceylon *Cordyceps*. *Transactions of the British Mycological Society*, *10*(1–2), 28–45.

Petch, T. (1933a). Entomogenous fungi from Madagascar. *Annales de Cryptogamie Exotique*, *6*, 230–235.

Petch, T. (1933b). Notes on entomogenous fungi. *Transactions of the British Mycological Society*, *18*(1), 48–75.

Petch, T. (1934). Notes on entomogenous fungi. *Transactions of the British Mycological Society*, *19*, 160–194.

Petch, T. (1937). Notes on entomogenous fungi. *Transactions of the British Mycological Society*, *21*(1–2), 34–67.

Petch, T. (1942). Notes on entomogenous fungi. *Transactions of the British Mycological Society*, *25*, 250–264.

Qin, Y., Chen, L., Liang, X. J., Mi, Y. N., Xiao, J., & Zhang, S. H. (2016). HPLC fingerprint of *Ophiocordyceps xuefengensis*. *Journal of Hunan University of CM*, *32*, 47–50.

Quandt, C. A., Kepler, R. M., Gams, W., Araújo, J. P. M., Ban, S., Evans, H. C., . . .Spatafora, J. W. (2014). Phylogenetic-based nomenclatural proposals for Ophiocordycipitaceae (Hypocreales) with new combinations in *Tolypocladium*. *IMA Fungus*, *5*, 121–134.

Reddy, G. V. P., Tangtrakulwanich, K., Wu, S. H., Miller, J. H., Ophus, V. L., Prewett, J., . . .Jaronski, S. T. (2014). Evaluation of the effectiveness of entomopathogens for the management of wireworms (Coleoptera: Elateridae) on spring wheat. *Journal of Invertebrate Pathology*, *120*, 43–49.

Rehner, S. A., Minnis, A. M., Sung, G. H., Luangsa-ard, J. J., Devotto, L., & Humber, R. A. (2011). Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. *Mycologia*, *103*, 1055–1073.

Ren, Y. F., & Zhu, R. (2013). A review of drag research and prevention and treatment of kidney disease about the Chinese traditional medicine, Chanhua. *Henan Traditional Chinese Medicine*, *33*, 781–784.

Robene, I., Jouen, E., Pastou, D., Payet-Hoareau, M., Goble, T., Linderme, D., . . .Costet, L. (2015). Description and phylogenetic placement of *Beauveria hoplocheli* sp. nov. used in the biological control of the sugarcane white grub, *Hoplochelus marginalis*, on Reunion Island. *Mycologia, 107*, 1221–1232.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*, 406–425.

Samson, R. A. (1974). *Paecilomyces* and some allied hyphomycetes. *Studies in Mycology*, *6*, 1–119.

Samson, R. A., Evans, H. C., & Hoekstra, S. (1982). Notes on entomogenous fungi from Ghana, VI. *Mycology*, *85*(4), 589–605.

Samson, R. A., & Evans, H. C. (1985). New and rare entomogenous fungi from Amazonia. *Proceedings of the Indian Academy of Sciences (Plant Science)*, *94*, 309–317.

Sanjuan, T., Tabima, J., Restrepo, S., Læssøe, T., Spatafora, J. W., & Franco-Molano, A. E. (2014). Entomopathogens of Amazonian stick insects and locusts are members of the *Beauveria* species complex (*Cordyceps* sensu stricto). *Mycologia*, *106*, 260–275.

Sanjuan, T. I., Franco-Molano, A. E., Kepler, R. M., Spatafora, J. W., Tabima, J., Vasco-Palacios, A. M., . . .Restrepo, S. (2015). Five new species of entomopathogenic fungi from the Amazon and evolution of neotropical *Ophiocordyceps*. *Fungal Biology, 119*(10), 901–916.

Sato, H., & Shimazu, M. (2002). Stromata production for *Cordyceps militaris* (Clavicipitales: Clavicipitaceae) by injection of hyphal bodies to alternative host insects. *Applied Entomology and Zoology*, *37*(1), 85–92.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., . . .Miller, A. N. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences, 109*(16), 6241–6246.

Scorsetti, A. C., Elíades, L. A., Stenglein, S. A., Cabello, M. N., Pelizza, S. A., & Saparrat, M. C. (2012). Pathogenic and enzyme activities of the entomopathogenic fungus *Tolypocladium cylindrosporum* (Ascomycota: Hypocreales) from Tierra del Fuego, Argentina. *Revista de Biología Tropical 60*(2), 833–841.

Sharma, S. K., Gautam, N., & Atri, N. S. (2015). Optimized extraction, composition, antioxidant and antimicrobial activities of exo and intracellular polysaccharides from submerged culture of *Cordyceps cicadae*. *BMC Complementary and Alternative Medicine, 15*, 446.

Shimizu, D. (1994). *Color iconography of vegetable wasps and plant worms*. Seibundo Shinkosha.

Shimizu, D. (1997). *Illustrated vegetable wasps and plant worms in color*. Ie-No-Hikari Association.

Shing, S. Z. (1975). Classfications of ‘Big Chan-Cao’ and ‘Small Chan-Cao’. *Acta Microbiologica Sinica*, *15*, 21–26.

Shinya, R., Watanabe, A., Aiuchi, D., Tani, M., Kuramochi, K., Kushida, A., & Koike, M. (2008). Potential of *Verticillium lecanii* (*Lecanicillium* spp.) hybrid strains as biological control agents for soybean cyst nematode: Is protoplast fusion an effective tool for development of plant-parasitic nematode control agents. *Japanese Journal of Nematology, 38*(1), 9–18.

Shrestha, B. (2011). Diversity of *Cordyceps* fungi in Nepal. *Nepal Journal of Science and Technology*, *12*, 103–110.

Shrestha, B., Zhang, W. M., Zhang, Y. J., & Liu, X. Z. (2012). The medicinal fungus *Cordyceps militaris*: Research and development. *Mycological Progress*, *11*(3), 599–614.

Shrestha, B., Tanaka, E., Hyun, M. W., Han, J. G., Kim, C. S., Jo, J. W., . . .Sung, G. H. (2016). Coleopteran and Lepidopteran hosts of the entomopathogenic genus *Cordyceps* sensu lato. *Journal of Mycology*. https://doi.org/10.1155/2016/7648219

Shrestha, B., Sung, G. H., & Sung, J. M. (2017a). Current nomenclatural changes in *Cordyceps* sensu lato and its multidisciplinary impacts. *Mycology*, *8*(4), 293–302.

Shrestha, B., Tanaka, E., Hyun, M. W., Han, J. G., Kim, C. S., Jo. J. W., . . .Sung, G. H. (2017b). Mycosphere Essay 19, *Cordyceps* species parasitizing hymenopteran and hemipteran insects. *Mycosphere*, *8*, 1424–1442.

Spatafora, J. W., Sung, G. H., Sung, J. M., Hywel-Jones, N. L., & White, J. J. F. (2007). Phylogenetic evidence for an animal pathogen origin of ergot and the grass endophytes. *Molecular Ecology*, *16*(8), 1701–1711.

Stensrud, O., Hywel-Jones, N. L., & Schumacher, T. (2005). Towards a phylogenetic classification of *Cordyceps*: ITS nrDNA sequence data confirm divergent lineages and paraphyly. *Mycological Research*, *109*, 41–56.

Su, C. H., & Wang, H. H. (1986). *Phytocordyceps*, a new genus of the Clavicipitaceae. *Mycotaxon*, *26*, 337–344.

Sufyan, M., Abbasi, A., Gogi, M. D., Arshad, M., Nawaz, A., & Neuhoff, D. (2017). Efficacy of *Beauveria bassiana* for the management of economically important wireworm species (Coleoptera: Elateridae) in organic farming. *Gesunde Pflanzen*, *69*(4), 197–202.

Sung, G. H., Hywel-Jones, N. L., Sung, J. M., Luangsa-ard, J. J., Shrestha, B., & Spatafora, J. W. (2007). Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. *Studies in Mycology*, *57*, 5–69.

Sung, G. H., Shrestha, B., Han, S. K., Kim, S. Y., & Sung, J. M. (2010). Growth and cultural characteristics of *Cordyceps cardinalis* collected from Korea. *Mycobiology*, *38*, 274–281.

Sung, G. H., Shrestha, B., Han, S. K., & Sung, J. M. (2011). Cultural characteristics of *Ophiocordyceps heteropoda* collected from Korea. *Mycobiology*, *39*, 1–6.

Swofford, D. L. (2002). *PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10*. Sinauer Associates.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*, 2725–2729.

Tang, A. M. C., Jeewon, R., & Hyde, K. D. (2007). Phylogenetic utility of protein (RPB2, β- tubulin) and ribosomal (LSU, SSU) gene sequences in the systematics of Sordariomycetes (Ascomycota, Fungi). *Antonie van Leeuwenhoek*, *91*, 327–349.

