BIODIVERSITY AND MOLECULAR PHYLOGENY OF ENTOMOPATHOGENIC FUNGI IN CHIANG MAI PROVINCE, THAILAND

OHNMAR MYO AUNG

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY IN BIOTECHNOLOGY IN PLANT PATHOLOGY

SCHOOL OF GRADUATE STUDIES

KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG

2008

KMITL-2008-AG-D-063-419

ชื่อวิทยานิพนธ์	ความหลากหลายทางชีวภาพและความสัมพันธ์ระดับโมเลกุลของเชื้อราที่ทำให้เกิดโรคกับแมลงในจังหวัดเชียงใหม่ ประเทศไทย
ชื่อนักศึกษา	Miss Ohnmar Myo Aung
รหัสประจำตัว	48065052
ปริญญา	ปรัชญาดุษฎีบัณฑิต
สาขาวิชา	เทคโนโลยีชีวภาพทางโรคพืช
พ.ศ.	2551
อาจารย์ที่ปรึกษาวิทยานิพนธ์	รศ.ดร. เกษม สร้อยทอง
อาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม	Prof. Dr. Kevin D. Hyde

บทคัดย่อ

วิทยานิพนธ์ฉบับนี้ได้วิจัยเพื่อศึกษาความหลากหลายทางชีวภาพและ phylogeny ของเชื้อราที่ทำให้เกิดโรคกับแมลงในประเทศไทย โดยได้ทำการสำรวจเชื้อราที่เป็นสาเหตุโรคของแมลงในพื้นที่ป่าอนุรักษ์และป่าที่ถูกใช้พื้นที่ (conserved and disturbed rainforests) และพื้นที่เกษตรกรรมในจังหวัดเชียงใหม่ ทำการเก็บตัวอย่างจากแมลงที่ตาย สัตว์มีปล้องและดินในพื้นที่ต่างๆ ระหว่างช่วงฤดูฝนปี พ.ศ. 2548 และ พ.ศ. 2549 จากการวิจัยพบเชื้อรา 34 ตัวอย่างซึ่งจัดอยู่ใน 15 สกุล (genera) เป็นราที่สืบพันธุ์แบบอาศัยเพศ (telemorphs) 18 ชนิด และราที่สืบพันธุ์แบบไม่อาศัยเพศ (anamorphs) 16 ชนิด โดยพบว่าเป็นราชนิดใหม่ 2 ชนิดคือ *Ophiocordyceps mrciensis* และ *Hymenostilbe furcata* พบราชนิดใหม่ครั้งแรกในประเทศไทย 2 ชนิดคือ *Cordyceps militaris* และ *Cordyceps militaris* var. *sphaerocephala* งานวิจัยนี้จึงได้จัดทำคำอธิบายและภาพประกอบของระยะการสืบพันธุ์แบบอาศัยเพศของ *Cordyceps militaris*, *C. militaris*. var. *sphaerocephala*, *C. nelumboides*, *Cordyceps* sp., *Hypocrella* sp., *Ophiocordyceps crinalis*, *O. dipterigena*, *O. elongata*, *O. filiformis*, *O. longissima*, *O. mrciensis*, *O. myrmecophila*, *O. nutans*, *O. oxycephala*, *O. pseudolloydii*, *O. sphecocephala*, *O. unilateralis* และ *Torrubiella hemipterigena* และระยะการสืบพันธุ์แบบไม่อาศัยเพศของเชื้อรา *Acremonium charticola*, *A. crassum*, *Aschersonia* sp., *Aspergillus* sp., *Beauveria bassiana*, *B. brongniartii*, *Cladosporium* sp., *Hymenostilbe furcata*, *Isaria cicadae*, *I. farinosus*, *I. fumosoroseus*, *I. tenuipes*, *Paecilomyces marquandii*, *Sporothrix insectorum*, *Stilbella buqueti* และ *Verticillium* sp. ราที่พบมากในมดได้แก่ *Ophiocordyceps myrmecophila* (22.6%) และ *O. unilateralis* (13.8%) ความหลากหลายของสายพันธุ์ (species) ในแมลงกลุ่ม Homoptera พบมากที่สุด รองลงมาคือแมลงในกลุ่ม Lepidoptera และ Hymenoptera ความหลากหลายของสายพันธุ์พบมากที่สุดในป่าที่ถูกใช้พื้นที่ รองลงมาคือ ป่าอนุรักษ์และพื้นที่การเกษตร

Cordyceps และ *Ophiocordyceps* พบกระจายทั่วไป 74.6% ในป่าอนุรักษ์ และ 61.3% ในป่าที่ถูกใช้พื้นที่ แต่ในพื้นที่การเกษตรพบ 1.6 %

การศึกษาความสัมพันธ์ของสิ่งอาศัยระหว่างรา *Beauveria, Cordydeps* และ *Paecilomyces* การวิเคราะห์ *Phylogenetic* โดยอาศัยพื้นฐานของระดับยีนในไรโบโซมและโปรตีน *(ribosomal and protein coding gene sequences* เชื้อรา *B. brongniartii (anomorph)* มีความสัมพันธ์ใกล้ชิดกับรา *C. militaris* ในขณะที่เชื้อรา *B. brongniartii* มีสิ่งอาศัย (host) ที่กว้างและไม่เฉพาะเจาะจง รา *Paecilomyces* จะเจริญเป็นกลุ่มก้อน เชื้อรา *Isaria* ไม่เฉพาะเจาะจงต่อสิ่งอาศัย แต่อย่างไรก็ตามราบางชนิดสามารถจะเจริญได้ในแมลงที่เฉพาะเจาะจงเท่านั้น เช่น *I. tenuipe* พบการเข้าทำลายเฉพาะดักแด้ของผีเสื้อ, *I. cicadae* เข้าทำลายเฉพาะแมลงกลุ่ม Homoptera (เพลี้ย) และ *I. rarinosus* เข้าทำลายเฉพาะดักแด้ของผีเสื้อ ราบางชนิดในกลุ่มของ *Ophiocordyceps* บางชนิดจะมีความเฉพาะเจาะจงต่อสิ่งอาศัยแตกต่างกันไป เช่น *O. pseudolloydii* เข้าทำลายมดในกลุ่ม formicine นอกจากนี้รา *Cordyceps* และ *Ophiocordyceps* ไม่มีความเกี่ยวข้องกับรากลุ่มที่สืบพันธุ์แบบไม่ใช้เพศได้แก่ *Beauveria, Paecilomyces* และ Nomuraea และกลุ่มที่สืบพันธุ์แบบใช้เพศได้แก่ *Elaphocordyceps* และ *Torrubiella* รา *Cordyceps* ประกอบด้วยเชื้อราที่มีความหลากหลายทางสัณฐานวิทยา รา *C. militaris, B. brongniartii, C. bifusispora* และ *Paecilomyces* มีความสัมพันธ์กับการสืบพันธ์แบบอาศัยเพศและไม่อาศัยเพศใน clades ของรา *Beauveria* และ *Paecilomyces*

การศึกษาเชื้อรา *Beauveria* ทั้งด้านสัณฐานวิทยาและในระดับโมเลกุลของดีเอ็นเอได้ศึกษาในรา *B. bassiana* และ *B. brongniartii* ซึ่งเป็นราไอโซเลทจากจังหวัดเชียงใหม่พบว่า โคนิเดีย (conidia) ของเชื้อรา *B. brongniartii* (ขนาด 2.2–6 ไมครอน) มีความยาวมากกว่าตัวอย่างเชื้อราจากประเทศบราซิล, ญี่ปุ่น, เกาหลี, สาธารณรัฐประชาชนจีน และฟิลิปปินส์ (ขนาด 2.3-4.2 ไมครอน) โคนิเดียของ *B. Bassiana* จากจังหวัดเชียงใหม่ (ขนาด 1.2-3X1-3 ไมครอน) มีขนาดเล็กกว่าโคนิเดียจากประเทศบราซิล, Commonwealth of Independent States (CIS), ฝรั่งเศส,สาธารณรัฐประชาชนจีน, โปแลนด์, สหรัฐอเมริกา และเวียดนาม (ขนาด 1.7-3.5x1.5-3.1 ไมครอน) อัตราส่วนความยาวต่อความกว้างของโคนิเดียมีความแตกต่างกันขึ้นอยู่กับสิ่งอาศัย อัตราส่วนความยาวความกว้างของโคนิเดียไอโซเลทจากจังหวัดเชียงใหม่แบ่งได้เป็น 3 กลุ่ม คือ เชื้อรา *B. brongniartii* ไอโซเลทจากแมลงปีกแข็ง (Coleoptera) มีขนาดระหว่าง 2-2.3, ไอโซเลทจากแมลงในกลุ่มผีเสื้อ (Lepidoptera) มีอัตราส่วนระหว่าง 1.8-2 และแมลงในกลุ่ม Diptera, Orthoptera และแมลงที่ไม่สามารถจัดจำแนกกลุ่มได้ มีอัตราส่วนระหว่าง 1.3-1.6 อัตราส่วนความยาวและความกว้างของโคนิเดียของเชื้อรา *B. brongniartii* ซึ่งแยกได้จากแมลงในกลุ่ม Diptera มีขนาด 1.3 ซึ่งเป็นขนาดที่เล็กกว่าโคนิเดียของเชื้อรา *B. bassiana*

นอกจากนี้ จากการวิเคราะห์ข้อมูลความสัมพันธ์ในระดับโมเลกุลจึงสามารถจำแนกกลุ่มของรา *Beauveria* ออกเป็น 5 กลุ่ม คือ กลุ่มของ *B. brongniartii* ไอโซเลทที่ได้จากจังหวัดเชียงใหม่ (A),

กลุ่มของ *B. brongniartii* ไอโซเลทจังหวัดเชียงใหม่และญี่ปุ่น เกาหลี สาธารณรัฐประชาชนจีน และฟิลิปปินส์ (B), กลุ่มของ *Beauveria* ชนิดอื่นๆ (*B. caledonica*, *B. amorpha* และ *B. vermiconia)* (C), กลุ่มของ *B. bassiana* จากประเทศ CIS, โปแลนด์ และโรมาเนีย (D), กลุ่มของ *B. brongniartii* ไอโซเลทจากจังหวัดเชียงใหม่, ประเทศบราซิล, ฝรั่งเศส, สาธารณรัฐประชาชนจีน และเวียดนาม (E)

คำสำคัญ: เชื้อราที่ทำให้เกิดโรคกับแมลง, ความหลากหลายทางชีวภาพ, Taxonomy, phylogeny

Thesis Title	Biodiversity and Molecular Phylogeny of Entomopathogenic Fungi in Chiang Mai Province, Thailand
Student	Ohnmar Myo Aung
Student ID.	48065052
Degree	Doctor of Philosophy
Program	Biotechnology in Plant Pathology
Year	2008
Thesis Advisor	Assoc. Prof. Dr. Kasem Soytong
Thesis Co-advisor	Prof. Dr. Kevin D. Hyde

ABSTRACT

The findings of a biodiversity and phylogeny study of entomopathogenic fungi in Thailand are presented in this thesis. A survey of entomopathogenic fungi was carried out in both conserved and disturbed rainforests and agricultural habitats in Chiang Mai Province. Dead insects, other arthropods, and soil samples were collected during the rainy seasons of 2005 and 2006. Thirty-four entomogenous taxa belonging to 15 genera (18 teleomorphs and 16 anamorphs) were encountered. *Ophiocordyceps mrciensis* and *Hymenostilbe furcata* are new to science. New records for Thailand include *Cordyceps militaris* and *Cordyceps militaris* var. *sphaerocephala*. Descriptions and illustrations of the sexual states of *Cordyceps militaris, C. militaris.* var. *sphaerocephala, C. nelumboides, Cordyceps* sp., *Hypocrella* sp., *Ophiocordyceps crinalis, O. dipterigena, O. elongata, O. filiformis, O. longissima, O. mrciensis, O. myrmecophila, O. nutans, O. oxycephala, O. pseudolloydii, O. sphecocephala, O. unilateralis,* and *Torrubiella hemipterigena* and anamorphs *Acremonium charticola, A. crassum, Aschersonia* sp., *Aspergillus* sp., *Beauveria bassiana, B. brongniartii, Cladosporium* sp., *Hymenostilbe furcata, Isaria cicadae, I. farinosus, I. fumosoroseus, I. tenuipes, Paecilomyces marquandii, Sporothrix insectorum, Stilbella buqueti* and *Verticillium* sp. are provided. *Ophiocordyceps myrmecophila* (22.6%) and *O. unilateralis* (13.8%) were dominant and only found on ants. Species diversity on Homoptera was highest, followed by Lepidoptera and Hymenoptera. The Highest species diversity occurred in disturbed rainforests, followed by conserved rainforests and agricultural habitats. *Cordyceps* and *Ophiocordyceps* species contributed to 74.6% of total taxa in conserved rainforests, 61.3% in disturbed forests but only 1.6% in agricultural habitats.

In order to investigate host-based relationships of *Beauveria, Cordyceps* and *Paecilomyces* species, a phylogenetic evaluation based on ribosomal and protein coding gene sequences was carried out. *Beauveria brongniartii* (anamorph) was closely related to *Cordyceps militaris*. Host

specificity was not evident among the *Beauveria brongniartii* isolates which had a wide host range. All *Paecilomyces* species clustered as a distinct group. Host specificity was not found among the *Isaria* isolates, however, some species attacked specialized group of insects: *I. tenuipes* only infected lepidopteran pupae, *I. cicadae* infected only Homoptera and *I. farinosus* only infected lepidopteran pupa. Some *Ophiocordyceps* species were also restricted to their respective hosts; *Ophiocordyceps pseudolloydii* infected dolichoderine ants and *O. myrmecophila* and *O. unilateralis* infected formicine ants. In addition, *Cordyceps* and *Ophiocordyceps* species do not constitute a monophyletic group as some species clustered together with anamorphic *Beauveria*, *Paecilomyces*, *Nomuraea* and teleomorphic *Elaphocordyceps* and *Torrubiella* species. *Cordyceps* comprises several strongly supported clades, characterized by species possessing divergent morphological characters. The phylogenetic affinities of *Cordyceps militaris* and *Beauveria brongniartii* and *C. bifusispora* and *Paecilomyces* clearly indicated teleomorph/ anaomorph relationships in the *Beauveria* and *Paecilomyces* clades.

To overcome difficulties in the systematics of *Beauveria* species, morphology and DNA molecular data were studied using *Beauveria bassiana* and *B. brongniartii* isolates from Chiang Mai Province. The conidia of *B. brongniartii* were longer (2.2-6 μm) than those of conidia in collections from Brazil, Japan, Korea, People Republic of China and Philippines (2.3-4.2 μm). Conidia of *B. bassiana* from Chiang Mai were smaller (1.2-3 × 1-3 μm) than those of conidia from Brazil, Commonwealth of Independent States (CIS), France, People Republic of China, Poland, USA and Vietnam (1.7-3.5 × 1.5-3.1 μm). The Length/width (l/w) ratios vary according to host. L/w ratios of the conidia of Chiang Mai isolates divided into three groups: *Bearuveria brongniartii* isolated from Coleoptera ranged between 2-2.3, Lepidoptera associated *B. brongniartii* ranged between 1.8-2 and other heterogeneous groups including Diptera, Orthoptera and unidentified insects were 1.3-1.6. The length/width ratio of the *B. brongniarti* isolated from Diptera (1.3) is less than the other *B. bassiana* isolates.

In addition, five major phylogenetic groups were established based on the phylogenetic analysis: a group containing only Chiang Mai *B. brongniartii* (A), a group of *B. brongniatii* from both Chiang Mai and, Japan, Korea, People Republic of China and Philippines (B), a group of other *Beauveria* species (*B. caledonica*, *B. amorpha* and *B. vermiconia)* (C), a group of only *B. bassiana* from CIS, Poland and Romania, a heterogeneous *B. bassiana* (D), *B. brongniartii* group from Chiang Mai and Brazil, France, People Republic of China and Vietnam (E).

Key words: entomopathogenic fungi, biodiversity, taxonomy, phylogeny

ACKNOWLEDGEMENT

I would like to express my utmost gratitude to my advisors, Assoc. Prof. Dr. Kasem Soytong and Prof. Kevin. D. Hyde for their invaluable guidance, supervision, and encouragement throughout this study.

I am extremely grateful to my main advisor Assoc. Prof. Dr. Kasem Soytong for accepting me as his student in KMITL and providing me all the kind necessary help, suggestions and encouragement during the study.

I will always be grateful to Prof. Kevin D. Hyde for introducing me the link between mycology and entomology. I also wish to express my deepest gratitude and appreciation to him for providing me the opportunity to do this project in Thailand, China and Hong Kong. Also his trust and support which let me work and learn in my own unconventional ways was highly precious in my education. I am also grateful to the Mushroom Research Foundation for postgraduate scholarship and financial support throughout my study.

I am especially indebted to Prof. Z.Q Liang for his generosity in teaching and sharing knowledge in the taxonomy of entomopathogenic fungi. I would also like to thank Prof. J.C. Kang for his advice on my project and molecular works and providing me the funding during my stay in China.

My sincere gratitude is due to Dr Rajesh Jeewon, the University of Hong Kong for his invaluable guide and teaching for molecular works and scientific writing.

I would like to extend my special thanks to Myanma Cotton and Sericulture Enterprise, Ministry of Agriculture and Irrigation, Myanmar for allowing me to study in Thailand and giving me study leave. I wish to express my deepest gratitude and appreciation to Dr. Pye Tin, Managing Director, Myanma Cotton and Sericulture Enterprise, for his kind permission and continuous support.

My special thanks to Dr. Else C. Vellinga and Dr. Janet Jennifer Luangsa-ard for their encouragement, moral support and kind assistance in many ways throughout the study.

Thanks also go to all the friends whom I met in Guizhou University (namely, Han, Xiao, Zhangjie, Liang, Liu, Xie and Ronny), Hong Kong University (especially, Dam, Danny, Hongli, Ying, Jing, Subbu and Becky), and KMITL (namely, Kai, Tarn, Oam, Rung and Ae). I must also thank Helen Leung and Heidi Kong for giving me technical support and various assistances in the mycology and molecular lab in HKU.

Special acknowledgment is also given to all of my colleagues from MRC who sharing mycological knowledge and have provided me with emotional support during the past years. This includes Aod, Hahn, Lam, Huyen, Po Po, Rulin, Thida, Iman, Marivic, Joy, Nilam, Nook, Sittisack, Phongeun, Deer and Samantha, whose friendship and various kinds of help.

My acknowledgement will not be complete without mentioning my teachers from Myanmar Dr. Myint Thaung and Dr. Aung Kyi for their guidance and encouragement for my further study. I would like to thank all the people who have directly or indirectly helped me in completing this thesis.

More specifically, I will never forget to thank Dr. Thane Htay and Aung (Aung Swe, HKU), whose want me to be a PhD laureate. Because of their volition and Prof. Kevin D. Hyde's kindness, I will become a PhD holder and it is an unforgettable memory in my life.

Finally, I would like to express my deepest gratitude to my sisters and brothers, whose unconditional love and support has lit my way through the life. Specially, I thank my late parents Dr. Myo Aung and Daw Nu Nu San for their love and encouragement inspired my passion for learning when they were with me. It is to commemorate their love that I dedicate this thesis to them.

Ohnmar Myo Aung

CONTENTS

CONTENTS (continued)

CONTENTS (continued)

CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (continued)

Figure Page

LIST OF FIGURES (continued)

Figure Page

LIST OF FIGURES (continued)

Figure Page

LIST OF FIGURES (continued)

Figure Page

Chapter 1

INTRODUCTION

Large numbers of fungi are associated with various insects and other arthropods. These fungi can be grouped as entomogenous fungi and which include entomopathogens, mutualistic symbionts, ectoparasites and endoparasites (Evans. 1988). Generally, entomogenous fungi can be divided into two groups: necrotrophic fungi which act against insects (killing host cells and utilizing the nutrient source) and biotrophic fungi which form mutualistic associations with insects (living host cells required) parasites (Benjamin *et al.* 2004; Blackwell and Vega. 2005). The necrotrophic parasites are pathogenic to insects and those entomopathogenic fungi are particularly important for controlling insect pests. There are a large number of species of these fungi and their morphologies are specialized and therefore only a few specialists study them. It is not surprising, therefore, that our knowledge of many species is poor (Benjamin *et al.* 2004).

Tropical rainforests are characterized by high richness in entomopathogenic mycotaxa (Evans. 1982) and Thailand's rainforests are characterized by high richness in flora and fauna. Petch (1932) was first to record a number of entomopathogenic fungi in Thailand, however before 1989 less than 10 invertebrate pathogenic fungi had been recorded from Thailand (Petch. 1932, 1933, 1937; Roffey. 1968; Schumacher. 1982). Studies on entomopathogenic fungi from Thailand have been carried out since 1990 and published (see Hywel-Jones. 2001; Jones and Hyde. 2004; Luangsa-Ard *et al.* 2007) and more than 400 morphotaxa have been recorded (Luangsa-Ard *et al.* 2007). These findings are relatively high as compared to other countries in the region.

There is also little information about the relationship between entomopathogenic fungi and different arthropod hosts. All insect orders appear to be susceptible to fungal infections (Hajek and St. Leger. 1994). Specificity between host insects and pathogens has been reviewed by many researchers (see Fargues and Remaudiere.. 1977). Specificity in entomopathogenic fungi may also be determined by ecological relationships of fungi and microorganisms in the environment and by spatial-temporal coincidence between populations of the pathogen and host insect (Fargues and Remaudiere. 1977).

Entomopathogenic fungi, especially in the genus *Cordyceps* and its many proven or suspected anamorphs (e.g. *Hirsutella, Hymenostilbe*, *Nomuraea*, *Paecilomyces*, *Verticillium*) are particularly well presented in tropical rain forests (Evans. 1982; Samson *et al.* 1988). *Cordyceps*

species are however, rare in depleted rainforests and in agricultural land bordering these habitats (Samson *et al.* 1988). On the other hand, non-specialized pathogens such as *Beauveria* and *Metarhizium* are poorly represented in forest habitats, but commonly encountered by agricultural entomologists and have potential as biological control agents (Madelin. 1966; Samson *et al.* 1988).

Cordyceps is a widespread teleomorphic genus in the family *Clavicipitaceae* (Ascomycota, fungi), and now includes more than 400 species (Mains. 1958; Kobayasi. 1982; Stensrud *et al.* 2005). This figure is probably an underestimation considering the extant global diversity (Hawksworth and Rossman. 1997; Sung *et al.* 2007). Species included in *Cordyceps* are morphologically heterogenous. They are associated with different larval and adult stages of host from various insect orders (e.g. Lepidoptera, Coleoptera, Hymenoptera). A small number are parasitic on spiders, hart's truffles and hypogeous *Elaphomyces* species (Mains. 1957; Nikoh and Fukatsu. 2000; Shimizu. 1994). Most *Cordyceps* species are restricted to a single host species or a set of closely related host species (Kobayasi. 1941, 1982; Mains. 1957, 1958). The anamorphs of *Cordyceps* species include *Akanthomyces*, *Beauveria*, *Cephalosporium*, *Desmidiospora*, *Diademospora*, *Gibellula*, *Hirsutella*, *Hymenostilbe*, *Isaria*, *Paecilomyces*, *Penicillium*, *Pochonia*, *Scytalidium*, *Sphacelia*, *Sporotrichum*, *Stachybotrys*, *Syngliocladium*, *Tilachlidiopsis*, *Tolypocladium* and *Verticillium* species (http://www.cbs.knaw.nl/databases/ index.htm).

Beauveria (family *Clavicipitaceae*) includes approximately 7 species which parasitise insects (Kirk *et al.* 2001). This genus is a common soil-borne entomopathogen and is used as a biological control agent for insect pest management (Rehner and Buckley. 2005). Criteria generally used to classify *Beauveria* species are 1) conidiophores consisting of whorls and dense clusters of sympodial, short and globose or flask-shaped conidiogenous cells with apical denticulate rachi (giving a distinct zig-zag appearance), and 2) one-celled conidia (Samson *et al.* 1988). Traditionally, the main difference between the most common species, *B. bassiana* and *B. brongniartii*, is the shape and size of the conidia; the former having mainly spherical conidia and the latter with more cylindrical conidia (Brady. 1979). The first record of *Beauveria* as an anamorph of *Cordyceps* and the first discovery of *Cordyceps* as a teleomorph of *Beauveria* was proposed by Shimazu *et al.* (1988).

Paecilomyces is another anamorphic entomopathogenic genus which is parasitic to various lepidopteran insects (Fukatsu *et al.* 1997). Known teleomorphs are *Byssochlamys*, *Talaromyces* and *Thermoascus*, and includes approximately 50 species (Kirk *et al.* 2001). *Paecilomyces* is

characterized by the shape of the phialides that taper into a long distinct neck and by the divergent aggregation of whorls. Samson (1974) divided *Paecilomyces* into two sections. Sect. *Paecilomyces* includes some mesophilic and thermophilic species and have yellow-brown to brown colonies; several species have sexual states. Sect. *Isarioidea* includes mesophilic species and has white or other bright colonies and include entomopathogenic taxa. Luangsa-Ard *et al.* (2004) stated that the type species, *Paecilomyces variotii*, and thermophilic relatives belong to the *Eurotiales* (*Trichocomaceae*), while mesophilic species related to *Paecilomyces farinosus* are *hypocrealean* (*Clavicipitaceae* and *Hypocreaceae*). *Paecilomyces* have also been reported as anamorphs of various *Cordyceps* species (Chen and Xu. 1989; Huang *et al.* 2002; Kobayasi. 1981; Kobayasi and Shimizu. 1976; Liu *et al.* 2001; Pacioni and Frizzi. 1978; Shimazu *et al.* 1988).

Several phylogenetic studies of entomopathogenic fungi have been carried out using nuclear ribosomal DNA (nrDNA) sequences (Guadet *et al.* 1989; Hansen *et al.* 2001; Hibbett. 1992; LoBuglio *et al.* 1993; Holst-Jensen and Schumacher. 1997; Schumacher and Holst-Jensen. 1997; Nikoh and Fukatsu. 2000; Artjariyasripong *et al.* 2001; Liu *et al.* 2002; Stensrud *et al.* 2005). The results provided useful information for establishing anamorph-teleomorph connections and in the delineation of species within *Cordyceps* (Liu *et al.* 2002). A new classification system for *Cordyceps* and the *Clavicipitaceae* was refined based on the multigene sequence analyses (Sung *et al.* 2007). These studies increase the understanding of the taxonomy of entomopathogenic fungi, their relatedness and evolution (Luangsa-Ard *et al.* 2007).

In order to document the diversity and taxonomy of entomopathogenic fungi in northern Thailand, a general survey of entomopathogenic fungi was carried out in several rainforests in Chiang Mai Province. This survey mainly focused on relationships between entomopathogenic fungi from different arthropod hosts and habitats.

Cordyceps, Beauveria and *Paecilomyces* are frequently found on different hosts in forests of Chiang Mai Province, Thailand. Although phylogenetic studies have been carried in these genera, phylogenetic relationships among species of these taxa have not been emphasized. The phylogenetic relationships of *Beauveria, Cordyceps*, and *Paecilomyces* species were investigated using ITS/5.8SrDNA and beta-tubulin sequence analyses.

A number of arthropod hosts including Coleoptera, Diptera, Hemiptera, Homoptera, Hymenoptera, Lepidoptera, Orthoptera, soil and unidentified insects were infected by *Beauveria* species. Morphological identification indicated that only *B. bassiana* and *B. brongniartii* were

recorded in Chiang Mai Province. *Beauveria bassiana* is very well represented in Thailand both in agricultural land and forests and *B. amorpha* is a common pathogen of coleopteran insects (Luangsa-Ard *et al.* 2007). *Beauveria brongniartii*, however, has been reported as pathogen of Coleoptera (Keller *et al.* 1989). Because of the confusion over host-pathogen relationship and species identification, morphological measurements and simple molecular techniques were conducted to classify the Chiang Mai *Beauveria* isolates.

1.1 Objectives

The main objectives of this study were as follows.

1) To validate the taxonomy of the entomopathogenic fungi and describe collections and new species.
2) To improve our understanding of relationships between fungi and their arthropod hosts and habitats.
3) To investigate the biodiversity of arthropods associated fungi in Chiang Mai Province, Thailand and establish a collection of isolates.
4) To establish the phylogenetic relationships of collections and isolates.
5) To add our knowledge of these fungi in Thailand.

1.2 Hypothesis

The hypotheses to be tested in this study were as follows.

1) How diverse are arthropod associated fungi?
2) Can new species be validated and described?
2) Are the relationships between fungi and their arthropod hosts in Thailand specific?
3) What are the phylogenetic relationships of new isolates with allied genera?

Chapter 2

LITERATURE REVIEW

2.1 Historical background of study in entomogenous fungi

The first published record of fungi associated with insects was that of Reaumur (1726) and Du Halde (1736) with the mentioned of the "Chinese plant worm" (Hia Tsao Tom Tchom). This worm occurred on larva of noctuid species from which a stem-like vegetable growth emerged and nowadays it has been designated with the generic name *Cordyceps*. The species was named *Cordyceps sinensis* Berk. and has great medicinal value and was used by Chinese emperor's physicians (Steinhaus. 1956).

In the beginning of the 18th century, Christian Paulinus recorded entomogenous fungi as "certain trees in the island of Sombrero in the East Indies have large worms attached to them underground, in the place of roots" (Gray. 1858; Steinhaus. 1956). Insects parasitized by *Cordyceps* species are frequently known as "vegetable wasps", or "plant worms", or "awetos" in New Zeland, "Dong Chong Xia Cao" (meaning a herb in winter and a worm in summer) in China and "Tostu Kaso" in Japan (Steinhaus. 1956; Kobayasi. 1941; Liu *et al.* 2001).

During the 19th century, in Italy as well as in France, a certain silkworm disease known as "mal del segno" or "calcino" (or, in France, "muscardine") was destined to play an important role in the sericulture industry. In 1834, Bassi showed experimentally that the fungus *Beauveria bassiana* (Bals.) Vulli. was the infectious causal organism of silkworm disease (Steinhaus. 1956). The findings, by Bassi, which pre-empted Koch's postulates, and formed the basis for germ theory, are now valuable and important in all areas of pathology (Hywel-Jones. 2001).

Metchnikoff (1879) reported a natural infection of the wheat cockchafer, *Anisoplia austriaca* Hbst., by the green-muscardine fungus, *Metarhizium anisopliae* (Metch.). Roland Thaxter, one of the leading American mycologists of his time, and certainly one of the world's outstanding students of entomogenous fungi, is best known for two monographs: "The *Entomophthoreae* of the United States" (1988), and "Contributions toward a monograph of the *Laboulbeniaceae*" (5 Volumes. 1896-1931) (Steinhaus. 1956). These contributions are of monumental importance in mycology. Thaxter also studied other entomogenous fungi groups such as *Cordyceps*, *Isaria*,

Aschersonia, fungi imperfecti, and other insect associated fungi, but little of this work was published (Steinhaus. 1956).

2.2 Current knowledge concerning the diversity of entomogenous fungi

2.2.1 Global fungal biodiversity

There are *ca* 80,000 species of fungi presently known and 1.5 million are thought to exist (Hawksworth. 1991; Kirk *et al.* 2001). An index to Saccardo's monumental *Sylloge Fungorum* listed that 120,000 names of fungi were described by 1931 (Saccardo. 1882-1931, 1972; Reed and Farr. 1993) and many fungi have since been described. Hyde (2001) mentioned that most of the undescribed taxa are microfungi and they may occur in the poorly investigated areas (e.g. the tropical forests), and less explored niches, tissues, hosts, and habitats.

2.2.2 Biodiversity of entomogenous fungi

Entomogenous fungi are an enormous group of fungi among the fungi with estimation of 500,000 to 1.5 million species (Hywel-Jones. 1993; Rossman. 1994). Samson *et al.* (1988), Hawksworth *et al.* (1983), Hajek and St. Leger (1994), Glare and Milner (1991), McCoy *et al.* (1988) Roberts and Hajek (1992) and Hywel-Jones (2001) have written the excellent reviews on entomogenous fungi. According to Hawksworth *et al.* (1983) and Kirk *et al.* (2001) the number of entomogenous fungi may be about 1000 (Table-2.1). Samson *et al.* (1988) listed several genera of entomogenous fungi, however some genera such as *Acremonium*, *Aspergillus*, *Conidiobolus*, *Fusarium*, *Nectria*, *Paecilomyces*, *Sporothrix*, *Stilbella* and *Verticillium* were not assigned to entomopathogenic fungi. A reliable number of worldwide entomogenous fungi is about 700 species, *ca* 1% of the total number of fungus species known to date (Hawksworth. 1991).

Petch (1931-1944) made the first major revision of invertebrate pathogens and described 74 new species from Sri Lanka, although very few were illustrated. Petch (1948) listed 86 species from the British Isles and with sporadic additions to this list we may estimate that *ca* 100 species are known from Britain (0.8% of the UK inventory). J. Moureau (Zaire), H.C. Evans (Brazil, Ghana, Ecuador and the Galapagos Islands) and Y. Kobayasi (Japan) have also made the other important contributions in the tropical regions (Hywel-Jones. 2001).

2.2.3 Common genera of entomopathogenic fungi

The most commonly encountered entomopathogenic fungi belong to Zygomycotina, Ascomycotina and hyphmycetous anamorphs (Evans. 1988). Amongst the Zygomycotina, order Entomophthorales is the important pathogenic group (Evans. 1988) and Trichomycetes are obligate endoparasites which basically non-parasitic to insect hosts (Moss. 1979). The most common members of the invertebrate pathogens from the Ascomycotina belong to *Clavicipitales*, *Cordycipitaceae* and *Ophiocordycipitaceae* (*Hypocreales*) (Luangsa-Ard *et al.* 2007), e.g., *Cordyceps, Hypocrella, Nectria, Podonectria, Torrubiella* species. Most of the anamorphic hyphomycetes belong to Ascomycotina (Evans. 1988). *Aschersonia, Beauveria, Gibellula, Hirsutella, Hymenostilbe, Metarhizium, Nomuraea,* and *Paecilomyces* are commonest anamorphic genera and account for about 70% of currently accepted species (Table-2.1) (Evans. 1988; Keller. 1987, 1991, 1993; Keller and Eilenberg. 1993; Samson *et al.* 1988; Tzean *et al.* 1997). Among the entomopathogenic fungi, the genera *Claviceps, Cordyceps* and *Torrubiella* are most closely related to each other (Mains. 1958).

2.3 Some perspectives on fungal diversity of entomogenous fungi: Classical and molecular application

Classical fungal taxonomy based on morphology is a basic tool for classification of fungi at most higher levels. Morphological characters may still provide the best information regarding ecological, developmental, physiological characters, and isozymes, secondary compounds, and ubiquinones (Taylor. 1993; LoBuglio and Taylor. 1993; Peterson. 1993; Kuraishi *et al.* 1991; Cruickshank and Pitt. 1980). These characters may also be useful for identification and phylogenetic studies in diversity of specific entomopathogenic fungi. Modern infrageneric classifications of entomogenous fungi emphasized on *Cordyceps* have been primarily based on the taxonomic studies of Kobayasi (1941, 1982), Mains (1958) and Massee (1895) (Sung *et al.* 2007).

The evolutionists including Lamarck (1809), Darwin (1859) and Haeckel (1866) accepted the Linnean system (Mayr. 1983) and started the reconstruction of phylogenetic history (Moritz and Hillis. 1996). Currently, molecular techniques have also been widely used by mycologists for solving problems in fungal systematics. These techniques have been useful as tools for systematic

Table 2.1 The most common genera of entomogenous fungi with numbers of known species according to Hawksworth *et al.* (1983), Kirk *et al.* (2001) and Indexfungorum (www.index fungorum. org).

Genera	No. of species		
	Hawksworth *et al.* (1983)	**Kirk *et al*, (2001)**	**Indexfungorum (www.index-fungorum.org)**
Ascomycotina* and *Basidiomycotina			
Asexual state			
Aschersonia Mont.	20	20	75
Beauveria Vuill.	3	7	45
Fusarium Link	50	50	1286
Gibellula Cavara	1	10	36
Hirsutella Pat.	15	25	93
Hymenostilbe Petch	9	9	24
Metarhizium Sorokin	2	5	30
Nomuraea Maubl.	2	2	7
Paecilomyces Bainier	31	50	119
Tolypocladium W. Gams	3	10	15
Verticillium Nees	40	40	257
Sexual state			
Ascosphaera L.S. Olive & Spiltoir	6	11	29
Cordyceps (Fr.) Link	100	100	506
Hypocrella Sacc.	30	30	101
Nectria (Fr.) Fr.	200	28	1054
Podonectria Petch	7 or 8	8	11
Torrubiella Boud.	10	10	80
Zygomycotina*: *Entomophthorales			
Batkoa Humber	-	4	7
Conidiobolus Bref.	27	27	69

Table 2.1 (continued).

Genera	No. of species		
	Hawksworth *et al.* (1983)	Kirk *et al.*, (2001)	Indexfungorum (www.index-fungorum.org)
Entomophaga Batko	6	9	27
Entomophthora Fresen.	82	11	143
Erynia (Nowak. ex Batko) Remaud. & Hennebert	25	12	81
Furia (Batko) Humber	-	12	16
Massospora Peck	11	11	19
Neozygites Witlaczil	8	9	20
Pandora Humber	-	16	23
Zoophthora Batko	25	20	89
Watermolds: *Chytridiomycetes* and *Oomycetes*			
Coelomomyces Keilin	25	69	118
Myiophagus Thaxt. ex Sparrow	1	2	3
Lagenidium Schenk	21	1	53

studies, however they are fundamentally different from all the other approaches as the total genomic DNA is analyzed directly. Several scientists have conducted phylogenetic studies using ribosomal DNA (Artjariyasripong *et al.* 2001; Sung *et al.* 2001; Stensrud *et al.* 2005) to test and refine the classification of *Cordyceps* (Sung *et al.* 2007). Presently, the most extensive multigene phylogenetic analyses have been conducted to reassess the morphological traits used in the current classification system and to revise the classification of *Cordyceps* and *Clavicipitaceae* (Sung *et al.* 2007).

2.3.1 Anamorph-teleomorph connections

Various papers and resources have listed anamorph-teleomorph connections (Ellis. 1971, 1976; Kendrick and Dicosmo. 1979; Carmichael *et al.* 1980; Tubaki. 1981; Subramanian. 1983; Sivanesan. 1984; Sugiyama. 1987; Sutton and Hennebert. 1994; Kirk *et al.* 2001; Index

Table 2.2 *Cordyceps* species and its anamorphs.

Teleomorph	Anamorph	Reference(s)
Cordyceps albella Massee	*Penicillium albellum* (Massee) Petch	Petch (1931)
Cordyceps arachnophila J.R. Johnst.	*Gibellula aranearum* P. Syd.	Mains (1940)
Cordyceps arachnophila J.R. Johnst.	*Hymenostilbe kobayasii* Koval	Ref. unknown
Cordyceps atewensis Samson, H.C. Evans & Hoekstra	*Hirsutella atewensis* Samson, H.C. Evans & Hoekstra	Samson, Evans and Hoekstra (1982)
Cordyceps australis Speg.	*Hymenostilbe melanopoda*	Samson, Evans and Hoekstra (1982)
Cordyceps bokyoensis Kobayasi	"cf. *Nalanthamala* sp."	Ref. unknown
Cordyceps chlamydosporia H.C. Evans	*Pochonia chlamydosporia* (Goddard) Zare & W. Gams	Ref. unknown
Cordyceps clavulata (Schwein.) Ellis & Everh.	*Hymenostilbe lecaniicola* (Jaap) Mains	Ref. unknown
Cordyceps clavulata (Schwein.) Ellis & Everh.	*Isaria cicadae* Miq.	Ref. unknown
Cordyceps clavulata (Schwein.) Ellis & Everh.	*Verticillium* sp.	Ref. unknown
Cordyceps cucumispora var. cucumispora H.C. Evans & Samson	*Hirsutella ovalispora* var. *ovalispora* H.C. Evans & Samson	Evans and Samson (1982)
Cordyceps cucumispora var. dolichoderi H.C. Evans & Samson	*Hirsutella ovalispora* var. *dolichoderi* H.C. Evans & Samson	Evans and Samson (1982)
Cordyceps cylindrica Petch	*Isaria atypicola* Yasuda	Kobayasi and Shimizu (1977)
Cordyceps dipterigena Berk. & Broome	*Hymenostilbe dipterigena* Petch	Mains (1958) Brady (1979)
Cordyceps entomorrhiza (Dicks.) Fr.	*Hirsutella eleutheratorum* (Nees) Petch	Mains (1958)

Table 2.2 (continued).

Teleomorph	Anamorph	Reference(s)
Cordyceps erotyli Petch	*unnamed phialidic anamorph*	Mains (1959)
Cordyceps forquignonii Quél.	*Hymenostilbe muscarium* Petch	Petch (1938)
Cordyceps gracilis (Grev.) Durieu & Mont.	*Paraisaria dubia* (Delacr.) Samson & B.L. Brady	Ref. unknown
Cordyceps gunnii (Berk.) Berk.	*Paecilomyces gunnii* Z.Q. Liang	Liang (1985)
Cordyceps heteropoda Kobayasi	*Tolypocladium* sp.	Nakagiri and Ito (1999)
Cordyceps humberti C.P. Robin	*Hirsutella saussurei* (Cooke) Speare	Mains (1951)
Cordyceps kniphofioides var. *dolichoderi* H.C. Evans & Samson	*Hirsutella stilbelliformis* var. *dolicheroderi* H.C. Evans & Samson	Evans and Samson (1982)
Cordyceps kniphofioides var. *kniphofioides* H.C. Evans & Samson	*Hirsutella stilbelliformis* var. *stilbelliformis* H.C. Evans & Samson	Evans and Samson (1982)
Cordyceps kniphofioides var. *monacidis* H.C. Evans & Samson	*Hirsutella stilbelliformis* var. *monacidis* H.C. Evans & Samson	Evans and Samson (1982)
Cordyceps kniphofioides var. *ponerinarum* H.C. Evans & Samson	*Hirsutella stilbelliformis* var. *ponerinarum* H.C. Evans & Samson	Evans and Samson (1982)
Cordyceps kyusyuensis A. Kawam.	*Sporotrichum formosanum* Kobayasi	Ref. unknown
Cordyceps lloydii var. *lloydii* H.S. Fawc.	*Hymenostilbe formicarum* Petch	Mains (1958) Evans and Samson (1984)

Table 2.2 (continued).

Teleomorph	Anamorph	Reference(s)
Cordyceps memorabilis (Ces.) Ces.	*Paecilomyces farinosus* (Holmsk.) A.H.S. Br. & G. Sm.	Pacioni and Frizzi (1978) Domsch, Gams and Anderson (1980) Farr, Bills, Chamuris and Rossman (1989)
Cordyceps militaris (L.) Link	*Cephalosporium* sp.	Kobayasi (1941) Dennis (1978)
Cordyceps myrmecophila Ces.	*Tilachlidiopsis catenulata* Papierok & Charp.	Ref. unknown
Cordyceps nelumboides Kobayasi & Shimizu	*Hirsutella* sp.	Kobayasi and Shimizu (1977)
Cordyceps nutans Pat.	*Hymenostilbe ghanensis* Samson & H.C. Evans	Samson and Evans (1975)
Cordyceps odonatae	*unnamed synnematal phialidic hypho*	Mains (1959)
Cordyceps odonatae Kobayasi	*Hymenostilbe odonatae* Kobayasi	Ref. unknown
Cordyceps ophioglossoides (Ehrh.) Link	*Verticillium* sp.	Gams (1971)
Cordyceps pistillariiformis Berk. & Broome	*Hirsutella lecaniicola* (Jaap) Petch	Petch (1948)
Cordyceps polyarthra Möller	*Isaria dussii* Pat.	Mains (1955)
Cordyceps rubripunctata Moureau	*Hirsutella rubripunctata* Samson, H.C. Evans & Hoekstra	Samson, Evans and Hoekstra (1982)
Cordyceps sinensis (Berk.) Sacc.	*Hirsutella sinensis* X.J. Liu, Y.L. Guo, Y.X. Yu & W. Zeng	Ref. unknown

Table 2.2 (continued).

Teleomorph	Anamorph	Reference(s)
Cordyceps sinensis (Berk.) Sacc.	*Paecilomyces sinensis* Q.T. Chen, S.R. Xiao & Z.Y. Shi	Chen, Xiao and Shi (1987)
Cordyceps sinensis (Berk.) Sacc.	*Scytalidium hepiali* C. Lan Li	Ref. unknown
Cordyceps sinensis (Berk.) Sacc.	*Stachybotrys* sp.	Ref. unknown
Cordyceps sobolifera (Hill ex Watson) Berk. & Broome	*Isaria cicadae* Miq.	Mains (1951) Mains (1955) Mains (1958)
Cordyceps sp.	*Beauveria* sp.	von Arx (1981)
Cordyceps sp.	*Diademospora sp.*	Ref. unknown
Cordyceps sp.	*Gibellula* sp.	de Hoog (1977) von Arx (1974)
Cordyceps sp.	*Sphacelia* sp.	de Hoog (1977) von Arx (1974)
Cordyceps sphecocephala (Klotzsch ex Berk.) Berk. & M.A. Curtis	*Hymenostilbe sphecophila* (Ditmar) Petch	Petch (1948) Mains (1951)
Cordyceps sphingum (Tul. & C. Tul.) Berk. & M.A. Curtis	*Isaria pistillariiformis* Pat.	Petch (1931)
Cordyceps stylophora Berk. & Broome	*Hirsutella stylophora* Mains	Mains (1951) Mains (1958)
Cordyceps subsessilis Petch	*Tolypocladium inflatum* W. Gams	Hodge, Krasnoff and Humber (1996)
Cordyceps thaxteri Mains	*Akanthomyces aranearum* (Petch) Mains	Mains (1939) Mains (1958)
Cordyceps trinidadensis Mains	*unnamed synnematal phialidic hypho*	Mains (1959)

Table 2.2 (continued).

Teleomorph	Anamorph	Reference(s)
Cordyceps tuberculata (Lebert) Maire	*Akanthomyces pistillariiformis* (Pat.) Samson & H.C. Evans	Samson, Evans and Hoekstra (1982)
Cordyceps unilateralis (Tul.) Sacc.	*Desmidiospora myrmecophila* Thaxt.	Ref. unknown
Cordyceps unilateralis (Tul.) Sacc.	*Hirsutella acerosa* H.C. Evans & Samson	Ref. unknown
Cordyceps unilateralis (Tul.) Sacc.	*Hirsutella formicarum* Koval	Ref. unknown
Cordyceps unilateralis (Tul.) Sacc.	*Hirsutella sporodochialis* H.C. Evans & Samson	Ref. unknown
Cordyceps variabilis Petch	*Syngliocladium* sp.	Hodge, Humber and Wozniak (1998)

Fungorum. 2007; Shearer *et al.* 2007; The Anamorph-Teleomorph Database. 2007) and mentioned that determination of anamorph-teleomorph connections have been difficult as both morphs cannot easily be cultured or induced to sporulate on artificial media. Shenoy *et al.* (2007) reported that one fifth of the known fungi lack sexual morphologies and are yet to be connected to their teleomorphs. More than 50 records of anamorph-teleomorph connections can be found in The Anamorph-Teleomorph Database (2007). The anamorphs of *Cordyceps* have been studied over a long time (Liu *et al.* 2001). A single *Cordyceps* species, however, can be associated with different anamorphic genera (Liu *et al.* 2001). Table 2.2 lists *Cordyceps* anamorph-teleomorph connections recorded in The Anamorph-Teleomorph Database (2007). Establishment of the connections between anamorphs and the teleomorph of *Cordyceps* species based on morphological and cultural characteristics were first reported by Petch (1921). Mycologists have since been trying to establish connections (Kobayasi. 1941, 1982; Mains. 1951, 1958; Gams. 1971; Samson. 1974; Samson and Evans. 1982; Liang. 1985, 1991; Liu *et al.* 2001). Liang (1991), however, reported that to induce cultures to produce mature stromata was the most effective way

Liu *et al.* (2001) and Liang and Liu (1991) also reported that microcyclic conidiation is the another effective method for determining anamorph-teleomorph connections (Liu *et al.* 2001). DNA sequence-data can also provide evidence of anamorph-teleomorph connections (Shenoy *et al.* 2007).

2.3.2 The importance of phylogenetic study in understanding host relationships

Various associations of insects and fungi have been found in the environment. The associations include many types of interaction among diverse taxa of these groups of organisms (Blackwell and Jones. 1997). Generally, entomopathogenic fungi are more or less host-specific especially the endoparasite can attack a specific group of host organisms or sometimes only a particular host insects and arthropods (Nikoh and Fukatsu. 2000). In this regard, endoparasites should develop their mechanisms to attack the host in the particular environment. It is important to understand the factors involved in evolution of new fungus-host relationships (Nikoh and Fukatsu. 2000). Molecular techniques have been well developed over the past 20 years and add to our knowledge of the entomopathogenic fungi and host relationships (Blackwell and Vega. 2005). Phylogenetic analyses can help proving the evolutionary development of symbioses and also contribute to the understanding of entomopathogenic species numbers which vary according to species concepts used (Blackwell and Jones. 1997).

Blackwell and Jones (1997) showed that a number of ascomycete lineages are closely related to hosts. Interestingly, the most basal of the ascomycetes such as *Taphrina, Protomyces, Schizosaccharomyces, Saitoella* and *Pneumocystis*, however, are not related with insects (Blackwell and Jones. 1997). They also suggested that complete taxon sampling can provide the tracking of interactions between ascomycetes and arthropod hosts.

2.3.3 Systematics of entomopathogenic fungi and related hosts by using nrDNA sequences and COI DNA barcodes

Phylogenetic studies have enlightened both mycologists and entomologists about the study of fungus-insect associations. Analyses of molecular data can help both scientists to identify the entomopathogenic fungi (Guadet *et al.* 1989; Hansen *et al.* 2001; Hibbett. 1992; LoBuglio *et al.* 1993; Holst-Jensen and Schumacher. 1997; Schumacher and Holst-Jensen. 1997; Nikoh and Fukatsu. 2000; Artjariyasripong *et al.* 2001; Liu *et al.* 2002; Blackwell and Vega.

2005; Stensrud *et al.* 2005). Host affiliation is valuable and essential in the classification of entomopathogenic fungi particularly for *Cordyceps* species (Massee. 1895; Kobayasi. 1982). The classification of arthropods infected by entomopathogens however, has been problematic due to the difficulty in identifying mummified immature hosts. Some *Cordyceps* species are restricted to specific hosts (Hymenoptera, Hemiptera and some Diptera) at the family level. However, it is difficult to prove fungus-host associations below the family level due to the difficulties of identifying the mummified immature insects.

Identification plays an important role in systematics of organism or a species (Shenoy *et al.* 2007). Several attempts have been tried to combine molecular data into identification systems (Shenoy *et al.* 2007). A new and interesting identification system for animals based on diversity of the mitochondrial gene Cytochrome Oxidase I (COI) was proposed by Hebert *et al.* (2002). Currently, DNA barcoding is a promising method that can be used as a tool for host identification using short and standardized gene regions as internal species tags. This advanced system gives fast and precise answers in species identification (Hebert and Gregory. 2005). There are some evidence in efficacy of DNA barcoding system for some major groups such as birds (Hebert *et al.* 2004b), fish (Ward *et al.* 2005), lepidopteran insects (Hebert *et al.* 2004a; Hajibabaei *et al.* 2006), and marine algae (Saunders. 2005). Research has been conducted to employ various gene regions such as nuclear large ribosomal subunit (Kurtzman and Robbnett. 1998), internal transcribed spacer (ITS) (Berch *et al.* 2002; Cunnington *et al.* 2003; Druzhinina *et al.* 2005; Koljalg *et al.* 2005), partial beta-tubulin gene sequences (Samson *et al.* 2004) and partial elongation factor 1-alpha (EF-1α) sequences (Geiser *et al.* 2004) for identifying fungi to species. Patterns of sequence divergences in the full COI gene in the Kingdom *Fungi* and fungus-like Oomycota have been examined by Seifert *et al.* (2007). Their results revealed that divergences in COI gene in fungi are considerable, unlike in plants (Kress *et al.* 2005). COI variation in the genus *Penicillium* subgenus *Penicillium* and closely related species has also been investigated by Seifert *et al.* (2007). They concluded that COI gene has lower sequence divergence than the beta-tubulin gene, however the latter provided a higher degree of taxonomic resolution (Seifert *et al.* 2007; Shenoy *et al.* 2007). Currently, the mycologist, has preferred the ITS to CO1 as the most appropriate gene for DNA barcoding in fungi (Unpublished Report of the All Fungi Barcode of Life Planning Workshop. 2007). The primers for this gene are very prosperous and have a greater range in phylogenetic signal than mitochondrial genes. The use of DNA barcoding to identify mummified

insects and ITS nu-rDNA to identify the associated fungi will provide new data on the association between entomopathogenic and insect hosts.

2.4 Studies on bioactive compounds derived from entomogenous fungi

Bioassays and activity-guided fractions of extracts from broths of entomopathogenic fungi and chemical structure elucidation have shown that they are excellent sources of chemical versatility (Tanticharoen. 2003). Entomopathogenic fungi produce different kinds of novel metabolites exhibiting various biological activities ranging from activity against malarial parasites, fungi, virus, mycobacteria and tumor cell lines. Among the entomopathogenic fungi, the genera *Cordyceps*, *Hypocrella* and *Torrubiella* have already demonstrated their potential as sources of novel medicinal compounds (Nisbet and Porter. 1989; Hywel-Jones. 2001).

Cordyceps species have extensively been reported to have natural medicinal properties, such as acting as a hemostatic, a mycolytic, an anti-asthmatic, or an expectorant (Tang and Eisenbrand. 1992; Namba. 1993). *Cordyceps* species have been found as parasites of a variety of underground stages insects in the mountains of China, Korea, Japan, and Thailand (Namba. 1993; Ahn *et al.* 2000; Hywel-Jones. 2001). The medical properties, relating to *Cordyceps* spp. have been explored in many ways. Cultured mycelia have been compared with naturally occurring *Cordyceps* sp. and it was found that the effectiveness of mycelia is equal to that of wild species (Wang and Shiao. 2000).

Cordyceps species have been shown to be a source of many bioactive compounds such as Cordycepin (Cunningham *et al.* 1951) and other anti-bacterial and anti-tumor adenosine derivatives (Furuya *et al.* 1983), Ophicordin and anti-fungal agents shown to have anti-tumor activity (Yamada *et al.* 1984; Ohmori *et al.* 1986), an immunopotentiating galactotryptomannan, and L-tryptophan (Zhang *et al.* 1991).

Cordyceps sinensis is parasitic on lepidopteran larvae, and has a long history of ethnomedical use. It has been used as popular remedy in Traditional Chinese Medicine for hundreds of years (Chen *et al.* 1997; Liu *et al.* 2001). Evidence for using this fungus is documented in the Qing dynasty *Bencao* Congxin (New compilation of Materia Medica) in 1757 (Buenz *et al.* 2005). It has gained prominence as a tonic for world record breaking Chinese athletes (Pegler *et al.* 1994; Steinkraus and Whitfield. 1994). The species is commonly available

in Chinese herbalists and drinks containing extracts are common in Asian markets (Hywel-Jones. 2001). Adenosine, ergosteryl- β-glucopyranoside, 22-dihydroergosteryl- β-D-glucopyranoside, and twelve fatty acids were isolated from the fruiting bodies of *Cordyceps sinensis* by TLC and reverse phase HPLC (Shiao *et al.* 1989). According to their findings, the profiles of nucleosides and nitrogen bases in *C. sinensis* and allied entomopathogenic fungi, e.g., *C. memorabilis* (Lesat.) Sacc., *C. militaris, C. phingum* Berk., and Curt., *Paecilomyces cicadae, P. farinosus, P. javanicus,* and an unidentified *Cordyceps* species, are very similar. The profile might be used as standards for typification of the commercial products of these medicinal fungi (Shiao *et al.* 1994).

Different studies revealed that *C. sinensis* possesses important pharmacological activities capable of modulating immune responses and bioactive metabolites and promotion of natural vitality (Zhu *et al.* 1998a,b). An active fraction in the ascostroma of *C. sinensis* has been isolated and its structures identified (Tzean *et al.* 1997). The steroid derivative, termed "HI-A", which might be used for the suppression of activated mesangical cells and IgA nephropathy in humans was detected (Lin *et al.* 1996). *In vivo,* polysaccharides with peptide-moiety from *C. sinensis* have been shown to modulate the immune response, inhibit tumor growth, relieve hyperglycemia and arrhythmia, block platelet aggregation, and promote erythropiesis (Chen *et al.* 1997; Yamada *et al.* 1984). Growth inhibitors (CS-36-39 and CS-48-5), other than Cordycepin and polysaccharides, have also been isolated from the fruiting bodies of *C. sinensis.* The active fractions significantly inhibit the growth of the erythro leukemia (K 562) and also some tumor and transformed cell lines. These fractions act as immunomodulatory agents (Tzean *et al.* 1997). They significantly inhibit the blastogenesis, natural killer cell activity, and interleukin-2 (IL-2) production of human mononuclear cells stimulated by phytohemagglutinin (Kuo *et al.* 1994, 1996).

Among pharmacological studies, anti-tumor effects are of major interest to scientists. *Cordyceps sinensis* has been used as medicines to modulate immune responses, inhibit the growth of tumor cells, enhance hepatic energy, promote the secretion of adrenal hormones, and possesses hypotensive and vasorelaxant (Huang *et al.* 2001b). The studies also illustrated that *C. sinensis* can enhance reproductive activity, restore the impaired reproductive function and enhance libido in humans (Zhu *et al.* 1998a). However the evidences for influencing the reproductive system, or even directly affecting sexual hormone release, such as testosterone from Leydig cells and estrogen and progesterone from granulose or theca cells is still lacking (Huang *et al.* 2001b).

Determination of the effect of *C. sinensis* on steroidogenesis in purified mouse Leydig cells has been carried out. *Cordyceps sinensis* extracts stimulated normal mouse Leydig cell steroidogenesis in a dose-dependent relationship (Huang *et al.* 2001b).

Kuo *et al.* (1994) reported that growth inhibitors against tumor cells are composed in the fruiting body of *Cordyceps sinensis*, besides Cordycepin and polysaccharides (Bok *et al.* 1999). *Cordyceps sinensis* is also useful for the general debility treatment after sickness and for elderly (Chang and But. 1987). Aqueous phosphate buffer saline (PBS) extracts of *Cordyceps sinensis* were used to evaluate the effect of *C. sinensis* on the smooth vascular muscle and whether it could be used for treatment of hypertension (Chiou *et al.* 2000). *Cordyceps sinensis* contains a polypeptide macro-molecule that significantly reduces mean arterial pressure of rats by inducing a direct endothelium-dependent vasorelaxant effect through stimulating the production of nitric oxide and endothelium-derived hyperpolarizing factor (Chiou *et al.* 2000).

Cordyceps sinensis has various medicinal properties including a tonic supplement for sexual dysfunction (Zhu *et al.* 1998b). *Cordyceps sinensis* can stimulate Leydig cells and granulose-lutein cells to produce sex steroids (Huang *et al.* 2000, 2001a,b, 2004a,b; Hsu *et al.* 2003a; Chen *et al.* 2005). *Cordyceps sinensis* mycelium can activate steroidogenesis both in mouse Leydig cells, MA-10 Leydig tumor cells and human granulose-lutein cells and also enhance the cyclic-AMP-protein-kinase-A signal pathways, but not protein kinase C, to regulate steroidogenesis in purified mouse Leydig cells (Huang *et al.* 2000, 2001a,b, 2004a,b; Hsu *et al.* 2003a). Chen *et al.* (2005) also confirmed that *C. sinensis* can vitalize the cyclic-AMP-protein-kinase-A signal pathway and to adujst steroidogenesis in purified mouse Leydig cells (Hsu *et al.* 2003b). However it cannot activate the protein kinase C (Hsu *et al.* 2003b). This fungi also increased StAR protein and aromatase expression to induce 17 β-estradiol in human granulose-lutein cells (Huang *et al.* 2004a,b). The mechanism through which *C. sinensis* upregulates steroidogenesis in MA-10 mouse Leydig tumor cells however, remains elusive.

There are some remarkable challenges relating to research surrounding *C. sinensis*, such as difficulties in identification of the various *Cordyceps* species and many conflicting reports of medicinal abilities in literatures (Buenz *et al.* 2005). Recently, reports on the ethnomedical uses of *C. sinensis* are limited to the application as a general tonic in China (Huang *et al.* 1981; Jiang. 1991; Hanssen and Schadler. 1982) and as an aphrodisiac in Nepal (Bhattarai. 1989, 1992a,b, 1993, 1994). However, some interesting reports have been focused on the biological functions of *C. sinensis* and its ability to modify apoptotic homeostasis (Buenz *et al.* 2005). The term

"apoptosis", or programmed cell death, is an essential event in organism development (Vaux and Korsmeyer. 1999; Hidalgo and Ffrench-Constant. 2003) and homeostatis (Cory and Adams. 2002; Kucharczak *et al.* 2003); however, it is becoming clear that numerous disorders such as stroke (Zheng *et al.* 2003), myocardial infarction (Krijnen *et al.* 2002), and HIV (Buenz and Badley, 2004) incorporate apoptosis in their etiology and pathogenesis (Buenz *et al.* 2005). The alteration of the apoptotic pathway of *C. sinensis* is not so straightforward. The inhibiting and inducing of apoptosis which extracted from *C. sinensis* resulted a phenomenon level of observation. Treatment with *C. sinensis* gave decreased caspase-3 activity (Shahed *et al.* 2001.)

The cordycepic acid (mannitol-D), the principle single marker compound of *C. sinensis* does not necessarily guarantee the presence of other potentially active compounds (Buenz *et al.* 2005).

Cordyceps militaris is also a popular medicinal entomopathogenic species that has been widely used in Traditional Chinese Medicine for a long time (Yu *et al.* 2004; Won and Park 2005). It possesses similar bioactive compounds and functions to those of *C. sinensis* (Yu *et al.* 2004). *Cordyceps militaris* is widely used due to folklore, which are not based on scientific findings (Won and Park. 2005). Only a few pharmacological and biochemical actions of *C. militaris* have been reported (Won and Park. 2005). Nan *et al.* (2001) reported that extracellular biopolymers from mycelial liquid cultures of *C. militaris* produced antii-fibrotic effects on fibrotic rats induced by a bile duct ligation and scission operation. This fungus also inhibited proliferation of cultured human glomerular mesangial cells induced by low-density lipoprotein (Wu *et al.* 2000) and produced Cordycepin (3′-deoxyadenosine), which can inhibit the growth of various tumor cells (Johns and Adamson. 1976; Muller *et al.* 1977; Kodama *et al.* 2000).

Cordycepin can be also useful as a new preventive agent against various diseases caused by clostridia (Ahn *et al.* 2000) and possesses larvicidal activity against *Plutella xylostella* via a direct effect rather than an inhibitory action on chitin synthesis (Kim *et al.* 2002). *Cordyceps militaris* extracts have significant anti-angiogenesis and anti-tumor growth properties ***in vivo*** and ***in vitro*** (Yoo *et al.* 2004). *Cordyceps militaris* extracts possess anti-inflammatory and anti-angiogenic activites, which are supported by its inhibitory activity on inducible nitric oxide (iNOS) expression (Won and Park. 2005).

The effect of oxygen supply on Cordycepin production was conducted in submerged cultivation of *C. militaris* by using a 5-L turbine-agitated bioreactor (TAB) (Mao and Zhong. 2004). The proposed two-stage dissolved oxygen (DO) control strategy significantly enhanced the Cordycepin production and productivity (Mao and Zhong. 2004). This is also a good evidence for

the effective large-scale production of bioactive compound from entomopathogenic fungi (Mao and Zhong. 2004).

Cordyceps militaris water extract has beneficial effects on insulin utilization by increasing the glucose disposal rate in skeletal muscles without altering the insulin secretion capacity of pancreatic β-cells. However, *Paecilomyces tenuipes* possesses rather deleterious effects on treating diabetes; it suppressed the insulin secretion capacity of pancreatic β-cells, whole body glucose disposal in rats, and glucose utilization in skeletal muscles (Choi *et al.* 2004).

Besides *Cordyceps sinensis* and *C. militaris*, some other entomopathogenic fungi can produce natural products with various biological activities which are useful in pharmacology and biological control programmes. Isaka *et al.* (2000) reported that two unique anhydrides: Cordyanhydrides A and B were isolated and identified from a culture broth of the insect pathogenic fungus *Cordyceps pseudomilitaris* although these compounds are rare in nature. A small amount of epidithiodike-topiperazine was isolated from *Hirsutella kobayasii*, however further biological testing cannot be done due to lack of materials. Another bioactive compound cyclohexadepsipeptide, named Hirsutellide A, was isolated from a cell extract of the entomopathogenic fungus *Hirsutella kobayasii* and exhibited anti-mycobacterial and low anti-malarial activities with no toxic effects (Isaka *et al.* 2000). Hajek and St. Leger (1994) have listed the many classes of secondary metabolites produced by a few invertebrate pathogenic fungi which have been commonly isolated and screened (Hywel-Jones. 2001).

Fruiting bodies of *Paecilomyces japonica*, a new strain, was compared with *C. sinensis*, a wild form of *Cordyceps* in anti-tumor and immuno-stimulating activities (Shin *et al.* 2003). Based on their findings, *P. japonica* was found to possess similar biological activities to those of *C. sinensis*. Thus *P. japonica* could be substituted as a new health food and new type of alternative drug (Shin *et al.* 2003).

Chen *et al.* (1995) reported that a novel bioactive compound desmethyldestruxin B2, extracted from *Metarhizium anisopliae,* has been found to be suppressing the hepatitis B virus surface antigen production in human hepatoma cells.

In Thailand, some, 2200 isolates of invertebrate pathogenic fungi have been made and screened for their bioactivity in a series of assay: anti-malaria, TB, anti-bacterial and fungal, HIV/AIDS (Jones. 2004). Ten new bioactive compounds has been selected and characterized from the member of the *Hypocreales* (Table 2.3). Disarapong (2003) researched the anti-fungal properties of *Hypocrella scutata* and reported that 25 of 35 isolates exhibited activity (73.5%).

Eighteen isolates (53%) were active against *Microsporum gypseum,* while one (3%) was active against *Cladosporium* sp., *Alternaria* sp. and *Curvularia* sp. and six (17.6%) were active against all four test fungi (Jones. 2004).

Table 2.3. List of new bioactive or novel compounds isolated and characterized from Thai Entomogenous fungi according to Jones (2004).

Fungus	New compound	Activity	Reference
Cordyceps unilateralis	Naphthoquinine	--	Kittakoop *et al.* 1999
Cordyceps pseudomilitaris	Cordyanhydrides A and B	--	Isaka *et al.* 2000
Paecilomyces tenuipes	Cyclodepsipeptides	Anti-mycobacterial and anti-plasmodial	Nilanonta *et al.* 2000
Cordyceps nipponica	N-hydroxy-N-methoxy-2-pyridones: Cordypyridones A-D	Anti-malarial	Isaka *et al.* 2001
Cordyceps pseudomilitaris	Eleven bioxanthracenes	Evaluated for their anti-malarial activity	Jaturapat *et al.* 2001
Hirsutella kobayasi	Hirsutellide A	Anti-mycobacterial	Vongvanich, *et al.* 2003
Verticillium heipterigenum	Enniatins H and I	Anti-malarial Anti *T. tuberculosis* Weakly oxiccytotoxic	Nilanonta *et al.* 2003
Verticillium heipterigenum	Bisdethiodi(methylthio)-dimethylhyalodendrin 1-demethylhyal odendrin tetrasulfide	Inactive Anti-malarial Both cyto	Nilanonta *et al.* 2003

Table 2.3 (continued).

Fungus	New compound	Activity	Reference
Hypocrella discoidea	Various novel compounds(+)	All are cytotoxic	Watts *et al.* 2003
H. tamurai	Rugulsin; skyrin	Towards Sf (insect cell lines)	
Aschersonia samoensis			
A. badia			
A. tamurai			
Verticillium heipterigenum	Novel asochlorin glycoside	Cytotoxic	Seephonkai *et al.* 2004

2.5 Entomogenous fungi as biocontrol agents

Recently, many entomopathogenic fungi have been considered to be useful as biocontrol agents. These fungi have been useful for the crop pest protection program, and some have been produced as commercial products (Thungrabeab *et al.* 2006). The application of microorganisms in integrated pest management system was proposed by pioneers in invertebrate pathology such as Agostino Bassi, Louis Pasteur, and Elie Metchnikoff (Steinhaus. 1956, 1975). In the late 19th century, the experiments relating to entomopathogenic fungi as biocontrol agents were carried out by various scientists (Lacey *et al.* 2001). However, the usefulness of these fungi in insect pest control system is limited due to their lack of broad-spectrum activity (Jones. 2004).

Steinkraus and Hollingsworth (1994) reported the application of natural epizootics in integrated pest management (IPM) with an example of the regulation of the cotton aphid *Aphis gossypii* Glover, a significant sucking pest of cotton. They also mentioned about the entomophthoralean fungus *Neozygites fresenii* (Nowakowski) Batko which often reduces or eliminates the requirement for chemical control of this pest (Steinkraus *et al.* 1991, 1995). In fact, most species of entomophthoralean fungi have been relatively difficult to produce and their primary conidia are short lived. Lacey *et al.* (2001) suggested that development of effective methods for production of resting spores and competent mycelia of entomophthoralean species can support the utility of these fungi.

Anamorphic entomopathogenic fungi exhibit as broad spectrum pesticide in pest control program and can be commercially produce especially for controlling homopterous pest insects. Several species can be potentially produced on artificial media with low cost and possessed good shelf lives. This is an advantage for using fungi as biocontrol agent in pest protection. *Aschersonia aleyrodis, Beauveria bassiana, Hirsutella thompsonii, Metarhizium anisopliae* and *Verticillium lecanii* are currently marked as potential biocontrol agents in the Brazil, England, USA, and USSR.

These fungi have been investigated for use to control a broad range of insect pests, including whiteflies, aphids, thrips, termites, grasshoppers and locusts, beetles, and others (McCoy *et al.* 1988; Ferron *et al.* 1991; Fargues and Maniania. 1992; Khan *et al.* 1993; Zimmermann. 1993; Devi. 1994; Milner and Prior. 1994; Feng *et al.*, 1994; Goettel *et al.* 1995, 2000; Lacey *et al.* 1996; Keller *et al.* 1997; Milner. 1997). Currently, some entomopathogenic fungi such as *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii, Paecilomyces fumosoroseus* and experimental isolates of *Metarhizium flavoviride, Nomuraea rileyi,* and *Aschersonia aleyrodis* are used as commercial products or in process. Despite their somewhat broader host range, the Hyphomycetes still provide a degree of selectivity (McCoy *et al.* 1988; Goettel *et al.* 1990).

Trichomycetes, an another group of arthropod-associated fungi, may be useful as biological control agents, however, so little is known that one cannot even speculate of their value to humanity.

2.6 Future outlook

Entomogenous fungi can be associated with many invertebrate taxonomic groups. However, the accurate data regarding these fungi has been still needed to document. The number of entomogenous species may be in excess of 1000 (Samson *et al.* 1988; Hawksworth *et al.* 1983), although probably less, since several of the genera listed by Samson *et al.* (1988) contain many species which are not invertebrate pathogens. Many taxonomists described a number of taxa associated with arthropods, however, the data relating entomogenous fungi have been scattered. For example, Hawksworth *et al.* (1983) listed 100 *Cordyceps,* 10 *Torubiella* species, while Kobayasi (1982) listed 282 *Cordyceps,* 59 *Torubiella* species and 75 species of other genera. Due

to the list of synonyms, the record of entomogenous taxa in *Indexfungorum* is found to be huge number (Table 2.4).

Table 2.4. Numbers of known entomogenous species according to Hawksworth *et al.* (1983), Kirk *et al.* (2001) and Indexfungorum (www.indexfungorum.org).

Fungi	Number of species		
	Hawksworth *et al.* (1983)	Kirk *et al.* (2001)	Indexfungorum (www.indexfungorum.org)
Lower fungi	227 or 228	164 or 165	597
Higher fungi	368 or 369	203	2079
Imperfect fungi	416	619	3138
Total	**1011 or 1013**	**986 or 987**	**5814**

The identification of fungal species is commonly based on micromorphological methods and biochemical testing methods (Bastola *et al.* 2004). Numerous methods are available to construct phylogenetic trees that predict the relatedness between microbial species (Nei 1996, Rogers and Swofford. 1998) and the probable evolution of their genetic traits (Tateno *et al.* 1982). A number of molecular techniques have been employed in the detection, identification and phylogenetic analysis of fungal populations (Green *et al.* 2004).

Successful use of entomopathogenic fungi as microbial control agents will ultimately depend on the many factors such as use of the right propagule, formulated in an optimal manner and applied at an appropriate dosage and time. Timing will depend on the presence of susceptible host stages, favorable environmental conditions, and compatible scheduling with other agricultural practices (i.e., linked with irrigation, avoiding fungicides, etc.). Further improvement in the microbial control activity of entomopathogenic fungi can be expected by their combination with other interventions and technologies, use of other biological control agents, use of environmental manipulation to favor the infection processes, and use of targeted pests to aid in the dissemination of fungus.

Generally, the entomogenous fungi have restricted host ranges (Hywel-Jones. 2001). For example, *Hypocrella* (*Clavicipitales*) species are restricted to two families of the Homoptera

(*Aleyrodidae* and *Coccidae*). *Torrubiella* species with *Gibellula* anamorphs are specifically infected on hunting spiders (Samson and Evans. 1973, 1992). However, *Pseudogibellula formicarum* (Mains) Samson and Evans is exceptional as it has been linked with several insect species (Samson and Evans. 1973; Samson *et al.* 1989). Several *Cordyceps* species known from ants (Hymenoptera) are specific to particular complexes or tribes of ants (Evans and Samson. 1982, 1984). The prospects of genetic engineering for improvement of entomopathogenic fungi have steadily increased within the past decade (Ferron *et al.* 1991; Riba *et al.* 1994; St. Leger and Roberts. 1997). Developments in the molecular biology of entomopathogenic fungi will provide the tools for elucidating the mechanisms of pathogenesis and in the future for producing recombinant fungi with enhanced virulence (Charnley *et al.* 1997). The application of molecular techniques in the study of entomopathogenic fungi is presented in detail by St. Leger and Joshi (1997).

Chapter 3

RESEARCH AND METHODOLOGY

3.1 Study areas

A general survey of entomopathogenic fungi was carried out in rainforests of Chiang Mai Province, Thailand at 15-day intervals during the rainy seasons from June 2005 to October 2006. This survey was carried out in the two different types of rainforests: conserved rainforests (Doi Inthanon National Park, Doi Suthep-Pui National Park, Mae Sae National Park, Mokfa Waterfall, New Waterfall and Geyser Pong Dueb Hot Spring) and disturbed rainforests (Mushroom Research Centre, Pha Daeng Village and Tung Joaw Village). In order to compare different non-forest habitats, two agricultural habitats; Mae Lod Coffee Plantation and Mae Ma Lei Village mango orchard were included in this study (Fig 3.1).

3.1.1 List of collecting sites

3.1.1.1 Conserved rainforests

CS-1: Doi Inthanon National Park, at 25 km marker on Highway 1009, North 18° 32.54' East 98° 33.51'.

CS-2: Doi Suthep-Pui National Park, North 18° 48.62' East 98° 54.6'.

CS-3: Mokfa Waterfall, located near 18 km marker on Highway 1095.

CS-4: New Waterfall, located near 36 km marker on Highway 1095.

CS-5: Geyser Pong Dueb Hot Spring.

CS-6: Mae Sae National Park, Located near 50 km marker on Highway 1095.

3.1.1.2 Disturbed rainforests

CS-7: Mushroom Research Centre (MRC), Bahn Pha Deng, North 19° 07.123′ East 98° 44.009'.

CS-8: Pha Daeng Village,

CS-9: Tung Joaw Village, North 19° 8.07' East 98° 38.9'

3.11.3 Agricultural habitat

CS-10: Mae Ma Lei Village, Mango orchard, Located near 20 km marker on Highway 1095.

CS-11: Mae Lod Village, Coffee plantation.

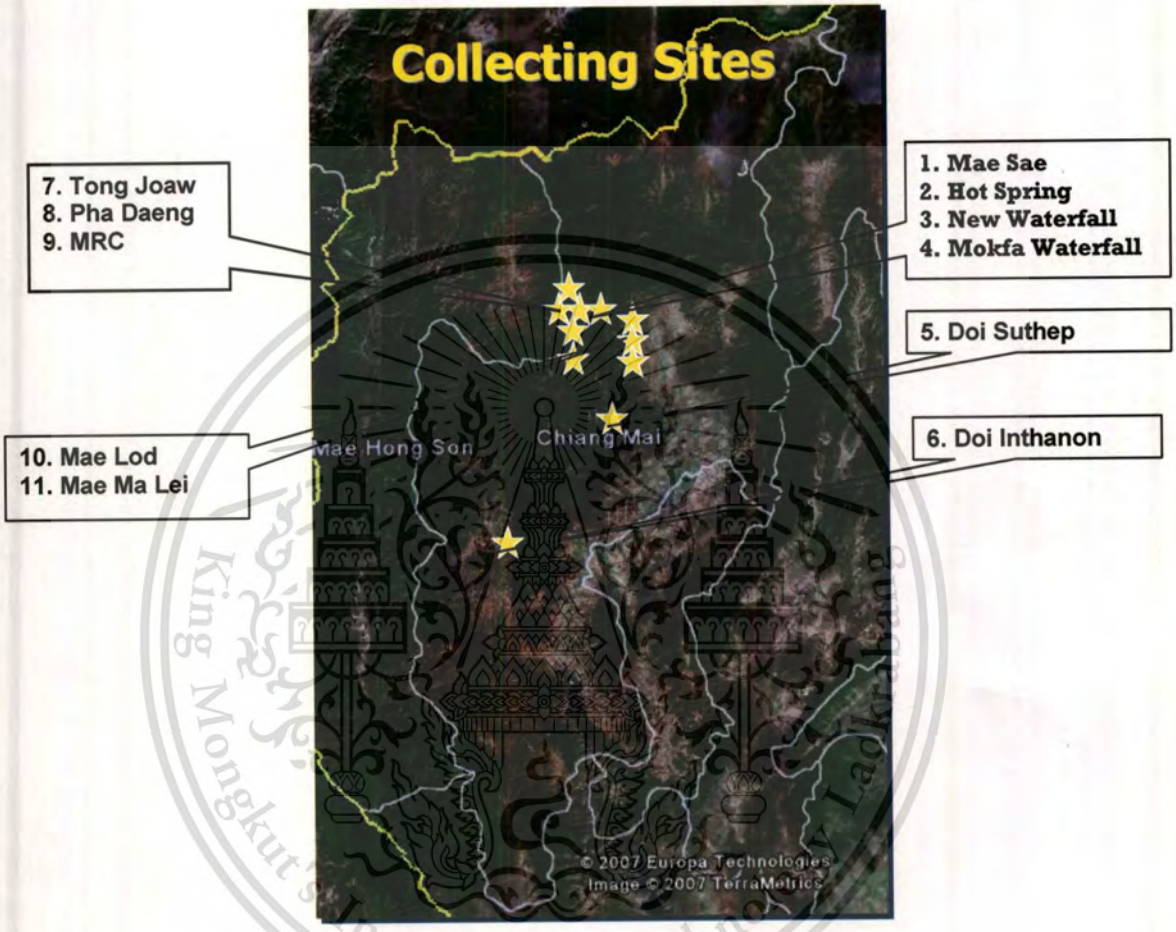


Fig. 3.1 Map of Chiang Mai Province, Thailand showing the collecting sites of entomopathogenic fungi.

3.2 Collecting and Isolation

Special attention was directed towards different arthropod hosts. In addition, soil close to dead insects was sampled and the data was incorporated. Soil, litter, herbaceous plants, and tree leaves were examined for dead insects and other arthropods, which were collected and transported the same day to the laboratory in plastic containers. The samples were examined and microscopic measurements made of the fungi. Details of methods used can be found in Lacey and Brooks

(1997). Soil sampling and isolation method used in this study followed Goettel and Inglis (1997). Single spore isolations were made according to Choi *et al.* (1999). Subcultures were made onto Czapek agar, potato dextrose agar, and Sabouraud dextrose agar according to Brown and Smith (1957) and Samson (1974). Collections are deposited in Mae Fah Luang University Herbarium (MFLUH), Chiang Rai, Thailand and representative strains are maintained in the International Fungal Research and Development Centre (IFRD), The Research Institute of Resource Insects, Chinese Academy of Forestry, Kunming, PR China.

3.3 Entomopathogenic fungi from Chiang Mai Province, Thailand

3.3.1 Taxonomy

After incubation at 25-26°C for 14 days, the colony characters, conidiogenous structures, and other biological features were recorded. Identification of species of entomopathogenic fungi followed Samson *et al.* (1988), Kobayasi (1981), Kobayasi and Shimizu (1983), Luangsa-Ard *et al.* (2005) and Sung *et al.* (2007).

3.3.2 DNA extraction, PCR amplification and sequence analysis

Two different protocols for DNA extractions were used in this study. For the dry specimens, fruit bodies were surface-sterilized in 75% ethanol and cut using a clean razor to obtain uncontaminated fungal tissue. The tissue was ground well into powder in a 1.5 ml microfuge tube with plastic mortar. DNA was extracted from this fungal tissue with E.Z.N.A Forensic DNA Kit (Omega bio-tek product code: D3591-01). For the anamorphic taxa, isolates were grown on potato dextrose agar (PDA) for two to four weeks and total genomic DNA was extracted from fresh mycelium using the protocol as outlined by Jeewon *et al.* (2003) and Lacap *et al.* (2003).

Polymerase chain reaction (PCR) amplification products were obtained with the use of two pairs of primers, universal primers ITS4 and ITS5 (described by White *et al.* 1990) and β-tubulin BT2A and BT2B (Glass and Donaldson. 1995; O'Donnell and Cigelnik. 1997), respectively. ITS sequences were subjected to a nucleotide-nucleotide BLAST (Altschul *et al.* 1997) of the NCBI sequence database (http://www.ncbi.nlm.nih.gov/). The β-tubulin sequences were also used in cases where ITS sequences did not provide adequate BLAST results.

3.4 Diversity of entomopathogenic fungi in rainforests of Chiang Mai Province, Thailand

3.4.1 Data analysis

The frequency of occurrence for each species was calculated by the following formula.

$$\% \text{ Occurrence frequency of taxon A} = \frac{\text{occurrence of taxon A}}{\text{total number of all species}} \times 100$$

The Shannon's diversity and Simpson's diversity indices were applied to evaluate the diversities of entomogenous fungi on different orders of host arthropods and in different study sites (Hayek and Buzas. 1997). Evenness indices were estimated to establish the closeness of equability of species present (Gotelli and Colwell. 2001). Index of similarity was calculated using Sørensen's formula to determine the similarity in species occurrences (Odum. 1971). The similarity values range from 0 to 1 (1 meaning very similar, 0 indicating no similarity) by using the following formula.

$S'=2C/(A+B)$

Where S′ is the degree of similarity, A and B are the number of species at host/sites A and B, respectively and C is the number of species common to both collections.

3.5 Host based relationships of entomopathogenic fungi (*Beauveria, Cordyceps,* and *Paecilomyces*): A phylogenetic evaluation based on ribosomal and protein coding gene sequences

3.5.1 Materials

Entomopathogenic fungi collected from the selected rain forests in Chiang Mai Province, Thailand and isolates generated from the collections were used in this study. Isolates of *Beauveria* and *Paecilomyces* from different hosts, field collected specimens of *Cordyceps* species

were used for DNA extraction. Soil, underneath of *Cordyceps militaris* specimens were sampled and isolated on PDA.

3.5.2 DNA extraction, PCR, and sequencing

Total genomic DNAs were extracted from fresh mycelium and field collected specimens using the protocol as outlined by Jeewon *et al.* (2004), Cai *et al.* (2005, 2006) and Photita *et al.* (2005).

To obtain target DNA, PCR amplification was performed using the primer pairs ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (described by White *et al.* 1990) and β-tubulin BT2A (5'-GGTAACCAAATCGGTGCT GCTTTC-3') and BT2B (5'-ACCCTCAGTGTAGT GACCCTTGGC-3') (Glass and Donaldson. 1995; O'Donnell and Cigelnik. 1997), respectively. The amplification reaction was performed in a 50 µl reaction volume as outlined by Cai *et al.* (2005, 2006).The PCR thermal cycle consisting of 95° C for 3min, followed by 30 cycles of denaturation at 95° C for 1 min, annealing at 52° C for 50 s and elongation at 72° C for 1 min, with a final extension step of 72° C for 10 min. PCR products were checked on 1% agarose electrophoresis gels stained with ethidium bromide. PCR products were then purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (Amersham product code: 27-9602-01). DNA sequencing was performed using the primers mentioned above in an Applied Biosystem 3730 DNA Analyser at the Genome Research Centre, The University of Hong Kong.

3.5.3 Sequence alignment and phylogenetic analyses

For each fungal strain, sequences generated from the ITS/5.8S and β-tubulin gene together with reference sequences obtained from GenBank were aligned using CLUSTALX (1.83) (Thompson *et al.* 1997). Bioedit (Hall. 1999) was used to obtain an assembled sequence. Information regarding fungal specimens and culture used in this study is shown in Table 4.6. Alignment was manually adjusted to allow maximum alignment and minimize gaps. Phylogenetic analyses based on maximum parsimony (MP) were performed for single gene dataset (ITS and β-tubulin) as well as for a combination of the two genes in PAUP * 4.0b10 (Swofford. 2002). Ambiguously aligned regions were excluded from all analyses. Datasets were initially analyzed using weighted and unweighted parsimony. All characters were unordered. The heuristic search option was used, ignoring invariant and uninformative characters. Random addition of sequences

with tree bisection-reconnection (TBR) branch swapping was performed. MulTrees option was in effect, and zero-length branches were collapsed. Weighted parsimony analyses were conducted in which changes among transitions, transversions, and gaps were subjected to a symmetric stepmatrix generated using STMatrix ver.2.2 (François Lutzoni and Stefan Zoller, Biology Department, Duke University) as described by Miller and Huhndorf (2004). Clade stability was assessed in a bootstrap analysis with 1000 replicates, each with ten replicates of random stepwise addition of taxa.

MrModeltest 2.2 (Nylander. 2004) was used to get the best-fit model of substitution for maximum likelihood (ML) analyses. Bayesian posterior probabilities (PP) (Rannala and Yang. 1996; Zhaxybayeva and Gogarten. 2002) were calculated by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v3.0. (Huelsenbeck and Ronquist. 2001) using above model of evolution. The implemented model was the same as that for ML analysis. Six simultaneous Markov chains were run for one million generations (resulting in 10,000 trees). The first 2,000 trees which represented the burn-in phase of the analysis were discarded. The remaining 8,000 trees were used for calculating the PP in the majority rule consensus tree. Trees were viewed in Treeview (Page. 1996).

3.6 Morphological and phylogenetic study of *Beauveria* spp. from Chiang Mai Province, Thailand

3.6.1 Morphological study

Entomopathogenic fungi collected from the selected rain forests in Chiang Mai Province, Thailand were examined for infection of *Beauveria* species by means of direct observation and microscopic measurement. In order to study morphological characteristics of these fungi, single spore isolation and conidial isolation were made according to Choi *et al.* (1999). The subcultures were made onto Czapek agar according to Brown and Smith (1957) and Samson (1974). After incubation at 25-26°C for 14 days, the colony characters and other biological features including conidiogenous structure, conidia size, hyphae size and rachis length were measured and recorded. Twenty numbers of biological features from 17 isolates were measured and statistically analyzed.

3.6.1.1 Analysis of morphology data

Data were analyzed using analysis of variance ($P < 0.05$) with SPSS software version 13.0 (SPSS Inc., Chicago, USA) (Kirkpatrick and Feeney. 2006).

3.6.2 Phylogenetic study

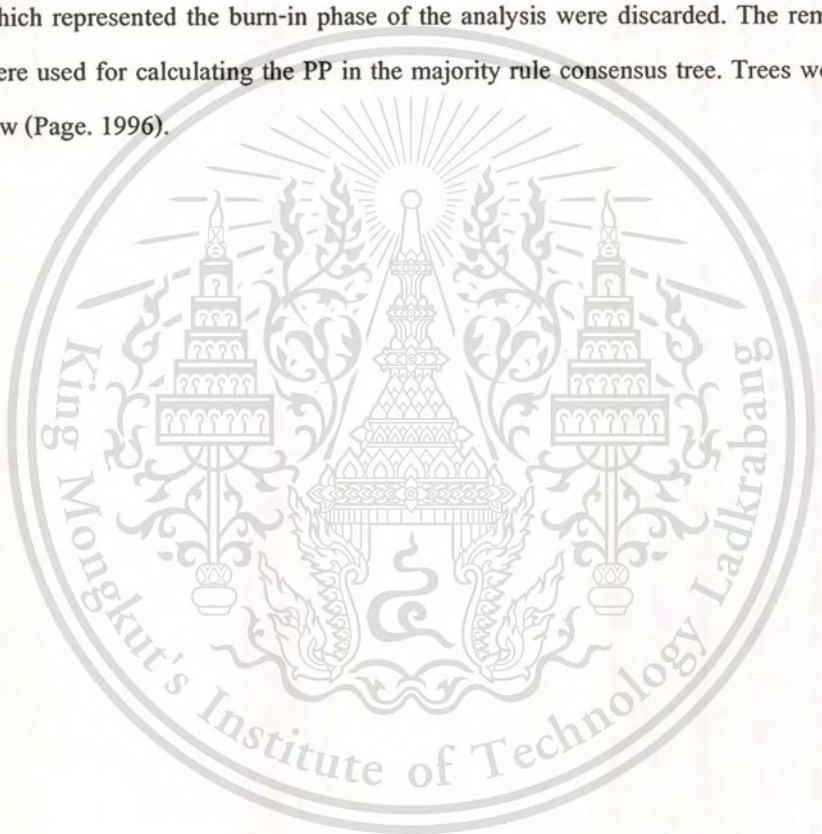
Fungal isolates were also grown on potato dextrose agar (PDA) for two to four weeks and total genomic DNA was extracted from fresh mycelium using the protocol as outlined by Jeewon *et al.* (2004), Cai *et al.*(2005, 2006) and Photita *et al.* (2005).

To obtain target DNA, PCR amplification was performed using the primer pair ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (described by White *et al.* 1990). The amplification reaction was performed in a 50 µl reaction volume as outlined by Cai *et al.* (2005, 2006).The PCR thermal cycle consisting of 95° C for 3min, followed by 30 cycles of denaturation at 95° C for 1 min, annealing at 52°C for 50 s and elongation at 72°C for 1 min, with a final extension step of 72°C for 10 min. PCR products were checked on 1% agarose electrophoresis gels stained with ethidium bromide. PCR products were then purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (Amersham product code: 27-9602-01). DNA sequencing was performed using the primers mentioned above in an Applied Biosystem 3730 DNA Analyser at the Genome Research Centre, The University of Hong Kong.

For each fungal strain, sequences generated from the ITS/5.8S gene together with reference sequences obtained from GenBank were aligned using CLUSTALX (1.83) (Thompson *et al.* 1997). Bioedit (Hall. 1999) was used to obtain an assembled sequence. Information regarding fungal specimens and culture used in this study is shown in Table 4.6. Alignment was manually adjusted to allow maximum alignment and minimize gaps. Phylogenetic analyses based on maximum parsimony (MP) were performed for ITS in PAUP * 4.0b10 (Swofford. 2002). Ambiguously aligned regions were excluded from all analyses. Datasets were initially analyzed using weighted and unweighted parsimony. All characters were unordered. The heuristic search option was used, ignoring invariant and uninformative characters. Random addition of sequences with tree bisection-reconnection (TBR) branch swapping was performed. MulTrees option was in effect, and zero-length branches were collapsed. Weighted parsimony analyses were conducted in which changes among transitions, transversions, and gaps were subjected to a symmetric stepmatrix generated using STMatrix ver.2.2 (François Lutzoni and Stefan Zoller, Biology

Department, Duke University) as described by Miller and Huhndorf (2004). Clade stability was assessed in a bootstrap analysis with 1000 replicates, each with ten replicates of random stepwise addition of taxa.