Taylor, J. W. (2011). One Fungus = One Name: DNA and fungal nomenclature twenty years after PCR. *IMA Fungus*, *2*(2), 113–120.

Teng, S. C. (1936). Additional fungi from China IV. *Sinensia*, *7*, 752–823.

Tzean, S. S., Hsieh, L. S., Chen, J. L., & Wu, W. J. (1992). *Nomuraea viridulus*, a new entomogenous fungus from Taiwan. *Mycologia*, *84*, 781–786.

Vega, F. E., Goettel, M. S., Blackwell, M., Chandler, D., Jackson, M. A., Keller, S., . . .Roy, H. E. (2009). Fungal entomopathogens: New insights on their ecology. *Fungal Ecology*, *2*, 149–159.

Wan, Y. H., Cai, K. M., & Wei, S. (2013). Herbal research and biology of ‘Chaihua’. *Journal of Jiangsu Forestry Science & Technology*, *40*, 50–53.

Wang, Y. B. (2016). *Studies on phylogeny of Polycephalomycetaceae fam. nov., with microbial diversities of Polycephalomyces multiramosus and its host* (Doctor dissertation). Yunnan University.

Wang, X. L., & Yao, Y. J. (2011). Host insect species of *Ophiocordyceps sinensis*: A review. *ZooKeys*, *127*, 43–59.

Wang, L., Zhang, W. M., Hu, B., Chen, Y. Q., & Qu, L. H. (2008). Genetic variation of *Cordyceps militaris* and its allies based on phylogenetic analysis of rDNA ITS sequence data. *Fungal Diversity*, *31*, 147–155.

Wang, W. J., Wang, X. L., Li, Y., Xiao, S. R., Kepler, R. M., & Yao, Y. J. (2012). Molecular and morphological studies of *Paecilomyces sinensis* reveal a new clade in clavicipitaceous fungi and its new systematic position. *Syst. Biodivers.*, *10*, 221–232.

Wang, H. L., Zhang, J., Sit, W. H., Lee, C. Y. J., & Wan, J. M. F. (2014a). *Cordyceps cicadae* induces G2/M cell cycle arrest in MHCC97H human hepatocellular carcinoma cells: A proteomic study. *Chinese Medicine*, *9*, 1–12.

Wang, L., Li, H. H., Chen, Y. Q., Zhang, W. M., & Qu, L. H. (2014b). *Polycephalomyces lianzhouensis* sp. nov., a new species, co-occurs with *Ophiocordyceps crinalis*. *Mycological Progress*, *13*, 1089–1096.

Wang, Y. B., Yu, H., Dai, Y. D., Chen, Z. H., Zeng, W. B., Yuan, F., . . .Liang, Z. Q. (2015). *Polycephalomyces yunnanensis* (Hypocreales), a new species of *Polycephalomyces* parasitizing *Ophiocordyceps nutans* and stink bugs (hemipteran adults). *Phytotaxa*, *208*(1), 34–44.

Wen, T. C., Zhu, R. C., Kang, J. C., Huang, M. H., Tan, D. B., Ariyawansha, H., . . .Liu, H. (2013). *Ophiocordyceps xuefengensis* sp. nov. from larvae of *Phassus nodus* (Hepialidae) in Hunan Province, southern China. *Phytotaxa, 123*(1), 41–50.

Wen, T. C., Xiao, Y. P., Li W. J., Kang, J. C., & Hyde, K. D. (2014). Systematic analyses of *Ophiocordyceps ramosissimum* sp. nov., a new species from a larvae of Hepialidae in China. *Phytotaxa*, *161*, 227–234.

Wen, T. C., Zha, L. S., Xiao, Y. P., Wang, Q., Kang, J. C., & Hyde, K. D. (2015). *Metacordyceps shibinensis* sp. nov. from larvae of Lepidoptera in Guizhou Province, southwest China. *Phytotaxa*, *226*(1), 51–62.

Wen, T. C., Xiao, Y. P., Zha, L. S., Hyde, K. D., & Kang, J. C. (2016b). Multigene phylogeny and morphology reveal a new species, *Ophiocordyceps tettigonia*, from Guizhou Province, China. *Phytotaxa*, *208*, 141–151.

Wen, T. C., Zha, L. S., Kang, J. C., & Hyde, K. D. (2016c). Some entomological issues in studying entomogenous fungi. *Mycosystema*, *35*(11), 1303–1309.

Wen, T. C., Zha, L. S., Kang, J. C., & Hyde, K. D. (2017). Problems and prospects of research and development of *Cordyceps militaris*. *Mycosystema*, *36*(1), 14–27.

White, T. J., Bruns, T., Lee, S., & Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White (Eds.), *PCR protocols: A guide to methods and applications*. New York: Academic Press.

Xiao, Y. P., Hongsanan, S., Hyde, K. D., Brooks, S., Xie, N., Long, F. Y, & Wen, T. C. (2019). Two new entomopathogenic species of *Ophiocordyceps* in Thailand. *MycoKeys*, *47*, 53–74.

Yahagi, N. (2008). Illustrated catalogue of Japanese *Cordyceps* (entomogenous fungi): The Yahagi collection of Japanese *Cordyceps* stored in the Tohoku University Museum. *Bulletin of the Tohoku University Museum*, (8), 29–89.

Yan, J. Q., & Tolgor, B. (2015). *Cordyceps ningxiaensis* sp. nov., a new species from dipteran pupae in Ningxia Hui Autonomous Region of China. *Nova Hedwigia*, *100*, 251–258.

Yang, S. B. (2004). *The taxonomic diversity and conservation of Cordyceps Fr. from Changbai Mountains* (Master thesis). Jilin Agricultural University.

Yokoyama, E., Arakawa, M., Yamagishi, K., & Hara, A. (2006). Phylogenetic and structural analyses of themating-type loci in Clavicipitaceae. *FEMS Microbiology Letters*, *264*, 182–191.

Yu, S. J., Chai, X. Y., & Fan, M. Z. (2015). Analysis of nutritional and bioactive components of solid-state fermented substrate of *Isaria cicadae*. *Food & Machinery*, *31*, 155–158.

Yuan, L. P. (2014). The Xuefeng Mountain, a famous Chinese mountain. *Forest & Humankind*, *8*, 42–49.

Zha, L. S., Wen, T. C., Kang, J. C., & Hyde, K. D (2015). The genus *Bolivaritettix* in Thailand (Orthoptera: Tetrigidae: Metrodorinae), with three new species and one new record. *Entomological News*, *125*, 136–146.

Zha, L. S., Huang, S. K., Xiao, Y. P., Boonmee, S., Eungwanichayapant, P. D., McKenzie, E. H. C., . . .Wen, T. C. (2018). An evaluation of common *Cordyceps* (Ascomycetes) species found in Chinese markets. *International Journal of Medicinal Mushrooms*, *20*, 1149–1162.

Zha, L. S., Wen, T. C., Huang, S. K., Boonmee, S., Eungwanichayapant, P. D., & Hyde K. D. (2019a). Taxonomy and biology of *Cordyceps qingchengensis* sp. nov. and its allies. *Phytotaxa*, *416*(1), 14–24.

Zha, L. S., Wen, T. C., Jeewon, R., Xie, Z. M., Boonmee, S., Eungwanichayapant, P. D., . . .Hyde, K. D. (2019b). Xuefeng *Cordyceps*: Insights into species diversity, life cycle and host association. *Current Science*, *117*(5), 839–846.

Zha, L. S., Xiao, Y. P., Jeewon, R., Zu, X., Wang, X., Boonmee, S., . . .Wen, T. C. (2019c). Notes on the medicinal mushroom Chanhua (*Cordyceps cicadae* (Miq.) Massee). *Chiang Mai Journal of Science*, *46*(6), 1023–1035.

Zha, L. S., Kryukov, V. Y., Ding, J. H., Jeewon, R., & Chomnunti, P. (2021). Novel taxa and species diversity of *Cordyceps* sensu lato (Hypocreales, Ascomycota) developing on wireworms (Elateroidea and Tenebrionoidea, Coleoptera). *MycoKeys*, *78*, 79–117.

Zhang, W. M., Li, T. H., Chen, V. Q., & Qu, L. H. (2004). *Cordyceps campsosterna*, a new pathogen of *Campsosternus auratus*. *Fungal Diversity*, *17*, 239–242.

Zhang, X. Y. (2013). *Study on Cordyceps species and its allies from bamboo forest* (Master thesis). Anhui Agricultural University.

Zhang, S. L., He, L. M., Chen, X., & Huang, B. (2013). *Beauveria lii* sp. nov. isolated from *Henosepilachna vigintioctopunctata*. *Mycotaxon*, *121*, 199–206.

Zhang, S. H., Cai, P., Chen, L., Liang, X. J., Qin, Y., Zhu, R. C., . . .Huang, H. Y. (2015). Identification of chemical constituents in *Ophiocordyceps xuefengensis* sp. nov. by HPLC-Q-TOF-MS/MS. *Chinese Traditional and Herbal Drugs*, *46*, 817–821.

Zheng, B., Xie, F. Y., Cai, G. H., Zhu, R. C., Li, K., Gao, S. Q., . . .Hao, X. Y. (2015). Effects of *Ophiocordyceps xuefengensis* on proliferation of DC-CIK cells and activity of killing HepG-2 cells by DC-CIK cells. *Chinese Journal of Immunology*, *31*, 189–192.

Zhou, Y. M., Zou X., Qu, J. J., & Liang, Z. Q. (2015). A new record of the genus *Ophiocordyceps* from China. *Microbiology China*, *42,* 1710–1716.

Zhu, H. F., Wang, L. Y., & Han, H. X. (2004). *Fauna sinica, Insecta, vol. 38, Lepidoptera, Hepialidae, Epiplemidae.* Science Press.

Zhu, R., Zheng, R., Deng, Y. Y., Chen, Y. P., & Zhang, S. W. (2014). Ergosterol peroxide from *Cordyceps cicadae* ameliorates TGF-β1-induced activation of kidney fibroblasts. *Phytomedicine*, *21*, 372–378.

Zhu, R. C., Tan, D. B., Qin, Y., Liang, X. J., Zhang, S. H., & Huang, H. Y. (2015). Study on dynamic changes of adenosine in *Ophiocordyceps xuefengensis* from different harvest time. *Modern Chinese Medicine*, *17*, 1180–1183.

Zimmermann, G. (2008). The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): Biology, ecology and use in biological control. *Biocontrol Science and Technology*, *18*(9), 865–901.

APPENDIX

APPENDIX

PUBLICATIONS

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RESEARCH ARTICLE

MycoKeys

Novel taxa and species diversity of *Cordyceps* sensu lato (Hypocreales, Ascomycota) developing on wireworms (Elateroidea and Tenebrionoidea, Coleoptera)

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Abstract

Species of *Cordyceps* sensu lato (Hypocreales, Sordariomycetes) have always attracted much scientific attention for their abundant species diversity, important medicinal values and biological control applications. The insect superfamilies Elateroidea and Tenebrionoidea are two large groups of Coleoptera and their larvae are generally called wireworms. Most wireworms inhabit humid soil or fallen wood and are often infected with *Cordyceps* s.l. However, the species diversity of *Cordyceps* s.l. on Elateroidea and Tenebrionoidea is poorly known. In the present work, we summarise taxonomic information of 63 *Cordyceps* s.l. species that have been reported as pathogens of wireworms. We review their hosts and geographic distributions and provide taxonomic notes for species. Of those, 60 fungal species are accepted as natural pathogens of wireworms and three species (*Cordyceps militaris*, *Ophiocordyceps ferruginosa* and *O. variabilis*) are excluded. Two new species, *O. borealis* from Russia (Primorsky Krai) and *O. spicatus* from China (Guizhou), are described and compared with their closest allies. *Polycephalomyces formosus* is also described because it is reported as a pathogen of wireworms for the first time. Phylogeny was reconstructed from a combined dataset, comprising SSU, LSU and TEF1-α gene sequences. The results, presented in this study, support the establishment of the new species and confirm the identification of *P. formosus*.

Keywords

Two new species, Elateridae, molecular phylogeny, *Ophiocordyceps*, taxonomy, Tenebrionidae

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Taxonomical clarification of *Tetrix gibberosa* (Wang & Zheng), a high-backed pygmy grasshopper species from eastern PR China (Orthoptera: Tetrigidae)

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Abstract

Tetrix gibberosa (Wang & Zheng) is a high-backed pygmy grasshopper species from eastern PR China. Due to its reduced hind wings and pleomorphism (length changes of hind wings and the hind pronotal process, which is generally called macropterous and brachypterous morphs), the species have been described into different species which involve several taxonomically confused genera. This study clarifies its taxonomy and distribution and provides ecological information for the species. At the same time, we comment the relationships of related genera in the subfamily Tetriginae, including *Tetrix* Latreille, *Exothotettix* Zheng & Jiang, *Alulatettix* Liang, *Aalatettix* Zheng & Mao, *Formosatettix* Tinkham, and *Formosatettixoides* Zheng. Additionally, we report for the first time that nematodes can parasitize pygmy grasshoppers.

New synonyms are proposed: *Tetrix gibberosa* (Wang & Zheng, 1993) = *Alulatettix bulbosus* Zheng & Zhong, 2001, **syn. nov.**, = *Exothotettix jiangxiensis* Liang & Jia, 2008, **syn. nov.**, = *Tetrix glochinota* Zhao, Niu & Zheng, 2010, **syn. nov.**, = *Alulatettix nigromarginalis* Zhang, Deng & Zha, 2014, **syn. nov.**, = *Alulatettix flavotibialis* Zhang, Deng & Zha, 2014, **syn. nov.**.

Key words: Tetrigoidea, Tetriginae, taxonomy, synonym, pleomorphism

Introduction

We are doing taxonomic revisions to the family Tetrigidae from Southeast Asia. In this family, the shape and size of flying organs are usually different among individuals, and these have always been treated as valid taxonomic traits. But in practice, fore and hind wings of many Tetrigidae species are reduced and even absent; they are partially or completely covered by pronota and can not be observed if not uncovering the pronota. As a result, flying organs of many related species have been incorrectly described, which made numerous confusions to the taxonomy of the family. For this reason and on the basis of taxonomical practices, Zha *et al.* (2016, 2017, 2020) discussed the evolutionary relationship between tegmen (fore wing) and the tegminal sinus, and they classified the flying organs of the family into four types (normal, abbreviated, vestigial and apterous). Following these theories, taxonomic confusions of those taxa with reduced or absent flying organs have been or will be clarified.

Another typical taxonomic confusion of the family is the pleomorphism of hind wings and the hind pronotal process (also called macropterous and brachypterous morphs). On the basis of practical observations and morphological evidence of some species, Zha *et al.* (2021) outlined the intraspecific variation relationship of hind wings and the hind process (with the extension of hind process, the hind wings will gradually become longer than the process itself; on the contrary, with the reduction of hind process, the hind wings will gradually become shorter than the process). This pleomorphism has already been proved on many species of *Phaesticus* Uvarov, *Tetrix* Latreille, and *Thoradonta* Hancock (Storozhenko & Dawwrueng, 2015; Ding *et al.*, 2021; Zha *et al.*, 2021).

Tetrix gibberosa (Wang & Zheng) is a common pygmy grasshopper species distributed in eastern PR China. With reduced hind wings and the pleomorphism of hind wings and the hind pronotal process, the species have been

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Taxonomic revision of *Phaesticus* Uvarov and synonymy with *Flatocerus* Liang & Zheng syn. nov. (Orthoptera: Tetrigidae)

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Abstract

The genus *Phaesticus* Uvarov, 1940 is revised here with updated generic characteristics and a key to the species. New synonyms and a new combination are proposed: 1) *Phaesticus* Uvarov, 1940 = *Flatocerus* Liang & Zheng, 1984 **syn. nov.**; 2) *P. mellerborgi* (Stål, 1855) = *P. insularis* (Hancock, 1907) **syn. nov.**, = *P. carinatus* Zheng, 1998 **syn. nov.**, = *P. azemii* Mahmood, Idris & Salmah, 2007 **syn. nov.**, = *F. brachynotus* Liang, Chen & Chen, 2008 **syn. nov.**, = *P. uvarovi* Storozhenko & Dawwrueng, 2015 **syn. nov.**; 3) *P. moniliantennatus* (Günther, 1940) = *F. nankunshanensis* Liang & Zheng, 1984 **syn. nov.**, = *F. wuyishanensis* Zheng, 1991 **syn. nov.**, = *F. guizhouensis* Wang, 1992 **syn. nov.**, = *F. daqingshanensis* Zheng & Jiang, 1998 **syn. nov.**, = *F. dentifemura* Zheng, 2003 **syn. nov.**; 4) *P. hainanensis* (Liang, 1988) **comb. nov.**, = *F. hainanensis* Liang, 1988. Intraspecific variation in the lengths of the hind pronotal processes and hind wings in the family Tetrigidae are discussed in detail.