MrModeltest 2.2 (Nylander. 2004) was used to get the best-fit model of substitution for maximum likelihood (ML) analyses. Bayesian posterior probabilities (PP) (Rannala and Yang. 1996; Zhaxybayeva and Gogarten. 2002) were calculated by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v3.0. (Huelsenbeck and Ronquist. 2001) using above model of evolution. The implemented model was the same as that for ML analysis. Six simultaneous Markov chains were run for one million generations (resulting in 10,000 trees). The first 2,000 trees which represented the burn-in phase of the analysis were discarded. The remaining 8,000 trees were used for calculating the PP in the majority rule consensus tree. Trees were viewed in Treeview (Page. 1996).



Chapter 4

RESULTS

4.1 Entomopathogenic fungi from Chiang Mai Province, Thailand

Thirty four taxa belonging to 15 genera of entomopathogenic fungi from Chiang Mai Province, Thailand are described and discussed in this thesis. These include 34 taxa, 18 teleomorphs and 16 anamorphs. *Ophiocordyceps mrciensis* and *Hymenostilbe furcata* are new to science. New records for Thailand include *Cordyceps militaris* and *Cordyceps militaris var. sphaerocephala*. Sexual states found and detailed are *C. militaris*, *C. militaris*. var. *sphaerocephala*, *C. nelumboides*, *Cordyceps* sp., *Hypocrella* sp. *Ophiocordyceps crinalis*, *O. dipterigena*, *O. elongata* , *O. filiformis*, *O. longissima*, *O. mrciensis*, *O. myrmecophila*, *O. nutans*, *O. oxycephala*, *O. pseudplloydii*, *O. sphecocephala*, *O. unilateralis*, and *Torrubiella hemipterigena*. Anamorphic states found and provided with descriptions and illustration are *Acremonium charticola*, *A. crassum*, *Aschersonia* sp., *Aspergillus* sp., *Beauveria bassiana*, *B. brongniartii*, *Cladosporium* sp., *Hymenostilbe furcata*, *Isaria cicadae*, *I. farinosus*, *I. fumosoroseus*, *I. tenuipes*, *Paecilomyces marquandii*, *Sporothrix insectorum*, *Stilbella buqueti* and *Verticillium* sp. In order to help further elucidate the taxonomy of taxa, BLAST results of sequence data of the treated species are presented.

4.1.1 Sequence analysis

Sequence data obtained from the PCR products are deposited in GenBank (Table 4.6). BLAST searches resulted in associations with known fungal species or orders. The expected values (E-values) are given for BLAST searches. The E-value indicates the statistical significance of a given pairwise alignment and reflects the size of the database and the scoring system. These results are discussed in the descriptive notes below each treated species, albeit not possible to extract DNA from some taxa.

4.1.2 Taxonomy

4.1.2.1 Teleomorphs

4.1.2.1.1 ***Cordyceps*** Fr., Observ. Mycol. 2 (revis.): 31 (1818) emend. G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora

= *Phytocordyceps* C.H. Su & H.H. Wang, Mycotaxon 2: 338 (1986).

Stroma or subiculum pallid or brightly pigmented, fleshy. *Perithecia* superficial to completely immersed, ordinal in arrangement. *Asci* hyaline, cylindrical with thickened ascus apex. *Ascospores* hyaline, cylindrical multiseptate, disarticulating into part-spores or non disarticulating, rarely possessing a thread-like structure connecting the fusiform ends.

Anamorphs: *Beauveria*, *Isaria*, *Lecanicillium*, *Mariannaea*-like, *Microhilum*, *Simplicillium*.

Type species: *Cordyceps militaris* (L.: Fr.) Fr., Observ. Mycol. 2(revis.): 317 (1818).

Reference: Sung *et al.* 2007.

Cordyceps militaris (L.) Link, Handbuck zur Erkennung der Nutzbarsten und am Häufigsten Vorkommenden Gewächse 3: 347 (1833). (Fig. 4.1)

≡ *Clavaria militaris* L., Sp. Plantarum: 1182 (1753).

≡ *Hypoxylon militare* (L.) Mérat, Nouv. Fl. Envir. Paris: 137 (1821).

≡ *Xylaria militaris* (L.) Gray, Nat. Arr. Brit. Pl. (London): 10 (1821).

≡ *Sphaeria militaris* (L.: Fr.) Fr., Syst. Mycol. 2: 32 (1823).

≡ *Torrubia militaris* (L.: Fr.) Tul. & C. Tul., Sel. Fung. Carpol. 3: 6 (1865).

Ascostroma solitary or sometimes several, usually arising from the head, but sometimes from articulations of the pupa, yellowish-orange, usually simple, 2-5 cm. high; clavate, 1-2 cm. long, 3-5 mm. thick, tuberculose. *Perithecia* subconical, 400-570 × 250-325 µm. *Asci* 150-300 × 4-5 µm. *Ascospores* 1 µm or less in diam., breaking up into short segments 2-6 µm long.

Host: Larva/pupa, Lepidoptera.

Material examined: THAILAND, Chiang Mai Province, Doi Inthanon National Park, along Highway 1009 at 25 km marker, N18°32.56' E098°33.51', 1073 m alt., on larva/ pupa Lepidoptera, 14 June 2006 O.M. Aung MFLU873, MFLU874 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123' East 98° 44.009', 2 September 2006, O.M. Aung MFLU918, MFLU919 (MFLUH); *ibid.*, 14 September 2006, O.M. Aung MFLU953, MFLU954, MFLU955 (MFLUH); *ibid.*, 15 September 2006, O.M. Aung MFLU956, (MFLUH); *ibid.*, 19 September 2006, O.M. Aung MFLU958, MFLU959, (MFLUH); *ibid.*, 30 September 2006, O.M. Aung 967, (MFLUH); *ibid.*, 2 October 2006, O.M. Aung MFLU969, (MFLUH).

Remarks: The collections are very similar to *C. militaris*. There are eight *Cordycep* species which are morphologically very close to *C. militaris* such as *C. kyusyuensis* A. Kawam., *C. roseostromata* Kobayasi & Shimizu, *C. pruinosa* Petch, *C. takaomontana* Yakush. & Kumaz., *C. pseudomilitaris* Hywel-Jones & Sivichai, *C. bassiana* Z.Z. Li, C.R. Li, B. Huang & M.Z. Fan, and *C. brongniartii* Shimazu. This species is easily confused with *C. kyusyuensis*. The main differences between these two species are in morphology. *Cordyceps militaris* has shorter ascostromata (20-40 × 1-5 mm vs. 20-70 × 1.5-2 mm) (Kobayasi. 1982). This is the new record of *C. militaris* for Thailand.

Blasting the ITS sequence of this species shows it to be similar (E-value = 0.0) to a *Cordyceps militaris* sequence from GenBank (AJ536564; 98% similarity), as well as. *C. brongniartii* (AB258367, AB237659, AY245628; 98% similarity), *B. brongniartii* (AB027381; 98% similarity), and *C. bassiana* (AY334542, 98% similarity). Dendrograms from phylogenetic analysis of the entomopathogenic taxa collected in this study are shown in Section 4.3. The sequences of the Thailand collections clusters with *B. brongniartii*. Further collections are needed to confirm the teleomorph-anamorphs connection between this *Cordyceps militaris* and *B. brongniartii*.

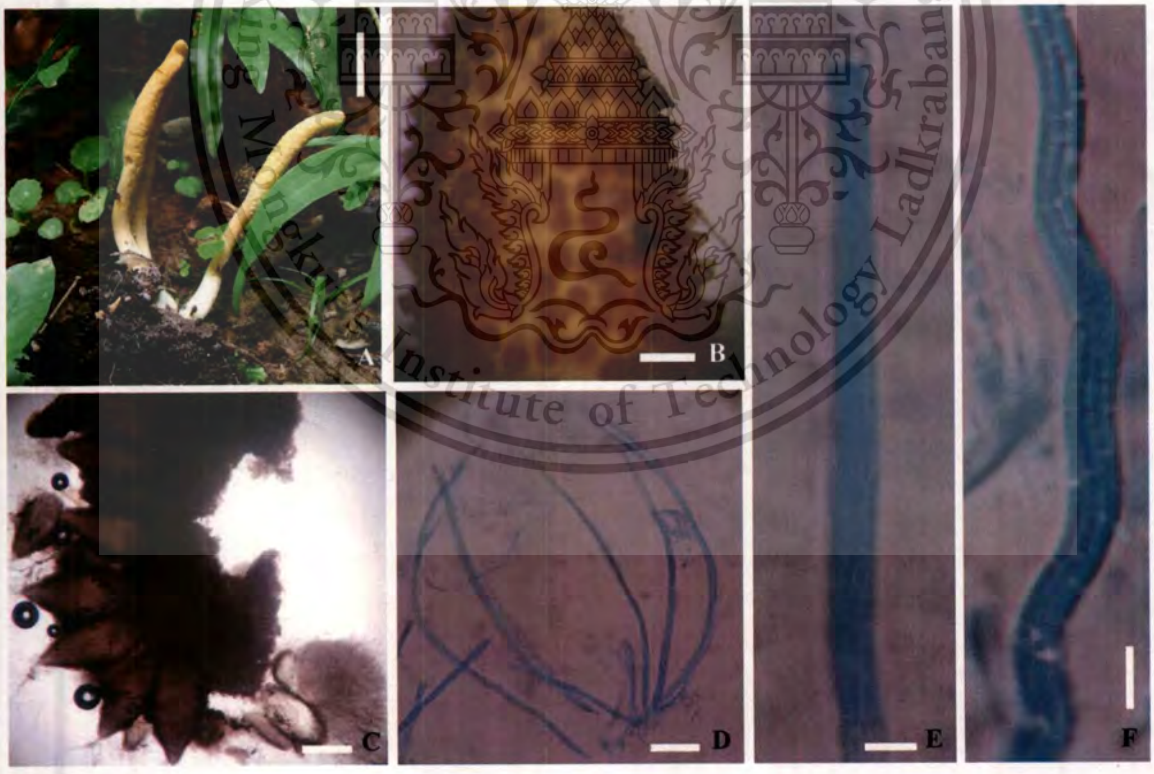


Fig. 4.1 *Cordyceps militaris*. **A.** Ascostromata arising from infected host caterpillar. **B.** Fertile part. **C.** Semi immersed perithecia. **D.** Asci. **E.** Tip of ascus. **F.** Ascospores breaking into partspores. Bars: A = 10 mm, B = 1 mm, C = 250 µm, D = 50 µm, E & F = 5 µm.

Cordyceps militaris* var. *sphaerocephala J.C. Schmidt, Myc. Heft. 1: 106 (1817). (Fig. 4.2)

Ascotromata stipitate, arising from various parts of the caterpillar host, some capitate. *Stipes* cylindrical to clavate, simple or occasionally branched, 2-32 mm long, 0.5-2 mm wide, wider towards the fertile apex, 1-1.5 mm long, 0.15-0.2 mm thick, light yellow to reddish-yellow, become dark-brown to dark with age. *Fertile part* terminal, irregular, some hemispherical or subglobose, 1-3 mm wide, 1-2 mm thick, yellow to light orange, surface roughened by erumpent, light brown to dark brown ostioles. *Perithecia* narrowly ovoid, pseudoimmersed, 875-1175 × 250-350 µm. *Asci* cylindrical, 8-spored, 150-480 × 3-5 µm, *ascus cap* ± 2 µm high and 3 µm wide. *Ascospores* filiform, multispetate, hyaline, breaking into secondary partspores. *Partspores* cylindrical to barrel shape, 3-5 × 1-2 µm.

Host: Caterpillar, Lepidoptera.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Geyser Pong Dueb Hot Spring, on Lepidoptera larva, 26 September 2006 O.M. Aung MFLU963 (MFLUH).

Remarks: This single specimen is identified as *C. militaris* var. *sphaerocephala*, because it is consistent with the description and illustration by Petch (1944) and Shimizu (1994).

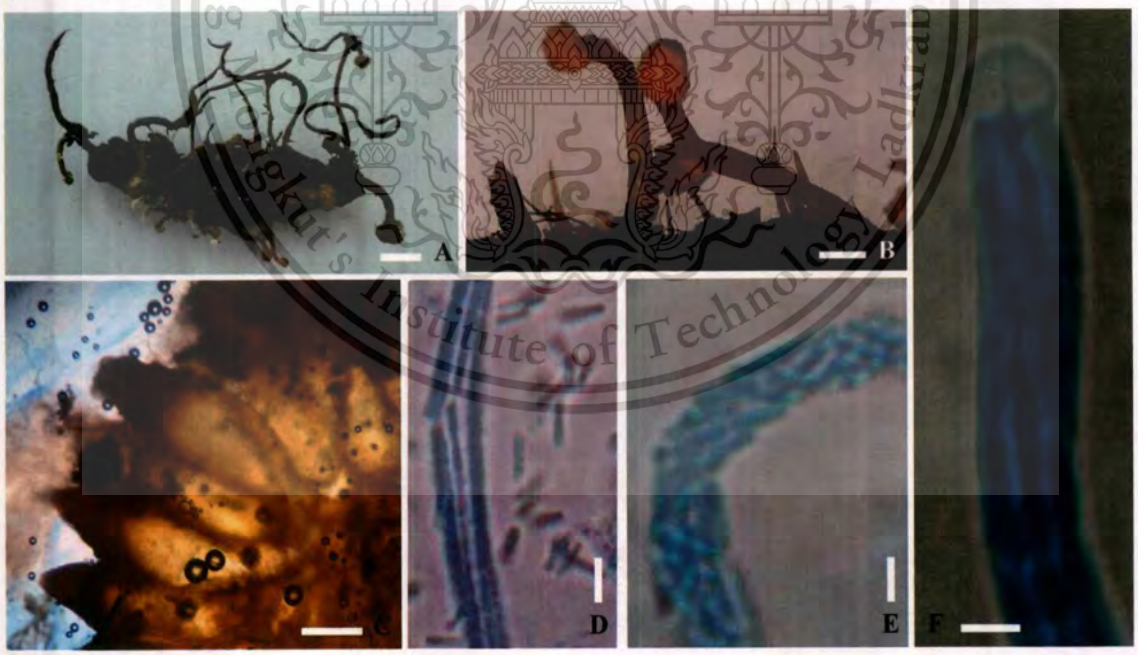


Fig. 4.2 *Cordyceps militaris* var. *sphaerocephala*. **A.** Numerous ascostromata arising from infected host caterpillar. **B.** Fertile part. **C.** Semi immersed perithecia. **D.** Ascospores breaking into partspores. **E.** Partspores. **F.** Tip of ascus. Bars: A = 10 mm, B = 5 mm, C = 250 µm, D & E= 5 µm, F = 2.5 µm.

Blasting the ITS sequence of this species shows it to be similar (E-value = 0.0) to a *Cordyceps ramosopulvinata* sequence from GenBank (AB027372), *Paecilomyces* sp. (AB044644), *Cordyceps crinalis* (EF495104), *Cordyceps kanzashiana* (AB027371), and *Cordyceps lianzhouensis* (EU149921). This sequence is the first sequence for this species deposited at the GenBank. Dendrograms from phylogenetic showed that this species is clustered together with *Cordyceps ramosopulvinata* in ITS sequence analysis while β-tubulin sequences are not available in GenBank. ITS sequence analysis also revealed that all taxa clustered together in the Clade D are residual species of *Cordyceps* species, which have been provisionally retained within *Cordyceps* s. l. by Sung *et al.* 2007.

Cordyceps nelumboides Kobayasi & Shimizu, Kew Bull. 31: 557 (1977). (Fig. 4.3)

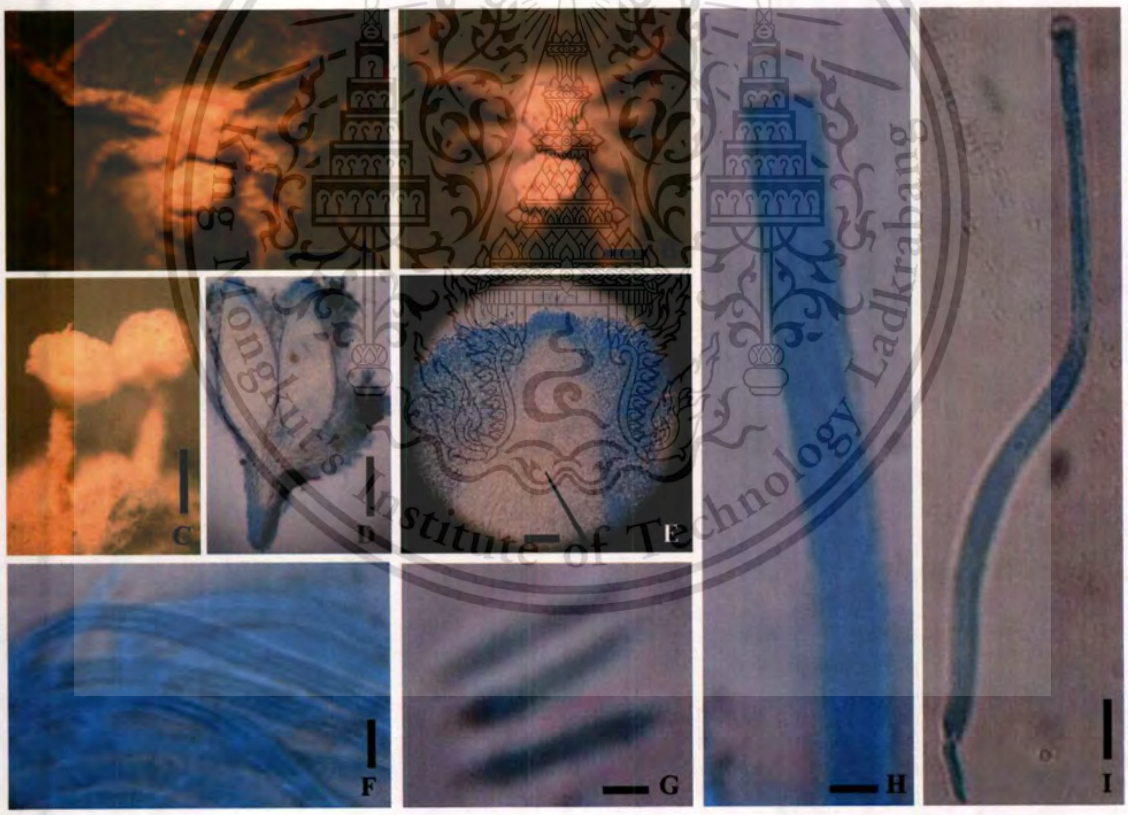


Fig. 4.3 *Cordyceps nelumboides*. **A.** Infected spider. **B.** Fertile part. **C.** Two ascostromata arising from infected host. **D.** Perithecia. **E.** Pseudoimmersed perithecia. **F.** Ascospores. **G.** Partspores. **H.** Tip of ascus. **I.** Ascus. Bars: A = 2.5 mm, B & C = 2 mm, D = 150 μm, E = 50 μm, F = 5 μm, G & H = 2.5 μm, I = 15 μm.

Infected spider covered with grayish-white mycelial mat. *Ascotromata* stipitate, two, arising from dorsal part of spider, some capitate, *Stipes* cylindrical to clavate, 2-3 mm long, 0.5-2 mm wide, greyish-white. *Fertile part* terminal, irregular, hemispherical to subglobose, 1-3 mm wide, 1-2 mm thick, greyish white, surface roughened by erumpent, ostioles 40 µm wide and 20 µm high. *Perithecia* narrowly ovoid, pseudoimmersed, 430 × 220 µm. *Asci* cylindrical, 8-spored, 75-190 × 3-4 µm, asci cap about, 2 µm high and 5 µm wide. *Ascospores* hyaline, breaking into secondary partspores. *Partspores* cylindrical to fusiform, 7-9 × 1.5-2 µm.

Host: Spider.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Pang Sa Det, Mae Sae village, N19°14.59' E98°38.45', 962m alt., on spider, 15 October 2006, O.M. Aung MFLU980 (MFLUH).

Remarks: The single specimen is identified as *Cordyceps nelumboides* but perithecia are shorter (430 × 220 µm vs. 535-545 × 180-190 µm) and asci are also shorter (75-190 × 3-4 µm vs. 400-450 × 5-6 µm) (Kobayasi and Shimizu. 1976).

I was not able to obtain DNA from this specimen.

Cordyceps sp. (Fig. 4.4)

Ascotromata arising from various parts of the caterpillar host, *Stipes* cylindrical to clavate, some flat, simple or occasionally branched, discrete or aggregated, 6-13 mm long, 1-5 mm wide, pinkish-white when young, turning red at the base, pink to white at the tip, becoming red with age. *Fertile part* irregular. *Perithecia* irregular, some obliquely immersed, 250-400 × 125-225 µm. *Perithecial wall* 40-60 µm. *Asci* cylindrical, 6-8-spored, 80-205 × 3-5 µm, *asci cap* about, 2 µm high and 3 µm wide. *Ascospores* filiform, multispetate, hyaline, not breaking into secondary partspores. Septate interval < 1 µm, like oil drops.

Host: Hairy Caterpillar about 25 mm × 10 mm.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', on Lepidoptera larva, 1 October 2006, O.M. Aung MFLU968 (MFLUH).

Remarks: A single specimen was found during the course of study and although it is probably a new species further collections are required before it can be described.

Blasting the ITS sequence of this species shows it to be similar to the sequnces of *Claviceps panicoidearum* (E-value = 4e-160), *Claviceps cynodontis* (E-value = 7e-157) and *Metarhizium flavoviride*. (E-value = 9e-156) from GenBank.

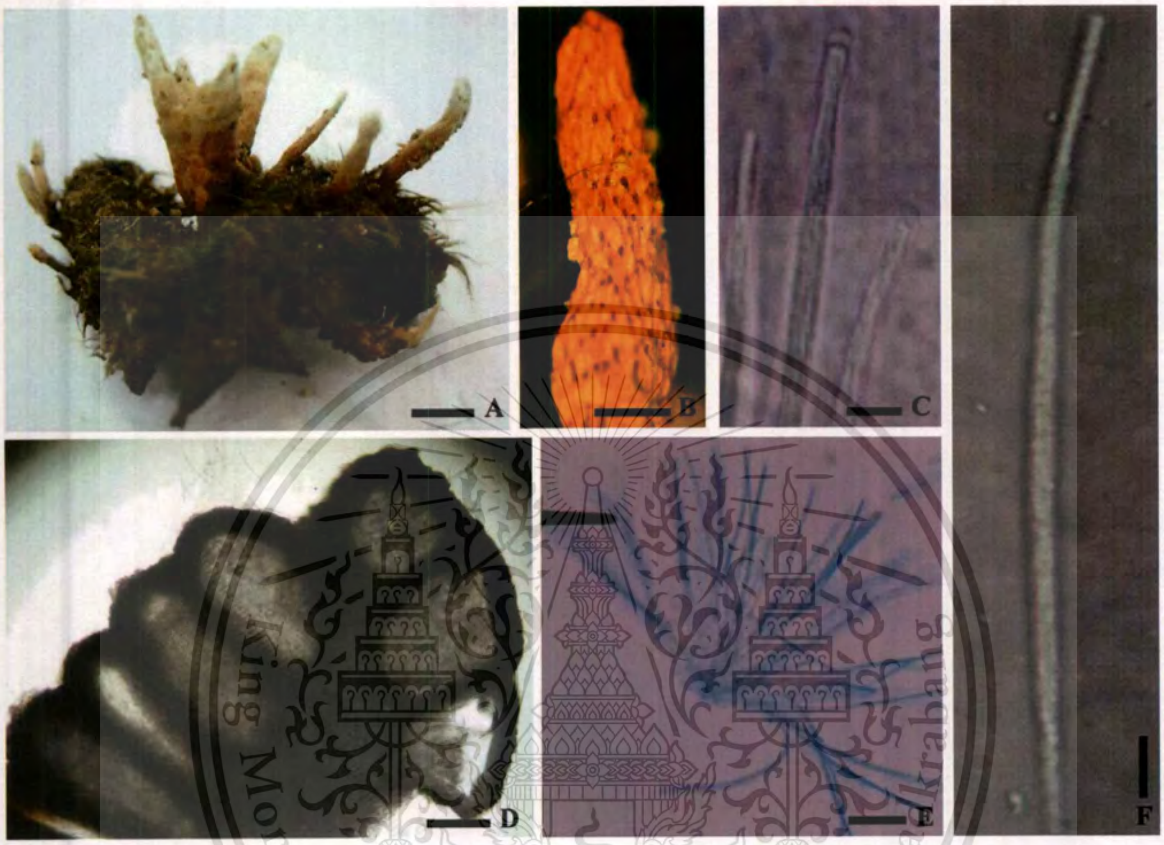


Fig. 4.4 *Cordyceps* sp. **A.** Ascostromata arising from infected caterpillar. **B.** Fertile part. **C.** Tips of asci. **D.** Perithecia. **E.** Asci. **F.** Ascus Bars: A = 5 mm, B = 3 mm, C = 10 µm, D = 125 µm, E = 50 µm, F = 15 µm.

4.1.2.1.2 ***Hypocrella*** Saccardo, Michelia 1: 322 (1878).

Ascostroma superficial, plate-like or cushion-shaped, producing internal ascocarps; ascocarps perithecial, globose to pyriform; *asci* cylindrical to filiform, with a prominent apical thickening, 8-spored; *ascospores* filiform, hyaline, multiseptate, disarticulating into cylindrical, truncate one-celled partspores at maturity.

Habitat: Parasitic on scale insects and whiteflies, Homoptera.

Type species: *Hypocrella discoidea* (Berk. & Br.) Sacc.

References: Petch. 1921; Samson *et al.* 1988.

Hypocrella sp. (Fig. 4.5)

Ascostroma superficial, cushion-shaped, producing internal ascocarp; ascocarps perithecial. *Perithecia* globose to pyriform, 600 × 350 µm. *Asci* cylindrical, with prominent apical thickening, 8-spored, 70-207.5 × 5-8 µm. *Ascospores* filiform, hyaline, multiseptate, disarticulating into cylindrical, truncate one-celled partspores at maturity.

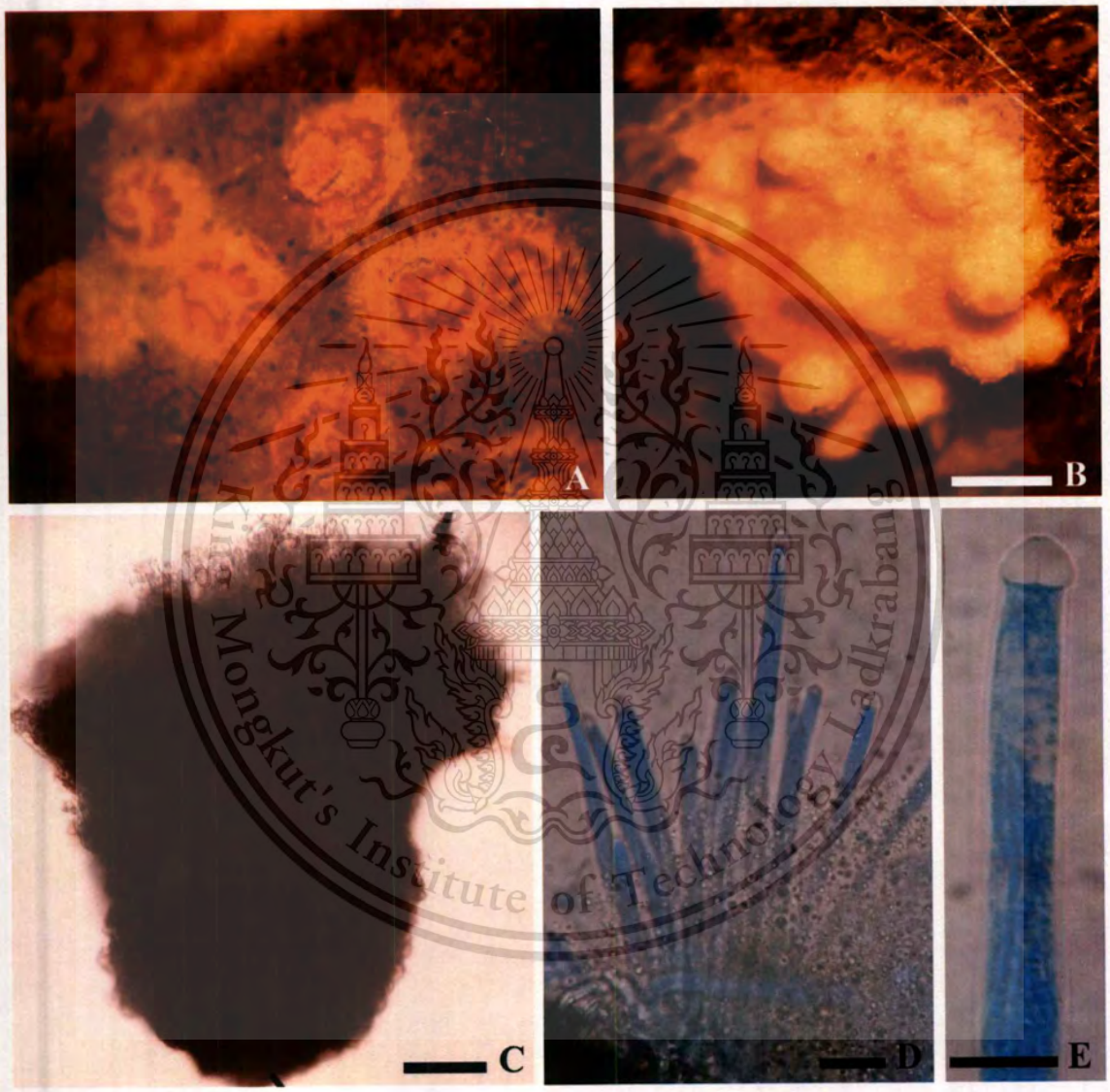


Fig. 4.5 *Hypocrella* sp. **A.** Infected insects on the substrate. **B.** Ascostroma. **C.** Perithecium. **D.** Asci. **E.** Tip of ascus. Bars: B = 1 mm, C = 100 µm, D = 30 µm, E = 10 µm.

Habitat: Infected homopteran insect attached to broad leaves.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', on whitefly, Homoptera, 12 October 2006, O.M. Aung MFLU1086 (MFLUH).

Note: The characteristics of this specimen is generally agree with the *Hypocrella* (Samson *et al.* 1988; Tzean *et al.* 1997).

I was not able to obtain DNA from this specimen.

4.1.2.1.3 ***Ophiocordyceps*** Petch, Trans. Brit. Mycol. Soc. 1: 73 (1931) emend. G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora.

= *Cordycepioideus* Stifler, Mycologia 33: 83 (1941).

Stromata or subiculum darkly pigmented or rarely brightly coloured, tough, fibrous, pliant to wiry, rarely fleshy, often with aperithecial apices or lateral pads. *Perithecia* superficial to completely immersed, ordinal or oblique in arrangement. *Asci* hyaline, cylindrical, usually with thickened ascus apex, rarely fusoid to ellipsoid. *Ascospores* usually cylindrical, multiseptate, disarticulating into part-spores or non-disarticulating.

Type: *Cordyceps blattae* Petch, Trans. Brit. Mycol. Soc. 1: 74. 1931.

Anamorphs: *Hirsutella*, *Hymenostilbe*, *Paraisaria*, *Synglíocladium*.

Reference: Sung *et al.* 2007.

Ophiocordyceps crinalis (Ellis ex Lloyd) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Studies in Mycology 57: 5-59 (2007). (Fig. 4.6)

≡ *Cordyceps crinalis* Ellis ex Lloyd, Mycol. Writ. : 912 (1920).

Ascotromata about 4, arising from various parts of the caterpillar host, slender, 5.5-7.5 cm long, 0.5-1 mm thick, simple or occasionally furcated above, tapering at the tip, dark brown.. *Fertile part* 20-30 mm long. *Perithecia* superficial, yellowish-brown to dark brown, flat at the base, ellipsoid to ovoid, 300-320 × 180-300 µm, almost scattered on the stipe, in some places crowded. *Asci* mostly cylindrical, some fusoid, 8-spored, 50-200 × 4-6 µm, *ascus cap* 2-3 µm high and 4-5 µm wide. *Ascospores* filiform, multispetate, nearly as long as asci, hyaline, not breaking into secondary partspores.

Host: Hairy Caterpillar, Lepidoptera.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Pang Sa Det, Mae Sae village, N19°14.59' E98°38.45', 962m alt., on Lepidoptera larva, 8 August 2006 O.M. Aung MFLU887 (MFLUH).

Remarks: A single specimen was found during the course of study. This species is easily confused with *O. filiformis*. The main differences between these two species are in size and shape of perithecia. Both species have superficial perithecia. *Cordyceps filiformis* has wider perithecia (380-450 vs. 300-330 in length) (Kobayasi. 1982). The shape of *Cordyceps crinalis* is ovoid but *O. filiformis* possesses ovate conic perithecia. This collection of *O. crinalis* has 4 ascostromata arising from various parts of host, but *O. filiformis* has only one ascostromata which arises from the ventral abdomen part of host.

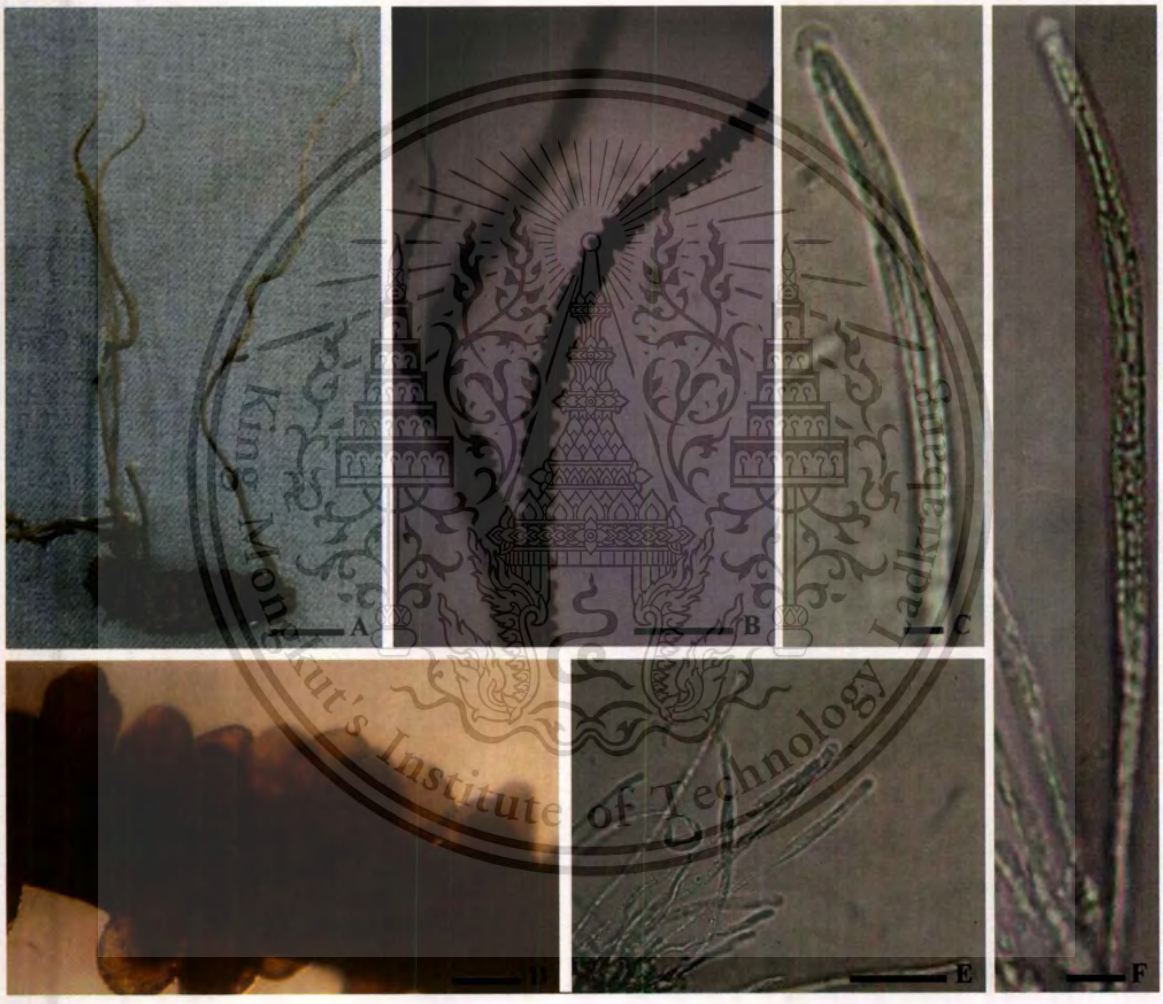


Fig. 4.6 *Ophiocordyceps crinalis*. **A.** Ascostromata arising from infected host caterpillar. **B.** Fertile part. **C.** Tip of ascus. **D.** Superficial perithecia. **E.** Asci. **F.** Ascus with ascospores. Bars: A = 10 mm, B = 5 mm, C = 10 µm, D = 250 µm, E = 20 µm, F = 50 µm.

Ophiocordyceps dipterigena (Berk. & Broome) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Studies in Mycology 57: 5-59 (2007). (Fig. 4.7)

≡ *Cordyceps dipterigena* Berk. & Broome, J. Linn. Soc. 14: 111 (1875).

= *Cordyceps muscicola* A. Möller, Phycomyceten u. Ascomyceten: 221 (1901).

= *Cordyceps surinamensis* Henn., Hedwigia 41: 169 (1902).

= *Cordyceps oumensis* Höhn., Sitzungsber. Kaiserl. Akad. Wiss. Wien 118: 309 (1909).

= *Cordyceps ouwensii* Höhn., Sitzungsber. Kaiserl. Akad. Wiss. Wien 118: 309 (1909).

= *Cordyceps thwaitesii* Lloyd, Mycol. Writ.: 1060 (1921).

= *Cordyceps opposita* Syd., Bot. Jahrb. Syst. 7: 325 (1922).

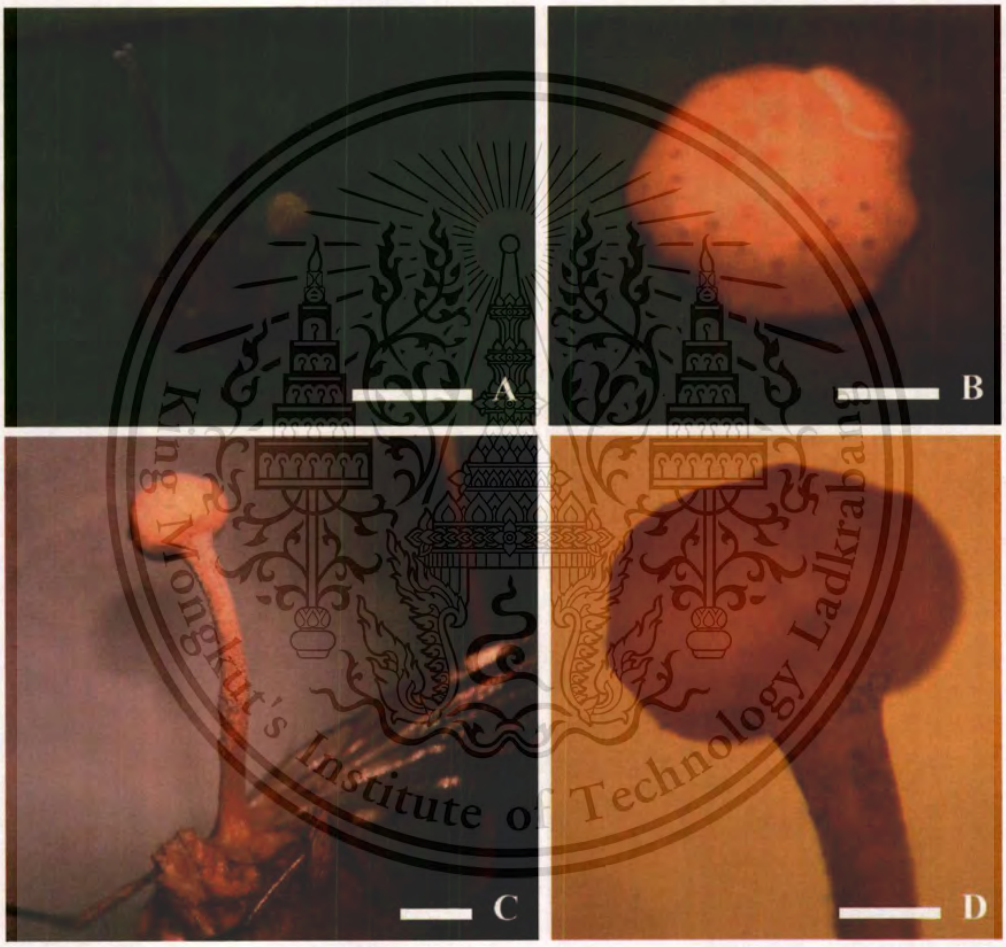


Fig. 4.7 *Ophiocordyceps dipterigena*. **A.** Ascostromata and synnemata arising from infected fly. **B.** Perithecia embedded in pseudoparenchymatous cortex. **C.** Ascostromata arising from infected host. **D.** Fertile part. Bars: A = 5 mm, B & D = 0.5 mm, C = 1 mm.

Ascotromata stipitate, two, arising from sides of the neck portion of the infected host, capitate, 3.5-5 mm long, light yellow to yellow, light orange. *Fertile part* terminal, flattened oblate globoid or flattened turbinate, orange-red to brownish-yellow, becoming light brown to brownish-orange with

age, 0.5-1.5 mm wide, 0.8-1 mm thick, surface roughened, ridged. *Stipe* cylindrical. *Perithecia* vertical, narrowly ovoid to obclavate, occasionally irregular, 500-920 × 160-200 µm, ostiolate, embedded in pseudoparenchymatous cortex. *Asci* cylindrical, 8-spored, up to 450 µm long, 4.5-6 µm wide, with a perforated refractive cap, 3-5.6 µm high and 5.5-7 µm wide. *Ascospores* hyaline, filiform, multispetate, finally breaking into one-celled secondary spores. *Secondary spores* cylindrical to fusiform, 5.5-18 × 1.5 µm.

Host: Fly, Diptera

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Pang Sa Det, Mae Sae village, N19°14.59' E98°38.45', 962m alt., on Diptera adult (fly), 15 October 2006 O.M. Aung MFLU979 (MFLUH).

Remarks: A single specimen was found during the study. The collected specimen resembles *Cordyceps dipterigena*. Kobayasi (1982) mentioned this species as special species with discoid fertile part and apical appendages.

Blasting the ITS sequence of this species shows it to be similar (E-value = 2e-62) to a *Cordyceps nutans* sequence from GenBank, as well as. *C. forquignoni* (AJ786562, 9e-61) and *Hymenostilbe odonatae* (AB104725, 9e-61). There are two records of sequence belong to *O. dipterigena* in GenBank. Both sequences, however, are not shown any similiarities in blast results. The results indicated that although this species is morphologically identical to known species, it might be genetically different between Thailand collection and GenBank record.

Ophiocordyceps elongata (Petch) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Studies in Mycology 57: 5-59 (2007). (Fig. 4.8)

≡ *Cordyceps elongata* Petch, Trans. Brit. Mycol. Soc. 21: 47 (1937).

Ascotromata stipitate, arising from the mouthpart of the caterpillar host buried in soil, 21 cm long, 0.75 mm wide. *Stipe* arising singly, hairy. *Hairs* 1-8 mm long, tapering at the tip, black. *Stalk* flexuose, longitudinally sulcate and twisted, pale brown to brown, nearly glabrous, about 1 mm diam. *Fertile part* 10 mm long × 1 mm wide, red, rough with red-brown ostioles, black with aging. *Perithecia* immersed, flat at the base, ellipsoid to cylindrical, 250-350 × 125-200 µm. *Asci* could not be found, possibly immature.

Anamorph: *Hirsutella*.

Host: Larva, Lepidoptera.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., New Waterfall, located near 36 km marker on Highway 1095, on Lepidoptera larva, 28 June 2006 O.M. Aung MFLU879 (MFLUH).

Remarks: A single specimen was found during the study. This collection is very similar to *C. elongata*, except that the fertile part is shorter (10 mm vs. 10-40 mm) (Petch. 1937; Kobayasi. 1982). The size of perithecia, however is almost the same (250-350 µm vs. 250-350 µm) Kobayasi (1982). The anamorph *Hirsutella* was also found on the specimen. *Hirsutella gigantea* Petch was recorded as anamorphic state of *C. elongata* (Petch. 1937).

I was not able to obtain DNA from this specimen.