Key words: Tetrigoidea, pygmy grasshopper, new combination, intraspecific variation, China

Introduction

The genera *Phaesticus* Uvarov, 1940 and *Flatocerus* Liang & Zheng, 1984 are morphologically closely similar. The name *Phaesticus* came from *Phaestus* Bolívar, 1887, originally established to include only *Tettix mellerborgi* Stål from Indonesia (Java) (Bolívar, 1887). Since the generic name '*Phaestus*' was previously occupied by two genera in the orders Hymenoptera (*Phaestus* Förster) and Coleoptera (*Phaestus* Jacoby), the names *Phaesticus* Uvarov, 1940 and *Lampranges* Blackith, 1992 were orderly proposed to replace the name *Phaestus* Bolívar. Considering the double generic names and the priority rule, Otte (1997) synonymized *Lampranges* with *Phaesticus*. Taxonomy of *Phaesticus* was dynamic. The genus was assigned to the section Tripetalocerae (originally assigned by Bolívar (1887), = Tripetalocerinae), subfamily Tetriginae (transferred by Otte (1997) without any comments), and subfamily Discotettiginae (Hancock, 1907; Günther, 1938; Tumbrinck, 2014). Skejo (2016) considered Discotettiginae as a synonym of the subfamily Scelimeninae based on its type genus *Discotettix* Costa, and this suggestion has been adopted in the current taxonomy of Tetrigidae (Cigliano *et al.*, 2021). As a result, *Phaesticus* has temporarily been placed in Scelimeninae, even though Skejo (2017) noted that it definitely does not belong to Scelimeninae. In other words, *Phaesticus* (and *Flatocerus*) were left without subfamily assignment. *Phaesticus* currently comprises six species distributed in Indonesia (Java, Sumatra), West Malaysia, Thailand, Singapore, northern Vietnam, PR China (Yunnan, Fujian), and probably India (Assam, Mizoram) (Zheng, 2005; Skejo, 2017).

The genus *Flatocerus* was established for *F. nankunshanensis* Liang & Zheng from Guangdong, PR China, and separated from *Phaesticus* mainly by the shape and number of broadened antennal segments (Liang & Zheng, 1984). Currently, the genus *Flatocerus* includes seven species, which were all reported in PR China (Hainan, Guangxi, Guangdong, Guizhou, Fujian, and Hunan).

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Two new species and one new synonymy of the pygmy grasshopper genus *Formosatettix* Tinkham, (Orthoptera: Tetrigidae) from China

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ABSTRACT

Formosatettix nanjiensis: Zha & Ding **sp. nov.** and *F. taibaiensis* Zha & Ding **sp. nov.** are described from Zhejiang and Shaanxi provinces of China, respectively. New synonymy is established: *Formosatettix tianmushanensis* Zheng & Li, 2001 = *Formosatettix zhejiangensis* Deng, 2019 **syn. nov.** An updated species key to the genus *Formosatettix* Tinkham, 1937 is provided.

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Introduction

The genus *Formosatettix* Tinkham, 1937 (Orthoptera: Tetrigidae: Tetriginae) was erected for two species, *F. arisanensis* Tinkham, 1937 (type species) and *F. karenkoensis* Tinkham, 1937, both from China (Taiwan, = Formosa) (Tinkham 1937). Nowadays it includes 68 species widely distributed in China (57 species), Russia (1 species), Korea (2 species), Japan (5 species), Pakistan (2 species), and Nepal (6 species) (Cigliano et al. 2019; Wei et al. 2019). *Formosatettix* is most similar to the genera *Alulatettix* Liang, 1993 (with 21 species from China and Japan) and *Formosatettixoides* Zheng, 1994 (with 11 species from China, Korea, and Nepal), but the former has conspicuous tegminal sinus (Liang 1993; Zhang et al. 2014), and the latter has visible tegmina and hind wings (not uncovering the pronotum; Zheng 1994; Zha and Zheng 2014), while in *Formosatettix* the tegminal sinus is absent and tegmina and hind wings are both invisible (not uncovering the pronotum).

In this paper, we describe two new members of *Formosatettix* from China and propose one new synonymy.

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Pygmy grasshoppers (Orthoptera: Tetrigidae) in Xinjiang, China: Species diversity and new synonyms

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Abstract: The species diversity of pygmy grasshoppers in Xinjiang Uygur Autonomous Region, China is determined. Three species, *Tetrix subulata* (Linnaeus, 1758), *Tetrix tartara* (Saussure, 1887), and *Tetrix tuerki* (Krauss, 1876), are recognized as distributed in Xinjiang. New synonyms are proposed: 1) *Tetrix subulata* (Linnaeus, 1758) = *Tetrix subulatoides* Zheng, Zhang, Yang & Wang, 2006, **syn. nov.**; 2) *Tetrix tartara* (Saussure, 1887) = *Tetrix fuhaiensis* Zheng, Zhang, Yang & Wang, 2006, **syn. nov.**, = *Tetrix jingheensis* Liang & Zheng, 1998, **syn. nov.**, = *Tetrix tartara subacuta* Bey-Bienko, 1951, **syn. nov.**, = *Tetrix xinjiangensis* Zheng, 1996, **syn. nov.**; and 3) *Tetrix tuerki* (Krauss, 1876) = *Tetrix torulosifemura* Deng, 2016, ***nomen nudum***. The distribution information for these three *Tetrix* species is updated. We exclude the distribution of *Tetrix bolivari* Saulcy, 1901 in China, and temporarily exclude the distribution of *Tetrix japonica* (Bolívar, 1887) in Xinjiang.

Key words: Tetrigoidea; Tetriginae; distribution

新疆地区蚱类昆虫物种多样性及种类修订（直翅目：蚱科）

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摘要：对新疆维吾尔自治区的蚱类昆虫物种多样性进行了修订和澄清。该地区共记录 3 种蚱：钻形蚱 *Tetrix subulata* (Linnaeus, 1758)、隆背蚱 *Tetrix tartara* (Saussure, 1887) 和土氏蚱 *Tetrix tuerki* (Krauss, 1876)。同物异名修订如下：1) 钻形蚱 *Tetrix subulata* (Linnaeus, 1758) = 拟钻形蚱 *Tetrix subulatoides* Zheng, Zhang, Yang & Wang, 2006, **syn. nov.**; 2) 隆背蚱 *Tetrix tartara* (Saussure, 1887) = 福海蚱 *Tetrix fuhaiensis* Zheng, Zhang, Yang & Wang, 2006, **syn. nov.**, = 精河蚱 *Tetrix jingheensis* Liang & Zheng, 1998, **syn. nov.**, = 亚锐隆背蚱 *Tetrix tartara subacuta* Bey-Bienko, 1951, **syn. nov.**, = 新疆蚱 *Tetrix xinjiangensis* Zheng, 1996, **syn. nov.**；3) 土氏蚱 *Tetrix tuerki* (Krauss, 1876) = 瘤股蚱 *Tetrix torulosifemura* Deng, 2016, ***nomen nudum***。本文更新了 3 种蚱的分布信息，排除了波氏蚱 *Tetrix bolivari* Saulcy, 1901 在中国、以及暂时排除日本蚱 *Tetrix japonica* (Bolívar, 1887) 在新疆的分布。

关键词：蚱总科；蚱亚科；分布

Introduction

Xinjiang Uygur Autonomous Region (34°25′–48°10′N, 73°40′–96°18′E, abbr. Xinjiang), the largest provincial level district (1.66 million km^2, 1/6 of the land area of China), is located in the northwest of China, and is the hinterland of the Eurasian Continent. Xinjiang is

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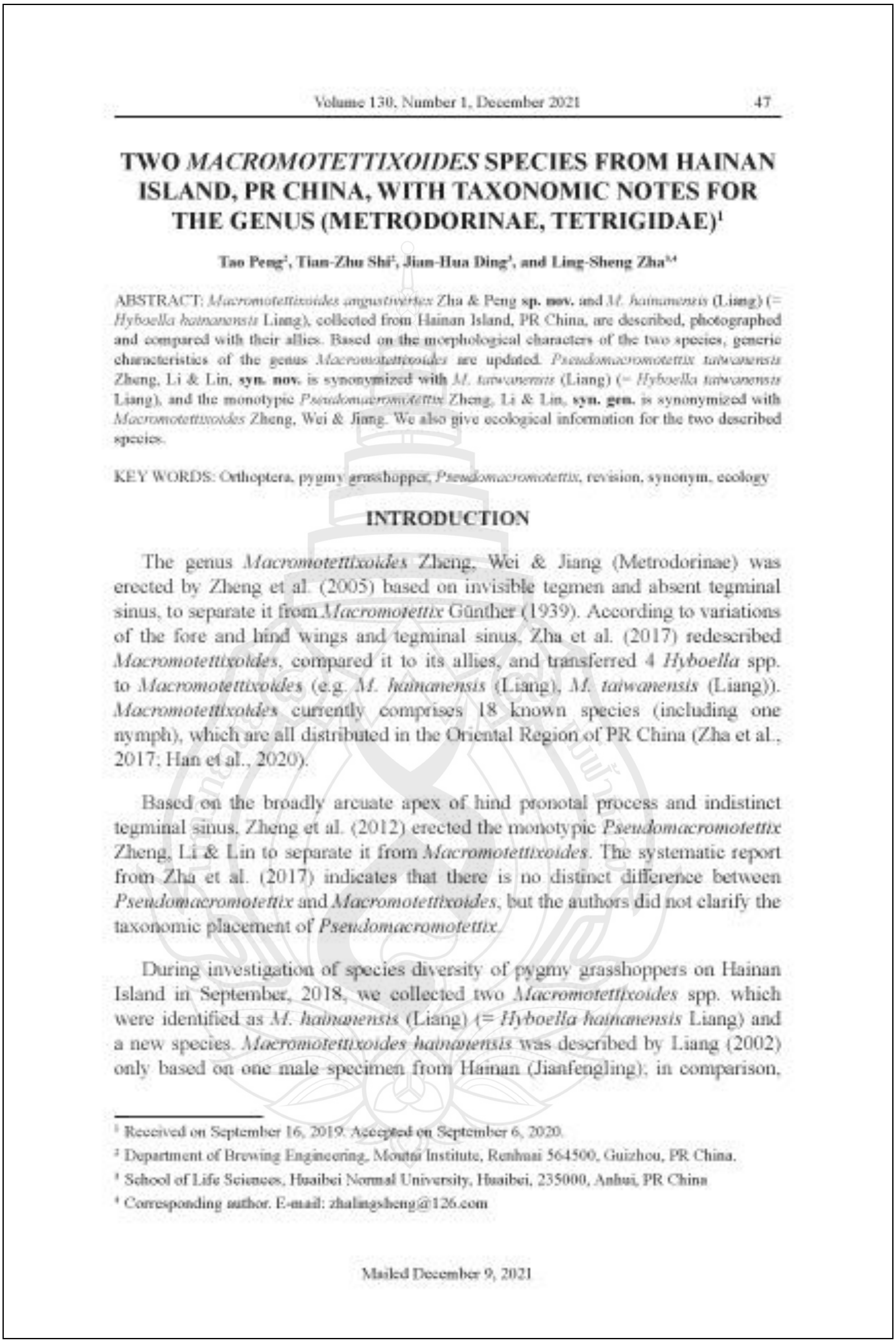

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TWO *MACROMOTETTIXOIDES* SPECIES FROM HAINAN ISLAND, PR CHINA, WITH TAXONOMIC NOTES FOR THE GENUS (METRODORINAE, TETRIGIDAE)[1]

Tao Peng[2], Tian-Zhu Shi[2], Jian-Hua Ding[3], and Ling-Sheng Zha[3,4]

ABSTRACT: *Macromotettixoides angustivertex* Zha & Peng **sp. nov.** and *M. hainanensis* (Liang) (= *Hyboella hainanensis* Liang), collected from Hainan Island, PR China, are described, photographed and compared with their allies. Based on the morphological characters of the two species, generic characteristics of the genus *Macromotettixoides* are updated. *Pseudomacromotettix taiwanensis* Zheng, Li & Lin, **syn. nov.** is synonymized with *M. taiwanensis* (Liang) (= *Hyboella taiwanensis* Liang), and the monotypic *Pseudomacromotettix* Zheng, Li & Lin, **syn. gen.** is synonymized with *Macromotettixoides* Zheng, Wei & Jiang. We also give ecological information for the two described species.

KEY WORDS: Orthoptera, pygmy grasshopper, *Pseudomacromotettix*, revision, synonym, ecology

INTRODUCTION

The genus *Macromotettixoides* Zheng, Wei & Jiang (Metrodorinae) was erected by Zheng et al. (2005) based on invisible tegmen and absent tegminal sinus, to separate it from *Macromotettix* Günther (1939). According to variations of the fore and hind wings and tegminal sinus, Zha et al. (2017) redescribed *Macromotettixoides*, compared it to its allies, and transferred 4 *Hyboella* spp. to *Macromotettixoides* (e.g. *M. hainanensis* (Liang), *M. taiwanensis* (Liang)). *Macromotettixoides* currently comprises 18 known species (including one nymph), which are all distributed in the Oriental Region of PR China (Zha et al., 2017; Han et al., 2020).

Based on the broadly arcuate apex of hind pronotal process and indistinct tegminal sinus, Zheng et al. (2012) erected the monotypic *Pseudomacromotettix* Zheng, Li & Lin to separate it from *Macromotettixoides*. The systematic report from Zha et al. (2017) indicates that there is no distinct difference between *Pseudomacromotettix* and *Macromotettixoides*, but the authors did not clarify the taxonomic placement of *Pseudomacromotettix*.

During investigation of species diversity of pygmy grasshoppers on Hainan Island in September, 2018, we collected two *Macromotettixoides* spp. which were identified as *M. hainanensis* (Liang) (= *Hyboella hainanensis* Liang) and a new species. *Macromotettixoides hainanensis* was described by Liang (2002) only based on one male specimen from Hainan (Jianfengling); in comparison,

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SAUSSURELLA BREVIFRONS SP. NOV. FROM NORTHERN THAILAND, WITH TAXONOMIC NOTES FOR THE GENUS (ORTHOPTERA: TETRIGIDAE)[1]

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ABSTRACT: *Saussurella brevifrons* sp. nov. Zha is reported from northern Thailand. The species is similar to *S. decurva* and *S. javanica*, but differs from the former by its anterior margin of vertex not protruding forward, lower margin of anterior pronotal process lacking hooked tip, and acutely angled posterior angle of lateral lobe from the former, and from the latter by its wider vertex, larger body size and elongate posterior pronotal process. We describe it with photographs and ecological information. For the taxonomy of the genus *Saussurella*, we suggest a uniform standard for the measurement of the length of anterior pronotal process, and consider the variation of the anterior margin of vertex as an important taxonomical character.

KEY WORDS: Batrachideinae, ecological habit, pygmy locust, *Saussurella decurva*, Tetrigoidea

INTRODUCTION

Saussurella, as the type genus of the tribe Cassitettigini Yin in the subfamily Batrachideinae Bolivar (Orthoptera: Tetrigidae), was erected by Bolivar (1887) with *Saussurella cornuta* (Haan) (= *Acridium* (*Tetrix*) *cornutum* Haan) from Indonesia as its type species (Cigliano et al., 2019; Storozhenko, 2019). The genus can be easily distinguished from other genera of Batrachideinae by a narrow, long and horn-like anterior pronotal process, and a distinct longitudinal sulcus on the dorsal margin of the fore femur (Deng et al., 2017). The genus is comprised of 10 known species currently, which are widely distributed in the Southeast of Asia (Deng et al., 2017). Among them, *S. cornuta* and *S. decurva* (Brunner von Wattenwyl) were sequentially reported in Thailand (Blackith, 1992; Storozhenko and Dawwrueng, 2015).

Species of *Saussurella* are morphologically closely similar; their structural changes mainly come from the horn-like process of anterior pronotum and subgenital plates of female and male. In published literature however, measuring methods of the horn-like process are always confusing, which hampers the identification of the species to a large extent. We are looking forward to determining a uniform standard for the measurement of the horn-like process, and we will try to discover other important structural characters for this genus.

During investigating the species diversity of Tetrigidae in northern Thailand

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文庭池

贵州大学（西南药用生物资源教育部工程研究中心）教授，博导，中国菌物学会和中国微生物学会会员。主要从事真菌资源多样性与药用真菌生物技术的教学和科研工作，已发表新科 2 个，新属 3 个，新种 50 余个；主持国家自然科学基金和省部级科研项目共 6 项，已发表学术论文 40 篇，参编专著 3 部，获专利授权 6 项。

Species diversity and host associations of cordyceps (Hypocreales) parasitic on Orthoptera insects

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Abstract: Totally 25 cordyceps (*Cordyceps sensu lato*) species have been reported on Orthoptera insects. Their hosts and distributions are reviewed and taxonomical notes for species are given. Using combined ITS and *tef1-α* sequence dataset, molecular phylogeny of cordyceps species on Orthoptera insects was constructed. Current species were assigned to three clades: the *Beauveria*, the *Metarhizium* and the *Ophiocordyceps* clades. Based on ecology and habits of the six well known groups of Orthoptera, the relation between species diversity and host of Orthoptera cordyceps is discussed. The rhizomorphs of cordyceps, a special root-like structure situated somewhere between fruiting bodies and synnemata, were observed on *Beauveria* spp. and *Metacordyceps taii* (syn. *Metarhizium guizhouense*). *Beauveria grylli* **comb. nov.** ≡ *Cordyceps grylli* = *Beauveria loeiensis* and *Beauveria kirkii* **comb. nov.** ≡ *Cordyceps kirkii* are proposed. The hosts of *Beauveria grylli* and *Ophiocordyceps tettigonia* are corrected.