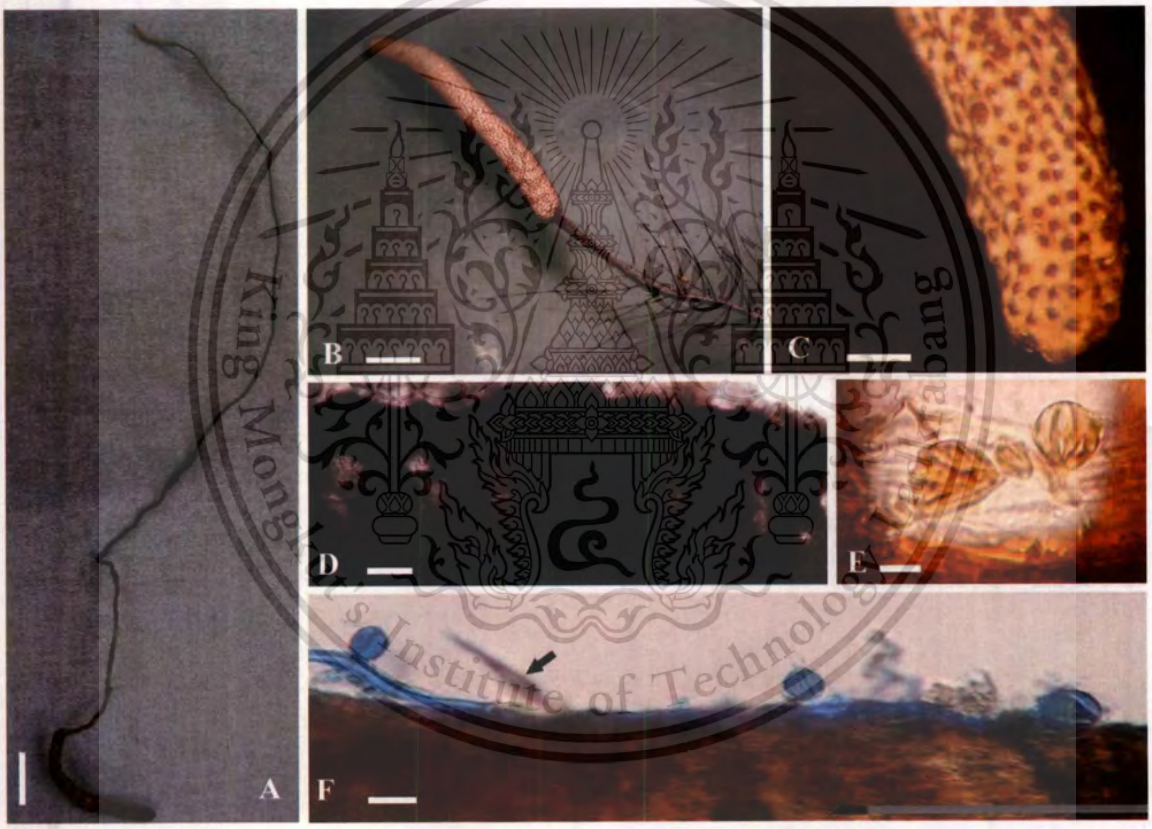


Fig. 4.8 *Ophiocordyceps elongata*. **A.** Ascostromata arising from infected host caterpillar. **B.** Fertile part. **C.** Tip of fertile part. **D.** Perithecia. **E.** Conidia. **F.** Conidiogenous cell. Bars: A = 20 mm, B = 2 mm, C = 0.5 mm, D = 100 µm, E = 10 µm, F = 20 µm.

Ophiocordyceps filiformis (Moureau) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Studies in Mycology 57: 5-59 (2007). (Fig. 4.9)

≡ *Cordyceps filiformis* Moureau, Mém. Inst. Roy. Colon. Belge 7: 14 (1949).

Ascotromata stipitate, arising from the ventral abdomen part of the caterpillar host, 13.5 mm long. *Stipe* arising singly at first and branching at 9 mm, tapering at the tip, brownish. *Perithecia* superficial, yellowish-brown to dark-brown, flat at the base, ellipsoid to ovate, almost scattered on the stipe, in some places crowded, 370-420 × 200-230 µm. *Asci* mostly cylindrical, some fusoid, 8-spored, 105-175 × 3-9 µm, ascus cap 2-4 µm high and 3-6 µm wide. *Ascospores* filiform, multispetate, hyaline, not breaking into secondary partspores. *Septate* 2-9 µm.

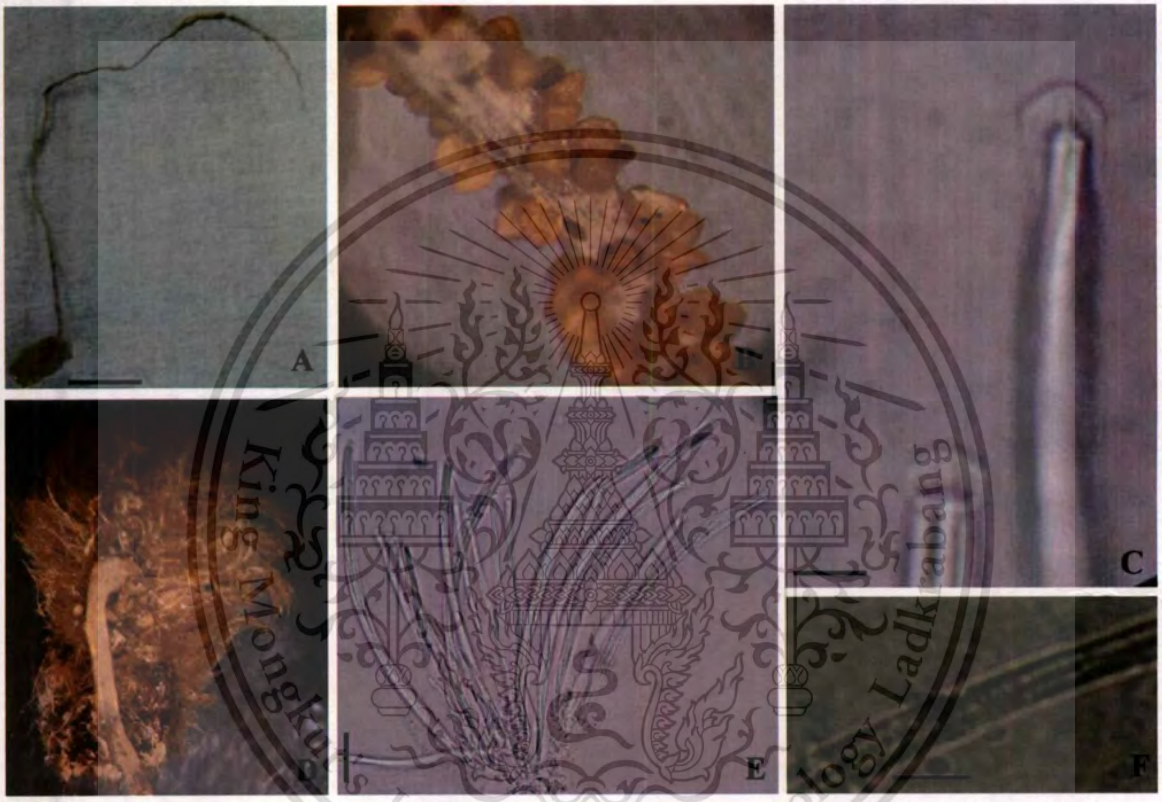


Fig. 4.9 *Ophiocordyceps filiformis*. **A.** Single ascostroma arising from infected host caterpillar. **B.** Superficial perithecia. **C.** Tips of asci. **D.** Ascostroma arising from ventral part of abdomen. **E.** Asci. **F.** Ascospores not yet breaking into partspores. Bars: A = 15 mm, B = 250 µm, C = 5 µm, E = 20 µm, F = 10 µm.

Host: Larva, Lepidoptera.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123' East 98° 44.009', on Lepidoptera larva, 16 August 2006 O.M. Aung MFLU896 (MFLUH).

Remarks: A single specimen was found during the course of study. This species is easily confused with *C. crinalis*. The differences between these species were explained in *C. crinalis*.

Blasting the ITS sequence of this species shows it to be similar to a number of sequnce of *Wallemia sebi* (E-value = 0.0) from GenBank. There is no reference sequence in GenBank for this species and it is the first deposited sequence.

Ophiocordyceps longissima (Kobayasi) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Studies in Mycology 57: 5-59 (2007). (Fig. 4.10)

≡ *Cordyceps longissima* Kobayasi, Bull. Natn. Sci. Mus. Tokyo 6: 300 (1963).

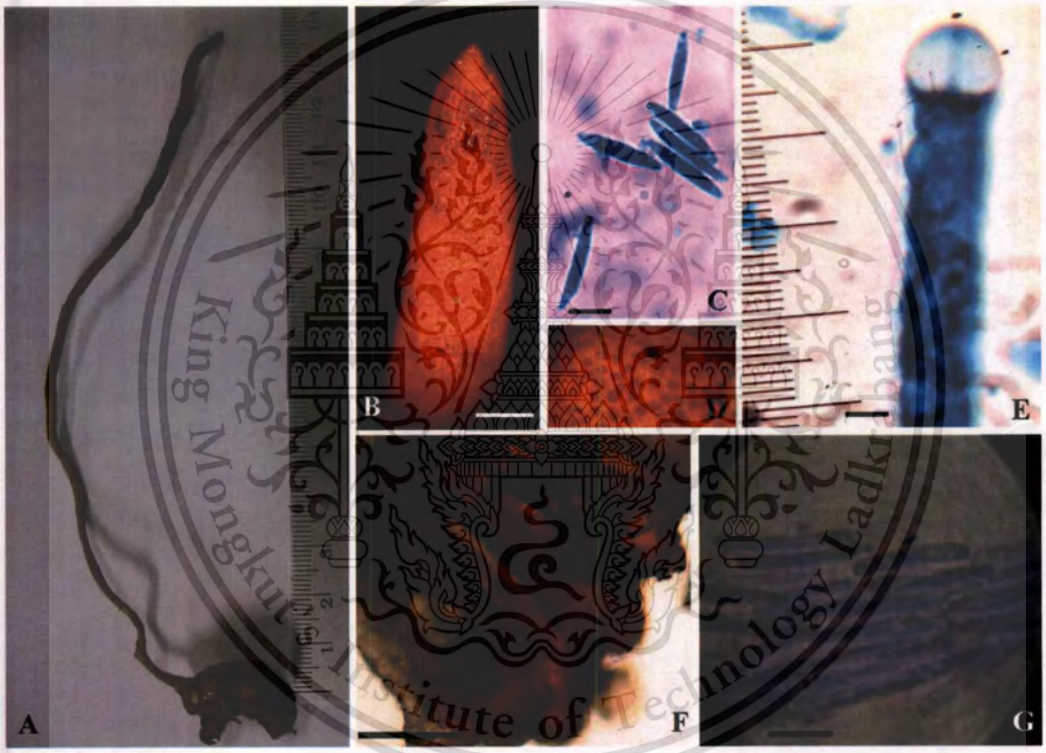


Fig. 4.10 *Ophiocordyceps longissima*. **A.** Ascostromata arising from infected host cicada. **B.** Fertile part. **C.** Partspores. **D.** Verrucose surface of fertile part. **E.** Tip of ascus. **F.** Perithecia. **G.** Ascospores breaking into partspores. Bars: B = 2 mm, C & G = 10 µm, E = 2 µm, F = 250 µm.

Ascostroma solitary, stipitate, arising singly from the forehead of infected cicada nymph. *Stipe* sterile, long flexuous, 15.3 cm long, 3 mm wide, pale red, cylindrical with terminal fertile part. *Fertile part* cylindrical, narrowly ovoid, orange-red, becoming brown with age, 4.5 cm long, 4 mm thick,

surface verrucose. *Perithecia* apical, immersed, flask-shaped, yellow to deep brown, 630 × 180 µm. *Asci* cylindrcial, 8-spored, 96-309 × 6-9 µm, with a perforated refractive cap, 3-3.6 µm high and 4.8 µm wide. *Ascospores* filiform, multiseptate, hyaline, breaking into secondary partspores. *Partspores* cylindrical, 3.6-20.4 × 1-1.8 µm.

Host: Cicada, Cicadae, Homoptera.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Geyser Pong Dueb Hot Spring, on Cicada nymph, Homoptera, 31 August 2005 O.M. Aung MFLU834 (MFLUH).

Remarks: A single specimen was found during the study. The specimen mostly agrees with *C. longissima*, except that the stipe is longer (15.3 cm × 3 mm vs. 10-13 cm × 1.5-2 mm) and partspores are two times longer (3.6-20.4 × 1-1.8 µm vs 9-11 × 1-1.2 µm) (Shimizu. 1994).

I was not able to obtain DNA from this specimen.

Ophiocordyceps mrciensis (Aung, J.C. Kang, Z.Q. Liang, Soytong & K.D. Hyde) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Studies in Mycology 57: 5-59 (2007). (Fig. 4.11)

≡ *Cordyceps mrciensis* Aung, J.C. Kang, Z.Q.Liang, Soytong & K.D. Hyde, Mycotaxon 97: 236 (2006).

Stromata arising from abdomen of infected spider, filiform, 5-12 mm long, light brown, branching. *Fertile part* black, with a 4 mm long sterile appendage. *Perithecia* superficial, elongate to ellipsoid, 210-375 × 150-180 µm, some with a short neck, 120 × 30 µm. *Asci* filiform, 8-spored, 135-305 × 9-15 µm; *caps of asci* 4.2-6.6 µm high, 5.4-8.4 µm wide. *Ascospores* filiform, 185-435 × 3-5 µm, not breaking into secondary ascospores, septate at 3.6-21 µm intervals.

Host: Spider, Arachnida.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123' East 98° 44.009', on spider, 17 September 2005, O.M. Aung MFLU831 (MFLUH).

Remarks: *Cordyceps mrciensis* was associated with a single infected spider, attached to a rotten bamboo culm, collected at the Mushroom Research Centre, Chiang Mai, Thailand. According to Mains (1954) only eight species of *Cordyceps* have been recorded in association with spiders. *Cordyceps mrciensis* can be distinguished from these known species in having stromata with a fertile

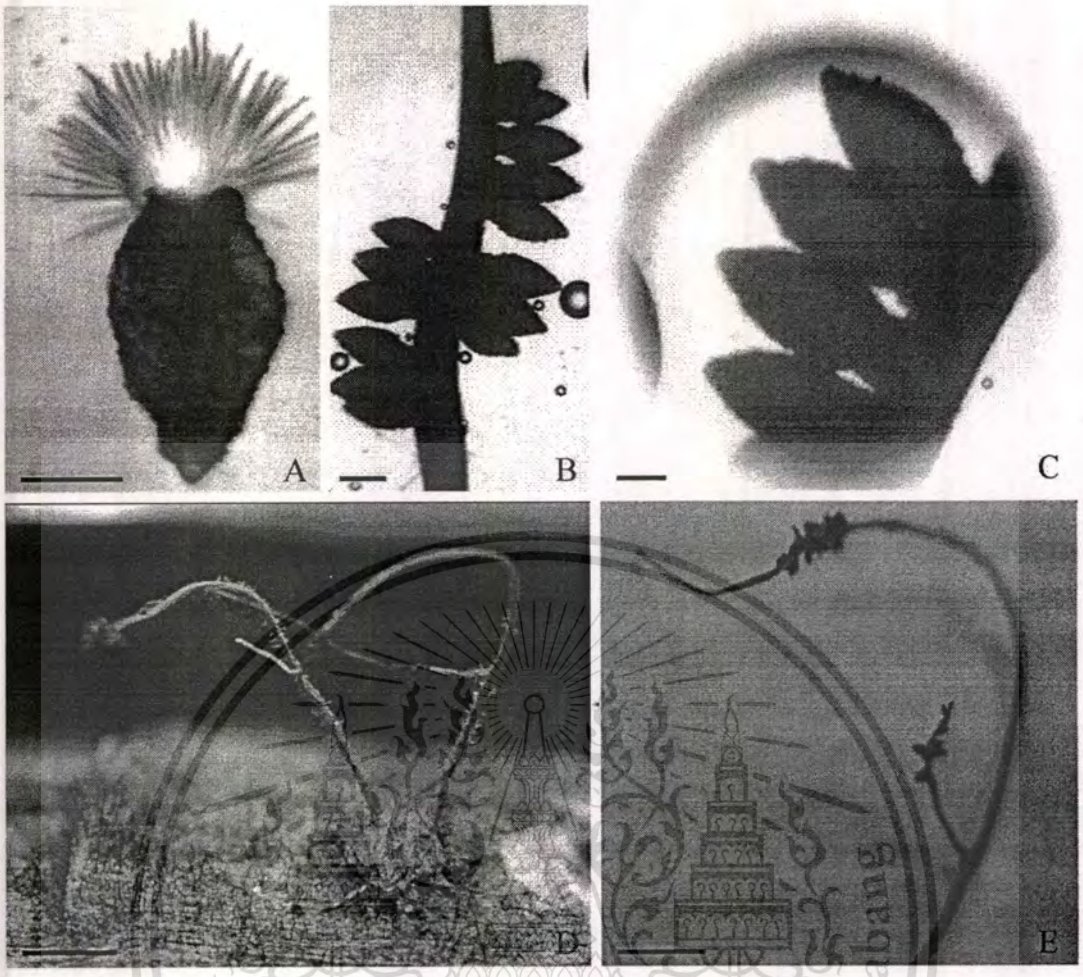


Fig. 4.11 *Ophiocordyceps mrciensis* (from **holotype**). **A.** A perithecium and asci. **B.** Superficial perithecia. **C.** Perithecia. **D.** Small spider bearing two stromata with superficial perithecia **E.** Appendage. Bars: A & C = 100 ìm, B = 200 ìm, D & E = 2.5 mm.

part and a stipe that continues as a distinctive sterile appendage, superficial perithecia and ascospores that do not break into partspores. There are only two species, *C. thaxteri* Mains and *C. engleriana* Henn., that have superficial perithecia. In *C. thaxteri* the perithecia are scattered, free, narrowly ovoid, and large (960-1200 × 300-360 µm, Mains. 1954). The perithecia of *C. engleriana* are also superficial, but crowded at the apex of the stromata and ovoid or flask-shaped (Mains 1954). *Cordyceps mrciensis* also has superficial perithecia but they are elongate to ellipsoid, small, 210-375 × 150-180 µm and some have short necks. The ascospores of *C. thaxteri* and *C. engleriana* break into partspores, whereas those of *C. mrciensis* do not. *Cordyceps caloceroides* Berk. & M.A. Curtis and *C. grenadensis* Mains, also associated with spiders, posses ascospores that do not break into secondary

partspores. *Cordyceps caloceroides* has immersed perithecia with slightly protruding ostioles, while *C. grenadensis* has partly imbedded, ovoid perithecia. The perithecia of *C. mrciensis* are entirely superficial and somewhat scattered on the stipe. Besides the above characters, the distinctive fertile part of the stroma with a distinctive sterile appendage is sufficient to distinguish *C. mrciensis* from the known *Cordyceps* species from spiders (Aung *et al.* 2006a).

I was not able to obtain DNA from this specimen.

Ophiocordyceps myrmecophila (Ces.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Studies in Mycology 57: 5-59 (2007). (Fig. 4.12)

≡ *Cordyceps myrmecophila* Ces., Bot. Zeitung 4: 877 (1846).

≡ *Torrubia myrmecophila* (Ces.) Tul. & C. Tul., Sel. Fung. Carpol. 3: 18 (1865).

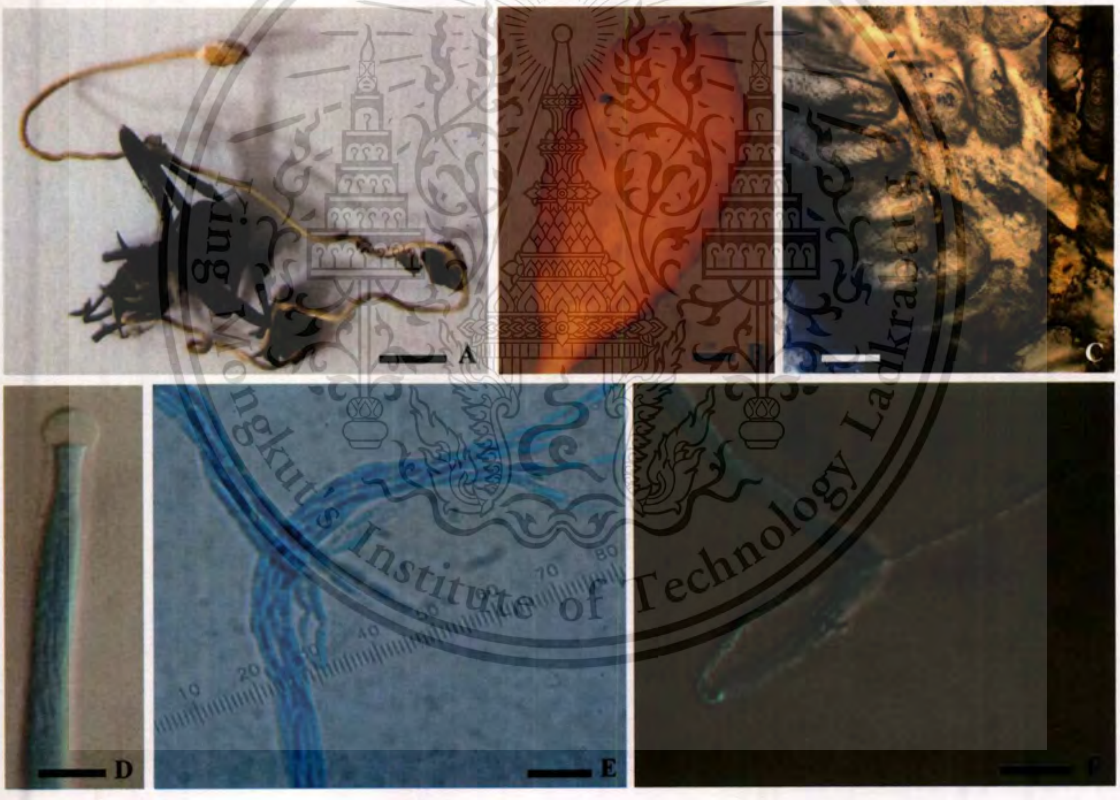


Fig. 4.12 *Ophiocordyceps myrmecophila*. **A.** Ascostroma arising from infected ant. **B.** Fertile part. **C.** Perithecia. **D.** Tip of ascus. **E.** Ascospores breaking into partspores. **F.** Tapering end of ascus. Bars: A = 5 mm, B = 0.5 mm, C = 125 µm, D = 5 µm, E = 20 µm, F = 10 µm.

Stromata arising singly from the cephalothorax portion of the host, capitate, with long stipe, slender, sometimes branching, 25-83 mm long, 0.3-0.5 mm thick, light yellow to yellow, light orange.

Fertile part ovoid, subglobose, 2-4 mm long, 1-1.5 mm thick, longitudinally irregularly rugose. *Perithecia* narrowly ovoid to obliquely immersed, 300-390 × 135-210 µm. *Asci* cylindrical, some tapering at the end, 8-spored, 150-288 × 3-4.5 µm, with a thickened perforated refractive, flattened hemispherical cap, 6-7.8 µm high and 3.6-4.2 µm wide. *Ascospores* filiform, multispetate, breaking into secondary partspores. *Partspores* barrel-shaped to cylindrical, slightly wider in middle, becoming longer near both ends, 1.2-14.4 × 0.9-1.8 µm.

Host: Adult Formicidae, Hymenoptera.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mokfa Waterfall, located near 18 km marker on Highway 1095, 25 June 2005, O.M. Aung MFLU818, MFLU819 (MFLUH); Chiang Mai Province, Doi Inthanon National Park, along Highway 1009 at 25 km marker, N18°32.56' E098°33.51', 1073 m alt., 27 June 2005, O.M. Aung MFLU820 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Pang Sa Det, Mae Sae village, N19°14.59' E98°38.45', 962m alt., 3 July 2005, O.M. Aung MFLU823, MFLU824 (MFLUH); *ibid.*, 17 July 2005, O.M. Aung MFLU826, MFLU827 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 9 July 2005, O.M. Aung MFLU825 (MFLUH); *ibid.*, 8 September 2005, O.M. Aung MFLU829 (MFLUH); *ibid.*, 17 September 2005, O.M. Aung MFLU830 (MFLUH); *ibid.*, 26 June 2006, O.M. Aung MFLU878 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mokfa Waterfall, located near 18 km marker on Highway 1095, 7 July 2006, O.M. Aung MFLU881 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 25 July 2006, O.M. Aung MFLU882 (MFLUH); *ibid.*, 5 August 2006, O.M. Aung MFLU886 (MFLUH); *ibid.*, 16 August 2006, O.M. Aung MFLU897, MFLU898, MFLU900 (MFLUH); *ibid.*, 19 August 2006, O.M. Aung MFLU901 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Geyser Pong Dueb Hot Spring, 23 August 2006, O.M. Aung MFLU903, MFLU904 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 24 August 2006, O.M. Aung MFLU916 (MFLUH); Chiang Dao, 25 August 2006, O.M. Aung MFLU917 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 02 September 2006, O.M. Aung MFLU920 (MFLUH); Chiang Mai Province, Mae Taeng Distr., New Waterfall, located near 36 km marker on Highway 1095, 05 September 2006, O.M. Aung MFLU921 (MFLUH); Chiang Mai Province, Doi Inthanon National Park, along Highway 1009 at 25 km marker, N18°32.56' E098°33.51', 1073 m alt., 06 September 2006, O.M. Aung MFLU927, MFLU928, MFLU929, MFLU930, MFLU931, MFLU932, MFLU933, MFLU934, MFLU935, MFLU936, MFLU937, MFLU938, MFLU939, MFLU940,

MFLU941, MFLU942, MFLU943, MFLU944, MFLU945, MFLU946, MFLU947, MFLU948, MFLU949 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 14 September 2006, O.M. Aung MFLU950, MFLU951, MFLU952 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mokfa Waterfall, located near 18 km marker on Highway 1095, 17 September 2006, O.M. Aung MFLU957 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 19 September 2006, O.M. Aung MFLU960 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Geyser Pong Dueb Hot Spring, 26 September 2006, O.M. Aung MFLU964, MFLU965, MFLU966 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 8 October 2006, O.M. Aung MFLU973, MFLU974, MFLU975 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Pang Sa Det, Mae Sae village, N19° 14.59' E98° 38.45', 962m alt., 15 October 2006, O.M. Aung MFLU976, MFLU977, MFLU978 (MFLUH).

Remarks: The collections resemble *C. myrmecophila* but morphological characters are variable. Blasting the ITS sequence of this species shows it to be similar (E-value = 2e-114) to *Cordyceps myrmecophila* sequences (AF122042 and AJ536562) from GenBank, as well as. *C. nutans* (4e-90) *C. ampullacea* (6e-88), *C. formicarum* (1e-84), *C. forquignoni* (7e-62), *C. oxycephala* (3e-60), *C. sphecocephala* (3e-60). The similarities values between Thailand collections and GenBank sequences are low (about 88% and 90%). Although morphological characters and similarities have found in some variation, Thailand collections and GenBank species are clustered together in ITS sequence analysis.

Ophiocordyceps nutans (Pat.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Studies in Mycology 57: 5-59 (2007). (Fig. 4.13)

≡ *Cordyceps nutans* Pat., Bull. Soc. Mycol. France 3: 127 (1887).

≡ *Cordyceps bicephala* subsp. *nutans* (Pat.) Moureau, Mém. Inst. Roy. Colon. Belge 7: 47 (1949).

Ascostroma stipitate, arising laterally from the thorax portion of infected hemipteran bug, rarely arising from the end of the abdomen. *Stipe* sterile, long flexuous, ca. 3.8-11 cm long, 1.5-3 mm wide, somewhat black, becoming orange to red towards the apex, with terminal fertile part, cylindrical, narrowly ovoid, becoming brown with age, 1-23 mm long, 0.5-2.5 mm thick, surface verrucose. *Perithecia* embedded except ostioles, narrowly ovoid, obliquely immersed, 735-945 × 105-225 µm, usually with long neck. *Asci* cylindrcial, 8-spored, 94.5-150 × 4.5-6.0 µm, with a perforated

apical ring, 3-4.8 µm high and 5.4-6 µm wide. Ascospore filiform, multiseptate, hyaline, breaking into secondary partspores. Partspores cylindrical, with rounded ends, 4.2-11.4 × 1.2-1.8 µm.

Host: Pentatomid bug, Hemiptera.

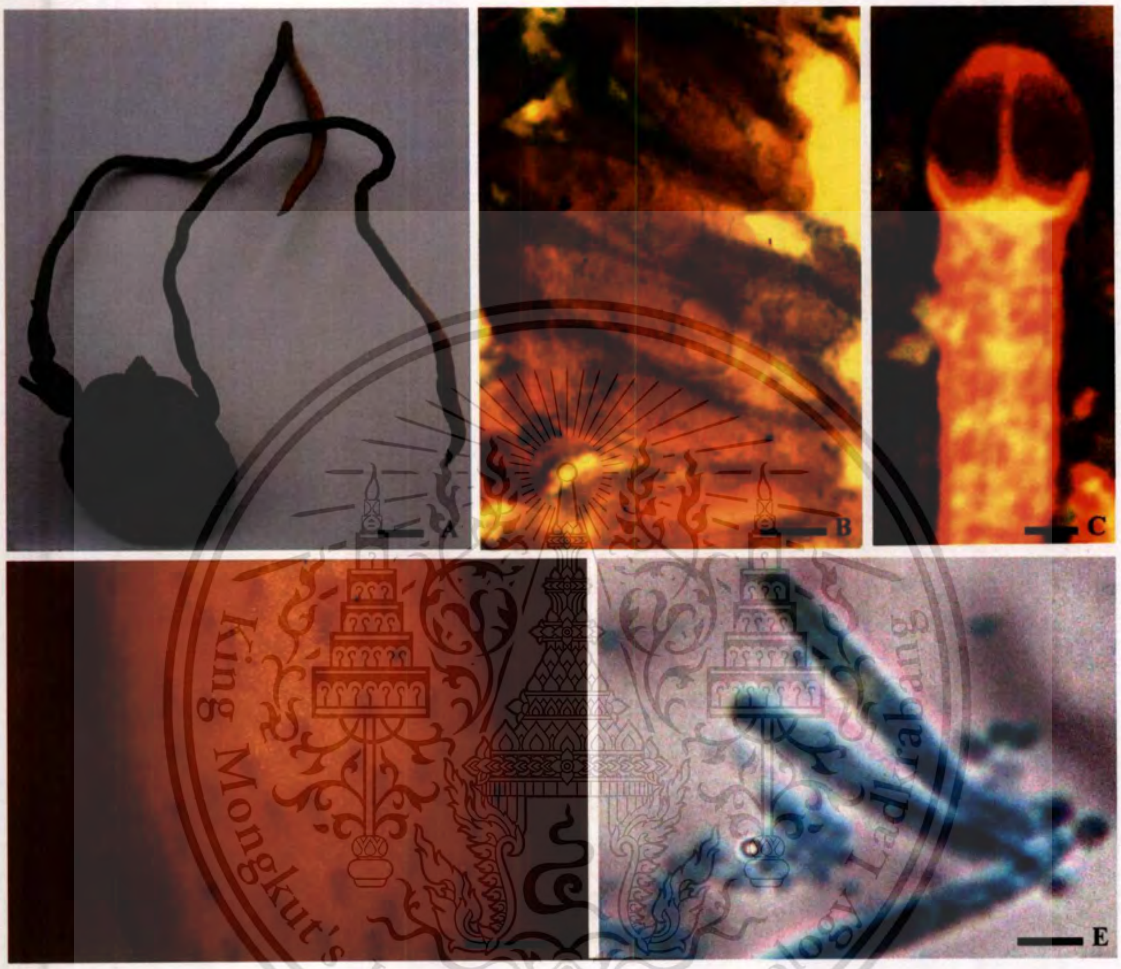


Fig. 4.13 *Ophiocordyceps nutans*. **A.** Infected Pentatomid bug. **B.** Perithecia. **C.** Tip of ascus. **D.** Immersed perithecia. **E.** Partspores. Bars: A = 10 mm, B = 150 µm, C = 2 µm, D = 0.5 mm, E = 2 µm.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Tung Joaw village, N19°08.07' E.098°38.09', 1423 m alt., on adult Hemiptera, 15 June 2005, Huyen T. Le MFLU810 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 16 July 2005, O.M. Aung MFLU812, MFLU813 (MFLUH); *ibid.*, 16 October 2005, O.M. Aung MFLU815 (MFLUH); *ibid.*, 7 November 2005, O.M. Aung MFLU811 (MFLUH); Chiang Mai Province, Doi Suthep-Pui National Park, Sangasahasri Lane to Huai Kok Ma village, N18°48.62' E098°54.60', 1150 m alt., 21 July 2005, O.M. Aung MFLU814 (MFLUH); Chaing Rai, 2

August 2006, O.M. Aung MFLU883, MFLU884 (MFLUH); Chiang Mai Province, Doi Suthep-Pui National Park, Sangasahasri Lane to Huai Kok Ma village, N18°48.62' E098°54.60', 1150 m alt.,10 August 2006, O.M. Aung MFLU895 (MFLUH); Chiang Mai Province, Doi Inthanon National Park, along Highway 1009 at 25 km marker, N18°32.56' E098°33.51', 1073 m alt., 06 September 2006, O.M. Aung MFLU922, MFLU923 (MFLUH).

Remarks: The collections are identical to *C. nutans* (Tzean *et al.* 1997; Luangsa-Ard *et al.* 2007).

Blasting the ITS sequence of this species shows it to be similar to a number of *Cordyceps nutans* sequence (AJ786583, AJ309367, AJ536560, AJ536558, AB176462 and AB176463) with different E-values (2e-120, 2e-114, 5e-114, 2e-112, 8e-23 and 3e-22, respectively) from GenBank. The similarities values between Thailand collections and GenBank sequences are low (about 73-79%). Although morphological characters and similarities have found in some variation, Thailand collections and GenBank species are clustered together in ITS sequence analysis. There might be some genetic variation between different specimens from different geographical distribution.

Ophiocordyceps oxycephala (Penz. & Sacc.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Studies in Mycology 57: 5-59 (2007). (Fig. 4.14)

≡ *Cordyceps oxycephala* Penz. & Sacc., Malpighia 11: 521 (1897).

≡ *Cordyceps sphecocephala* f. *oxycephala* (Penz. & Sacc.) Kobayasi, Trans. Mycol. Soc. Japan 23: 361 (1982).

Stromata arising singly from the cephalothorax portion of infected wasp, with long stipe, slender, 27-90 mm long, 0.5 mm thick, light yellow to yellow. *Fertile part* cylindrical to clavate, 8-11 mm long, 1 mm thick, longitudinal irregular rugose. *Perithecia* narrowly ovoid to flask-shaped, obliquely immersed, 780-900 × 210-225 µm. *Asci* cylindrical, 8-spored, 262.5-315 × 3-5 µm, with a distinctive cap, 4.2-9 µm high and 5.4-6.6 µm wide, cap is very high and it cannot easily be stained. *Ascospores* filiform, multispetate, breaking into secondary partspores. Young *partspores* cylindrical, mature partspores fusiform, 4.8-13.8 × 0.6-1.8 µm.

Host: Wasp, Vespidae, Hymenoptera.

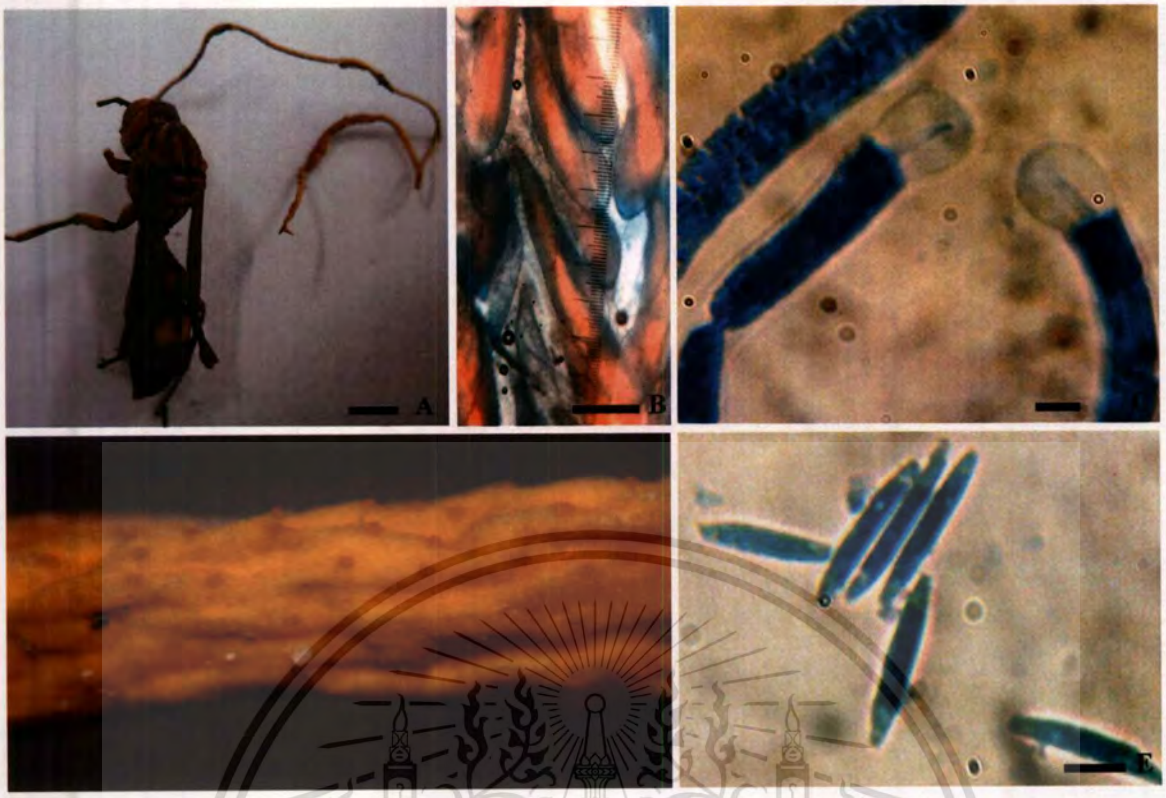


Fig. 4.14 *Cordyceps oxycephala*. **A.** Infected wasp. **B.** Perithecia. **C.** Tips of asci. **D.** Fertile part. **E.** Partspores. Bars: A = 10 mm, B = 300 µm, C = 5 µm, D = 0.25 mm, E = 5 µm.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 7 September 2005, O.M. Aung MFLU832 (MFLUH); *ibid*., 17 September 2005, O.M. Aung MFLU833 (MFLUH); Chaing Rai, 2 August 2006, O.M. Aung MFLU884 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 16 August 2006, O.M. Aung MFLU899 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Geyser Pong Dueb Hot Spring,23 August 2006, O.M. Aung MFLU902 (MFLUH); Chiang Mai Province, Doi Inthanon National Park, along Highway 1009 at 25 km marker, N18°32.56' E098°33.51', 1073 m alt., 06 September 2006, O.M. Aung MFLU924, MFLU925 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 22 September 2006, O.M. Aung MFLU962 (MFLUH); *ibid*., 8 October 2006, O.M. Aung MFLU972 (MFLUH); Chiang Mai Province, Doi Suthep-Pui National Park, Sangasahasri Lane to Huai Kok Ma village, N18°48.62' E098°54.60', 1150 m alt., 5 July 2006, O.M. Aung MFLU989, MFLU990 (MFLUH).

Remarks: The collections are similar to *C. sphecocephala* but the stromata with appendage is the important character to differentiate these species.

Blasting the ITS sequence of this species shows it to be similar (E-value = 0.0) to a *Cordyceps oxycephala* sequence from GenBank (AJ536553; 99 % similarity), as well as *C. polyarthra* (AJ536548, 99 % similarity), *C. sphecocephala* (AJ536550; 98 % similarity), *C. nutans* (AJ536561, 98% similarity), *C. irangiensis* (AY646400; 93 % similarity) and *Hymenostilbe aurantiaca* (AJ786596; 93 % similarity). Interestingly, *C. polyarthra* from GenBank was found to be 99% similarity with Thailand collection and also cluster together in the ITS sequence analysis. *Cordyceps polyarthra*, however, attacked lepidopteran insect and morphological characters are clearly dissimilar to *C. oxycephala*.

Ophiocordyceps pseudolloydii (H.C. Evans & Samson) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Studies in Mycology 57: 5-59 (2007). (Fig. 4.15)

≡ *Cordyceps pseudolloydii* H.C. Evans & Samson, Trans. Brit. Mycol. Soc. 82: 133 (1984).

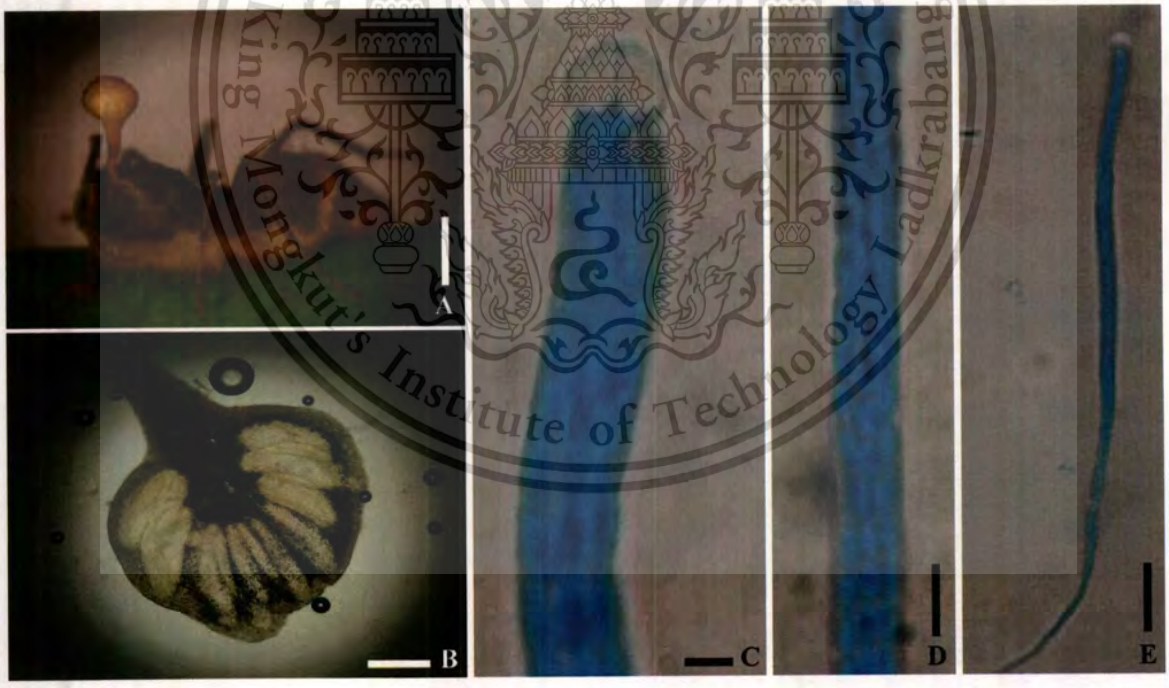


Fig. 4.15 *Ophiocordyceps pseudolloydii*. **A.** Ascostroma arising from infected ant. **B.** Perithecia. **C.** Tip of ascus. **D.** Ascospores with septates. **E.** Ascus. Bars: A = 1.5 mm, B = 200 µm, C = 2.5 µm, D & E = 20 µm.

Ascotromata stipitate, arising singly from the dorsal neck portion of the host, capitate, with short stipe, clavate, wider towards fertile apex, 1-1.5 mm long, 0.15-0.2 mm thick, light yellow to yellow, light orange. *Fertile part* terminal, hemispherical or subglobose, 0.5-0.8 mm wide, 0.3-0.5 mm thick, yellow to light orange, upper surface roughened. *Perithecia* narrowly ovoid to flask-shaped, obliquely immersed, 320-370 × 80-130 µm. *Asci* cylindrical, some tapering at the ends, 8-spored, 40-190 × 3-7.5 µm, with a flattened hemispherical cap, 2-45 µm high and 5-6 µm wide. *Ascospores* filiform, multispetate, do not break into secondary spores, septate interval and size is 1-12.5 × 0.75-1 µm.

Host: Ant, Hymenoptera.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 4 September 2006, O.M. Aung MFLU867, MFLU868, MFLU869 (MFLUH); *ibid.*, 14 September 2006, O.M. Aung MFLU970 (MFLUH); *ibid.*, 22 September 2006, O.M. Aung MFLU961 (MFLUH); *ibid.*, 7 October 2006, O.M. Aung MFLU970 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Pang Sa Det, Mae Sae village, N19°14.59' E98°38.45', 962m alt., 15 October 2006, O.M. Aung MFLU981, MFLU982 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mae Lod coffee plantation, Highway 1095 at 19 km marker, 15 October 2006, O.M. Aung MFLU988 (MFLUH).

Remarks: The collection is identical to *C. pseudolloydii* (Tzean *et al.* 1997). Only two different characteristics are 1) ascostromata are not branched and 2) ascospores do not break into secondary spores. The ascospores appear easy to break, however, the part spores were not observed under the microscope.

Blasting the ITS sequence of this species shows it to be similar to a number of *Cordyceps myrmecophila* sequence with different E-values (1e-115, 7e-94, 6e-63, 2e-62) as well as *C. tricentri* (E-value = 6e-69) and *C. nutans* (E-value = 2e-68) from GenBank. This is the first sequence for this species deposited in GenBank and also a reason why this sequence is similar to *C. myrmecophila* which has similar morphological character.

Ophiocordyceps sphecocephala (Klotzsch ex Berk.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Studies in Mycology 57: 5-59 (2007). (Fig. 4.16)

≡ *Sphaeria sphecocephala* Klotzsch ex Berk., J. Bot. (Hooker) 2: 206 (1843).

≡ *Torrubia sphecocephala* (Klotzsch ex Berk.) Tul. & C. Tul., Sel. Fung. Carpol. 3: 18 (1865).

≡ *Cordyceps sphecocephala* (Klotzsch ex Berk.) Berk. & M.A. Curtis, *in Berkeley*, J. Linn. Soc., Bot. 10: 376 (1868).

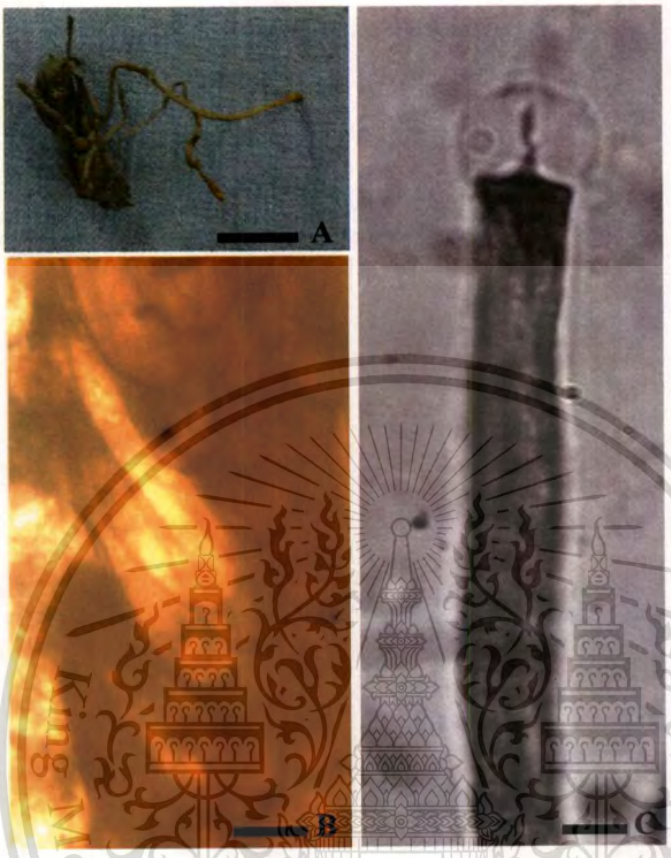


Fig. 4.16 *Ophiocordyceps sphecocephala*. **A.** Ascostroma arising from infected wasp. **B.** Perithecia. **C.** Tip of ascus. Bars: A = 15 mm, B = 200 µm, C = 3 µm.