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RESEARCH ARTICLE

Two new species of the genus *Formosatettix* Tinkham, 1937 (Orthoptera, Tetrigidae) from Guizhou and Chongqing, PR China

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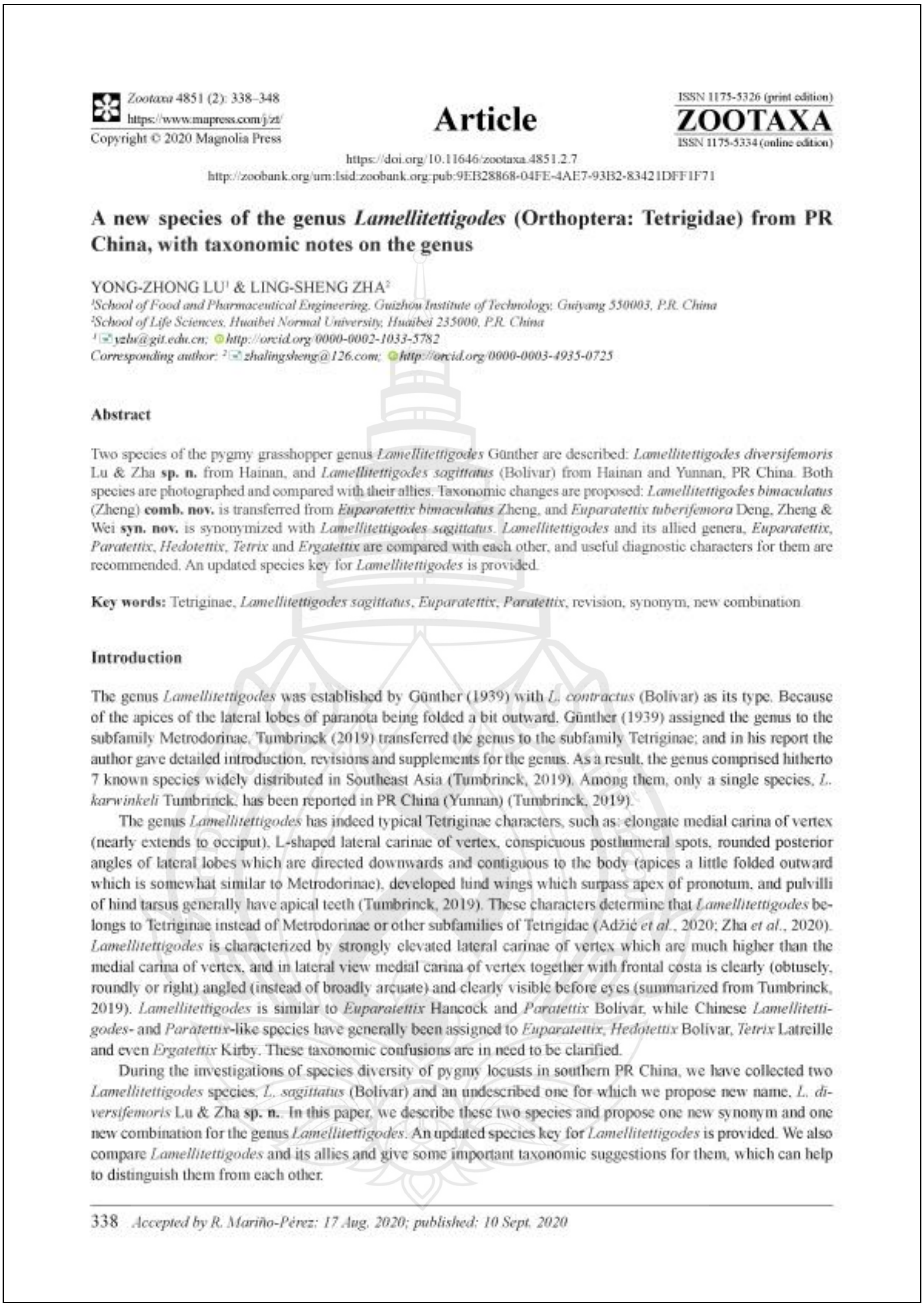
Abstract

Two new pygmy grasshopper species are described from PR China and are assigned to *Formosatettix* Tinkham, 1937, a large Asian tetrigin genus composed of species with reduced tegmina and hind wings: *F. leigongshanensis* Zha & Ding, **sp. nov.** from Guizhou and *F. wulongensis* Zha & Ding, **sp. nov.** from Chongqing. We provide descriptions of morphology and habit, supplemented with photographs. Flying organs of the genus *Formosatettix* are discussed and the genus is compared with other Asian genera with reduced flying organs, such as *Formosatettixoides* Zheng, 1994 and *Alulatettix* Liang, 1993 in Tetriginae, *Deltonotus* Hancock, 1904, *Epitettix* Hancock, 1907 and *Pseudepitettix* Zheng, 1995 in Cladonotinae, and *Macromotettixoides* Zheng, Wei & Jiang, 2005 and *Pseudomacromotettix* Zheng, Li & Lin, 2012 in Metrodorinae.

Keywords
ecology, habit, Karst Region, taxonomy, Tetriginae, Tetrigoidea

Introduction

The genus *Formosatettix* Tinkham, 1937 (subfamily Tetriginae) was originally established for only two species from Taiwan, China – *F. arisanensis* Tinkham, 1937 (type species) and *F. karenkoensis* Tinkham, 1937, but today it is a large genus composed of 68 known species in China, Japan, Korea, Nepal, Pakistan and Russia (Wei et al. 2019, Cigliano et al. 2020). *Formosatettix* is similar to the Tetriginae genera *Alulatettix* Liang, 1993 and



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A new species of the genus *Lamellitettigodes* (Orthoptera: Tetrigidae) from PR China, with taxonomic notes on the genus

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Abstract

Two species of the pygmy grasshopper genus *Lamellitettigodes* Günther are described: *Lamellitettigodes diversifemoris* Lu & Zha **sp. n.** from Hainan, and *Lamellitettigodes sagittatus* (Bolivar) from Hainan and Yunnan, PR China. Both species are photographed and compared with their allies. Taxonomic changes are proposed: *Lamellitettigodes bimaculatus* (Zheng) **comb. nov.** is transferred from *Euparatettix bimaculatus* Zheng, and *Euparatettix tuberifemora* Deng, Zheng & Wei **syn. nov.** is synonymized with *Lamellitettigodes sagittatus*. *Lamellitettigodes* and its allied genera, *Euparatettix*, *Paratettix*, *Hedotettix*, *Tetrix* and *Ergatettix* are compared with each other, and useful diagnostic characters for them are recommended. An updated species key for *Lamellitettigodes* is provided.

Key words: Tetriginae, *Lamellitettigodes sagittatus*, *Euparatettix*, *Paratettix*, revision, synonym, new combination

Introduction

The genus *Lamellitettigodes* was established by Günther (1939) with *L. contractus* (Bolivar) as its type. Because of the apices of the lateral lobes of paranota being folded a bit outward, Günther (1939) assigned the genus to the subfamily Metrodorinae. Tumbrinck (2019) transferred the genus to the subfamily Tetriginae; and in his report the author gave detailed introduction, revisions and supplements for the genus. As a result, the genus comprised hitherto 7 known species widely distributed in Southeast Asia (Tumbrinck, 2019). Among them, only a single species, *L. karwinkeli* Tumbrinck, has been reported in PR China (Yunnan) (Tumbrinck, 2019).

The genus *Lamellitettigodes* has indeed typical Tetriginae characters, such as: elongate medial carina of vertex (nearly extends to occiput), L-shaped lateral carinae of vertex, conspicuous posthumeral spots, rounded posterior angles of lateral lobes which are directed downwards and contiguous to the body (apices a little folded outward which is somewhat similar to Metrodorinae), developed hind wings which surpass apex of pronotum, and pulvilli of hind tarsus generally have apical teeth (Tumbrinck, 2019). These characters determine that *Lamellitettigodes* belongs to Tetriginae instead of Metrodorinae or other subfamilies of Tetrigidae (Adžić *et al.*, 2020; Zha *et al.*, 2020). *Lamellitettigodes* is characterized by strongly elevated lateral carinae of vertex which are much higher than the medial carina of vertex, and in lateral view medial carina of vertex together with frontal costa is clearly (obtusely, roundly or right) angled (instead of broadly arcuate) and clearly visible before eyes (summarized from Tumbrinck, 2019). *Lamellitettigodes* is similar to *Euparatettix* Hancock and *Paratettix* Bolivar, while Chinese *Lamellitettigodes*- and *Paratettix*-like species have generally been assigned to *Euparatettix*, *Hedotettix* Bolivar, *Tetrix* Latreille and even *Ergatettix* Kirby. These taxonomic confusions are in need to be clarified.

During the investigations of species diversity of pygmy locusts in southern PR China, we have collected two *Lamellitettigodes* species, *L. sagittatus* (Bolivar) and an undescribed one for which we propose new name, *L. diversifemoris* Lu & Zha **sp. n.**. In this paper, we describe these two species and propose one new synonym and one new combination for the genus *Lamellitettigodes*. An updated species key for *Lamellitettigodes* is provided. We also compare *Lamellitettigodes* and its allies and give some important taxonomic suggestions for them, which can help to distinguish them from each other.

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Ophiocordyceps tianshanensis sp. nov. on ants from Tianshan mountains, PR China

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Abstract

Ophiocordyceps tianshanensis is a novel species introduced from Xinjiang Uygur Autonomous Region, China. The taxon was discovered with its sexual and asexual specimens on *Camponotus japonicus* (Formicidae) under the bark of humid and decayed wood of *Picea schrenkiana*. With irregularly distorted stromata and distributed in the cold and high-altitude Tianshan Mountains, the species can be seperated from other extant *Ophiocordyceps* species. Morphological and multigene phylogenetic analyses support its novelty. We provide additional ecological data on this cordyceps species from northwestern China.

Keywords: new species, *Camponotus japonicus*, entomopathogenic fungi, ecology, multigene phylogeny, taxonomy, zombie-ant fungi

Introduction

Insects are the largest group of all organisms, currently comprising nearly 940,000 species distributed among 26 orders of the class Insecta. Ants belong to the family Formicidae (10,210 species) in the superfamily Vespoidea (21,862 species) of the order Hymenoptera (118,110 species) (https://www.catalogueoflife.org/). Ants are eusocial insects and widely distributed from arctic to tropical ecosystems; their colonies vary from a few dozen (Jahyny *et al.* 2002) to millions of individuals (Currie *et al.* 2003), and in tropical forests, it has been estimated that they may account for as much as 50 % of animal biomass (Hölldobler & Wilson 2009). They build dry nests in soil, under stones or logs, inside logs, hollow stems or even in plant seeds (Hölldobler & Wilson 1990), and they often use dry vegetation to build their nests (Hölldobler & Wilson 1977). As terrestrial insects, ants are unable to tolerate extremely humid environments where they might be parasitized by entomopathogenic fungi.

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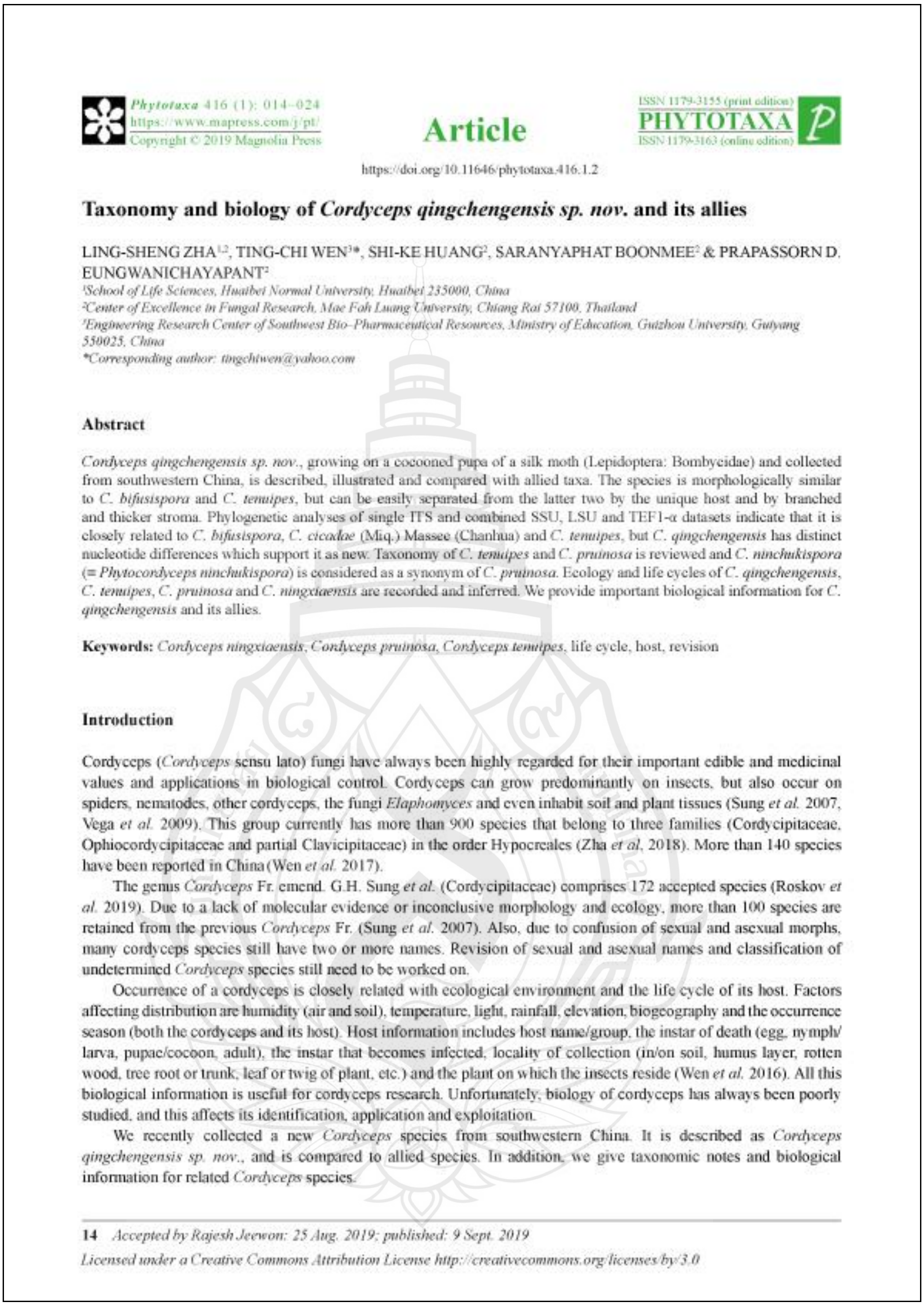

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Taxonomy and biology of *Cordyceps qingchengensis sp. nov*. and its allies

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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-α datasets indicate that it is closely related to *C. bifusispora*, *C. cicadae* (Miq.) Massee (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* (≡ *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.

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Xuefeng Cordyceps: insights into species diversity, life cycle and host association

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Cordyceps (= *Cordyceps* sensu lato) fungi are rather well known as entomopathogens and also for their medicinal importance in curing many diseases. Many cordyceps species with widespread distribution throughout China have been recorded earlier. However, their taxonomy, hosts, life cycles and the plants on which the host insects reside in are poorly understood, and have sometimes led to confusion in the proper identification of the species. In this study, Xuefeng Cordyceps collected from Xuefeng Mountain, Hunan, China are identified and an assessment of their life cycles, proper hosts and plants they are associated with is made through morphological and DNA sequence-based analyses. Morphological characterization reveals that *Ophiocordyceps xuefengensis* from this region as commonly accepted by mycologists and local people. Species collected also include *Beauveria bassiana* (sexual and asexual), *Metacordyceps taii* (syn. of *Metarhizium guizhouense*), *Ophiocordyceps macroacicularis* and *Ophiocordyceps ramosissimum*. Our result challenges previous taxonomic arrangement with regard to host identity. The hosts, previously mistaken for larvae of *Endoclita nodus*, are corrected herein as larvae of *Endoclita davidi* (Lepidoptera: Hepialidae). Ribosomal DNA sequence analyses based on the ITS regions also confirm that *Clerodendrum cyrtophyllum* (Lamiaceae) is the plant on which the host insects reside. Morphological illustrations, where appropriate, are given for the cordyceps species, hosts and plants. The infection mechanism and life cycle of the cordyceps are also outlined.

Keywords: *Clerodendrum cyrtophyllum*, cordyceps, *Endoclita davidi*, traditional Chinese medicine.

XUEFENG Mountain (literal meaning 'mountain with snowy top', because its top is covered with snow for most of the year) is located in west-central Hunan Province, China. It is 350 km long and 80–120 km wide with altitude varying between 240 and 1934 m amsl (Figure 1). The annual average temperature in this region is 12.7°C (varying from –10.1°C to 27.3°C), with relative humidity of more than 87% and abundant rainfall (annual average rainfall of 1810 mm). With heavy fog and frost, winter is rather dry and cold while summer is humid and cool[1].