Stromata slender, arising singly from the cephalothorax portion of infected wasp, with long stipe, slender, 3-5 cm long, 0.5 mm thick, yellowish-brown. *Fertile part* yellowish fusoid head, 3-4.5 mm long, 1.3 mm thick, longitudinal irregular rugose. *Perithecia* colloid, completely embedded, obliquely immersed, 850-950 × 200-250 µm. *Asci* cylindrical, 8-spored, 450.600 × 4-5 µm, with a distinctive cap, about 5 µm in height and width. *Ascospores* filiform, multispetate, breaking into secondary partspores. *Partspores* fusiform, 10-12 × 1-1.5 µm.

Host: Wasp, Vespidae, Hymenoptera.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 8 October 2006, O.M. Aung MFLU971 (MFLUH).

Remarks: The single collection is identical to *C. sphecocephala*.

Blasting the ITS sequence of this species shows it to be similar (E-value = 0.0) to a *Cordyceps sphecocephala* sequence from GenBank (AY646401; 97% similarity), as well as. *C. irangiensis* (AY646400 and AJ786566; 97% similarity), *Hymenostilbe aurantiaca* (AJ786596; 98% similarity), *C. oxycephala* (AJ536553, 94% similarity) and *C. nutans* (AJ536561; 93% similarity).

Ophiocordyceps unilateralis (Tul. & C. Tul.) Petch, Trans. Brit. Mycol. Soc. 16: 74. 1931.

(Fig. 4.17)

≡ *Torrubia unilateralis* Tul. & C. Tul., Sel. Fung. Carpol. 3: 18 (1865).

≡ *Cordyceps unilateralis* (Tul. & C. Tul.) Sacc., Syll. Fung. 2: 570 (1883).

= *Torrubia formicivora* Tul. & C. Tul., Sel. Fung. Carpol. 3: 18. (1865).

≡ *Cordyceps formicivora* (Tul. & C. Tul.) J. Schröt., Krypt.-Fl. Schlesien 3: 276 (1894).

Ascostroma solitary, stipitate, simple, arising from the cephalothorax region of the infected formicine ant, slender, sometimes curved, occasionally dichotomously branched. *Stipe* cylindrical, acute to acuminate, tomentose, dark-brown, brownish-grey to greyish-brown towards the apex, 6.5-15.3 mm long, 0.3-0.7 mm thick, composed of compact longitudinal more or less interwoven brown hyphae, bearing one to three lateral perithecial cushions, pulvinate, 0.6-1.4 mm in diam., greyish-brown and dark-brown to black, surface roughened due to papillate ostioles. *Perithecia* ovoid, densely aggregated, 160-260 × 100-170 µm, embedded in brown pseudoparenchymatous tissue, consisting of loosely or tightly interwoven septate, brown hyphae, perithecial wall 17-21 µm thick. *Asci* cylindrical, 8-spored, 140-216 × 7.9-10.4 µm, with a thickened perforated refractive cap, 3.3-5.6 µm thick, 5.6 µm wide. *Ascospores* cylindrical with subulate ends, 88-192 × 2.4-4 µm, multiseptate, not breaking into partspores at maturity.

Host: Ant, Hymenoptera.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Pang Sa Det, Mae Sae village, N19°14.59' E98°38.45', 962m alt., 18 June 2006, O.M. Aung MFLU843, MFLU844, MFLU845, MFLU846 (MFLUH); *ibid*, 8 August 2006, O.M. Aung MFLU848, MFLU849, MFLU850, MFLU851, MFLU852, MFLU853, MFLU854 (MFLUH); *ibid*., 18 June 2006, O.M. Aung MFLU875, MFLU876, MFLU877 (MFLUH); Chiang Mai Province, Mae Taeng Distr., New Waterfall, located near 36 km marker on Highway 1095, 28 June 2006, O.M. Aung MFLU880 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Pang Sa Det, Mae Sae village, N19°14.59' E98°38.45', 962m alt., 8 August 2006, O.M. Aung MFLU888, MFLU889, MFLU890, MFLU891,

MFLU892, MFLU892, MFLU894 (MFLUH); *ibid.*, 23 August, O.M. Aung MFLU905, MFLU906, MFLU907, MFLU908, MFLU909, MFLU910, MFLU911, MFLU912, MFLU913, MFLU914, MFLU915 (MFLUH); Chiang Mai Province, Doi Inthanon National Park, along Highway 1009 at 25 km marker, N18°32.56' E098°33.51', 1073 m alt., 06 September, O.M. Aung MFLU926 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Pang Sa Det, Mae Sae village, N19°14.59' E98°38.45', 962m alt., 15 October 2006, O.M. Aung MFLU983, MFLU984, MFLU985, MFLU986, MFLU987 (MFLUH).

Remarks: The specimens are identical to *C. unilateralis* (Tzean *et al.* 1997).

I was not able to obtain DNA from this specimen.

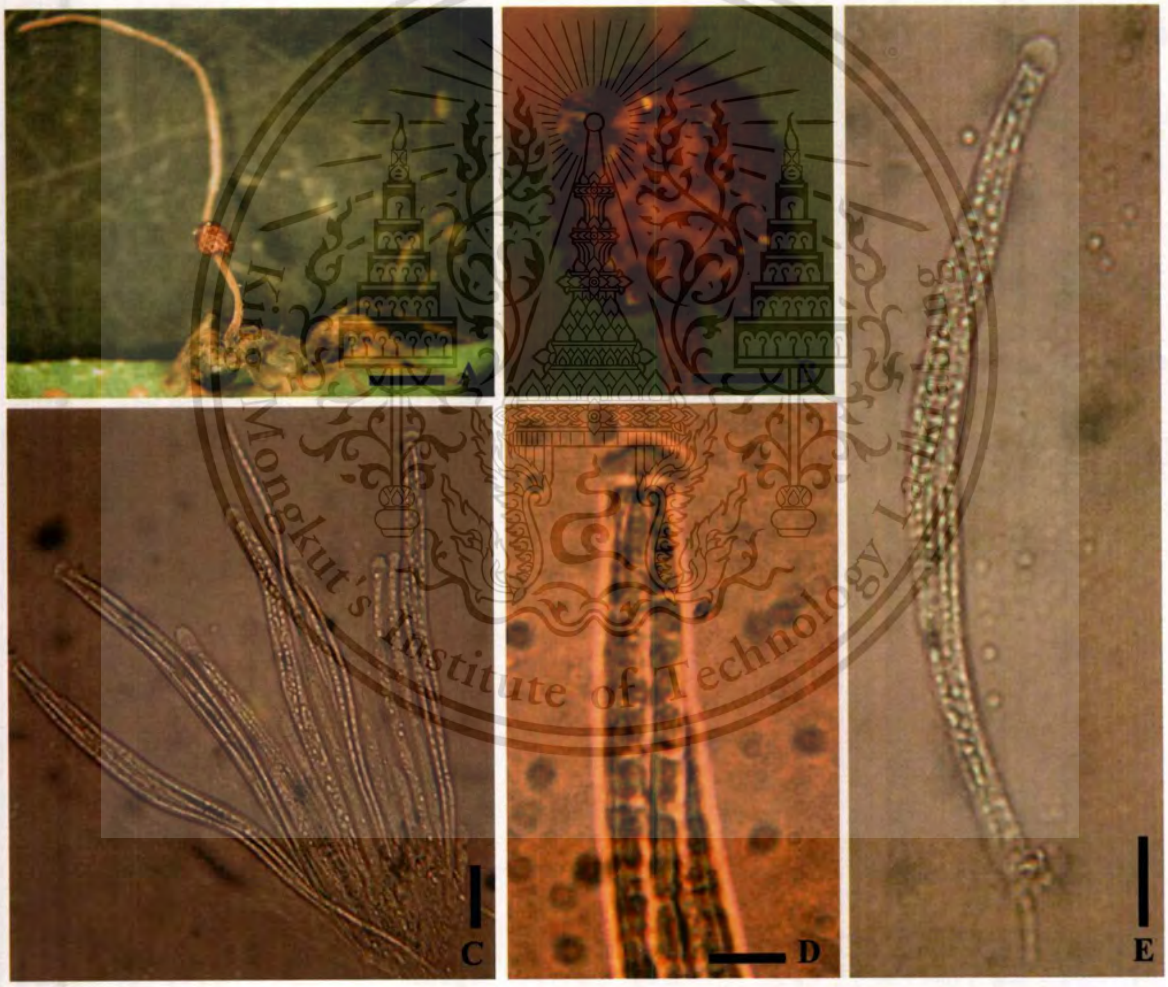


Fig. 4.17 *Ophiocordyceps unilateralis*. **A.** Ascostroma arising from infected ant. **B.** Fertile part with perithecial cushion. **C.** Asci. **D.** Tip of ascus. **E.** Ascus. Bars: A = 2.5 mm, B = 0.5 mm, C = 30 µm, D = 5 µm, E = 20 µm.

4.1.2.1.4 ***Torrubiella*** Boudier, Revue Mycologique 7: 226-227 (1885).

Ascocarps perithecial, mostly flask-shaped, solitary or in aggregately developing on thin weft of hyphae (subiculum), light to bright coloured, wall often covered with anamorphic conidiogenous structures; *asci* long cylindrical, 8-spored, apex conspicuously thickened, penetrated by a fine canal; *ascospore* filiform, multiseptate, disarticulating into cylindrical, truncate one-celled partspores at maturity.

Habitat: Parasitic on insects or spiders.

Type species: *Torrubiella aranicida* Boudier

References: Petch. 1923; Samson *et al.*1988.

Torrubiella hemipterigena Petch details (Fig. 4.18)

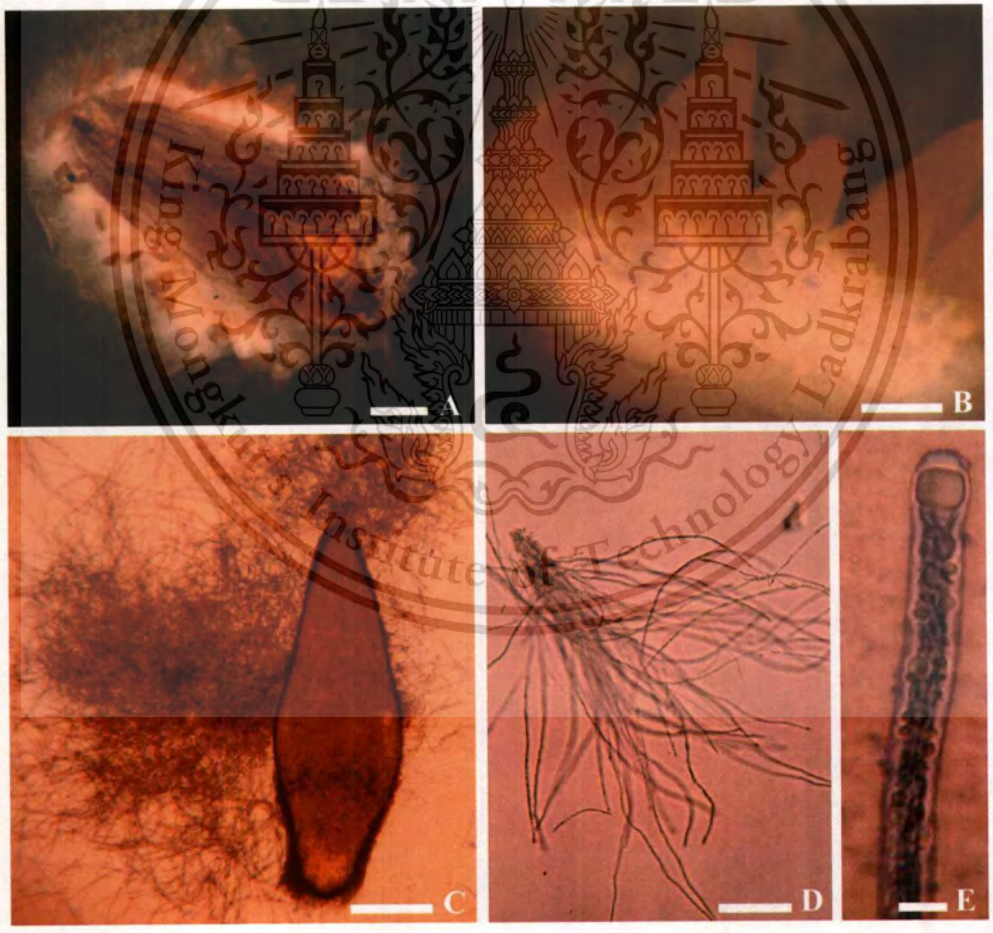


Fig. 4.18 *Torrubiella hemipterigena*. **A.** Infected insects surrounded by mycelial mat. **B.** Perithecia arising from mycelial mat. **C.** Perithecium. **D.** Asci. **E.** Tip of ascus. Bars: A = 1 mm, B & C = 200 µm, D = 100 µm, E = 5 µm.

Infected hopper surrounded by pulvinate mycelial mat, white. *Perithecia* arising directly from the mycelial mat or subiculum, superficial, scattered, elongated conoid, (nearly fusiform), slightly attenuated above, about 920 × 300 µm, yellowish-brown to brown. *Asci* immature, cylindrical, slender, 90-510 × 4-7 µm, *asci cap* 4-6 µm high and 5-7 µm wide. *Ascospores* filiform, multispetate, not breaking into secondary spores.

Host: Hopper, Homoptera, 7 × 4 mm in size.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Pang Sa Det, Mae Sae village, N19°14.59' E98°38.45', 962m alt., on adult Homoptera, 8 August 2006, O.M. Aung MFLU1001 (MFLUH).

Remarks: The specimen is similar to *T. hemipterigena* but perithecia are longer (920 × 300 µm vs. 800 × 300 µm) and asci are shorter (90-510 × 4-7 µm vs. 750 × 4 µm) (Petch. 1932).

I was not able to obtain DNA from this specimen.

4.1.2.2 **Anamorph taxa**

4.1.2.2.1 ***Acremonium*** Link ex Fr., Mag. Ges. naturf. Fr. Berlin 3:15 (1809).

Conidiophores mostly simple, erect, awl-shaped, septate at base, arising from mycelium or mycelial strand; *conidiogenous cells* phialidic, producing conidia in slime heads or occasionally in short chains; *conidia* hyaline or brightly coloured, one-celled or rarely two-celled, ellipsoid, oblong, or fusoid.

Habitat: Saprobes or parasites of insects, nematodes, fungi, or plants.

Lectotype species: *Acremonium alternatum* Link per S.F. Gray.

References. Gams. 1971; Samson *et al.*1988.

Acremonium charticola (J. Lindau) W. Gams, *Cephalosporium-artige Schimmelpilze* (Stuttgart): 46 (1971). (Fig. 4.19)

Infected grasshopper covered with dense, black mycelium. *Colonies* on Czapek agar growing fast, covering all surface of the media within 14 days at 25°C, loose floccose; creamy white. *Reverse* white at center, changing green to white at the margin. Odour absent. *Vegetative hyphae* branched, septate, smooth-walled, hyaline, 1.2-3 µm wide. *Conidiophores* erect, septate, branched, 1.6-2.4 µm wide, bearing solitary or whorls of 2-4 phialides. *Phialides* subulate, awl-shaped, occasionally 1-septate, orthropic or plagiotropic, 12-72 × 1.2-2.4 µm. *Conidia* in short chains, ellipsoidal to ovoid,

occasionally cylindrical, 3-13.2 × 1.2-3 µm, sometimes slightly apiculate at one or both ends, smooth-walled, hyaline, catenulate. Chlamydospores absent.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', on grasshopper, Orthoptera, 17 August 2005 O.M. Aung MFLU1088 (MFLUH).

I was not able to obtain DNA from this specimen.

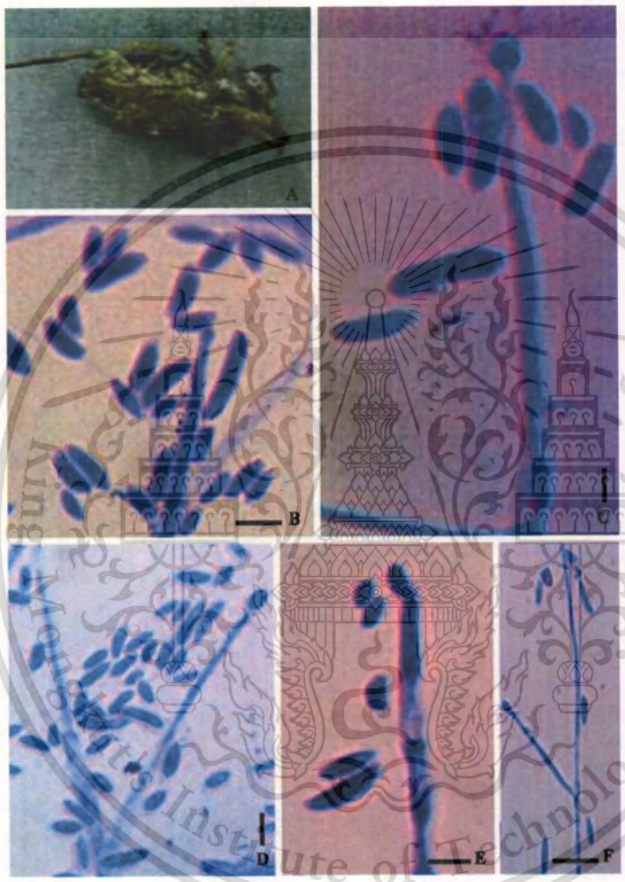


Fig. 4.19 *Acremonium charticola.* **A.** Infected orthopteran insect. **B-F.** Conidiophores, phialides and conidia. Bars: B & E = 30 µm, C = 25 µm, D = 40 µm, F = 10 µm.

Acremonium crassum Petch ref mising (Fig. 4.20)

Infected cicada covered with dense, brownish-white mycelium. *Colonies* on Czapek agar growing fast, covering all surface of the media within 14 days at 25°C, loose floccose; creamy-white. Reverse white at center, changing green to white at the margin. Odour absent. Vegetative hyphae branched, septate, smooth-walled, hyaline, sometimes fasciculate, 1-5 µm wide. *Conidiophores* phialidic, erect, septate, 2.2-3.6-2.4 µm wide, bearing phialides singly, or in whorls of 2 to 3.

Phialides subulate, awl-shaped, orthropic or plagiotropic, rarely 1-septate, 12-21 × 1.2-1.8 µm. *Conidia* produced singly in false heads, oval or cylindrical, occasionally slightly curved, both ends rounded, rarely one-septate, 3-5 × 1.2-3 µm, Chlamydospores absent.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', on adult cicada, Hemiptera, 25 June 2005, O.M. Aung MFLU835 (MFLUH).

I was not able to obtain DNA from this specimen.

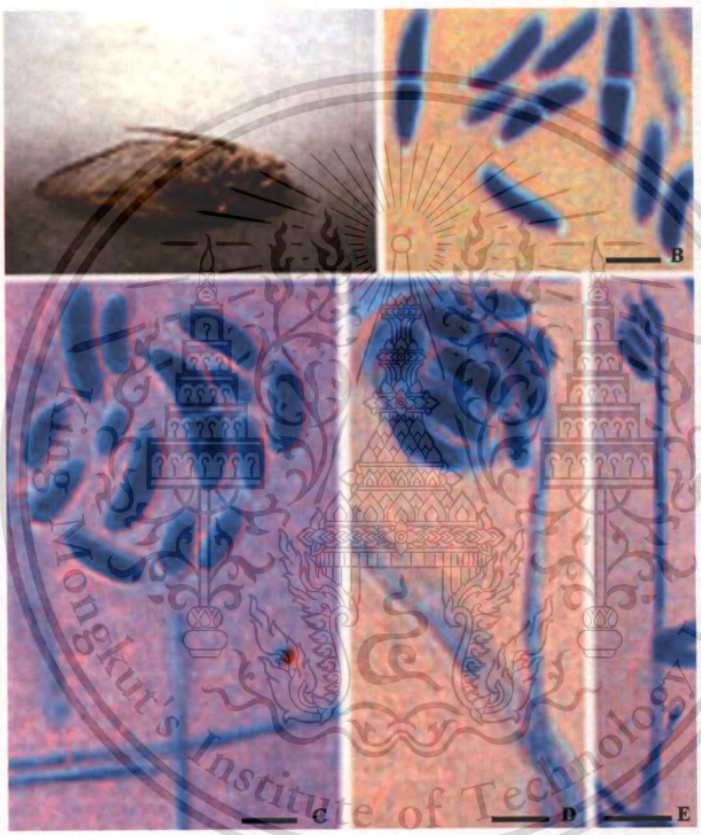


Fig. 4.20 *Acremonium crassum*. **A.** Infected cicada. **B-C.** Conidia. **D-E.** Phialides and conidia. Bars: B, C & E = 10 µm, D = 15 µm.

4.1.2.2.2 ***Aschersonia*** Montagne, Ann. Sci. Nat., Ser. 3, Bot., X., P. 122 (1848).

Pycnidia containing conidiophores and/or paraphyses, formed in hemispherical or cushion-shaped stroma; *conidiophore* slender, branched, consisting of thin-walled, usually awl-shaped conidiogenous cells; *conidia* one-celled, hyaline, smooth, mostly fusoid.

Type species: *Aschersonia taitensis* Montagne

References: Petch. 1921; Samson *et al.*1988.

Aschersonia sp. (Fig. 4.21)

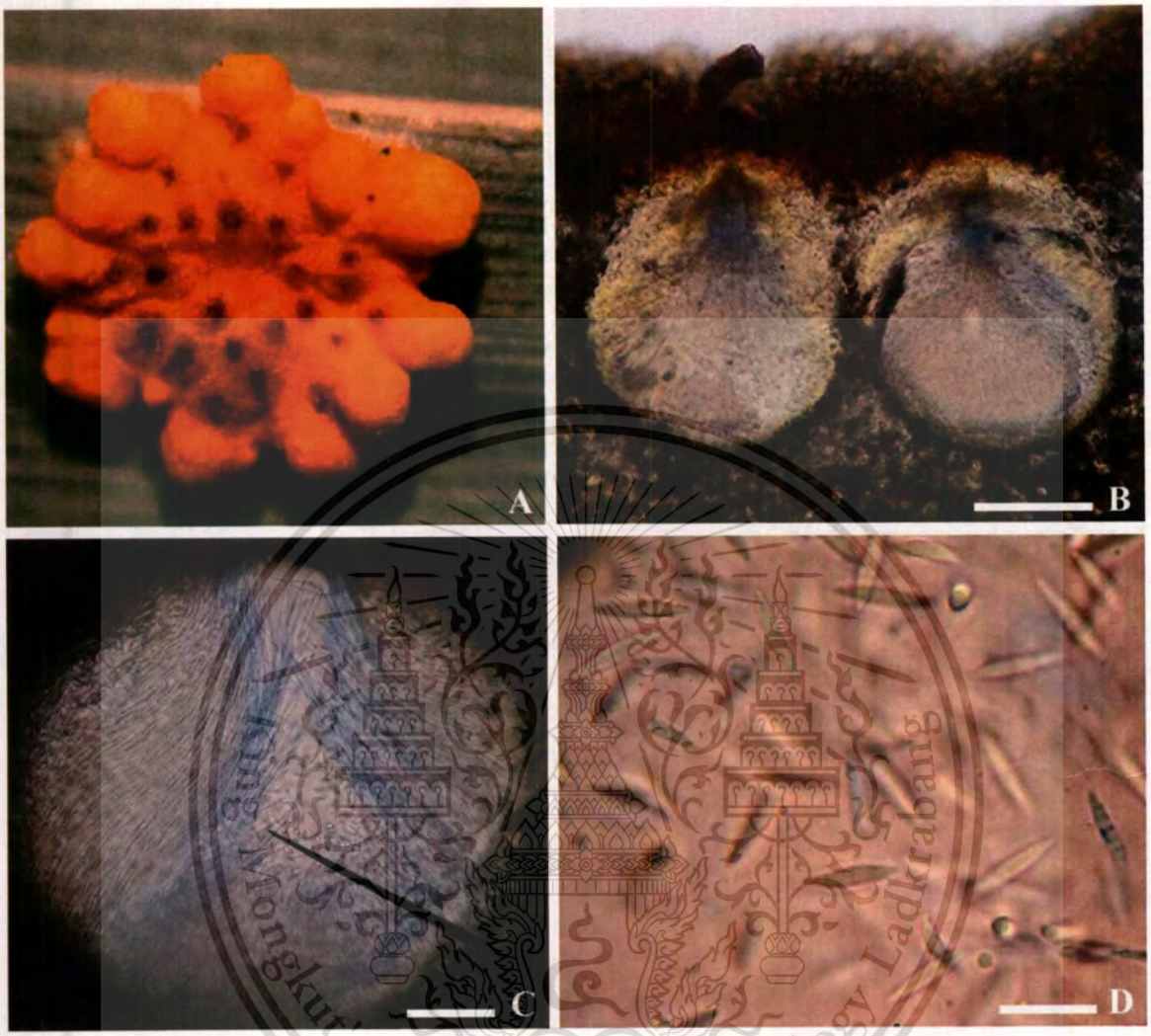


Fig. 4.21 *Aschersonia* sp. **A.** Infected insects on the substrate. **B.** Immersed pycnidia. **C.** Paraphyses. **D.** Conidia. Bars: B = 10 µm, C = 50 µm, D = 15 µm.

Pycnidia yellowish-brown, immsersed, globose to subglobose, 160-280 µm high and 160-230 µm wide. *Paraphyses* 65-100 × 1-2 µm *Conidiophores* slender, branched, thin-walled. *Conidiogenous cells* awl-shaped. *Conidia* one-celled, hyaline, smooth, fusoid 6-15 × 2-3 µm.

Teleomorph: *Hypocrella*, Asci immature.

Habitats: Infected insect attached to bamboo leaves, Homoptera.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., New Waterfall, located near 36 km marker on Highway 1095, on whiteflies, Homoptera, 13 July 2006, O.M. Aung MFLU1085 (MFLUH).

Remarks: The specimen is similar to *A. badia* but pycnidia are smaller (160-230 × 160-230 µm vs. 260-360 × 160-300 µm) and length of pycnidiospores are shorter (6-15 µm vs. 8.3-18.3 µm) (Tzean. 1997).

I was not able to obtain DNA from this specimen.

4.1.2.2.3 ***Aspergillus*** Micheli, Nova Plantarum Genera p. 212 (1929).

Conidiophores erect, unbranched, smooth or roughened, swelling terminally to form a globose, subglobose, ovoid to clavate vesicles, which bears phialides (uniseriate) or metuale (biseriate); *phialide* flask-shaped, producing a long, dry, basipetal chain of conidia; conidia one-celled, smooth or roughened, hyaline to dark pigmented.

Habitat: Mostly saprobic, a few species parasitic on insects.

Type species: *Aspergillus glaucus* (Mich. Ex L.: Fr.) Link

References: Gams and Samson. 1985; Klich and Pitt. 1988; Raper and Fennell. 1965.

***Aspergillus* sp.** (Fig. 4.22)

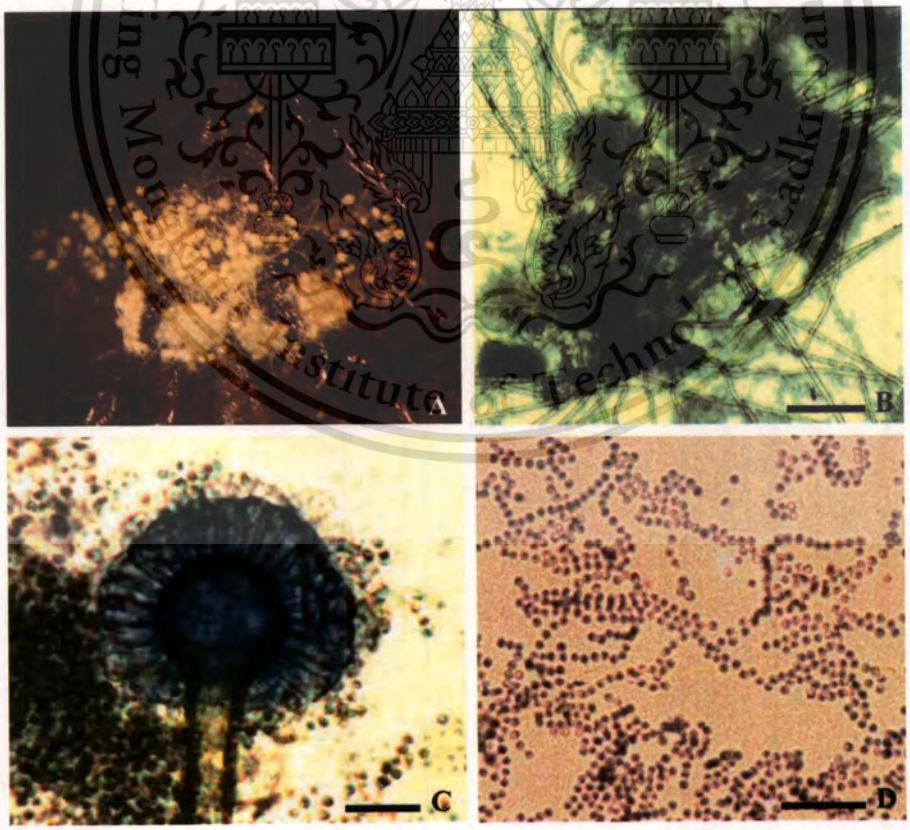


Fig. 4.22 *Aspergillus* sp. **A.** Infected beetle. **B.** Conidiophores and conidial heads. **C.** Aspergilla. **D.** Conidia. Bars: B = 150 µm, C & D = 15 µm.

Conidiophores erect, unbranched, smooth, swelling terminally to form a globose, subglobose, ovoid to clavate vesicles, which bears phialides metuale (biseriate); *phialide* flask-shaped, producing a long, dry, basipetal chain of conidia; conidia one-celled, smooth or roughened, hyaline to dark pigmented. Mostly saprobic, a few species parasitic on insects.

Colonies on Czapek agar growing rather slowly, attaining a diameter of 10-25 mm within 14 days at 25°C, powdery, yellow, irregular margin, notate, scatter colony on media. Reverse yellow to pale brown. *Conidiophores* erect, unbranched, smooth, swelling terminally to form a globose, subglobose vesicles, which bears metuale (biseriate). *Phialide* flask-shaped, 9.1-12.3 × 4.3-5.1 µm, producing a long, dry, basipetal chain of conidia. *Conidia* one-celled, roughened, hyaline, globose to subglobose, 1.2-2.5 µm diameter.

Host: Adult Coleoptera and Isoptera.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', on adult Coleoptera, 24 June 2006, O.M. Aung MFLU991 (MFLUH); *ibid*., on adult Isoptera, 16 July 2006, O.M. Aung MFLU1029 (MFLUH).

I was not able to obtain DNA from this specimen.

4.1.2.2.4 ***Beauveria*** Vuillemin, Bull. Soc. Bot. Fr. 59: 40 (1912).

Conidiophores mostly simple, often funiculose, or rarely synnematous, consisting of a swollen globose, subglobose or flask-shaped basal portion, and a long, slender geniculate, denticulate rachis, with a distinct zig-zag appearance; conidiogenous cells often in dense clusters, or scattered in whorls, arising from undifferentiated hyphae, or from short, somewhat inflated stalk cells; conidia one-celled, hyaline, smooth, thin-walled, globose to ellipsoidal.

Type species: *Beauveria bassiana* (Bals.) Vuillemin

Reference: De Hoog. 1972.

Beauveria bassiana (Bals.-Criv.) Vuill., Bull. Soc. bot. Fr. 12: 40 (1912). (Fig. 4.23)

Infected host covered with dense mycelium, typically pulvinate, granular-pulverulant, sometimes funiculose. *Colonies* on Czapek agar growing moderately fast, attaining a diameter of 25-40 mm within 14 days at 25°C, floccose, white, irregular margin, scatter colony on media. Reverse yellow at center, changing white to the margin. Odour absent. Hyphae smooth, hyaline, 1-3 µm wide, bearing groups of swollen lateral cells, globose, ellipsoid to subcylindrical, 4-6.8 × 2.9-4.8 µm, further branching, giving rise to smaller swollen cells or 1-3 conidiogenous cells in the first or second order, occasionally conidiogenous cells occurring in small groups on lateral cells, solitary arising from

the hyphae. *Conidiogenous* cells consisting of a globose to subglobose, 1.2-1.8 µm diameter to 2-3 × 1.2-2.4 µm in size, terminal cells mostly slender, forming a well developed rachis, up to 14µm, 0.6-1.2 µm wide, geniculate or irregularly bent, denticulate, forming zig-zag structure, denticles thinner than the rachis, about 0.4 µm long. *Conidia* globose, 1.2-1.8 µm diameter.

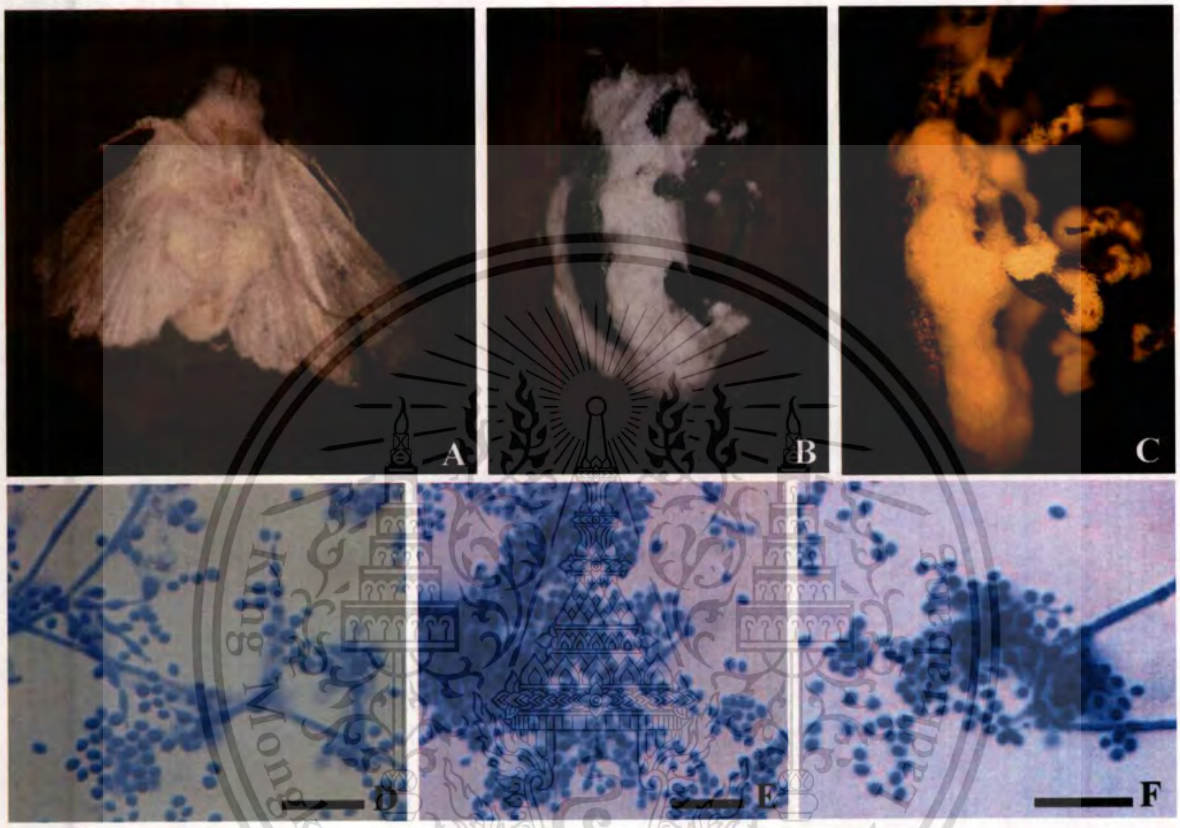


Fig. 4.23 *Beauveria bassiana*. **A.** Infected moth, Noctuidae. **B-C.** Infected beetle, *Cerambycidae*. **D-F.** Conidiophores, phialides, rachis and conidia. Bars: D = 10 µm, E & F = 5 µm.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', on adult Coleoptera, 9 August 2005, O.M. Aung MFLU809 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mae Lod coffee plantation, Highway 1095 at 19 km marker, on adult Coleoptera, 15 October 2006, O.M. Aung MFLU993 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', on adult Coleoptera, 16 August 2006, O.M. Aung MFLU996 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mokfa Waterfall, located near 18 km marker on Highway 1095, on adult Lepidoptera, 7 July 2006, O.M. Aung MFLU999 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Ban Pha Deng village, on adult Homoptera, 26 November 2006, O.M. Aung MFLU1002 (MFLUH);

Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', on adult ant, Hymenoptera, 24 June 2006, O.M. Aung MFLU847 (MFLUH).

Remarks: Blasting the ITS sequence of this species shows it to be similar (E-value = 0.0) to *Beauveria bassiana* sequences from GenBank (AB027382, AJ560691, AJ560690, AJ560687, AJ560685, and AJ560679; 99% similarity), as well as. *C. bassiana* (AJ564813, AJ564812, AJ564810, and AJ564809; 99% similarity). Detailed discussion has been presented in section 5.3.

Beauveria brongniartii (Sacc.) Petch , Trans. Br. mycol. Soc. 10(4): 249 (1926) (Fig. 4.24)

Mycelium covering the infected host, particularly abundant on ventral surface, lanose or granular-pulverulent, white to orange-white. *Colonies* on Czapek agar growing moderately fast, attaining a diameter of 35-40 mm within 14 days at 25°C, cottony, white, round. *Reverse* white. *Hyphae* smooth, hyaline, 1-3 µm wide, bearing groups of swollen lateral cells, globose, cylindrical to subcylindrical, 1.8-9 × 0.6-1.8 µm, further branching, giving rise to numerous smaller swollen cells or 1-6 conidiogenous cells in the first or sometimes in the second order. *Conidiogenous* cells consisting of globose to subglobose basal part, 2.8-4 × 2.8-3.6 µm, and terminal cells mostly slender, forming a well developed rachis, up to 14 µm, 0.8-1.2 µm wide, geniculate or irregularly bent, denticulate, denticles thinner than the rachis, about 0.4 µm long. *Conidia* subglobose, oblong to ellipsoidal, hyaline, smooth-walled, base slightly apiculate, 2.4-4.8 × 0.6-1.8 µm and 1.2-1.8 µm in diameter.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', on adult bug, Hemiptera, 10 July 2005, O.M. Aung MFLU836, (MFLUH); *ibid.*, on adult beetle, Coleoptera, 7 July 2005, O.M. Aung MFLU807 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Pang Sa Det, Mae Sae village, N19°14.59' E98°38.45', 962m alt., on adult beetle, Coleoptera, 17 July 2005, O.M. Aung MFLU808 (MFLUH); Chiang Mai Province, Mae Taeng Distr., New Waterfall, located near 36 km marker on Highway 1095, on adult Coleoptera, 28 June 2006, O.M. Aung MFLU992 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Geyser Pong Dueb Hot Spring, on adult Coleoptera, 15 July 2006, O.M. Aung MFLU994 (MFLUH); *ibid.*, on adult Coleoptera, 8 August 2006, O.M. Aung MFLU995 (MFLUH); *ibid.*, on adult Coleoptera, 23 August 2006, O.M. Aung MFLU997 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', on adult Coleoptera, 22 September 2006, O.M. Aung MFLU998 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Geyser Pong Dueb Hot Spring, on adult Diptera, 15 July 2006, O.M. Aung MFLU1000 (MFLUH); Chaing Rai, on adult Orthoptera, 2 August 2006, O.M. Aung MFLU1004 (MFLUH);

Chiang Mai Province, Mae Taeng Distr., Mokfa Waterfall, located near 18 km marker on Highway 1095, Unidentified insect, 25 June 2006, O.M. Aung MFLU1030 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Geyser Pong Dueb Hot Spring, on adult Hemiptera, 23 August 2006, O.M. Aung MFLU1032 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', Soil, 14 September 2006, O.M. Aung MFLU952 (MFLUH); *ibid.*, Soil, 2 October 2006, O.M. Aung MFLU969 (MFLUH).

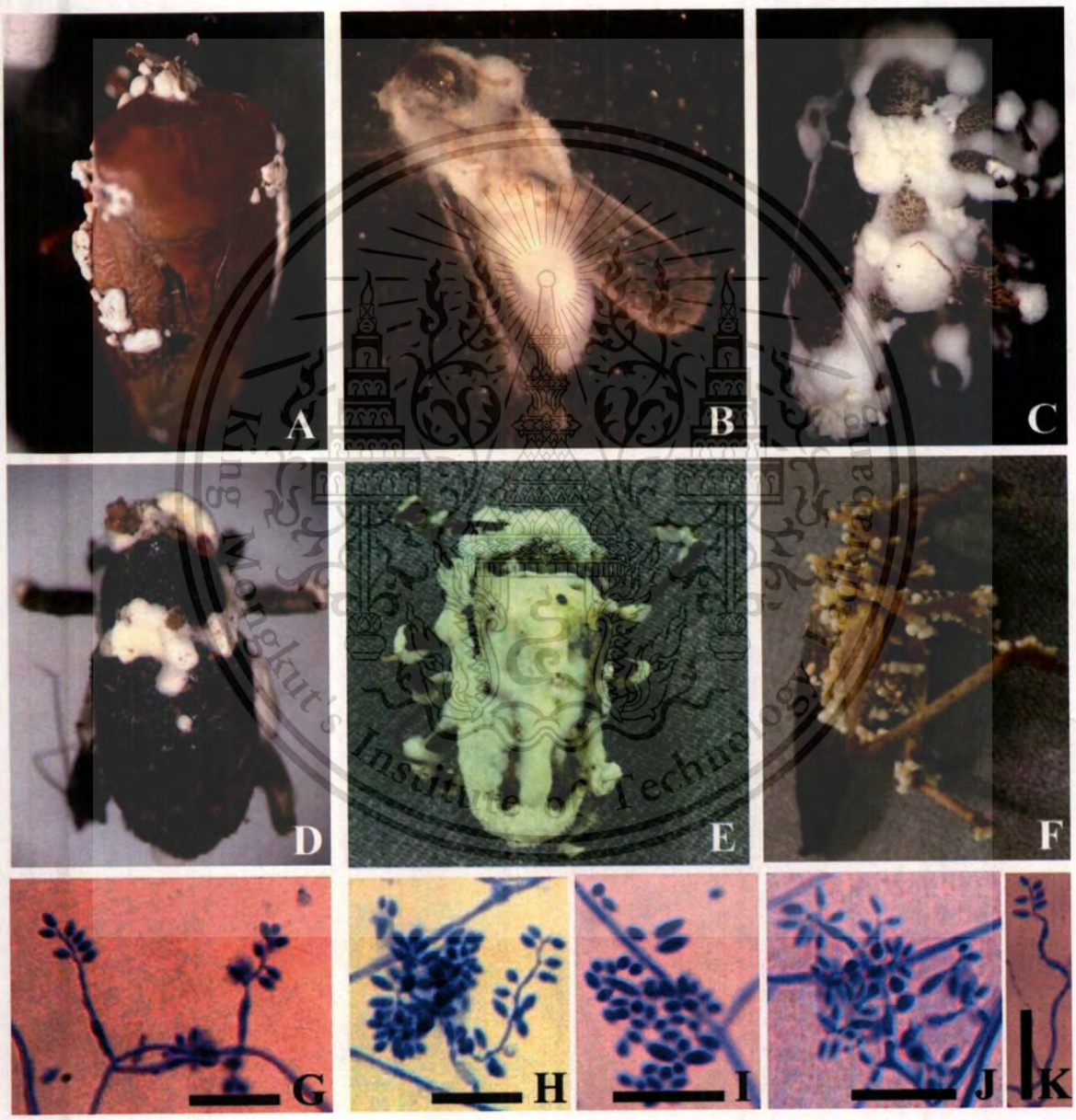


Fig. 4.24 *Beauveria brongniartii*. **A.** Infected bug, Pentatomidae. **B.** Infected fly, Diptera. **C-E.** Infected beetles. **F.** Infected grasshopper. **G-K.** Conidiophores, phialides, rachis and conidia. Bars: G-K = 10 μm.

Remarks: Blasting the ITS sequence of this species shows it to be similar (E-value = 0.0) to a *Beauveria brongniartii* sequence from GenBank (AB027381; 98 % similarity), as well as. *C. miliraris* (AJ536564, 99 % similarity), *C. brongniartii* (AB258367, AY245628, AB237659, AB106649; 98 % similarity), and *C. bassiana* (AY532027, AY334542, 99% similarity). Morphological and phylogenetic study between *Beauveria* species is discussed in section 5.3.

4.1.2.2.5 ***Cladosporium*** Link ex Fries; Link, Magazin Ges. naturf. Freunde, Berlin 7: 37 (1815).

Colonies effuse or occasionally punctiform, often olivaceous but also sometimes grey, buff, brown or dark blackish-brown, velvety, floccose or hairy. *Mycelium* immersed and often also superficial. Stroma sometimes present. *Setae* and *hyphopodia* absent. *Conidiophores* macronematous or semimacronematous and sometimes also micronematous; *macronematous conidiophores* straight or fleuxous, mostly unbranched or with branches restricted to the apical region forming a stipe and head, olivaceous brown or brown, smooth or verrucose. *Ramo-conidia* often present. *Conidiogenous cells* polyblastic, usually integrated, terminal and intercalary but sometimes discrete, sympodial, more or less cylindrical, cicatrized, scars usually prominent. *Conidia* catenate as a rule but sometimes solitary especially in species with large conidia, often in branched chains, acropleurogenous, simple, cylindrical, doliiform, ellipsoidal, fusiform, ovoid, spherical or subspherical, often with a distinctly protuberant scar at each end or just at the base, pale to dark olivaceous brown or brown, smooth, verruculose or echinulate, with 0-3 or occasionally more septa.