Cordyceps (= *Cordyceps* sensu lato)[2] fungi are one of the most important groups of invertebrate pathogens[2,3]. Currently, they include more than 900 species[2] belonging to three families (Cordycipitaceae, Ophiocordycipitaceae and partial Clavicipitaceae) in the order Hypocreales[4]. To date, more than 140 species are reported from China[5]. For a long time, Cordyceps research mainly focused on species diversity, medicinal values and biological control[2–5], however, biology, ecology and association with hosts are rather poorly understood.

The common name 'Xuefeng Cordyceps', previously known as 'immortal herbs', had originally been recorded in the middle or late Tang Dynasty (about 1200 years ago), and the species has been intensively used to cure various cancers and other diseases, improve the physique and extend human longevity[6]. Although considered as a precious traditional Chinese medicine (TCM) by local Yao people, this common name was not formally reported until *Ophiocordyceps xuefengensis* T. C. Wen *et al.*, the first species was introduced[7,8]. Molecular phylogenetic analysis indicated that *O. xuefengensis* is closely related with the important TCM *Ophiocordyceps sinensis* (Berk.) G. H. Sung *et al.* (Dong Chong Xia Cao; winter worm, summer herb)[7], also their hosts are all Hepialidae larvae[9]. From then on, the common name Xuefeng Cordyceps came into use, in spite of the second species *O. ramosissimum* T. C. Wen *et al.* being reported, which shares the same host insect that resides on the same plant as the former[10].

Rather than a precious TCM, to a greater extent Xuefeng Cordyceps represent a kind of Chinese traditional culture. In this study, we analyse the species diversity of Xuefeng Cordyceps, and identify their host insects and plants on which the insects reside. We also study the biology and ecology of Xuefeng Cordyceps and their host insects. To recognize the regional cordyceps correctly, and inherit the Chinese traditional culture properly, this study provides important taxonomic, biological and ecological information.

Explorations were made in the Xuefeng Mountain region. Specimens, including the cordyceps, host insects (larvae and pupae), and plants on which the insects resided were observed in the wild and brought to the laboratory for identification. A camera (Nikon Coolpix P520) and a compound microscope (Nikon Eclipse 80i) connected with a camera (Cannon EOS 600D) were used to take photographs. Measurements were made using Tarosoft (R) Image Frame Work software.

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Contributed Paper

Notes on the Medicinal Mushroom Chanhua (*Cordyceps cicadae* (Miq.) Massee)

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ABSTRACT

Chanhua fungus is an important Traditional Chinese Medicine and has a history of use for about 1,500 years. Chanhua has commonly been referred to *Cordyceps cicadae* S.Z. Shing, *Isaria cicadae*, *I. sinclairii*, *Ophiocordyceps sobolifera* and by other names. This has resulted in taxonomic confusion and nomenclatural problems. The biology, ecology and host affiliation of Chanhua are also poorly understood, and these restrict further development and application in various areas, especially in traditional medicine. In this paper, we reviewed the medicinal history and distribution of Chanhua and reinvestigated its phylogenetic relationships with allied species. Based on results obtained we clarified its taxonomy and reviewed its host and its biological and ecological aspects. The phylogeny based on ITS sequence data indicates that Chanhua is an independent species of the genus *Cordyceps*. Although Chanhua, *I. cicadae* and *I. sinclairii* have generally been accepted as the same species, the latter two lack DNA sequence data from their type localities. *C. cicadae* S.Z. Shing (syn. *C. zhejiangensis*), which has been treated as the sexual morph of Chanhua for over 40 years, has been reported to be *Tolypocladium paradoxum*, and the sexual morph of Chanhua is considered to be *C. kobayasii* (syn. *C. cicadae-sm*). We propose to use the scientific name *C. cicadae* (Miq.) Massee, together with the Chinese common name Chanhua, to describe or record this important medicinal mushroom.

Keywords: biology, distribution, *Isaria cicadae*, *Isaria sinclairii*, phylogeny, taxonomy

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PUBLICATIONS

Zha, L. S., Kryukov, V. Y., Ding, J. H., Jeewon, R., & Chomnunti, P. (2021). Novel taxa and species diversity of *Cordyceps* sensu lato (Hypocreales, Ascomycota) developing on wireworms (Elateroidea and Tenebrionoidea, Coleoptera). *MycoKeys*, *78*, 79–117. https://doi.org/10.3897/mycokeys.78.61836

Zha, L. S., Skejo, J., Mao, B. Y., & Ding, J. H. (2021). Taxonomic revision of *Phaesticus* Uvarov and synonymy with *Flatocerus* Liang & Zheng syn. nov. (Orthoptera: Tetrigidae). *Zootaxa*, *4965*(3), 501–514. https://doi.org/10.11646/zootaxa.4965.3.5

Ding, W. J., Ding, J. H., Zhang, H. J., & **Zha, L. S.** (2021) Taxonomical clarification of *Tetrix gibberosa* (Wang & Zheng), a high-backed pygmy grasshopper species from eastern PR China (Orthoptera: Tetrigidae). *Zootaxa*, *5082*(3), 278–285. https://doi.org/10.11646/zootaxa.5082.3.5

Ding, J. H., Pan, Z. X., & **Zha, L. S.** (2021). Two new species and one new synonymy of the pygmy grasshopper genus *Formosatettix* Tinkham, 1937 (Orthoptera: Tetrigidae) from China. *Oriental Insects*, *55*(3), 425–446. https://doi.org/10.1080/00305316.2020.1822944

Ding, J. H., Wang, M., Wu, X. M., & **Zha, L. S.** (2021) Pygmy grasshoppers (Orthoptera: Tetrigidae) in Xinjiang, China: Species diversity and new synonyms. *Entomotaxonomia*, *43*(3): 168–177. https://doi.org/10.11680/entomotax.2021029

Peng, T., Shi, T. Z., Ding, J. H., & **Zha, L. S.** (2021). Two *Macromotettixoides* species from Hainan island, PR China, with taxonomic notes for the genus (Metrodorinae, Tetrigidae). *Entomological News*, *130*(1), 47–60. https://doi.org/10.3157/021.130.0104

Zha, L. S., Chomnunti, P., Ding, J. H., & Zhang, H. J. (2020). *Saussurella brevifrons* sp. nov. from northern Thailand, with taxonomic notes for the genus (Orthoptera: Tetrigidae). *Entomological news*, *129*(4), 367–376. https://doi.org/10.3157/021.129.0404

Zha, L. S., Ye, L., Huang, S. K., Boonmee, S., Eungwanichayapant, P. D., Hyde, K. D., . . .Wen, T. C. (2020). *Cordyceps* fungi (Hypocreales) growing on Orthoptera insects. *Mycosystema*, *39*(4), 707–722. https://doi.org/10.13346/j.mycosystema.190195

Zha, L. S., Wu, X. M., & Ding, J. H. (2020). Two new species of the genus *Formosatettix* Tinkham, 1937 (Orthoptera, Tetrigidae) from Guizhou and Chongqing, PR China. *ZooKeys*, *936*, 61–76. https://doi.org/10.3897/zookeys.936.49552

Lu, Y. Z., & **Zha, L. S.** (2020). A new species of the genus *Lamellitettigodes* (Orthoptera: Tetrigidae) from PR China, with taxonomic notes on the genus. *Zootaxa*, *4851*(2), 338–348. https://doi.org/10.11646/zootaxa.4851.2.7

Wei, D. P., Wanasinghe, D. N., Hyde, K. D., Mortimer, P. E, Xu, J, C., To-Anun, C., . . .**Zha, L. S.** (2020). *Ophiocordyceps tianshanensis* sp. nov. on ants from Tianshan mountains, PR China. *Phytotaxa*, *464*(4): 277–292. https://doi.org/10.11646/phytotaxa.464.4.2

Zha, L. S., Wen, T. C., Huang, S. K., Boonmee, S., Eungwanichayapant, P. D., & Hyde K. D. (2019). Taxonomy and biology of *Cordyceps qingchengensis* sp. nov. and its allies. *Phytotaxa*, *416*(1), 14–24. https://doi.org/10.11646/phytotaxa.416.1.2

Zha, L. S., Wen, T. C., Jeewon, R., Xie, Z. M., Boonmee, S., Eungwanichayapant, P. D., . . .Hyde, K. D. (2019). Xuefeng *Cordyceps*: Insights into species diversity, life cycle and host association. *Current Science*, *117*(5), 839–846. https://doi.org/10.18520/cs/v117/i5/839–846

Zha, L. S., Xiao, Y. P., Jeewon, R., Zu, X., Wang, X., Boonmee, S., …Wen, T. C. (2019). Notes on the medicinal mushroom Chanhua (*Cordyceps cicadae* (Miq.) Massee). *Chiang Mai Journal of Science*, *46*(6), 1023–1035.