Lectotype species: *Cladosporium herbarum* (Pers.) Link ex S.F. Gray.

Reference: Ellis. 1971.

Cladosporium sp. (Fig. 4.25)

Colonies on Czapek agar growing fast, covering all surface of the media within 14 days at 25°C, olive-green to olivaceous-brown. *Reverse* greenish-black. *Conidiophores* mostly macronematous, straight or flexuous, often geniculate. *Ramo-conidia* 7-8 × 2-5 µm, smooth. *Conidia* mostly subglobose or ellipsoidal to fusiform.

Host: Larva, Lepidoptera.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Ban Pha Deng village, on Lepidoptera larva, 26 November 2006 O.M. Aung MFLU1003 (MFLUH).

Remarks: The collection resembles genus *Cladosporium*. *Cladosporium herbarum* was previously identified as a controlling candidate against cashew whitefly, *Aleurodicus coccis* and was

also effective as a bio-control agent against three species of whiteflies, namely; *Bemisia* sp., *Aleurothrixus* sp. and *Dialeurodes* sp. on various plant hosts in Venezuela (Carvalho *et al.* 1972; Rojas *et al.* 1998). Three species of *Cladosporium* (*C. uredinicola*; *C. cladosporioides* and *C. chlorocephalum*) can also be found as biocontrol agents of *Bemisia* spp., *Aphis gossypii* and *Empoasca* sp. (Abdel-Baky *et al.* 1998). The collection was associated with Lepidoptera lava. However, due to insufficient collections, further studies are needed to confirm the association between this fungus and Lepidoptera.

Blasting the ITS sequence of this species shows it to be similar (E-value = 0.0) to *Cladosporium cladosporioides* sequences from GenBank (98-99% similarity), as well as. *Cladosporium oxysporum* (99% similarity), *Cladosporium* sp. (98-99% similarity), and Uncultured soil fungus (98-99% similarity).

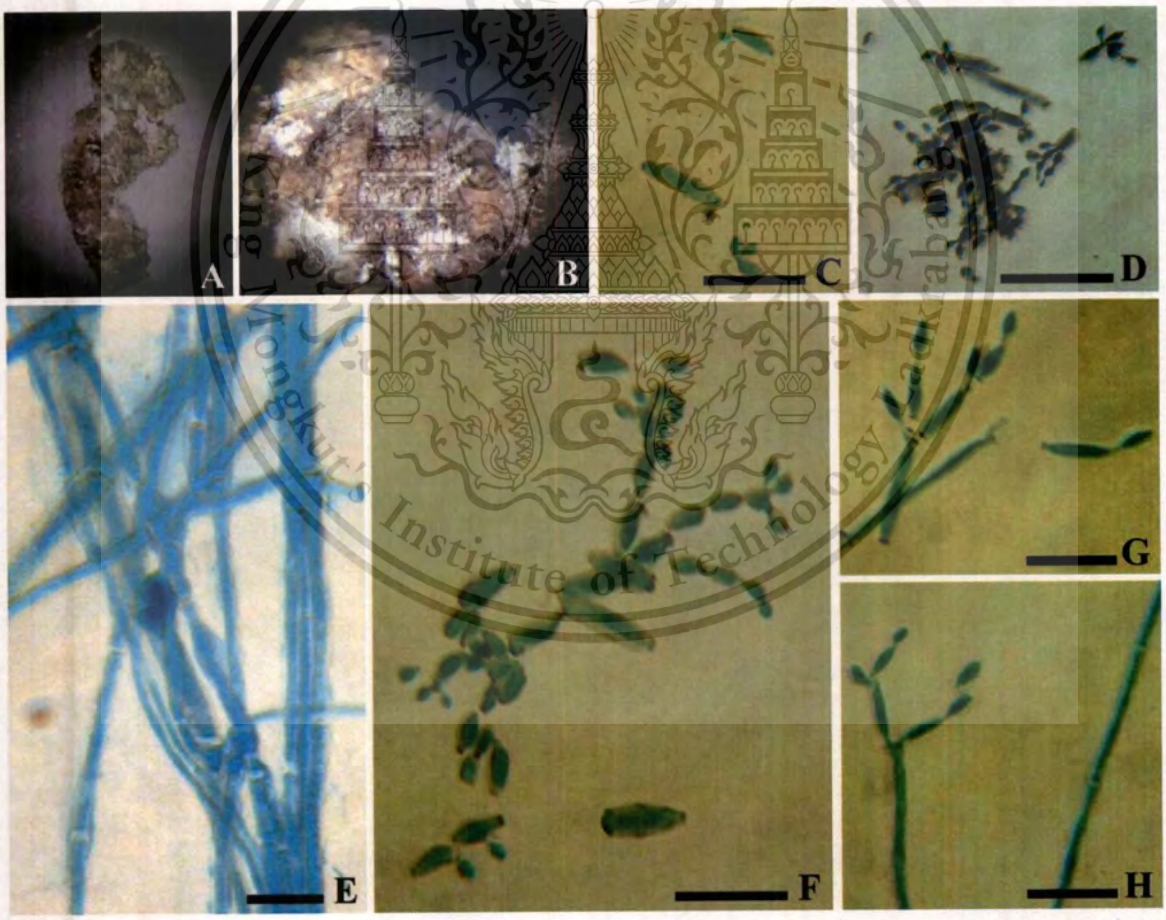


Fig. 4.25 *Cladosporium* sp. **A-B.** Infected larva. **C, D, F, G, H** Ramo-conidia and conidia. **D.** Hyphae. Bars: C-H = 10 μm.

4.1.2.2.6 ***Hymenostilbe*** Petch, Naturalist. Hull. 102 (1931).

Synnemata cylindrical, composed of longitudinally aligned closely compact hyphae; conidiogenous cells hymenial, covering the synnema; broadly cylindrical, polyblastic, bearing solitary conidia on short denticles; conidia one-celled, smooth, hyaline. Parasitic on insect or spiders.

Type species: *Hymenostilbe muscarium* Petch

Reference: Samson and Evans. 1975.

Hymenostilbe furcata Aung, J.C. Kang, Z.Q. Liang, Soytong & K.D. Hyde, Mycotaxon 97: 243 (2006). (Fig. 4.26)

Fig. 4.26 *Hymenostilbe furcata* (from **holotype**). A. Detached conidia. B. Infected Hemipteran insect with synnemata. C. Conidia D. Conidiogenous cell with forked denticles and conidium. E. Conidiogenous cells forming a hymenium-like layer. Bars: A, C, D & E = 5 µm, B = 5 mm.

Synnemata slender, 10-14 mm long, 94-120 µm wide, arising from head and thorax of insect, cylindrical, white; central core of parallel hyphae composed of cells 3-55 × 2.5-4 µm; covered by an outer hymenium-like layer of conidiogenous cells with basal cells 7.5-20 × 2.5-5 µm. *Conidiogenous* cells 5-18 × 3.5-6.5 µm, polyblastic, clavate or cylindrical, apically with 2-7 furcellate denticles, 0.6-2.4 µm. *Conidia* 8.5-15 × 3- 4.5 µm, solitary, smooth, hyaline, fusiform.

Remarks: *Hymenostilbe furcata* was collected only once on a hemipteran nymph in the rain forests in Thailand. It can be separated from *H. sulphurea* and *H. nutans*, which also occur on hemipteran insects, by the creamy white synnemata and the two to seven, forked denticles on the conidiogenous cells. *Hymenostilbe sulphurea* Samson & H.C. Evans has sulphur-yellow synnemata and subglobose to ellipsoidal, rough-walled conidia, while *H. furcata* has smooth, fusiform conidia. *Hymenostilbe nutans* has fusoid conidia but they are smaller than those of *H. furcata* (6-10 × 3.2-4 µm vs. 8.5-15 × 3-4.5 µm). The conidiogenous cells of *H. furcata* are clavate or cylindrical while those of *H. nutans* are cylindrical, apically pointed and the denticles are crowded at the apex. The conidiogenous cells of *H. furcata* are 5-18 µm long × 3.5-6.5 µm wide, whereas those of *H. nutans* are 15-24 µm long × 4.5-6.5 µm wide. Those of *H. sulphurea* are cylindrical to clavate, 15-25 × 5-6.5 ìm and the denticles are crowded at the apex (Samson and Evans. 1975; Aung *et al.* 2006b).

I was not able to obtain DNA from this specimen.

Material examined: THAILAND, MRC, on Hemiptera, 25 June 2005, O.M. Aung MFLU837 (MFLUH).

4.1.2.2.7 ***Isaria*** Pers., Syn. Meth. Fung.: 687 (1801): Fr., Syst. Mycol. 3: 270 (1832); *nom. cons. prop.*

Conidiophores mono- or synnematous, usually consisting of several verticillate branches, each bearing a dense whorl of phialides. *Synnemata* often branched with apical sporulating structures. *Phialides* consisting of a cylindrical or swollen basal portion, terminating in a thin often long neck, producing divergent conidial chains. *Conidia* one- or rarely two-celled, smoothwalled, hyaline. *Colonies* bright coloured, white, yellow, pale green, pink, red or purple. Hyphae hyaline to slightly pigmented, rough- or smooth-walled. *Chlamydospores* present in some species.

Teleomorph: *Cordyceps*, often absent in culture.

Type species : *Ramaria farinosa* Holmsk. 1781.

Isaria cicadae Miquel, Bull. Sci. phys. nat. ne´erl., se´r. 2, 10:378 (1838). (Fig. 4.27)

≡ *Paecilomyces cicadae* (Miquel) Samson, Stud. Mycol. 6: 52 (1974).

Colonies on Czapek agar growing moderately fast, attaining a diameter of 20-30 mm within 14 days at 25°C, floccose with zonate overgrowth. *Reverse* cream coloured. *Odour* absent. Vegetative hyphae smooth-walled, hyaline, 1.4-3 µm wide. Conidial structures mostly complex, consisting of erect conidiophores arising from submerged or laterally from aerial hyphae. *Conidiophores* mononematous, smooth-walled, hyaline, sometimes consisting of verticillate branches bearing whorls of up to 7 phialides. *Phialides* 7.2-26.6 × 2.4-4.5 µm, with mostly a ellipsoidal to cylindrical or rarely globose basal portion which tapers into a long distinct neck. *Conidia* mostly ellipsoidal to cylindrical, smooth-walled, hyaline, 4.9-16.7 × 2.7-3.4 µm with pointed base. Chlamydospores absent.

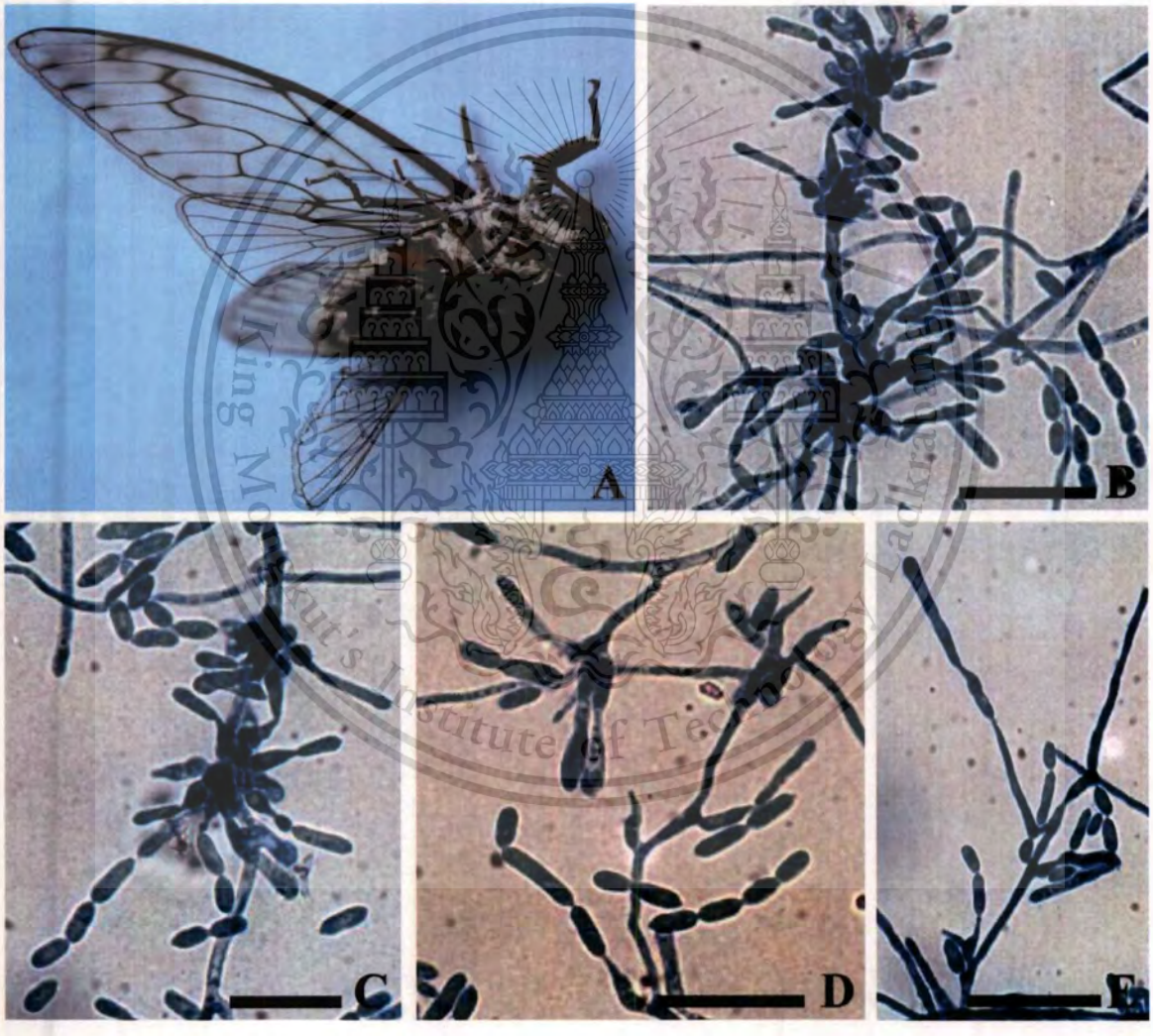


Fig. 4.27 *Isaria cicadae*. **A.** Infected cicada. **B-E.** Conidiophores, conidiogenous cells and conidia. Bars: B & E = 50 µm, C & D = 50 µm.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', on adult cicada, Homoptera, 14 June 2006, O.M. Aung MFLU872 (MFLUH); *ibid*., on adult Homoptera, 14 June 2006, O.M. Aung MFLU1031 (MFLUH).

Remarks: The collection is similar to *P. cicadae* but phialides and conidia are much larger than in Samson (1974): (7.2-26.6 × 2.4-4.5 µm vs. 4.2-6.5 × 2.5-3.5 µm) and (4.9-16.7 × 2.7-3.4 µm vs. 3.5-8 × 1.5-3.5 µm), respectively. Further collections are needed to establish if this is a new species.

Blasting the ITS sequence of this species shows it to be similar (E-value = 0.0) to *Paecilomyces cicadae* sequences from GenBank (AB085888, AB085887, AB085886, AB086630, AF368801; 99% similarity), as well as. *Cordyceps bifusispora* (AY245627; 98% similarity), *B. brongniartii* (AB027381; 98% similarity), *Cordyceps takaomontana* (AB044637, 98% similarity) and *Paecilomyces tenuipes* (AB086213; 97% similarity). Dendrogram from ITS analysis confirmed that homopteran associated fungal strains were clustered together in the same clade and they are separated from other *Paecilomyces* taxa with high bootstrap value (Fig. 4.36).

Isaria farinosa (Holmsk.) Fr., Syst. mycol. 3: 271 (1832): Fr. (Fig. 4.28)

≡ *Ramaria farinosa* Holmsk., K. Danske Vidensk. Selsks. Skr., Nye Samling 1: 279 (1781).

≡ *Paecilomyces farinosus* (Holmsk.: Fr.) A. H. S. Br. & G. Sm, Trans. Br. Mycol. Soc. 40: 50 (1957).

Colonies on Czapek agar growing moderately fast, attaining a diameter of 15-25 cm within 14 days at 25° C; consisting of a basal felt from which numerous conidiophores arise, in older strains with floccose aerial mycelium, forming synnemata; at first whitish-yellow, remaining so or turning bright yellow. Reverse cream to yellow, in fresh isolates often brightly yellow. *Odour* in distinct. *Vegetative hyphae* smooth-walled, hyaline, 1-2 µm wide. *Conidiophores* mainly arising from the submerged mycelium, with a floccose overgrowth arising as side-branches from aerial hyphae; conidiogenous structures smooth-walled, hyaline, consisting of verticillate branches with numerous whorls of. *Phialides* 4.2-7.8 × 1.2-3.0 µm, mostly ellipsoid, some are subglobose and cylindrical, with a swollen basal portion tapering into a distinct neck, about 0.5 µm wide. *Conidia* mostly globose, some ellipsoidal to fusiform, smooth-walled, hyaline, 1.2-2.4 × 1.2-1.8 µm. Chlamydospores absent.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Tung Joaw village, N19°08.07' E.098°38.09', 1423 m alt., on Lepidoptera larva, 16 October 2005, O.M. Aung MFLU841

(MFLUH); Chiang Mai Province, Mae Taeng Distr., Pang Sa Det, Mae Sae village, N19°14.59' E98°38.45', 962m alt., on Lepidoptera pupa, 18 June 2006, O.M. Aung MFLU1005 (MFLUH).

Remarks: The collection is similar to *P. farinosus* but phialides and conidia are shorter than in Samson (1974): (4.2-7.8 × 1.2-3 µm vs. 5-15 × 1.2-2.5 µm) and (1.2-2.4 × 1.2-1.8 µm vs. 2-3 × 1-1.8 µm), respectively. Further collections are needed to establish if this is a new species.

Blasting the ITS sequence of this species shows it to be similar (E-value = 0.0) to *Paecilomyces farinosus* sequences from GenBank (AB083033, AB080090, AB080088, AB080089, AF368784; 99% similarity), as well as. *Paecilomyces fumosoroseus* (AF461744, AF461743; 99% similarity), *Isaria farinosa* (AB080087; 99% similarity), and *Isaria farinosa* (AB233337, DQ888729, 98% similarity). ITS analysis shows that the sequences of *Isaria farinosa* and *Paecilomyces fumosoroseus* were found to separate group in *Isaria* clade with high bootstrap value (Fig. 4.36). Among them, *Isaria farinosa* from the Thailand collection does not cluster with other GenBank isolates.

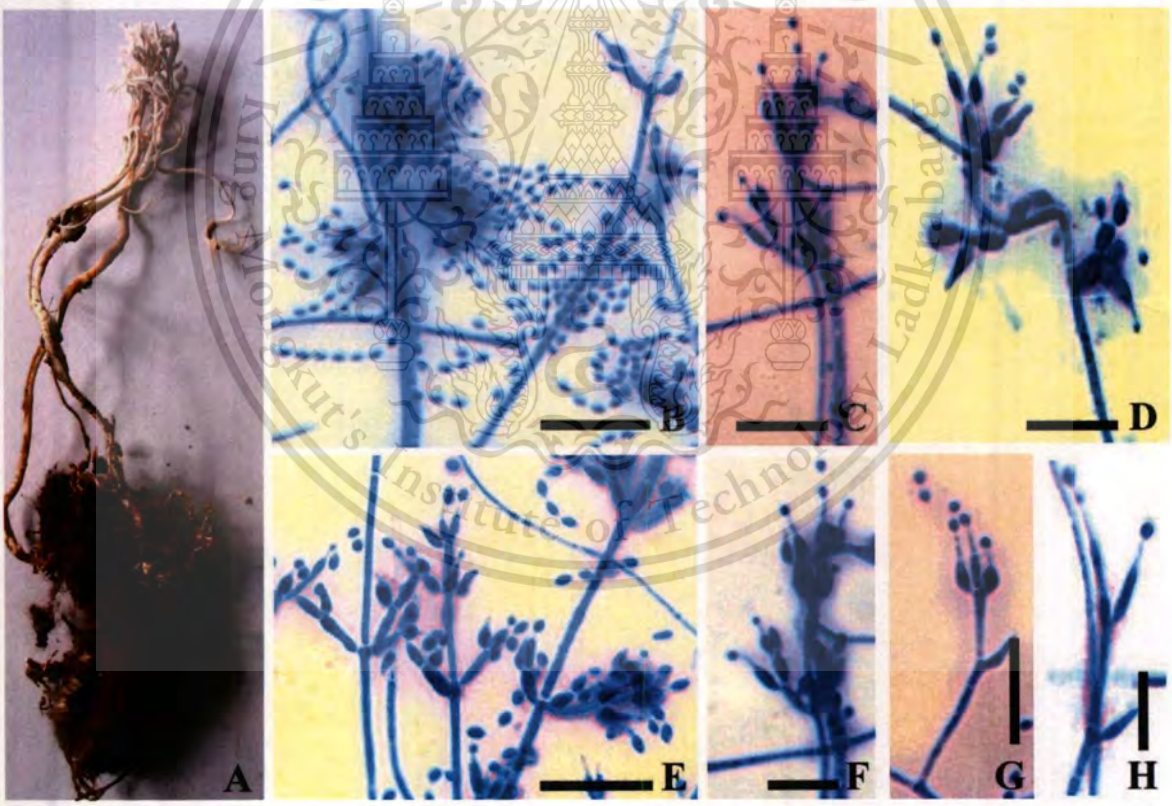


Fig. 4.28 *Isaria farinosa*. **A.** Infected caterpillar. **B-H.** Conidiophores, conidiogenous cells and conidia. Bars: B-G = 15 µm, H = 10 µm.

Isaria fumosorosea Wize, Bull. int. Acad. pol. Sci. Lett., classe sci. math. nat. 1904: 721 (1905). (Fig. 4.29)

≡ *Paecilomyces fumosorosea* (Wize) A.H.S. Br. & G. Sm., Trans. Br. mycol. Soc. 40: 50 (1957).

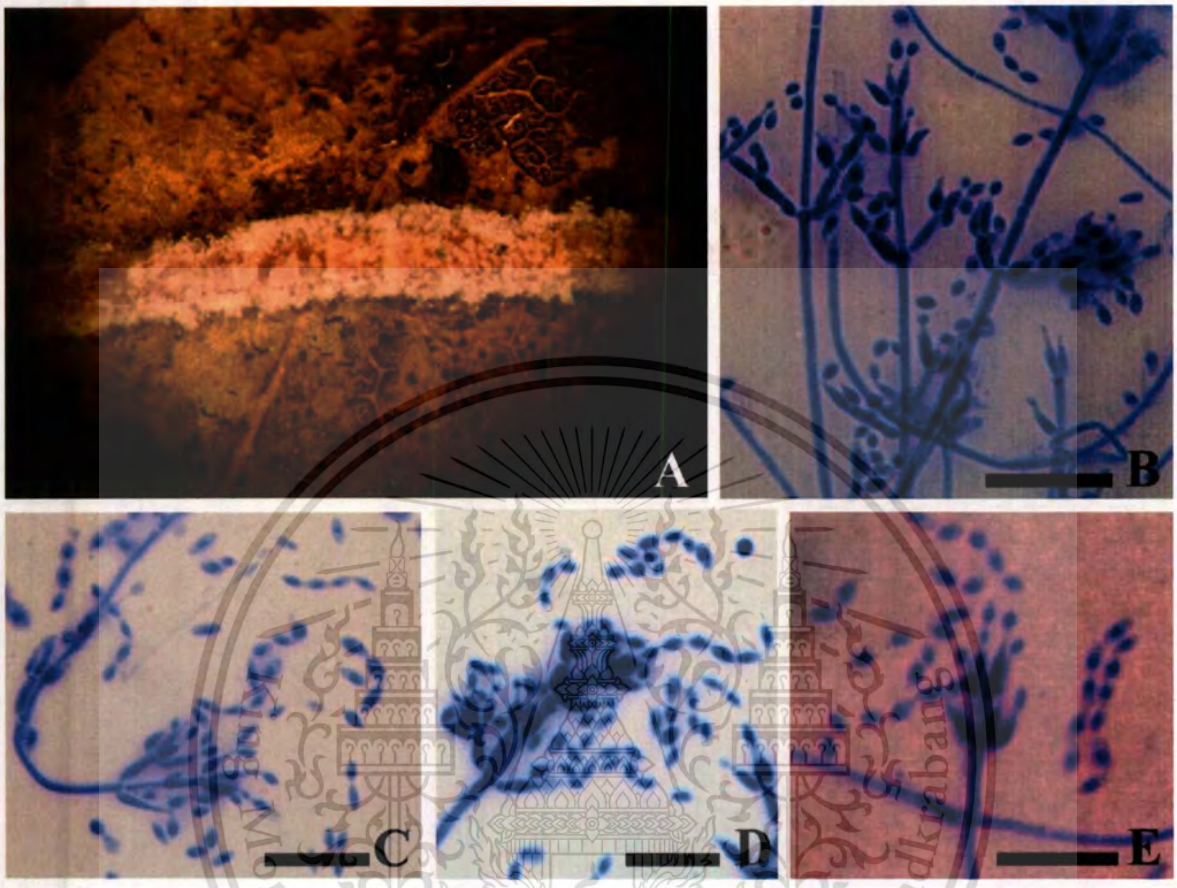


Fig. 4.29 *Isaria fumosorosea*. **A.** Infected caterpillar. **B-E.** Conidiophores, conidiogenous cells and conidia. Bars: B = 30 µm, C & E = 20 µm, D = 15 µm.

Colonies on Czapek agar growing moderately fast, attaining a diameter of 20-23 mm within 14 days at 25°C, consisting of a basal felt with raised floccose overgrowth, powdery when freshly isolated; white at first, remaining so or changing to pink shades, especially when sporulating abundantly. *Reverse* uncoloured or yellow. *Odour* absent. *Vegetative hyphae* smooth-walled, hyaline, 1-3 µm wide. Conidial structures mostly complex, consisting of erect conidiophores arising from submerged or laterally from aerial hyphae. *Conidiophores* mono- or synnematous, smooth-walled, hyaline, consisting of verticillate branches bearing whorls of 4 to 6 phialides. *Phialides* 4.2-6 × 1.2-2.4 µm, with mostly a ellipsoidal or rarely globose basal portion which tapers into a long distinct neck. *Conidia* mostly fusiform, some ellipsoidal, smooth-walled, hyaline, 1.2-4.2 × 1.2-1.8 µm. Chlamydospores absent.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Highway 1095 at 20 km marker, on Lepidoptera larva, 26 September 2005, O.M. Aung MFLU842 (MFLUH).

Remarks: The collection is similar to *P. fumosoroseus* but phialides are shorter (4.2-6 × 1.2-2.4 µm vs. 5-7.8 × 1-2 µm) Samson (1974). Further collections are needed to establish if this is a new species.

I was not able to obtain DNA from this specimen.

Isaria tenuipes Peck, Rep. N. Y. St. Bot. 31: 49 (1879). (Fig. 4.30)

≡ *Paecilomyces tenuipes* (Peck) Samson, Stud. Mycol. 6: 49 (1974).

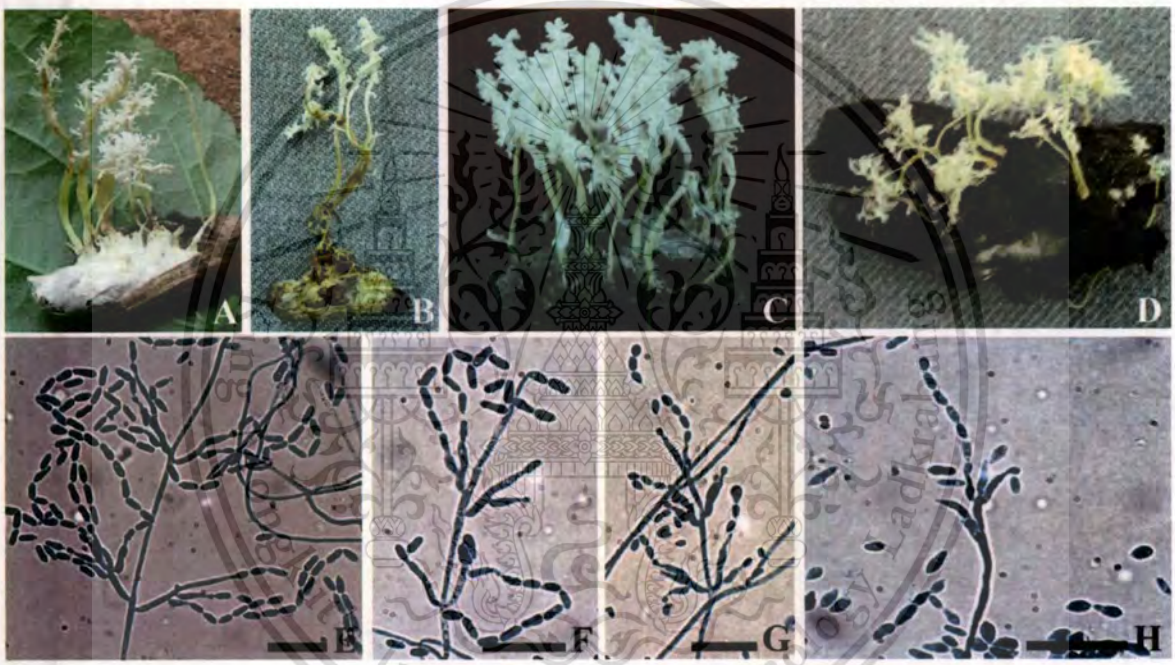


Fig. 4.30 *Isaria tenuipes*. **A-D.** Infected pupa. **E-H.** Conidiophores, conidiogenous cells and conidia. Bars: E & H = 30 µm, F & G = 15 µm.

Infected lepidopteran pupae covered with dense mycelial weft, white to yellowish white, bearing numerous synnemata. *Hyphae* hyaline, septate, smooth-walled, branched, 1-4.5 µm wide. *Synnemata* cylindrical, erect or prostrate, simple or irregularly branched, become greenish-yellow with age. 1.5-20 µm long, 0.5-5 µm wide, powdery, filamentous, white to yellowish-white. *Colonies* on Czapek agar growing slowly, attaining a diameter of 2 to 3 cm within 14 days at 25°C, consisting of a thin mycelial felt with numerous conidiophores giving the colony a powdery appearance; at first white, remaining so or turning cream, in fresh isolates conspicuous synnemata are produced, which

can reach 3 to 5 cm in length. *Reverse* cream. *Odour* absent or mushroom-like. *Conidiophores* arising primilarly from submerged hyphae, smooth-walled, hyaline, erect, mononematous or synnematous, 90-120 × 2.5-4 µm, consisting of verticillate branches with whorls of 2 to 6 phialides. *Phialides* smooth-walled, 4.5-6.5 × 2.5-3 µm, consisting of a globose basal portion, tapering into a thin neck. *Conidia* cylindrical, sometimes ovoid to broadly ellipsoidal, smooth-walled, hyaline, one-celled, measuring 2.4-9.1 × 1.4-3.7 µm. Chlamydospores absent.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., New Waterfall, located near 36 km marker on Highway 1095, 28 June 2006, O.M. Aung 1006 (MFLUH); *ibid.*, 13 July 2006, O.M. Aung MFLU1007 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 5 August 2006, O.M. Aung MFLU1008 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Geyser Pong Dueb Hot Spring, 8 August 2006, O.M. Aung MFLU1009, MFLU1011, MFLU1012, MFLU1013 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Pang Sa Det, Mae Sae village, N19°14.59' E98°38.45', 962m alt., August 2006, O.M. Aung MFLU1014 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Geyser Pong Dueb Hot Spring, 23 August 2006, O.M. Aung MFLU1015, MFLU1016, MFLU1017 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 24 August 2006, O.M. Aung MFLU1018 (MFLUH); Chiang Dao, 25 August 2006, O.M. Aung MFLU1019 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 02 September 2006, O.M. Aung MFLU1020 (MFLUH); Chiang Mai Province, Mae Taeng Distr., New Waterfall, located near 36 km marker on Highway 1095, 05 September 2006, O.M. Aung MFLU1021, MFLU1022 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', 22 September 2006, O.M. Aung MFLU1023 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Geyser Pong Dueb Hot Spring, 26 September 2006, O.M. Aung MFLU1024 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mae Lod coffee plantation, Highway 1095 at 19 km marker, 26 September 2006, O.M. Aung MFLU1025, MFLU1026, MFLU1027 (MFLUH); Chiang Dao Cave, 28 September 2006, O.M. Aung MFLU1028 (MFLUH).

Remarks: The collection is similar to *P. tenuipes* but conidia are longer (2.4-9.1 × 1.4-3.7 µm vs. 3-7.5 × 2-2.5 µm) Samson (1974).

Blasting the ITS sequence of this species shows it to be similar (E-value = 0.0) to *Isaria tenuipes* sequences from GenBank (AB086224, AB086223; 99% similarity), as well as. *Paecilomyces tenuipes* (AB086222, AB086221, AB086220, AB086219; 99% similarity) and *Cordyceps takaomontana*

(AB189447, AB189446, AB189445, AB189443; 99% similarity). *Isaria japonica* is the anamorphic taxa of *C. takaomontana* (Kobayasi and Shimizu. 1976; Kobayasi. 1981; Ito and Hirano. 1997) and is also synonym to *Paecilomyces tenuipes* (Samson. 1974). The blast results also also prove the previous findings.

4.1.2.2.8 ***Paecilomyces*** Bainier, Bull. Soc. Mycol. Fr. 23: 26 (1907).

Conidiogenous structures often mononematous, but mostly synnematous in insect parasitic species; *conidiophores* septate, erect, verticillate or irregularly branched, bearing whorls of conidiogenous cells; *conidiogenous cells* phialidic, usually consisting of a swollen base and an abruptly tapering, distinct, divergent slender neck; conidia in basipetal chains, one-celled, hyaline or slightly pigmented, smooth-walled or echinulate of various shapes. Saprobic or parasitic on nematodes or insects.

Type species: *Paecilomyces variotii* Bainier

Reference: Samson. 1974; Bissett. 1979.

Paecilomyces marquandii (Massee) S. Hughes, Mycol. Pap. 45: 30 (1951). (Fig. 4.31)

Colonies on Czapek agar growing moderately fast, attaining a diameter of 30-40 mm within 14 days at 25°C, consisting of a dense felt with a floccose overgrowth of aerial mycelium; at first white, becoming pale vinaceous to vinaceous near Dark Vinaceous Brown, changing with age to brown shades near Liver Brown (Ridgway, pl. 39; Rayner 5'"k, 7'm). *Reverse* white, exudate usually diffusing into the surrounding agar. *Odour* absent. *Vegetative hyphae* hyaline, smooth-walled, 1-3 µm wide. Conidial structures variable, mostly verticillate, sometimes synnematous, especially in fresh isolate; *conidiophores* hyaline, smooth-walled, arising from submerged hyphae or formed as sidebranches on the aerial hyphae, consisting of verticillate branches with numerous whorls. *Phialides* 4.2-6 × 1.2-2.4 µm, consisting of ellipsoidal to cylindrical basal portion, tapering into a distinct neck, about 1 µm wide. *Conidia* in dry divergent chains, mostly subgloboes, some short fusiform to seed of peach, 1.2-1.8 × 1.2-1.8 µm.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', on beetle, Coleoptera, 3 July 2005, O.M. Aung MFLU806 (MFLUH); *ibid.*, on sting bug, Hemiptera, 17 September 2005, O.M. Aung MFLU816 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Tung Joaw village, N19°08.07' E.098°38.09', 1423 m alt., on sting bug, Hemiptera, 16 October 2005, O.M. Aung MFLU817, (MFLUH); Chiang Mai Province, Mae Taeng Distr., Highway 1095 at 20 km marker, on Unidentified insect, 26

September 2005, O.M. Aung MFLU838 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mae Lod coffee plantation, Highway 1095 at 19 km marker, on Lepidoptera pupa, 26 September 2006, O.M. Aung MFLU1025 (MFLUH); *ibid.*, on adult Hemiptera, 26 September 2006, O.M. Aung MFLU1068-1078 (MFLUH); *ibid.*, on adult Hemiptera, 15 October 2006, O.M. Aung MFLU1079-1084 (MFLUH).

Remarks: The collection is similar to *P. marquandii* but phialides and conidia are shorter (4.2-6 × 1.2-2.4 µm vs. 8-15 × 1.2-2 µm); (1.2-1.8 × 1.2-1.8 µm vs. 3-3.5 × 2-2.2 µm) (Samson (1974). Further collections and appropriate molecular approach are needed to establish if this is a new species. I was not able to obtain DNA from this specimen.

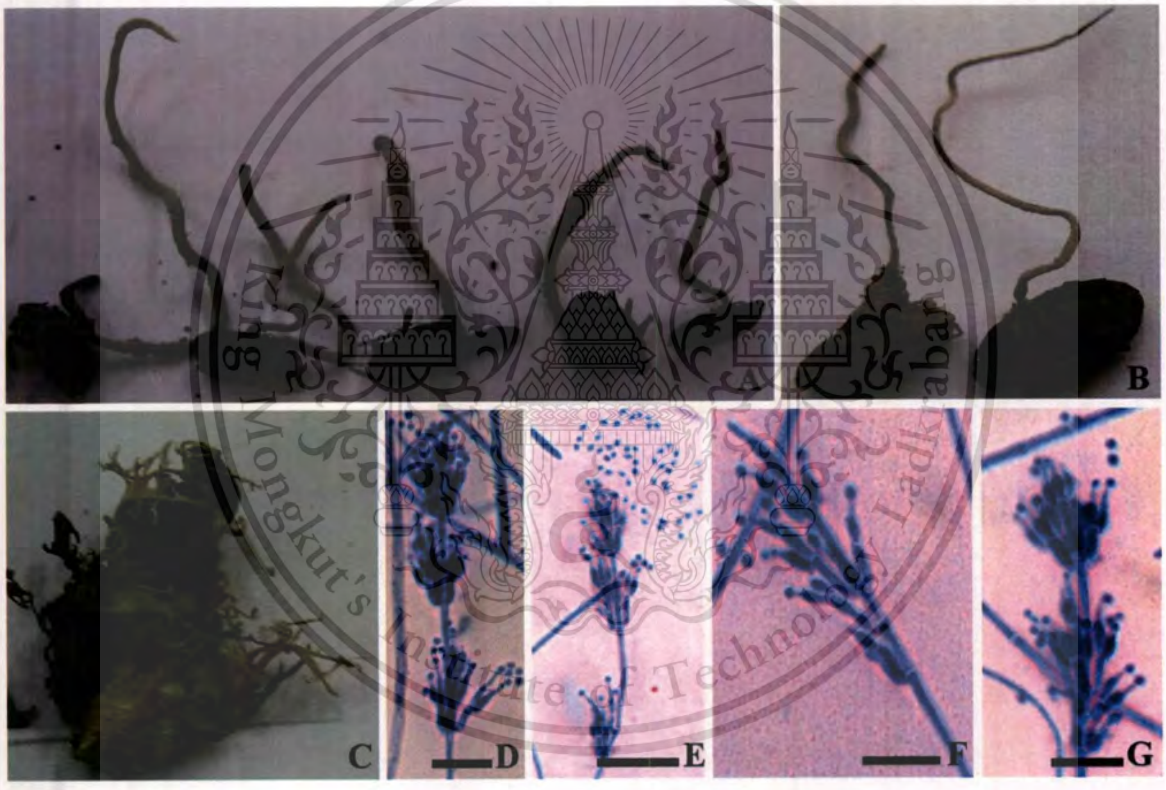


Fig. 4.31 *Paecilomyces marquandii*. **A.** Infected bugs from Mae Lod coffee plantation. **B.** Infected bugs from MRC. **C.** Infected pupa from Mae Lod coffee plantation. **D-G**. Conidiophores, conidiogenous cells and conidia. Bars: D-G = 15 µm.

4.1.2.2.9 ***Sporothrix*** Hektoen & Perkins ex Nicot & Mariot, Mycopath. Mycol. Appl. 49: 61 (1973).

Conidiophores mostly simple, mononematous, species on insects often synnematous; *conidiogenous cells* arising from undifferentiated cylindrical to linear, aseptate to septate hyphae,

apically or laterally scattered along the length, bearing a cluster of prominent denticles, or with a rather irregular, geniculate, denticulate rachis; *conidia* forming on denticles sympodial, one-celled, smooth-walled, apiculate, hyaline, ovoid, globose to fusiform. Saprobic or entomopathogenic; some species human pathogens.

Type species: *Sporothrix schenkii* Hektoen & Perkins.

Reference: De Hoog. 1974.

Sporothrix insectorum de Hoog & H.C. Evans, Studies in Mycology 7: 1-84 (1974). (Fig. 4.32)

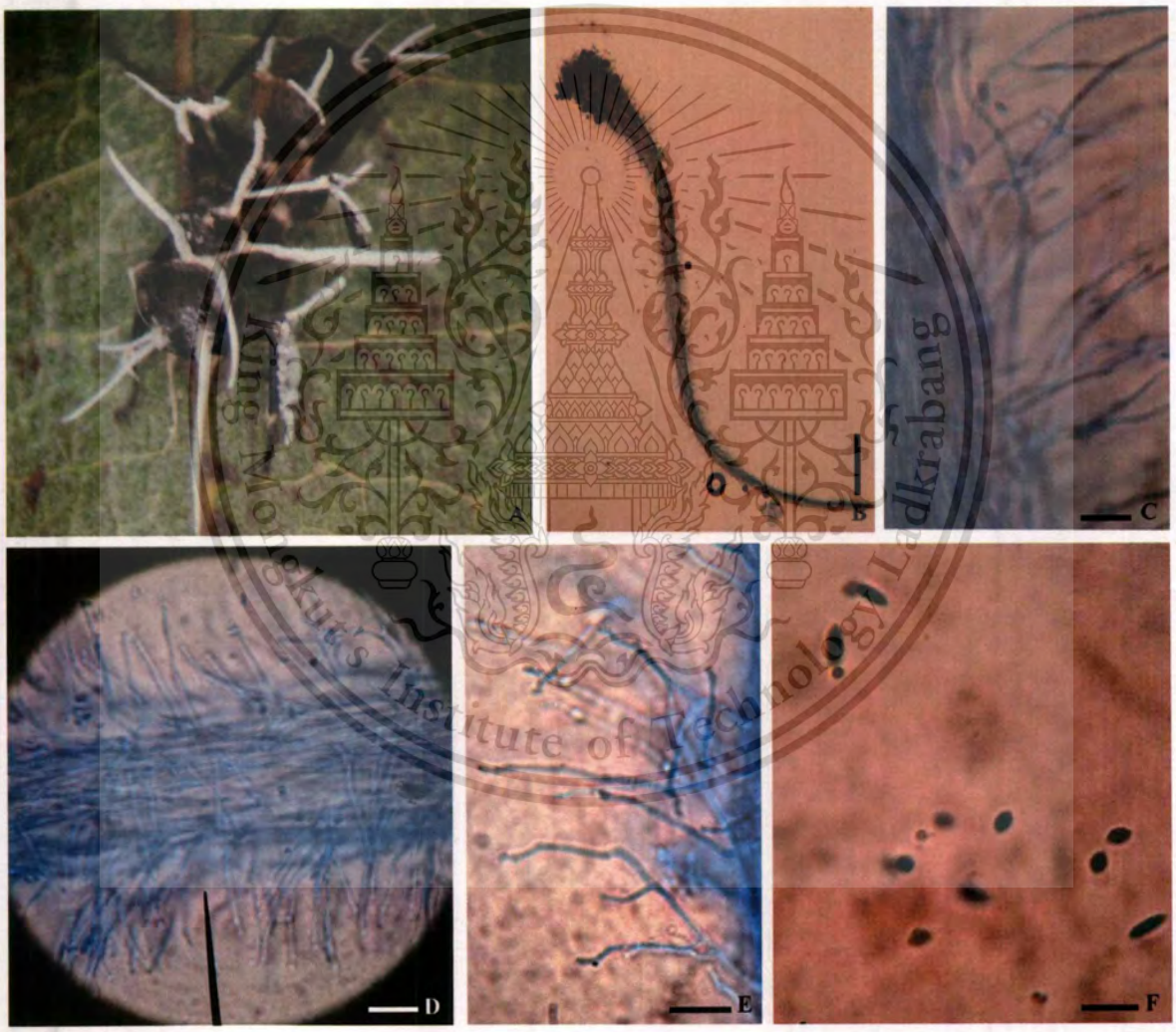


Fig. 4.32 *Sporothrix insectorum*. **A.** Infected ant. **B.** Synnemata. **C-D.** Conidiophores. **E.** Rachis. **F.** Conidia. Bars: B = 0.5 mm, C = 20 µm, D = 30 µm, E = 15 µm, F = 10 µm.

Synnemata arising from various parts of host ants. *Hyphae* septate, hyaline, smooth-walled, irregularly branched. *Synnemata* simple, cylindrical, pubescent, curved, occasionally dichotomously branched, 1-4 mm long × 40-100 µm wide, flat base, tapering to apex, consisting of longitudinal parallel hyphae. *Conidiophores* mono- or synnematous, septate, cylindrical to linear, integrate, erect or prostrate, simple, variable in length. *Conidiogenous cells* apically or laterally scattered along the length of conidiophore, bearing a rather irregular, geniculate, denticulate rachis, 20-30 µm long × 1-2 µm wide, denticle inconspicuous. Conidia forming on denticles, sympodial, subglobose, ellipsoidal, with a pointed base, end apiculate, hyaline, smooth-walled, one-celled, 2.5-5 µm × 1-2 µm.

Host: Ant, Formicidae, Hymenoptera.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Pang Sa Det, Mae Sae village, N19°14.59' E98°38.45', 962m alt., 23 August 2006, O.M. Aung MFLU855, MFLU856, MFLU857, MFLU858, MFLU859, MFLU860, MFLU861, MFLU862, MFLU863, MFLU864, MFLU865, MFLU866 (MFLUH).

Remarks: The abundant collections were found at the same places with *C. unilateralis*. There was no evidence about anamorph-connection between this species and *C. unilateralis*. Further collections are needed to confirm the relationship between both taxa.

I was not able to obtain DNA from this specimen.

4.1.2.2.10 ***Stilbella*** Lindau, Naturl Pflanzenfam. Teil. 1, Abt. 1, p. 489 (1900).

Conidiomata synnematous; *synnemata* cylindrical, simple or branched, with distinct terminal fertile head and sterile compact stipe; *conidiophores* concentrated in head, mono- bi-, or terverticillate; *conidiogenous cells* phialidic, cylindrical or subulate, forming a hymenial layer, producing conidia in mucoid head; *conidia* one-celled, smooth-walled, hyaline, globose to ellipsoid.

Habitat: Saprobic or parasitic on insects.

Lectotype species: *Stilbella fimeraria* (Pers.) Lindau = *Stilbella erythrocephala* (Ditm.) Lindau.

Reference: Seifert. 1985.

Stilbella buquetii **var. *buquetii*** (Mont. & C.P. Robin) Samson & H.C. Evans, in Samson, Evans & van de Klashorst, Proc. K. Ned. Akad. Wet., Ser. C, Biol. Med. Sci. 84(3): 290 (1981). (Fig. 4.33)

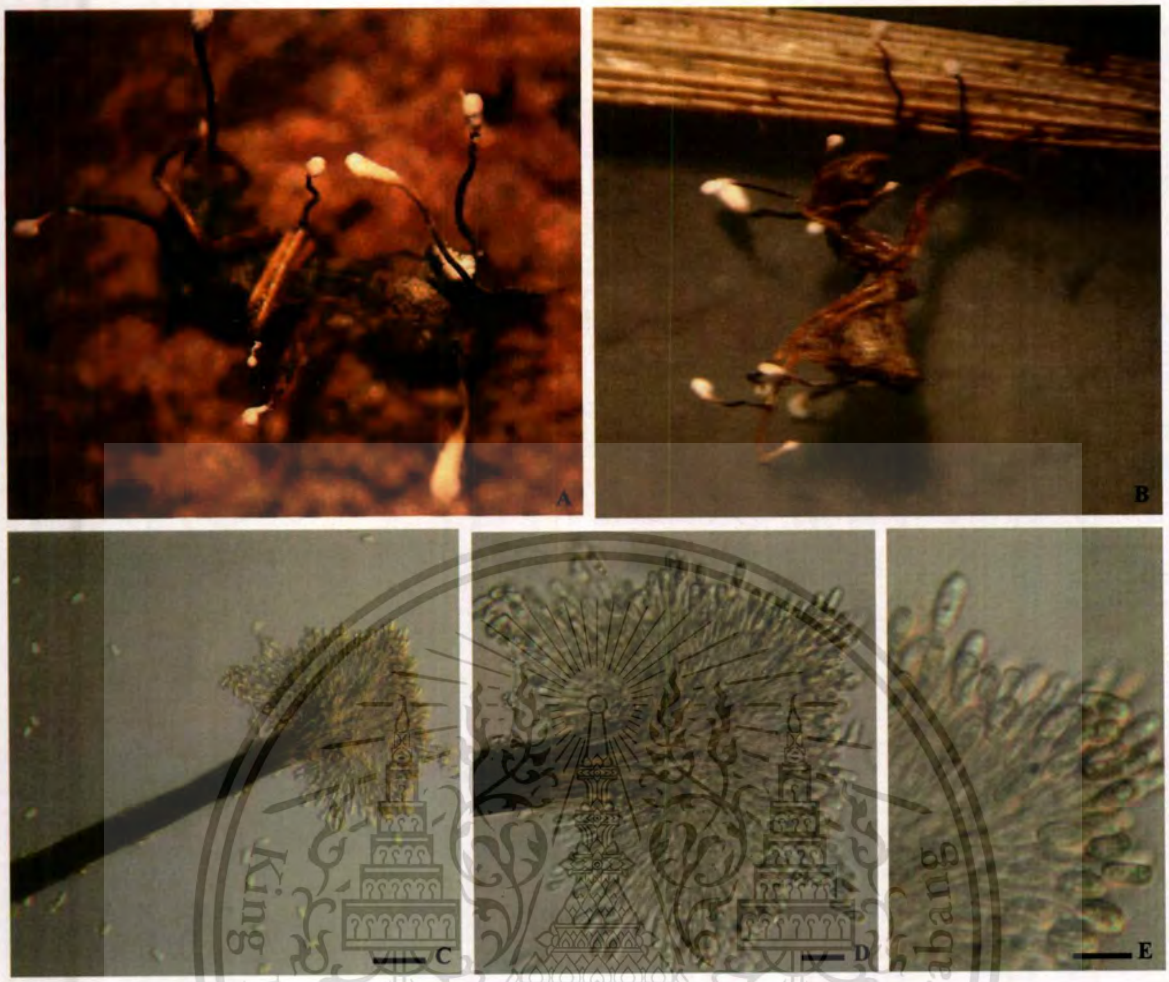


Fig. 4.33 *Stilbella buquetii*. **A-B.** Infected ant. **C.** Synnemata. **D.** Phialides. **E.** Conidia. Bars: C = 200 µm, D & E= 10 µm.

Host attached to substratum, integument covered with weft of mycelium, light brown. *Synnemata* solitary or numerous, arising from integument of head, cephalothorax and abdomen, simple, with distinct terminal fertile head, white, tomentose, minutely pruinose, 140-230 µm in diameter and sterile compact cylindrical stipe, flexuous, dark brown to black, 2.5-5 mm tall, 120-230µm wide, consisting of longitudinal, parallel septate hyphae, 3.2-5.5 µm wide. Conidiophores concentrated in head, mono-, bi-, or terverticillate. *Conidiogenous cells* phialidic, forming a hymenial layer, cylindrical or subulate, tapering into a short neck, 9.1-15.9 × 2.4-3.2 µm, hyaline, smooth-walled. *Conidia* produced singly, broadly obovoid to short obclavate, hyaline, smooth-walled, one-celled, ends apiculate, 4.8-7.9 × 2.4-5 µm.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', on Ant, Hymenoptera, 17 September 2005,

O.M. Aung MFLU840 (MFLUH); *ibid.*, on Ant, Hymenoptera, 6 October 2005, O.M. Aung MFLU871.

Remarks: The collections are identical to *Stilbella buquetii* var. *buquetii* (Tzean *et al.* 1997) and only found in Mushroom Resarch Centre.

I was not able to obtain DNA from this specimen.

4.1.2.2.11 ***Verticillium*** Nees, Syst. Pilze Schwämme p. 57. (1816).

Conidiophores slender, erect, attenuated toward the apex, branched, at least some of the branches or phialides verticillate; *conidiogenous cells* phialidic, mostly awl-shaped, sometimes base slightly swollen; *conidia* one-celled, ovoid to ellipsoid, hyaline, smooth-walled, borne singly, in small slime head, or occasionally in chains.

Habitat: Saprobes or parasites of plants, fungi, or insects.

Type species: *Verticillium tenerum* (Nees. ex Pers.) Link

Reference: Samson *et al.* 1988; Gams. 1971.

Verticillium sp. (Fig. 4.34)

Host covered by white, pulverulent to tomentose mycelial weft. *Colonies* on Czapek agar growing moderately fast, attaining a diameter of 50-60 mm within 14 days at 25°C, consisting of a floccose; white. *Reverse* creamy white. *Odour* absent. *Vegetative hyphae* moderately branched, septate, hyaline, smooth-walled, 1-3 µm wide. *Conidiophores* tall, erect, septate, bearing phialides singly or in whorls of 1-3. *Phialides* awl-shaped, hyaline, smooth, 10.8-37.2 × 1.2-1.8 µm. *Conidia* produced at the tip of phialides, often forming a mucoid false head, ellipsoidal, shortly curved cylindrical, 2.4-7.2 (9) × 1.2-2.4 µm. Chlamydospores absent.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng Distr., Pang Sa Det, Mae Sae village, N19°14.59' E98°38.45', 962m alt., On Ant, Hymenoptera, 28 June 2005, O.M. Aung MFLU805 (MFLUH); Chiang Mai Province, Mae Taeng Distr., Mushroom Research Centre, North 19° 07.123′ East 98° 44.009', On Ant, Hymenoptera, 10 October 2005, O.M. Aung MFLU835 (MFLUH).

Remarks: The culture characters can be identified as *Verticillium* sp. (Tzean *et al.* 1997) however species level identification is still confused. The infections were only found on hymenopterous ants.

I was not able to obtain DNA from this specimen.

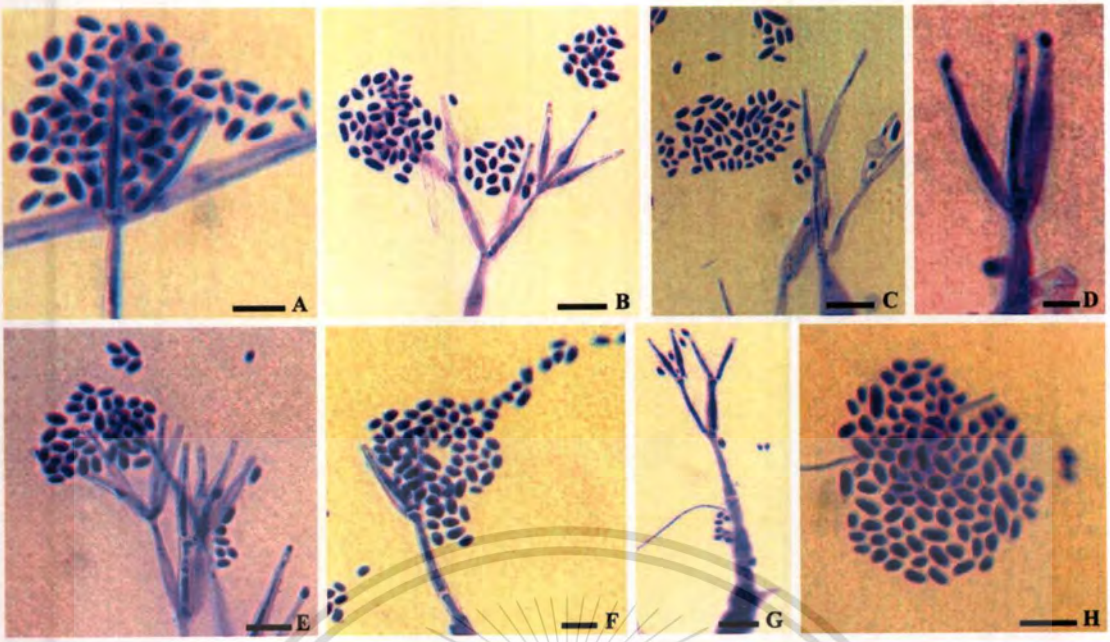


Fig. 4.34 *Verticillium* sp. **A-H.** Conidiophores, conidiogenous cells and conidia. Bars: A = 5 µm, B & C = 25 µm, D = 3 µm, E & F= 15 µm, G = 25 µm, H = 15 µm.

4.2 Diversity of entomopathogenic fungi in rainforests of Chiang Mai Province, Thailand

4.2.1 Biodiversity of entomopathogenic fungi

Thirty-four entomogenous taxa belonging to 15 genera were encountered during this study (Table 4.1). These were identified from 301 arthropod cadavers and two soil samples. The most common taxa were *Ophiocordyceps myrmecophila* and *O. unilateralis* (on Hymenoptera), *Isaria fumosoroseus* (Hemiptera), *Paecilomyces marquandii* (Coleoptera), and *I. tenuipes* (Lepidoptera). During this survey, two species (*Ophiocordyceps mrciensis* and *Hymenostilbe furcata*) were described as new species (Aung *et al.* 2006a, b).

4.2.2 Species diversity and similarities between hosts

Species diversity from different hosts, using the Shannon's and Simpson's indices, gave similar results (Table 4.2). The species diversity index for Homoptera was the highest, followed by Lepidoptera and Hymenoptera. The species richness (S) for Lepidoptera was highest, followed by Hymenoptera, Homoptera, Hemiptera, Coleoptera, Arachnida, Diptera, Orthoptera, Isoptera and soil (Table 4.2).

Similarity indices of fungal taxa between different hosts (Table 4.3) showed values between Coleoptera and Hymenoptera or between Coleoptera and Lepidoptera were higher than those between Hymenoptera and Lepidoptera, between Lepidoptera and Homoptera, between Homoptera and Hymenoptera, or between Homoptera and Coleoptera.

Table 4.1 Fungal taxa found on different insect orders and soil.

Taxa	N_i (individual no. of i^{th} species)											f
	AR	CO	DI	HE	HO	HY	IS	LP	OR	SO	UI	
Acremonium charticola									1			0.35
A. crassum					1							0.35
Aschersonia sp.					1							0.35
Aspergillus sp.	1	1					1					1.06
Beauveria bassiana		3			1	1		1				2.12
B. brongniartii		7	1	2					1	2	1	4.95
Cladosporium sp.								1				0.35
Cordyceps militaris								12				4.24
C. militaris. var. *sphaerocephala*								1				0.35
C. nelumboides	1											0.35
Cordyceps sp.								1				0.35
Hymenostilbe furcata				1								0.35
Hypocrella sp.					2							0.71
Isaria cicadae					3							1.06
I. farinosus								2				0.71
I. fumosoroseus				37								13.07
I. tenuipes								15				5.3
Ophiocordyceps crinalis								1				0.35
O. dipterigena			1									0.35
O. elongata								1				0.35
O. filiformis								1				0.35
O. longissima					1							0.35

Table 4.1 (continued).

Taxa	N_i (individual no. of i^{th} species)											*f*
	AR	CO	DI	HE	HO	HY	IS	LP	OR	SO	UI	
O. mrciensis	1											0.35
O. myrmecophila						64						22.61
O. nutans				11								3.89
O. oxycephala						11						3.89
O. pseudolloydii						9						3.18
O. sphecocephala						1						0.35
O. unilateralis						39						13.78
Paecilomyces marquandii		1		19							1	7.42
Sporothrix insectorum						12						4.24
Stilbella buquetii						3						1.06
Torrubiella hemipterigena				1								0.35
Verticillium sp.		1				1		1				1.06

AR: Archnida, CO: Coleoptera, DI: Diptera, HE: Hemiptera, HO: Homoptera, HY: Hymenoptera, IS: Isoptera, LP: Lepidoptera, OR: Orthoptera, SO: Soil, UI: Unidentified insect, *f*: Occurrence frequency.

Table 4.2 Summary of species diversity on different insect orders and soil.

	AR	CO	DI	HE	HO	HY	IS	LP	OR	SO	UI
Cordyceps and *Ophiocordyceps*	2	0	1	1	1	5	0	6	0	0	0
Other taxa	1	5	1	5	5	4	1	5	2	2	2
Species richness (*S*)	3	5	2	6	6	9	1	11	2	1	2
Individual numbers	3	13	2	71	9	141	1	37	2	2	2
Shannon index (*H*)	1.10	1.26	0.69	1.2	1.68	1.49	0	1.67	0.69	0	0.69
Simpson index (*1-D*)	0.67	0.64	0.50	0.63	0.79	0.70	0	0.72	0.5	0	0.5
Evenness (E_H)	1	0.71	1	0.55	0.89	0.49	1	0.48	1	1	1

AR: Archnida, CO: Coleoptera, DI: Diptera, HE: Hemiptera, HO: Homoptera, HY: Hymenoptera, IS: Isoptera, LP: Lepidoptera, OR: Orthoptera, SO: Soil, UI: Unidentified insect

Table 4.3 Similarity indices of fungal taxa between different hosts.

Hosts	Sørensen's index (S')		
	Lepidoptera	**Hymenoptera**	**Coleoptera**
Homoptera	0.12	0.1	0.18
Lepidoptera		0.2	**0.25**
Hymenoptera			**0.29**

Table 4.4 Summary of species diversity in different habitats.

	Conserved forests	**Disturbed forests**	**Agricultural Habitats**
Cordycep and *Ophiocordyceps*	12	9	1
Other taxa	9	16	4
Species richness (*S*)	21	25	5
Individual numbers	142	80	61
Shannon index (H')	2.08	2.73	1.03
Simpson index (*1-D*)	0.8	0.9	0.56
Evenness (E_H)	0.38	0.61	0.56

4.2.3 **Species diversity and similarities between different collecting sites**

The highest species diversity was found in disturbed rainforests, followed by conserved rainforests and agricultural habitats (Table 4.4). The highest individual number of fungi was found in conserved rainforests (142 individual records), followed by disturbed forest (80 individual records) and agricultural habitats (61 individual records). The greatest species richness was recorded in disturbed rainforests (25 taxa), followed by conserved rainforests (21 taxa) and agricultural habitats (5 taxa). *Cordyceps* and *Ophiocordyceps* species were the most abundant in conserved forest (12 taxa), followed by disturbed forest (9 taxa), and agricultural habitats (1 taxon) (Fig. 4.35).

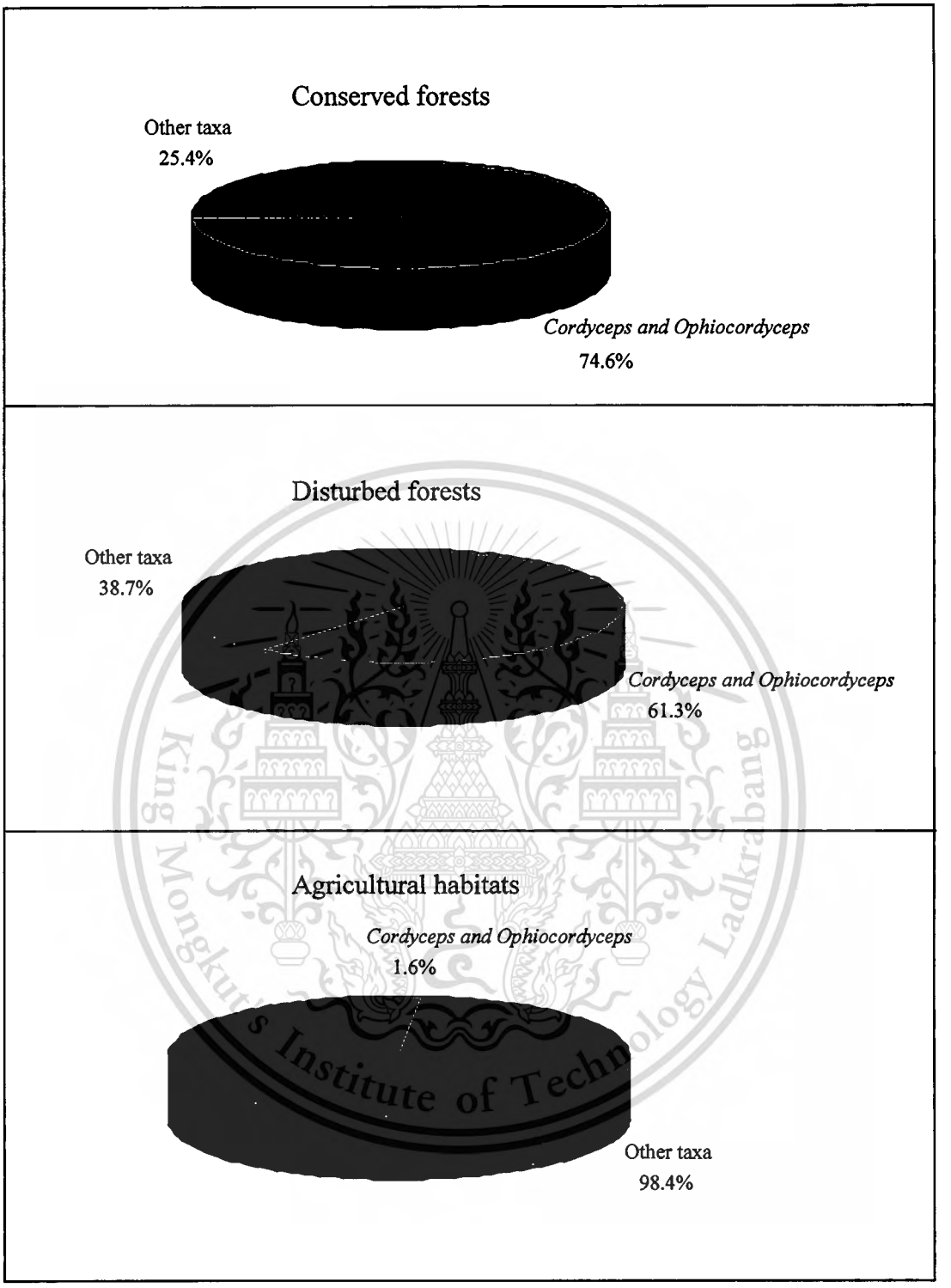


Fig. 4.35 Percentage of fungal records in different habitats.

The similarity index of the fungal taxa between the conserved rainforests and the disturbed rainforests was higher than that between the disturbed rainforests and the agricultural habitats, or the conserved rainforests and the agricultural habitats (Table 4.5).

Table 4.5 Similarity indices of fungal taxa between different collecting sites.

Habitat	Sørensen's index (S')	
	DF	AH
CF	**0.48**	0.23
DF		0.33

4.3 Host based relationships of *Beauveria*, *Cordyceps*, and *Paecilomyces*: A phylogenetic evaluation based on ribosomal and protein coding gene sequences

4.3.1 ITS Phylogeny

The ITS dataset consisted of 74 taxa with 736 characters, of which 146 characters were excluded in the analyses and alignment gaps were treated as missing data. Of the remaining 590 included characters, 108 characters were constant, 66 variable characters parsimony-uninformative, and 416 parsimony-informative characters (PIC). Forty two were newly generated sequences belonging to five anamorphic species [*Beauveria brongniartii* (11), *B. bassiana* (6), *Isaria cicadae* (3), *I. tenuipes* (8), and *I. farinosus* (1)] and 11 teleomorphic species [*C. militaris* (4), *C. militaris* cf. *sphaerocephala* (1), *C.* sp. (1), *O. crinalis* (1), *O. dipterigena* (1), *O. filiformis* (1), *O. myrmecophila* (2), *O. nutans* (2), *O. oxycephala* (1), *O. pseudolloydii* (1) and *O. sphaecocephala* (1)] (Table 4.6). The data were compared with 28 sequences of other ascomycetes from GenBank. *Hypocrea lutea* was chosen as the outgroup taxa for this analyses based on published reports (Glenn *et al.* 1996; Spatafora and Blackwell. 1993).

Based on MrModeltest 2.2, model GTR+G was chosen to be most appropriate for ML analyses and Bayesian analyses. ML resulted in a tree that was topologically similar to that from MP analyses. Transition weighted three times over transversion, and other parameters were as follows: shape parameter of 1.1036 and - ln likelihood = 6043.5732. Estimated base frequencies were as follows: A = 0.2933, C = 0.2247, G = 0.2249 and T = 0.2570. The trees generated from the ML and MP analyses were very similar in topology. The single tree generated from weighted parsimony (TL = 1547, CI = 0.594, RI = 0.865, RC = 0.514, HI = 0.406) is shown in Fig. 4.36.

Generally, four main Clades (A, B, C and D) were recovered (Fig. 4.36). Clade A, *Beauveria* clade, comprises *C. bassiana*, *C. brongniartii*, *C. militaris*, *B. bassiana*, and *B. brongniartii*. Clade B

can be referred as the *Isaria* clade. It consists of *I. cicadae*, *I. japonica*, *I. farinosus*, *I. fumosoroseus*, *I. tenuipes* and *C. bifusispora*. Clade C includes *O. irangiensis*, *O. sphaecocephala*. *C. polyarthra*, *O. oxycephala*, *O. myrmecophila*, *O. pseudolloydii*, *C. ampullacea*, *O. formicarum*, *O. nutans*, *O. crinalis*, *O. filiformis* and *O. dipterigena*, and *Hymenostilbe aurantiaca*. Clade D includes five entomogenous species namely *C. hawkesii*, *C. gunnii*, *C. ramosopulvinata*, *C. militaris* var. *sphaerocephala*, *Cordycep* sp. and two mycogenous species *E. ophioglossoides*, *E. valliformis*. To discuss tree outputs, clade A and B are strongly supported by 92% bootstrap (BS), 96% posterior probabilities (PP) and 99% BS, 96% (PP), respectively. Clade B received moderate support; 85% BS, 72% (PP) and Clade D is supported by low BS value 60% with high PP value 94% (Fig. 4.36).

4.3.2 β-tubulin Phylogeny

The β-tubulin dataset comprised 52 taxa with 383 characters and alignment gaps were treated as missing data. There were 153 PIC in this dataset. Forty two were newly generated sequences belonging to five anamorphic species [*Beauveria brongniartii* (11), *B. bassiana* (6), *I cicadae* (2), *I. farinosus* (1), and *I. tenuipes* (9)] and nine teleomorphic species [*C. militaris* (4), *C. militaris* cf. *sphaerocephala* (1), *C.* sp. (1), *O. crinalis* (1), *O. filiformis* (1), *O. myrmecophila* (1), *O. nutans* (2), *O. oxycephala* (1), and *O. sphaecocephala* (1)] (Table 4.6). The data were compared with 10 sequences of other ascomycetes from GenBank. *Hypocrea lixii* was chosen as the outgroup.

ML and Bayesian analyses were performed with MrModeltest 2.2 using HKY85+G+I model. Transition was weighted three times over transversion, and other parameters were as follows: shape parameter of 0.9556 and - ln likelihood = 3138.1929. Estimated base frequencies were as follows: A = 0.2057, C = 0.2962, G = 0.2170 and T = 0.2811. Generally, phylograms resulted were divided into three clades (A, B, and C) (Fig. 4.37). Among them, only clades A and B are supported by 62% BS and 77% BS. Clade A can also be further subdivided into two subclades: *Isaria* and *B. bassiana*. The latter clade (*B.* bassiana) received 78% BS, whereas the first Calde did not receive reliable branch support. Clade B comprised only *B. brongniartii* and *C. militaris* species with a BS value of 77%. Clade C comprised *Ophiocordyceps* and *Cordyceps* species with no supportive BS value. Phylogenetic placement of the some taxa including *O. sphecocephala*, *I. farinosus*, *O. oxycephala* and *C.* sp. were ambiguous as they do not cluster with other taxa. There are only 16 internal subclades which received >50% BS in this analysis. The single tree generated from weighted parsimony (TL = 654, CI = 0.595, RI = 0.756, RC = 0.449, HI = 0.405) is shown in Fig. 4.37.

4.3.3 Combined ITS and β-tubulin Phylogeny

The combined dataset with 52 taxa, has 1083 characters. Ambiguous regions (178) were excluded in all analyses, and there were 511 PIC in the included regions. MP, ML and Bayesian analysis were also performed. The trees generated from all analyses were not significantly different. ML tree was found as the best (Fig. 4.38). ML and Bayesian analyses were performed with MrModeltest 2.2 using SYM+G model. Transition was weighted three times over transversion, and other parameters were as follows: shape parameter of 0.7711 and - ln likelihood = 10099.3105. Estimated base frequencies were resulted as same (0.25). The Fig. 4.38 shows the tree generated from weighted parsimony (TL = 2043, CI = 0.575, RI = 0.752, RC = 0.432, HI = 0.425) is shown in Fig. 4.38.

Although there were different reference taxon sampling between datasets, phylogenies obtained were almost similar in species groupings (Figs 4.36-4.38). Phylograms from different optimality criteria can be generally divided into four clades. The tree resulting from the combined dataset, however, is much more resolved. There are 26 subclades which supported by > 50% BS while 16 subclades are supported by >50% BS in β-tubulin phylogeny. The inclusion of the more genes gave the better resolved for the trees than β-tubulin.

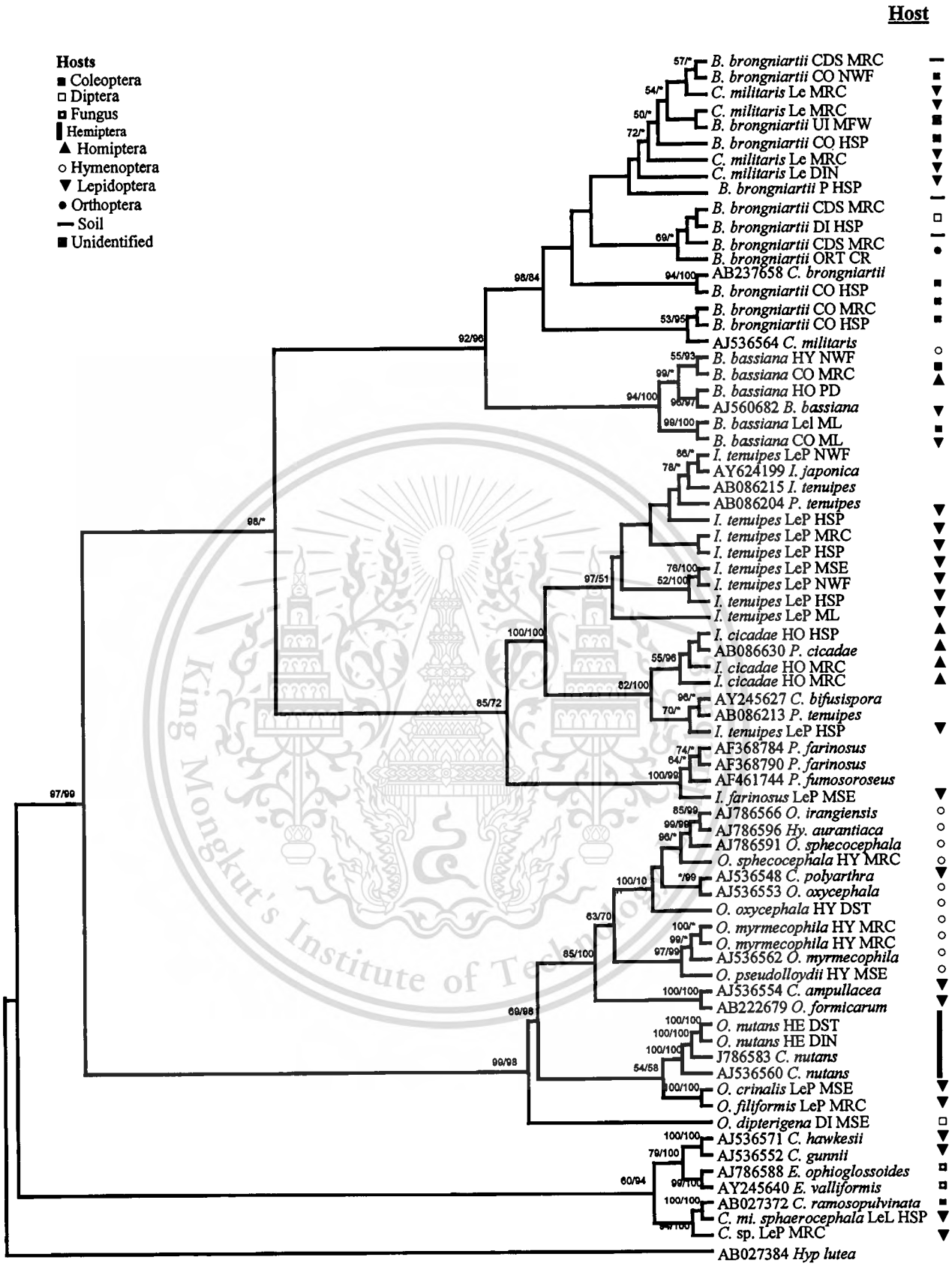


Fig. 4.36 Strict consensus of tree generated from maximum likelihood analysis of the ITS and 5.8S dataset of 70 taxa. Values before the backslash are parsimony BS (above 50%) while after are Bayesian posterior probabilities (above 50%). *B.*, *Beauveria*; *E.*, *Elaphocordyceps*; *C.*, *Cordyceps*; *Hy.*, *Hymenostilbe*; *I.*, *Isaria*; *O.*, *Ophiocordyceps*; *P.*, *Paecilomyces;* CO., ■ Coleoptera; DI., □ Diptera; ◘ Fungus; HE., ▌ Hemiptera.; HO., ▲ Homoptera; HY., ○ Hymenoptera; LE., ▼ Lepidoptera; ORT., ● Orthoptera; S., ▬ Soil; UI., ■ Unidentified insect. The designated outgroup was *Hypocrea lutea*.

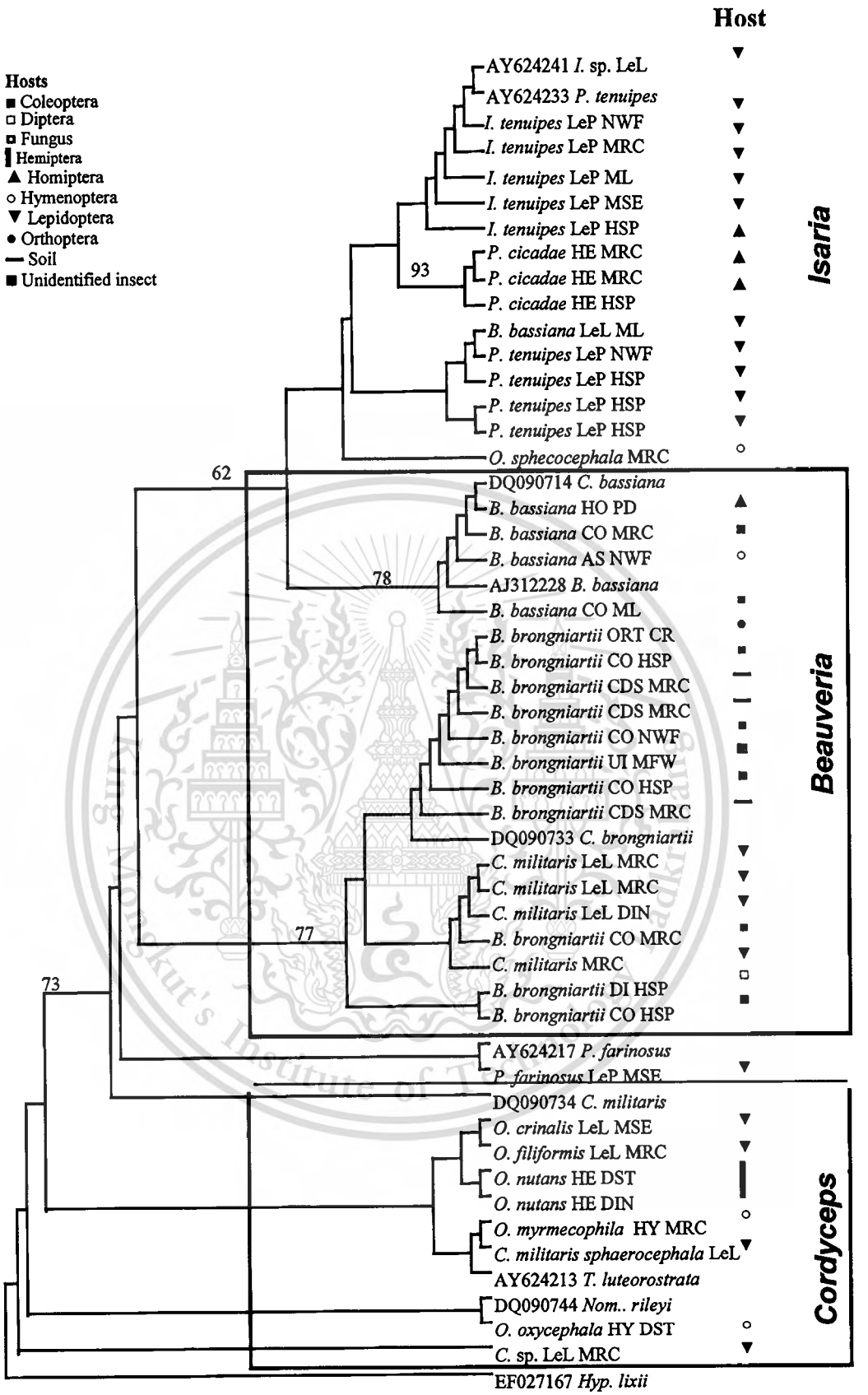


Fig. 4.37 Strict consensus of tree generated from maximum likelihood analysis of the β-tubulin dataset of 52 taxa. Numbers above the nodes represent the proportion of 1000 bootstrap replications. *B.*, *Beauveria*; *E.*, *Elaphocordyceps*; *C.*, *Cordyceps*; *Hy.*, *Hymenostilbe*; *I.*, *Isaria*; *O.*, *Ophiocordyceps*; *P.*, *Paecilomyces*; *T.*, *Torrubiella*; *Nom.*, *Nomuraea*; *Mar.*, *Mariannaea*; CO., ■ Coleoptera; DI., □ Diptera; ◘ Fungus; HE., ▌ Hemiptera.; HO., ▲ Homoptera; HY., ○ Hymenoptera; LE., ▼ Lepidoptera; ORT., ● Orthoptera; S., ▬ Soil; UI., ■ Unidentified insect. The designated outgroup was *Hypocrea lixii*.

Fig. 4.38 Strict consensus of tree generated from maximum likelihood analysis of the ITS/5.8S and β-tubulin combined dataset of 52 taxa. Values before the backslash are parsimony BS (above 50%) while after are Bayesian posterior probabilities (above 50%). *B.*, *Beauveria*; *E.*, *Elaphocordyceps*; *C.*, *Cordyceps*; *Hy.*, *Hymenostilbe*; *I.*, *Isaria*; *O.*, *Ophiocordyceps*; *P.*, *Paecilomyces; T.*, *Torrubiella*; *Nom.*, *Nomuraea*; *Mar.*, *Mariannaea* CO., ■ Coleoptera; DI., □ Diptera; ◘ Fungus; HE., ▌ Hemiptera.; HO., ▲ Homoptera; HY., ○ Hymenoptera; LE., ▼ Lepidoptera; ORT., ● Orthoptera; S., ▬ Soil; UI., ▩ Unidentified insect. The designated outgroup was *Mariannaea camptospora*. * Clades that received less thank 50% support.

Table 4.6 DNA sequences used in this study.

Taxon	Host	Geographic origin	Source of sequence	Voucher Information[a)]	GenBank accession no.	
					ITS	Bt
Beauveria bassiana	Hymenoptera	New Waterfall	This study	IFRD2019	EU573326	EU604124
B. bassiana	Coleoptera	Mushroom Research Centre	This study	IFRD2020	EU573327	EU604123
B. bassiana	Homoptera	Pha Daeng Village	This study	MFLU1002	EU573328	EU604122
B. bassiana	Lepidoptera	Mae Lod	This study	MFLU1028	EU573329	EU604134
B. bassiana	Coleoptera	Mae Lod	This study	IFRD201	EU573330	EU604125
B. bassiana	Storage pests	Kenya	GenBank;de Muro. *et al.*, unpubl		AJ560682	
B. brongniartii	Diptera	Geyser Pong Dueb Hot Spring	This study	MFLU1000	EU573310	EU604120
B. brongniartii	Lepidoptera (Larva)	Mushroom Research Centre	This study	IFRD2023	EU573312	EU604107
B. brongniartii	Orthoptera	Geyser Pong Dueb Hot Spring	This study	IFRD2024	EU573313	EU604113
B. brongniartii	Lepidoptera	Mushroom Research Centre	This study	MFLU956	EU573314	EU604111
B. brongniartii	Coleoptera	Geyser Pong Dueb Hot Spring	This study	IFRD2027	EU573325	EU604114
B. brongniartii	Coleoptera	Mushroom Research Centre	This study	IFRD2025	EU573323	EU604119
B. brongniartii	Coleoptera	Geyser Pong Dueb Hot Spring	This study	IFRD2026	EU573324	EU604121
B. brongniartii	Coleoptera	New Waterfall	This study	IFRD2021	EU573317	EU604110
B. brongniartii	Unidentified Insect	Mokfa Waterfall	This study	MFLU1030	EU573316	EU604109

Table 4.6 (continued).

Taxon	Host	Geographic origin	Source of sequence	Voucher Information[a)]	GenBank accession no.	
					ITS	Bt
B. brongniartii	Lepidoptera	Mushroom Research Centre	This study	IFRD2028	EU573318	EU604112
B. brongniartii	Lepidoptera (Pupa)	Geyser Pong Dueb Hot Spring	This study	MFLU1010	EU573322	EU604128
B.brongniartii	Coleoptera	Geyser Pong Dueb Hot Spring	This study	IFRD2022	EU573311	EU604108
Cordyceps ampullacea			GenBank; Chen *et al.*, (2004), unpubl		AJ536554	
C. bifusispora			GenBank; Kuo (2003), unpubl		AY245627	
C. brongniartii			GenBank; Watanabe *et al.* (2006)		AB237658	
C. cf takaomontana	Lepidoptera (Larva)	Thailand	GenBank; Luangsa-ard *et al.* (2005)		AY624198	AY624240
C. gunnii			GenBank; Chen *et al.*, (2003), unpubl		AJ536552	
C. hawkesii			GenBank; Chen *et al.*, (2003), unpubl		AJ536571	
Cordyceps. militaris	Lepidoptera (Larva)	Mushroom Research Centre	This study	MFLU956	EU573315	EU604116
C. militaris	Lepidoptera (Larva)	Mushroom Research Centre	This study	MFLU969	EU573320	EU604117

Table 4.6 (continued).

Taxon	Host	Geographic origin	Source of sequence	Voucher Information[a)]	GenBank accession no.	
					ITS	Bt
C. militaris	Lepidoptera (Larva)	Mushroom Research Centre	This study	MFLU954	EU573319	EU604118
C. militaris	Lepidoptera (Larva)	Doi Inthanon National Park	This study	MFLU873	EU573321	EU604115
C. militaris			GenBank; Chen *et al.*, (2003), unpubl		AJ536564	
C. militaris var. sphaerocephala	Lepidoptera (Larva)	Geyser Pong Dueb Hot Spring	This study		EU573344	EU604141
C. polyarthra			GenBank; Chen *et al.*, (2003), unpubl		AJ536548	
C. ramosopulvinata	Homoptera (Cicada)	Tsuruoka, Yamagata	GenBank; Nikoh and Fukatsu (2000)		AB027372	
C. sp.	Lepidoptera (Larva)	Mushroom Research Centre	This study	MFLU963	EU573345	EU604142
Elaphocordyceps. ophioglossoides			GenBank; Stensrud *et al.*(2005)		AJ786588	
E. valliformis			GenBank; Kuo (2003), unpubl		AY245640	
Hymenostilbe aurantiaca			GenBank; Stensrud *et al.* (2005)		AJ786596	

Table 4.6 (continued).

Taxon	Host	Geographic origin	Source of sequence	Voucher Information[a)]	GenBank accession no.	
					ITS	Bt
Hypocrea lutea		IFO9061	GenBank; Nikoh and Fukatsu (2000)		AB027384	
Isaria japonica	Lepidoptera (Larva)	Japan	GenBank; Luangsa-ard *et al.* (2005)		AY624199	AY624241
I. tenuipes			GenBank; Yokoyama *et al.* (2003)		AB086215	
Mariannaea camptospora	Forest soil	The Netherlands	GenBank; Luangsa-ard *et al.* (2005)		AY624202	AY624244
Metarhizium cylindrosporae	Homoptera (cicada)	Thailand	GenBank; Luangsa-ard *et al.* (2005)		AY624204	AY624247
Nomuraea rileyi	*Trichoplusia ni*	USA	GenBank; Luangsa-ard *et al.* (2005)		AY624205	AY624250
Ophiocordyceps. crinalis	Lepidoptera (Larva)	Mae Sae	This study	MFLU887	EU573352	EU604143
O. dipterigena	Diptera (Fly)	Mae Sae	This study	MFLU979	EU573346	
O. filiformis	Lepidoptera (Larva)	Mushroom Research Centre	This study	MFLU896	EU573353	EU604144

Table 4.6 (continued).

Taxon	Host	Geographic origin	Source of sequence	Voucher Information[a)]	GenBank accession no.	
					ITS	Bt
O. formicarum			GenBank; Yokoyama and Hara (2005), unpubl		AB222679	
O. irangiensis		Thailand/BIOTEC	GenBank; Stensrud *et al.*(2005)		AJ786566	
O. myrmecophila	Hymenoptera (Ant)	Mushroom Research Centre	This study	MFLU898	EU573349	
O. myrmecophila	Hymenoptera (Ant)	Mushroom Research Centre	This study	MFLU952	EU573350	EU604140
O. myrmecophila			GenBank; Chen *et al.*, (2003), unpubl		AJ536562	
O. nutans	Hemiptera	Doi Inthanon National Park	This study	MFLU923	EU573308	EU604105
O. nutans	Hemiptera	Doi Suthep-Pui National Park	This study	MFLU895	EU573309	EU604106
O. nutans			GenBank; Stensrud *et al.*(2005)		AJ786583	
O. nutans			GenBank; Chen *et al.*, (2003), unpubl		AJ536560	
O. oxycephala	Hymenoptera (Wasp)	Doi Suthep-Pui National Park	This study	MFLU990	EU573348	EU604145

Table 4.6 (continued).

Taxon	Host	Geographic origin	Source of sequence	Voucher Information[a)]	GenBank accession no.	
					ITS	Bt
O. oxycephala			GenBank; Chen *et al.*, (2003), unpubl		AJ536553	
O. pseudolloydii	Hymenoptera (Ant)	Mae Sae	This study	MFLU981	EU573351	
O. sphecocephala	Hymenoptera (Wasp)	Mushroom Research Centre	This study	MFLU971	EU573347	EU604146
O. sphecocephala			GenBank; Stensrud *et al.*(2005)		AJ786591	
Paecilomyces amoeneroseus	Coleopteran pupa	Ghana	GenBank; Luangsa-ard *et al.* (2005)		AY624168	AY624207
P. cicadae	Homoptera	Mushroom Research Centre	This study	IFRD2029	EU573331	EU604136
P. cicadae	Homoptera	Mushroom Research Centre	This study	IFRD2030	EU573333	EU604137
P. cicadae			GenBank; Yokoyama *et al.* (2004)		AB086630	
P. farinosus	Lepidoptera (Pupa)	Mae Sae	This study		EU573343	EU604126
P. farinosus			GenBank; Huang *et al.* (2001), unpubl		AF368784	

Table 4.6 (continued).

Taxon	Host	Geographic origin	Source of sequence	Voucher Information[a)]	GenBank accession no.	
					ITS	Bt
P. farinosus	Garden soil	UK	GenBank; Luangsa-ard *et al.* (2005)		AY624179	AY624217
P. farinosus			GenBank; Huang *et al.* (2001), unpubl		AF368790	
P. fumosoroseus			GenBank; Fargues *et al.* (2001), unpubl		AF461744	
P. fumosoroseus		France	GenBank; Luangsa-ard *et al.* (2005)		AY624184	AY624222
P. tenuipes	Hemiptera	Geyser Pong Dueb Hot Spring	This study	MFLU1032	EU573332	EU604138
P. tenuipes	Lepidoptera (Pupa)	Geyser Pong Dueb Hot Spring	This study	IFRD2031	EU573334	EU604139
P. tenuipes	Lepidoptera (Pupa)	Geyser Pong Dueb Hot Spring	This study	IFRD2032	EU573335	EU604131
P. tenuipes	Lepidoptera (Pupa)	New Waterfall,	This study	IFRD2033	EU573336	EU604135
P. tenuipes	Lepidoptera (Pupa)	Geyser Pong Dueb Hot Spring	This study	MFLU1011	EU573337	EU604127
P. tenuipes	Lepidoptera (Pupa)	Geyser Pong Dueb Hot Spring	This study	IFRD2034	EU573339	EU604133
P. tenuipes	Lepidoptera (Pupa)	New Waterfall	This study	IFRD2035	EU573338	EU604132
P. tenuipes	Lepidoptera (Pupa)	Mae Lod	This study	MFLU1009	EU573342	EU604129

Table 4.6 (continued).

Taxon	Host	Geographic origin	Source of sequence	Voucher Information[a)]	GenBank accession no.	
					ITS	Bt
P. tenuipes	Lepidoptera (Pupa)	Mushroom Research Centre	This study	MFLU1023	EU573340	EU604130
P. tenuipes	Lepidoptera (Pupa)	Geyser Pong Dueb Hot Spring	This study	IFRD2036	EU573341	
P. tenuipes			GenBank; Yokoyama *et al.* (2003)		AB086213	
P. tenuipes			GenBank; Yokoyama *et al.* (2003)		AB086204	
P. tenuipes	Lepidopteran pupa		GenBank; Luangsa-ard *et al.* (2005)		AY624196	AY624234
P. tenuipes			GenBank; Luangsa-ard *et al.* (2004), unpubl			AY624233
Torrubiella luteorostrata	Homoptera	Thailand	GenBank; Luangsa-ard *et al.* (2005)		AY624206	AY624237

[a)]MFLU, Mae Fah Luang University Herbarium (MFLUH), Chiang Rai, Thailand; IFRD, International Fungal Research and Development Centre (IFRD), The Research Institute of Resource Insects, Chinese Academy of Forestry, Kunming, PR China.

4.4 *Beauveria* entomopathogens from Thailand: Systematics based on morphology and DNA molecules.

4.4.1 Morphological characters

Morphological characters of the isolates of *Beauveria* are presented in Table 4.7. The results of comparison between *B. bassiana* and *B. brongniartii* indicate that isolates with conidia longer than 3.1 μm should be classified as *B. brongniartii*; isolates with shorter, spherical conidia are *B. bassiana*. An important comparative character was the length/width ratio of the conidia of *B. brongniartii* and *B. bassiana* and this ranged between 1.3-2.4 and 0.7-1.5, respectively. The length/width ratio of the *B. brongniarti* isolate from Diptera (1.3) is less than the *B. bassiana* isolates. The statistical significance was found among insect host, size of conidia and conidiogenous structure (Table 4.8). Width of conidia ($P = 0.000$) and conidiogenous structure ($P = 0.013$) give high significant values than length of these structures. The length of rachis is not an informative character. The multiple range tests results also indicated that there was an association between *B. bassiana* isolates and their insect hosts (Table 4.9). Based on the homogeneous subsets analysis, conidial size of the isolate from dipteran fly was found to be significantly different from the other isolates of different hosts in both Student-Newman-Keuls and Tukey HSD multiple range tests (Table 4.9). Conidial size, isolated from the soil was also significantly different from other isolates while isolates from Lepidoptera, Coleoptera, Orthoptera and unidentified host are not significantly different from each other. Hymenoptera and Homoptera hosts are found to be in another group.

Table 4.8 ANOVA table of the isolates of *Beauveria* species from different isolates.

Character	Sum of square	df	F	Sig.
Conidia (L)	3.240	1	4.989	.026
Conidia (W)	7.846	1	34.180	.000
Conidiogenous structure (L)	115.993	1	4.605	.033
Conidiogenous structure (W)	2.282	1	6.243	.013
Rachis (L)	3.090	1	0.350	.555

L = Length

W = Width

Table 4.9 Homogeneous subsets analysis of *Beauveria* isolates from different hosts.

Character	Tests	Hosts							
		DI	LE	S	CO	UI	ORT	HY	HO
Conidia (L)	Student-Newman-Keuls	5.01^{a}	3.38^{c}	4.06^{b}	2.9^{c}	3.28^{c}	3.26^{c}	2.26^{d}	2.25^{d}
	Tukey HSD	5.01^{a}	3.38^{c}	4.06^{b}	2.89^{cd}	3.28^{c}	3.26^{c}	2.26^{d}	2.25^{d}
Conidia (W)	Student-Newman-Keuls	2.58^{a}	2.13^{b}	2.14^{b}	1.77^{cd}	2.02^{b}	$2.01b^{c}$	1.56^{d}	1.62^{d}
	Tukey HSD	2.58^{a}	2.13^{b}	2.14^{b}	1.77^{bcd}	2.02^{b}	2.01^{bc}	1.56^{d}	1.62^{d}

4.4.2 **Molecular phylogeny**

The ITS dataset consisted of 38 taxa with 695 characters, of which 109 characters were excluded in the analyses and alignment gaps were treated as missing data. Sixteen newly generated sequences belonging to two species [*Beauveria brongniartii* (11) and *B. bassiana* (5)] were included in this study. The data were compared with 22 sequences of other *Beauveria* squences from GenBank. *Cordyceps ramosopulvinata*.was chosen as the outgroup taxon (Spatafora and Blackwell, 1993; Glenn *et al.*, 1996).

Based on MrModeltest 2.2, model HKY85+G was chosen to be most appropriate for ML analyses. ML analysis resulted in a tree that was topologically similar to that from MP analyses. Transition weighted 2.06 times over transversion, and other parameters were as follows: shape parameter of 0.4179 and - ln likelihood = 1684.9227. Estimated base frequencies were as follows: A = 0.24060, C = 0.30160, G = 0.26820 and T = 0.18960. The trees generated from the ML and MP analyses were very similar in topology. The single tree generated from weighted parsimony (TL = 170, CI = 0.871, RI = 0.927, RC = 0.807, HI = 0.129) is shown in Fig. 4.39.

ITS sequence analysis recognized three well defined clades designated here as *B. brongniartii* clade, *B. bassiana* clade and heterogenous clade (*other Beauveria* species + *B. bassiana* overseas clade). A sister-group relationship was found between *B. brongniartii* clade and heterogenous clade. *Beauveria brongniartii* clade comprises two major groups: a group containing only Chiang Mai *B. brongniartii* (A) and a group of *B. brongniatii* from both Chiang Mai and overseas (B). *Beauveria bassiana* clade includes only one group of *B. bassiana* from overseas and Chiang Mai isolates (E). The heterogenous group contains other *Beauveria* species

(*B. caledonica*, *B. amorpha* and *B. vermiconia)* are included in the clade C and a group of only *B. bassiana* from overseas are found in the clade D. (Fig. 4.39).

Table 4.7 Morphological characters of the *Beauveria* strains from Chiang Mai Province, Thailand.

Species (Strains)	Voucher Information[a)]	Host	Geographic origin	Hyphae width	Conidiogenous cell		Conida		Conidia length/ width ratio	Rachis
					Length	Width	Length	Width		
Beauveria bassiana	IFRD2019	Hymenoptera	New Waterfall	1.2-2.8	2.5-5	1.2-3	1.5-3	1.3-2.7	1.45	5-12.8
B. bassiana	IFRD2020	Coleoptera	Mushroom Research Centre	1.1-2.3	2.6-5.2	1.2-2.5	1.3-2.7	1.8-3	0.71.	2.9-11.1
B. bassiana	MFLU1002	Homoptera	Pha Daeng Village	1.4-2.4	2-4	1-2.8	1.2-2.9	1.3-2.7	1.39	4.8-12
B. bassiana	MFLU1028	Lepidoptera	Mae Lod	1.5-2.7	2.3-4.8	1.3-2.7	1.7-2.8	1.5-2.5	1.19	5.6-15
B. bassiana	IFRD201	Coleoptera	Mae Lod	1.3-2.8	2.6-4.5	1.8-3.1	1.8-3.1	1.4-3	1.42	7.2-16
B. brongniartii	MFLU1000	Diptera	Geyser Pong Dueb Hot Spring	1.9-3.4	2.8-9.3	1.8-3.5	2.2-4.3	1.8-3.2	1.26	2-6.2
B. brongniartii	IFRD2023	Lepidoptera	Mushroom Research Centre	2.1-2.9	2-8.4	1.7-2.9	4.2-5.6	1.8-3.1	2.08	3.1-9.5
B. brongniartii	IFRD2024	Orthoptera	Geyser Pong Dueb Hot Spring	1.3-3.3	4.2-5.8	1.4-3	2.2-4.3	1.4-2.6	1.62	5.7-15.4
B. brongniartii	MFLU956	Lepidoptera	Mushroom Research Centre	1.2-3.2	7.5-11.8	1.8-2.6	2.6-4.5	1.4-2.6	1.69	7.5-13.1
B. brongniartii	IFRD2027	Coleoptera	Geyser Pong Dueb Hot Spring	1.3-2.3	1.9-3.8	1.3-2.1	2.9-4.3	1.1-2.1	2.33	5.6-7.8

Table 4.7(continued).

Species (Strains)	Voucher Information[a)]	Host	Geographic origin	Hyphae width	Conidiogenous cell		Conida		Conidia length/ width ratio	Rachis
					Length	Width	Length	Width		
B. brongniartii	IFRD2025	Coleoptera	Mushroom Research Centre	1-2.7	1.9-13.8	1-2.8	1.9-3.7	0.8-1.8	1.95	2.5-7.8
B. brongniartii	IFRD2026	Coleoptera	Geyser Pong Dueb Hot Spring	1.1-3.4	6.5-12.5	1.8-3.4	2.8-5	1.3-2.6	2.01	2.7-13.3
B. brongniartii	IFRD2021	Coleoptera	New Waterfall	1.1-3.8	1.9-2.3	2.7-3.5	1.8-4.2	0.8-2	2.38	6.6-10.7
B. brongniartii	MFLU1030	Unidentified Insect	Mokfa Waterfall	1.9-3.4	2.8-9.3	1.9-3.5	2.2-4.3	1.8-3.2	1.62	2.6-11.1
B. brongniartii	IFRD2028	Lepidoptera	Mushroom Research Centre	1.4-3.2	4.1-16	1.3-2.4	2.4-6	1.4-2.9	1.59	4.2-11.9
B. brongniartii	MFLU1010	Lepidoptera	Geyser Pong Dueb Hot Spring	1.4-3.1	8.6-20.6	2.1-3.7	3.4-5	1.4-3	2.30	3.2-14.2
B.brongniartii	IFRD2022	Coleoptera	Geyser Pong Dueb Hot Spring	1.2-2.8	6.9-52.9	0.8-1.8	1.6-3.5	1.4-2.6	1.44	7.6-12.5

[a)]MFLU, Mae Fah Luang University Herbarium (MFLUH), Chiang Rai, Thailand; IFRD, International Fungal Research and Development Centre (IFRD), The Research Institute of Resource Insects, Chinese Academy of Forestry, Kunming, PR China.

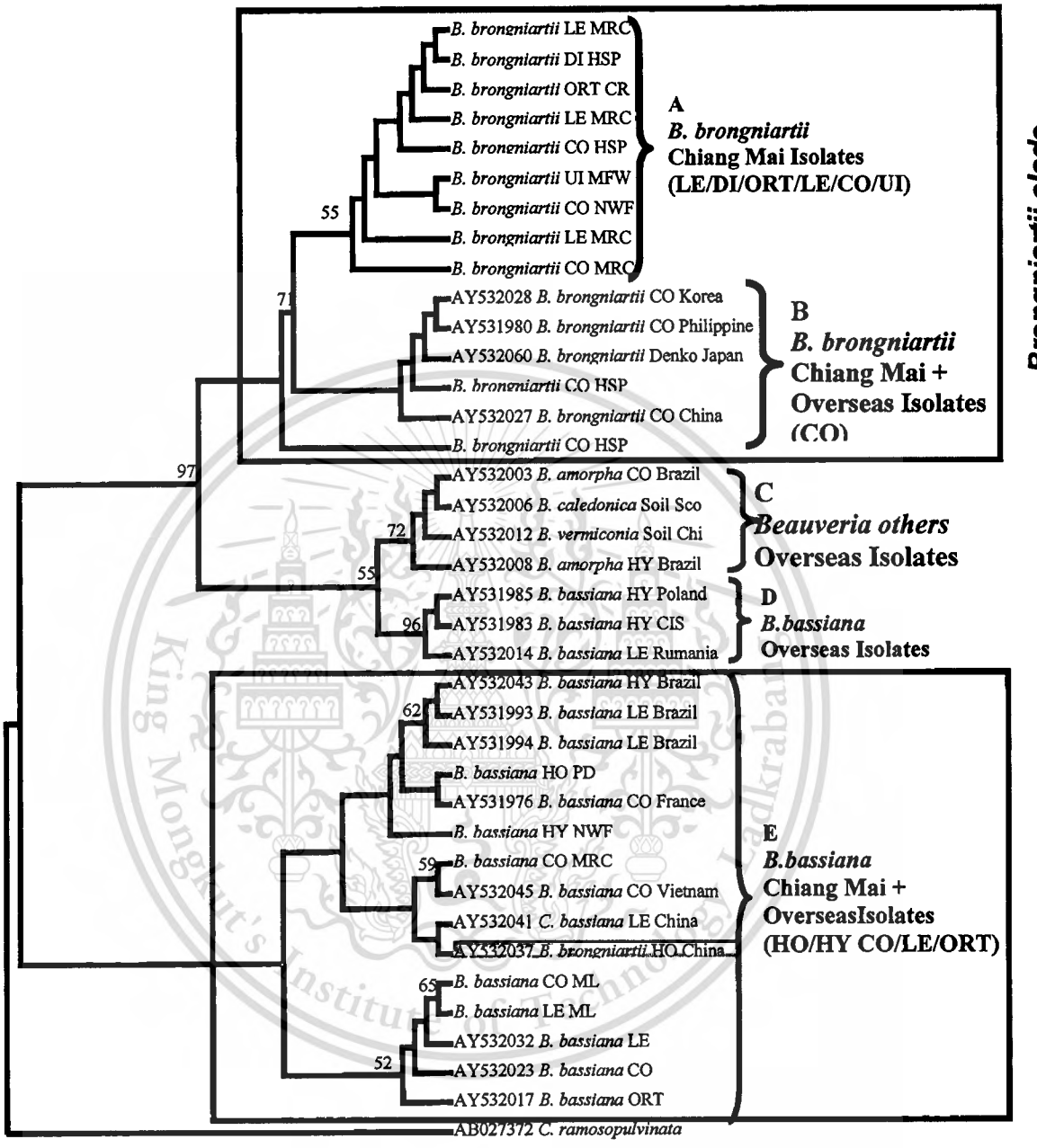


Fig. 4.39 Strict consensus of tree generated from maximum likelihood analysis of the ITS and 5.8S dataset. Numbers above the nodes represent the proportion of 1000 bootstrap replications. *B.*, *Beauveria*; *C.*, *Cordyceps*; LE., Lepidoptera; DI., Diptera; LE., Lepidoptera; S., Soil; CO., Coleoptera; UI., Unidentified insect; ORT., Orthoptera; HY., Hymenoptera; HO., Homoptera. The designated outgroup was *Cordyceps ramosopulvinata*.

Chapter 5

DISCUSSION

5.1 Diversity of entomopathogenic fungi in rainforests of Chiang Mai Province, Thailand

Entomopathogenic fungi are mainly found amongst the Zygomycetes (*Entomophthorales*) and Ascomycetes (*Clavicipitales*, *Hypocreales* and hyphomycetous anamorphs) (Evans. 1988). The *Entomophthorales* are commonly reported as pathogens of forest pests in temperate forest habitats (Burges. 1981), but are rare in tropical forests (Evans. 1982).

5.1.1 Occurrence frequency of entomopathogenic fungi

The occurrence frequencies of entomopathogenic fungi on arthropods in study areas were dominated by *Ophiocordyceps myrmecophila* (22.6%) and *O. unilateralis* (13.8%) on ants. This is not surprising as ants are the dominant arthropods in lowland tropical rainforests (Elton. 1973) and, therefore, are the most affected quantitatively by entomopathogenic fungi (Samson *et al.* 1988). Ants are infected at the adult stage, and the hard exoskeleton is not colonized by the fungus, thus sufficient salient taxonomic features are present to enable host identification at the generic or species level.

High occurrence frequencies can also be found in *Isaria fumosoroseus* (13%), *Paecilomyces marquandii* (7.4%), and *I. tenuipes* (5.3%). *Paecilomyces* incorporates many species (Liang *et al.* 2005), but only three species were found to be abundant in this study. Although *I. fumosoroseus*, a geographically widespread species, is reported as a pathogen of many insects (Lepidoptera, Coleoptera, Diptera and Homoptera) (Obornik *et al.* 2001), the fungus, however, was found only on Hemiptera in this study. *Paecilomyces marquandii* was the most frequently encountered pathogen on Hemiptera while *I. tenuipes* was recorded only from Lepidoptera pupa in this study. This finding is in agreement with Tzean *et.al.* (1997) that *Paecilomyces* species were recorded for different infected hosts of Lepidoptera, Coleoptera, Homoptera, Hymenoptera, Diptera Hemiptera, Orthoptera, or even Arachnida, the Lepidoptera however appears to occur on preferred hosts. Fukatsu *et al.* (1997) have also reported that *P. tenuipes* (sometimes referred to as *Isaria japonica* or other synonyms) parasitizes various Lepidoptera in larva and pupal stages.

5.1.2 Fungal diversity and similarities between hosts

Most entomopathogenic fungi have relatively broad host ranges, but apparently reoccur on some hosts (Tzean *et al.* 1997); they are well represented on plant sucking homopterans in tropical rainforests (Petch. 1925) and coccids and whiteflies with ascomycete infections are also prominent in tropical rainforests (Mains. 1958; Evans. 1982; Samson *et al.* 1988). Based on our findings, the species diversity on Homoptera was highest, followed by Lepidoptera and Hymenoptera, a result that is consistent with a previous report of invertebrate pathogenic fungi in Thailand. Jones (2004) pointed out that the most common host for invertebrate pathogenic fungi in Thailand was Homoptera, followed by Lepidoptera, *Araneae*, Coleoptera and Hymenoptera. The species diversity value of Lepidoptera in our study, however, was lower than for Homoptera but its species richness was highest among the hosts.

5.1.3 Fungal diversity and similarities between collecting sites

Observations of entomopathogenic fungi in tropical rainforests in both Africa and South America reported that richness of entomopathogenic fungi decreases as rainforests are exploited; whether this is due to the disappearance of the specific hosts or the loss of optimum conditions for infection, or a combination of both, is unknown (Samson *et al.* 1988). In our study the highest diversity of entomopathogenic fungi was found in disturbed forests while the conserved forests and agricultural habitats had low diversity values. This finding does not support Samson *et al* (1988). This is perhaps due to the fact that the disturbed rainforest comprises both forest habitats and agricultural habitats and both types of specialized and generalist entomopathogens are well presented in this environment.

Cordyceps species are usually found in undisturbed habitats where there is clean air, high humidity, and adequate shading by overhanging trees to help retain soil moisture levels (www.mushtech.org). A similar finding was observed in our study. *Cordyceps* and *Ophiocordyceps* species contributed 74.7% of total taxa in conserved rainforests, with 61.3% in disturbed forest and only 1.6% in agricultural habitats.

The similarity indices among different collection sites show that the similarity between conserved forest and disturbed forest was high. The two environments had 11 fungal species in common. Only three taxa in both conserved forests and agricultural habitats while 5 taxa were common to both disturbed forest and agricultural habitats. Three taxa, *B. bassiana*, *O.pseudolloydii* and *I. tenuipes* were found in all collection sites. *Beauveria bassiana* is one of the

most widely recognized and encountered of all entomopathogenic fungi due to its cosmopolitan distribution, easy recognition, and frequent appearance nature (Rehner. 2005). Generally, *Beauveria* and *Metarhizium* species are rarely encountered on insects in tropical rainforests, although *Beauveria* can be found colonizing insect remains in the soil (Evans. 1988). In the present study, *B. brongniartii* was isolated from the soil underneath dead insects. *Metarhizium* was not found in the current study.

5.1.4 Host specificity

Many entomopathogenic fungi are thought to be host specific. *Cordyceps* species most frequently attack Lepidoptera, Hymenoptera, Coleoptera and Orthoptera, and several life cycle stages of a particular host may be infected, but not necessarily by the same species of fungi (Benjamin *et al.* 2004). In our study, Hymenoptera, Lepidoptera, Hemiptera and Arachnida were mostly infected by *Cordyceps* and *Ophiocordyceps* species. Some *Cordyceps* species are obligately parasitic on ants and are important pathogenic fungi in tropical ecosystems (Evans and Samson, 1982). Disease appears to be maintained at a constant or enzootic level partly by the activities of the infected hosts (Evans and Samson. 1982, 1984). Infected ants escape from their normal ant trails and nests, radically modify their behavioral patterns to find selected niches. After infection with *Cordyceps*, ground-dwelling ponerine ants go up to vegetation and die in exposed positions, grasping the sub-stratum with legs and mandibles (Evans. 1988). Only *O. pseudolloydii* infection on dolichoderine ants was found at every collecting sites. The most abundant species, *O. myrmecophila* and *O. unilateralis*, infected formicine ants in the rainforests habitats. *Ophiocordyceps myrmecophila* was found both in conserved and disturbed rainforests while *O. unilateralis* was found only in conserved rainforests. This result is strongly indicative that there is a high degree of specificity within these associations, as a single *Cordyceps* species is typically confined to a single genus or tribe of ants (Samson *et al.* 1988). Host identification in the majority of cases however is rudimentary and thus the specific insect-fungal association has not been determined. The complete life cycles of many of the tropical forest *Cordyceps* species still require elucidation (Samson and Evans. 1973; Evans and Samson. 1982).

Based on my findings, a number of entomopathogenic fungi are found to be associated with different hosts including soils. The data obtained in this study also reveal the general conclusion of diversity and complexity of fungus-host associations, diversity and similarity of fungal taxa among different hosts and habitats. To add our knowledge of entomopathogenic fungi,

mycologists and entomologists must cooperate in broad research relating to studies of natural ecosystems.

5.2 Host based relationships of entomopathogenic fungi (*Beauveria, Cordyceps,* and *Paecilomyces*): A phylogenetic evaluation based on ribosomal and protein coding gene sequences

5.2.1 Molecular Phylogeny

In this study, we only focused on the host based relationship of the entomopathogenic genera: *Beauveria*, *Cordyceps* and *Paecilomyces*, those are frequently collected from the Chiang Mai Province, Thailand. The results of the analyses indicate the phylogenetic relationships of *Beauveria*, *Cordyceps* and *Paecilomyces*.

Phylogenies derived from DNA sequence analyses have been found to be important issues in classification of the anamorphs and their teleomorphs connections (Rehner and Samuels. 1995; Taylor. 1995; Jacobs and Rehner. 1998; Jeewon *et al.* 2002; Liu *et al.* 2002; Shenoy *et al.* 2006; Kodsueb *et al.* 2007). Our present results show that *Beauveria* and *Paecilomyces* under this investigation are closely related with high bootstrap value. These *Beauveria* and *Paecilomyces* are known as anamorphic genera of *Cordyceps*, which characterized morphologically by their conidiogenous cells that produce conidia in dry chains (Samson *et al.* 1988; Sung *et al.* 2007).

5.2.1.1 *Beauveria*

Beauveria, a well known cosmopolitan genus, commonly attacks a wide host range of arthropods especially Coleoptera and Lepidoptera (De Hoog. 1972). *Beauveria brongniartii*, however, commonly attacked Coleoptera and it is used as biocontrol agent for the European cockchafer, *Melolontha melolontha* (Keller *et al.* 1989). *Beauveria brongniartii* isolates included in this study were collected and cultured from various hosts such as Coleoptera, Lepidoptera, Orthoptera, soil and unidentified insects. Our finding is inconsistent with the previous findings (Keller *et al.* 1989; Rehner and Buckley. 2005). Host specificity was not found among the *Beauveria brongniartii* isolates and also can be regarded as wide host range. Phylogenetic analyses confirmed that isolates from the same host were not phylogenetically related. For example, *B. brongniartii* isolated from Coleoptera are phylogenetically different from each other. Each of these isolates clusters with other isolates from the different hosts (Fig. 4.39).

Phylogeny generated from combined dataset shows that *B. brongniartii* isolated from Coleoptera collected from Hot spring is closely related to the strain that isolated from the dipteran fly collected from Hot spring. The phylogeny resulted from ITS dataset, however, revealed that *B. brongniartii* isolated from Coleoptera collected from Hot spring is clustered together with the strain that isolated from the Coleoptera collected from Mushroom Research Centre.

Beauveria bassiana clade, sister to *B. brongniartii* clade, includes isolates cultured from the different hosts such as Coleoptera, Homoptera, Hymenoptera and Lepidoptera. All isolates generated in this study are not phylogenetically closed each other. There are two strains of *B. bassiana* isolated from Coleoptera and they, however, can be found in separate clades within *B. bassiana* clade. This finding supports that *B. bassiana* s.l. cannot be host specific but is an opportunistic invertebrate-pathogen capable of attacking a wide range of insect taxa (Rehner and Buckley. 2005). *Beauveria bassiana* is a cosmopolitan entomopathogenic fungus and is known to be a generalist with no strict host specificity (Li. 1988; Inglis *et al.* 2001; Wraight *et al.* 2003; Pathan *et al.* 2007). *Beauveria bassiana* has an opportunistic life style with both pathogenic and saprophytic habit.

Clade A is also the indicative of an anamorph affiliation to *Beauveria* and *Cordyceps*. The morphological characters of *Cordyceps* in this clade, collected from Chiang Mai Province, were identical to *C. militaris*. This species is a new record for Thailand. Based on the molecular analyses, the *C. militaris* specimens collected from Chiang Mai Province are phylogenetically related to *B. brongniartii* (Figs 4.36-4.38). During this study, soil samples closed to *C. militaris* specimens were collected and isolated on PDA media. *Beauveria brongniartii* were obtained from those samples. Interestingly, all the above isolates clustered together in clade A. This finding is in agreement with Sung *et al.* (2007) that *Beauveria* can be isolated from stromata of many *Cordyceps* taxa such as *C. bassiana*; *C. brongniartii*, *C. staphylinidaecola* and C. *sobolifera* and can be demonstrated that some *Beauveria* species are sexual (Rehner and Buckley. 2005). Three *Cordyceps* species, i.e. *C. brongniartii, C. sobolifera* and *C. bassiana* were reported to form anamorphic *Beauveria* spp. such as *B. brongniartii* (Shimazu *et al.* 1988), *B. basssiana* (Huang *et al.* 2002; Li *et al.* 2001) and *B. sobolifera* (Liu *et al.* 2001a). Zare and Gams (2001) reported that the new anamorph genus *Lecanicillium* has been referred to the imperfect state of *C. militaris*. Stensrud *et al.* (2005) reported that the conserved type *C. militaris* is closely related to *Beauveria* species in their phylogenetic study. The taxonomic relationship between *C. militaris* and *B. brongniarii* in this study, however, is unclear. *Cordyceps militaris* included in this study were

associated with lepidopteran larvae and those are clustered together with *B. brongniartii* isolated from Coleoptera to form a subclade within clade A (92% PP). *Beauveria brongniartii* isolated from soil are not closely related to *C. militaris* within such clade A. Therefore, further investigation is still needed to confirm *B. brongniartii* as an anamorph of *C. militaris*.

5.2.1.2 ***Paecilomyces***

Based on the previous phylogenetic studies, *Paecilomyces* isolates used in this study belong to the genus *Isaria* (Luangsa-Ard *et al.* 2005). *Isaria tenuipes* were collected from lepidopteran pupae and all these isolates were clustered together with: *I. japonica* (AY624198), *P. tenuipes* (AY624196), and *C. cf. takaomontana* (AY624198). Samson (1974) recognized *Paecilomyces* as two sections, i.e. *Paecilomyces* and *Isarioidea*. This study supports a close connection between the two genera. Samson (1974) assigned *Isaria japonica* as a synonym to *Paecilomyces tenuipes*. This arrangement was supported by Fukatsu *et al.* (1997) and Liang (1997). Kobayasi (1941) reported that the anamorph of *C. takaomontana* Yakush. & Kumaz. is *Isaria japonica* Yasuda. Chen and Xu (1989), on the other hand, stated that *P. tenuipes* is the anamorph of *C. polyarthra*. *Cordyceps polyarthra* in this study, however, is not related to *I. tenuipes* but is found to share a close affinity to *O. oxycephala*. Clade B consists *I. cicadae* subclade with high statistical support. The isolates included in this clade were cultured from Homoptera. One isolate of the *I. farinosa* cultured from lepidopteran pupa in Chiang Mai Province cluster together with *P. farinosus* (AY624179). *Isaria farinosa,* synonymized as *P. farinosus* by Samson (1974), has been reported to occur on six insect orders (Lepidoptera, Coleoptera, Hemiptera, Homoptera, Diptera, and Hymenoptera) and also on spiders (*Araneae*) (Sung *et al.* 2007). Based on my findings, host specificity was found among the *Isaria* collected from Chiang Mai Province. *I. tenuipes* only attacked the lepidopteran pupae, *I. cicadae* infected only Homoptera and *I. farinosus* attacked the lepidopteran pupa.

5.2.1.3 ***Cordyceps***

Morphological characterizations and host specificity have been used in identification of *Cordyceps* and related genera. Recent molecular phylogenetic studies, however, showed that host specificity was found to be of limited phylogenetic significance, and several host shifts were suggested to have occurred during the evolution of *Cordyceps* (Stensrud *et al.* 2005). Some groups of fungal are conserved the endoparasite-host interactions to some extent (Stensrud *et al.* 2005). In ITS phylogeny, *Cordyceps* species from clade C have been associated

with Hymenoptera, Hemiptera and Lepidoptera hosts. The hymenopteran parasites include *O. irangiensis, Hymenostilbe aurantiaca, O. sphecocephala. O. oxycephala, O. myrmecophila,* and *O. formicarum. Cordyceps polyarthra, C. ampullacea, O. crinalis, O. filiformis* are found as lepidopteran parasites, *O. dipterigena* attacked the Diptera and *O. nutans* attacked Hemiptera. However, this heterogenous clade is well supported (99% BS) by the both ML and MP analyses.

The *Cordyceps* species included in clade D are to be found as entomopathogens for insect orders Homoptera, Lepidoptera and some are mycogenous taxa (Sung *et al.* 2007) (Fig. 4.36). *Cordyceps militaris* var. *sphaerocephala,* collected from Hot Spring is closely related to *C. ramosopulvinata* with BS value 100%. The previous taxa infected lepidopteran insect however the later attacked cicada insect. Even though the *Cordyceps* taxa in this clade infected different host, they are phylogenetically related to each other. *Cordyceps hawkesii* and *C. gunnii,* which infected to lepidopterous insects, are also included in this clade. ITS phylogeny also resulted that *Cordyceps* species from this clade attacked the diverse groups of hosts. According to Sung *et al.* (2007), the classification of *Cordyceps* species in this clade is not confidently assigned in the new *Cordyceps* classification system.

Based on the phylogenies, I can conclude that *B. brongniartii* anamorphs are closely related to *C. militaris*. All *Paecilomyces* are recognized as a distinct group. Based on sequence analyses and morphology, some *Cordyceps* spp. are restricted to their respective hosts. In addition, *Cordyceps* species do not constitute a monophyletic group. There are several strongly supported clades, characterised by species possessing divergent morphological characters (Stensrud *et al.* 2005; Sung *et al.* 2007). The *Cordyceps* species sampled in this study were polyphyletic. This is in agreement with the previous studies (Artjariyasripong *et al.* 2001; Stensrud *et al.* 2005; Spatafora *et al.* 2007; Sung *et al.* 2007a, b). *Cordyceps militaris* is closely related to *Beauveria brongniartii* (Figs 4.36-4.39) and *C. bifusispora* is in grouped with *Paecilomyces*. It also clearly indicates the anamorphs and its teleomorphs affiliations in the *Beauveria* and *Paecilomyces* clades.

The phylogenies generated in this study reveal meaningful taxonomic insights into the systematics of *Beauveria, Cordyceps* and *Paecilomyces*. The present study reported three supported groups such as *Beauveria, Cordyceps* and *Paecilomyces* in all analyses. While essentially similar topologies were obtained from all datasets, analysis of ITS/5.8S rDNA gene provided much better resolution than others.

5.3 *Beauveria* entomopathogens from Thailand: Systematics based on morphology and DNA molecules.

Morphological study of *Beauveria* spp. from our study indicated that both *B. bassiana* and *B. brongniartii* are present in Chiang Mai Province, Thailand. The length of the conidia is the distinguishable character to identify between *B. bassiana* and *B. brongniartii.* Glare and Inwood (1998) suggested that conidia longer than 3 µm and length/width ratio 2 or greater were identified as *B. brongniartii.* The length of the conidia of *B. brongniartii* isolates in this study were longer than 3.1 µm. Results derived from the multiple range tests showed that there is an interaction between hosts and *Beauveria* spp. based on the average measurements of conidia, conidia structures and rachis length. The isolates were also compared with overseas published sequences for morphological characterization. The length of the conidia of *B. brongniartii* from Chiang Mai were longer (2.2-6 µm) than those of other countries' conidia (2.3-4.2 µm). The size of the conidia of *B. bassiana* from Chiang Mai were smaller (1.2-3 × 1-3 µm) than those of overseas' conidia (1.7-3.5 × 1.5-3.1 µm).

The distinction of the morphological criteria between *B. bassiana* species has always been difficult because of the comparatively large heterogeneity of spherical/globose-spored (Glare and Inwood. 1998). The form of the spore was the most important character for separating *Beauveria* species (Mugnai *et al.* 1989). Based on their research findings, *Beauveria bassiana* was relatively heterogeneous and with the regard to form of spores, *Beauveria brongniartii* isolates produced only ellipsoidal/cylindrical conidia, while *B. bassiana* produced both globose and ellipsoidal conidia. In some research, *Beauveria* isolates produced ellipsoidal conidia on the host, but only spherical conidia in culture (Townsend *et al.* 1995; Glare and Inwood. 1998). In this study *B. bassiana* isolates produced spherical conidia both from host and culture. *Beauveria brongniartii,* however, produced ellipsoidal conidia on the host and also ellipsoidal/spherical conidia in culture.

In this study, isolates within Chiang Mai *B. brongniartii* group (A) were cultured from a wide range of hosts including four insect orders, two soil samples and single specimen of unidentified insect and this fungus may not be host-specific. Glare and Inwood (1998) reported that the New Zealand group of *Beauveria* includes isolates from four hosts, all Coleoptera, but those fungi might not have host specificity as some strains have exhibited cross-infectivity. In this study, the conidial size is different between host and fungal strain. Length/width (L/w) ratios of the conidia were divided into three groups: *Bearuveria brongniartii* isolated from Chiang Mai Coleoptera

ranged between 2-2.25, Lepidoptera associated *B. brongniartii* ranged between 1.78-2 and other heterogeneous group including Diptera, Orthoptera and unidentified insects received 1.3-1.63.

Group B isolates represent *B. brongniartii* originated from Asia and all are isolated from coleopteran hosts. L/w ratio of the Chiang Mai *B. brongniartii* in this group ranged between 2-2.25. L/w ratio of the other countries' isolates ranged between1.5-1.88. Two *B. brongniartii* isolates from Hot spring are found this group, they are phylogenetically distinct from the other Chiang Mai isolates.

The other *Beauveria* species, Group C (*B. amorpha*, *B. vermiconia* and *B. caledonica*) were separated by phylogenetic characterization. The strains were isolated from Coleoptera, Hymenoptera and Soil. *Beauveria caledonica*, isolated from Scottish moorland soil, was originally described by (Bisset and Widden. 1988) and separated from *B. bassiana* and *B. amorpha* based on conidial shape and color (Glare and Inwood. 1998). There was no comparison study regarding the affinity of *B. brongniartii* to *B. caledonica*. Some research have been carried out to evaluate the distinction between *B. caledonica* and other species using morphological and biochemical comparisons (Mugnai *et al.* 1989) mitochondrial probes, specific primers and SSCP analysis (Hegedus and Khachatourians. 1996). In this study, it is found that *B. caledonica* is phylogenetically different from *B. brongniartii* and *B. bassiana* and it is clustered with *B. amorpha* and *B. vermiconia*.

Group D includes *B. bassiana* isolates form Hymenoptera and Lepidoptera. The L/w ratio of the conidia in this group ranged 1.1-1.13. Group E is the heterogeneous group of *B. bassiana* isolates from various hosts including Coleoptera, Hemiptera, Homoptera, Lepidoptera and Orthoptera. Only one *B. brongniartii* strain isolated from Homoptera from China cluster together in this clade. This finding also supported that *B. brongniartii* constitutes a complex of several or more cryptic species, which available evidence suggests is distributed across Eurasia (Rehner and Buckley. 2005). Results here generally corroborate the findings of Rehner and Berkley (2005) who pointed out that *B. bassiana* s.l. is not host specific but an opportunistic entomopathogen capable of attacking a wide range of insect taxa. The results also indicated that there was a certain association between *B. brongniartii* isolates and their geographical origins, but no clear correlation between those isolates and their insect hosts. *Beauveria brongniartii* from clade B has been associated with Coleoptera. Single isolate of the *B. brongniartii* associated with Homoptera did not, however, separated from heterogeneous group of *B. bassiana*.

Currently, the difficulties between host-pathogen and geographic distribution of *Beauveria* species have been recognized. Poprawski *et al.* (1988) reported that correlation between strain and geographic origin has occasionally been found. This is reinforced by evidence that closely related isolates originating from the same geographic region were isolated from taxonomically distant insect hosts (Rehner and Buckley. 2005).

It is also noted that interpretation of morphological features is sometimes difficult and equivocal, which led to different hypotheses on phylogenetic relationships (e.g. for *Paraperonospora*: Skalicky'.1966, Constantinescu. 1989; for *Bremiella*: Constantinescu. 1979). Therefore, morphology alone cannot be considered to be a phylogenetically informative for species characterisation.

The present study together with existing morphological data provides insight to the understanding of the phylogenetic relationships within *Beauveria* species. There are three findings in this study. First, morphological analysis of *Beauveria* isolates from Chiang Mai Province resulted in two species including *B. bassiana* and *B. brongniartii*. The results also strongly supported that there are interaction between host and fungal strains. In addition, six major phylogenetic groups were found in phylogenetic analysis.

Chapter 6

CONCLUSIONS AND SUGGESTIONS

6.1 Conclusions

A great deal of research on entomopathogenic fungi in Thailand has been carried out during the past 20 years. Most of the documented records concerning this fungal group have focused on descriptions of new species and the use of molecular techniques in understanding their phylogeny. Ecological studies and studies on host-fungal pathogen relationships are however still few. The present research has been carried out with focus on aspects of biodiversity, morphology, taxonomy and molecular phylogeny of entomopathogenic fungi in Chiang Mai Province, Thailand.

Entomopathogenic fungi in Thailand amount to about 400 known species (Luangsa-Ard *et al.* 2007). Most of these fungi belong to the families *Clavicipitaceae*, *Cordycipitaceae* and *Ophiocordycipitaceae* (Luangsa-Ard *et al.* 2007). To validate the taxonomy of the entomopathogenic fungi and describe collections and new species, collections were made in the two different kinds of rainforests in Chiang Mai Province. Agricultural habitats were also included in this study to compare the distribution of different taxa. Morphological descriptions of collected entomopathogenic fungi are provided in this thesis as well as some notes realating to particular species and collections. Two new species and two new records for Thailand are reported. A brief account also discusses with the blast result and sequence data outcomes. The fungal taxa described in this research mainly belong to *Clavicipitaceae*, *Cordycipitaceae* and *Ophiocordycipitaceae*. Besides these families *Eurotiaceae* and *Mycosphaerellaceae* can be found as minor groups.

This study also investigated the diversity of entomopathogenic fungi occurring on different hosts and in different habitats to improve the understanding of relationships between fungi and their arthropod hosts and habitats. Detecting all entomogenous fungi in selected ecological habitats is impractical. Therefore, the study emphasized on the entomopathogenic fungi and their diversity in conserved and disturbed forests. The research findings imply that species diversity on Homoptera was highest, followed by Lepidoptera and Hymenoptera. Highest species diversity was found in disturbed rainforests, followed by conserved rainforests and agricultural habitats.

The species richness of entomopathogenic fungi was highest among the Lepidoptera and in disturbed rainforests. The highest individual number was found on Hymenoptera and in the conserved rainforests. Entomopathogenic fungi were dominated by *Ophiocordyceps myrmecophila* (22.6%) and *O. unilateralis* (13.8%) on ants. *Cordyceps* and *Ophiocordyceps* species contributed 74.7% of total taxa in conserved rainforests, 61.3% in disturbed forests but only 1.6% in agricultural habitats.

There is also considerable interest in the relationship of host and fungal pathogens. Entomopathogenic fungi are host specific. For example, *Cordyceps* species can be found as important entomopathogenic fungi especially on ants in tropical ecosystems (Evans and Samson, 1982).

Phylogenetic analyses have been conducted to establish host based relationships of entomopathogenic fungi from Chiang Mai Province in the present study. The results from the taxonomy and diversity research indicated that *Cordyceps*, *Beauveria* and *Isaria* were frequently found from the different hosts from different habitats. Several phylogenetic studies have provided new insights into the associations between the Old World fungus-growing termites and their fungal crop (Blackwell and Vega. 2005). This is the best known example of evidence in using molecular techniques to prove an insect-fungal association. In this study, ITS/5.8S and β-tubulin gene analyses have shown that *Beauveria* and *Isaria* are confirmed as anamorphic genera of *Cordyceps*. The results also show that *Beauveria* isolates are not host specific and have wide host range. Isolates from the same host were not phylogenetically related to each other. Host specificity, however, was found among the *Isaria* isolates collected from Chiang Mai Province. *Isaria tenuipes* and *I. farinosus* particularly infected lepidopteran pupae and *I. cicadae* attacked only Homoptera. Updated phylogenetic studies revealed that host specificity was found to be of limited, and several host shifts occurred during the evolution of *Cordyceps* species (Stensrud *et al.* 2005). Sequence analyses describe in this study report that some *Cordyceps* spp. are host specific.

There is also one remarkable finding from this study regarding taxonomic relationship between *Beauveria brongniartii* and *Cordyceps militaris*. *Beauveria brongniartii* isolated from Coleoptera and *Cordyceps militaris* associated with lepidopteran larvae in this study were formed subclade within *Beauveria* clade with high bootstrap value. Although *Beauveria* has been recorded as an anamorph of *Cordyceps* (Shimazu *et al.* 1988) there is no evidence for the anamorph-teleomorph connection between *Beauveria brongniartii* and *Cordyceps militaris*.

This study also investigated the systematics of *Beauveria* isolates from Chiang Mai Province based on morphology and DNA molecules. The results generated from morphology study indicated that there are two *Beauveria* species: *B. bassiana* and *B. brongniartii* present in Chiang Mai Province, Thailand.

During this study, some difficulties have been encountered. Entomopathogenic fungi are comparatively small as compared to mushrooms and are relatively rare in ecosystems, thus the number of collections are very low. The studies relating to the application of entomopathogenic fungi as biocontrol agents could not be carried out due to time limitations.

6.2 Suggestions and Future Plans

Although numerous studies concerning entomopathogenic fungi such as taxonomy, diversity and molecular phylogeny have received attention, there are several gaps of information. For example, documentation of the geographical distribution of entomopathogenic fungi in different region and seasonal occurrence of these fungi should be carried out. Continued research relating to *Beauveria brongniartii* and *Cordyceps militaris* will greatly contribute to our understanding of anamorph-teleomorph connection in science. Even though the anamorphic state of *Cordyceps militaris* has been known as *Lecanicillium* (Zare Gams 2001), the *C. militaris* from Chiang Mai Province are notably related to *B. brongniartii*.

Fungal succession research on the naturally occurring infected insects will possibly be helpful in detection of anamorph-teleomorph connection.

Classification of the host arthropods has many problems and uncertainties (Evans, 1984) especially in mummified infected hosts. There must be exciting and endless possibilities for research in parallel approach of DNA barcoding for arthropod hosts and rDNA sequencing of fungal pathogens.

Research relating to the use of entomopathogenic fungi as biocontrol agents should be considered as different project.

BIBLIOGRAPHY

Abdel-Baky, N.F., Arafat, N.S. and Abdel-Salam, A.H. 1998. "Three *Cladosporium* spp. as promising biological control candidates for controlling whiteflies (*Bemisia* spp.) in Egypt". **Pakistan Journal of Biological Sciences.** 1: 188-195.

Ahn, Y.J., Park, S.J., Lee, S.G., Shin, S.C. and Choi, D.H. 2000. "Cordycepin: Selective growth inhibitor derived from liquid culture of *Cordyceps militaris* against *Clostridium* spp.". **Journal of Agricultural and Food Chemistry.** 48: 2744-2748.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. 1997. "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs". **Nucleic Acids Research.** 25: 3389-3402.

Artjariyasripong, S., Mitchell, J.I., Hywel-Jones, N.L. and Jones, E.B.G. 2001. "Relationship of the genus *Cordyceps* and related genera, based on parsimony and spectral analysis of partial 18S and 28S ribosomal gene sequences". **Mycoscience.** 42: 503-517.

Aung, O.M., Kang, J.C., Liang, Z.Q., Soytong, K. and Hyde, K.D. 2006a. "*Cordyceps mrciensis* sp. nov. from a spider in Thailand". **Mycotaxon.** 97: 235-240.

Aung, O.M., Kang, J.C., Liang, Z.Q., Soytong, K. and Hyde, K.D. 2006b. "A new entomopathogenic species, *Hymenostilbe furcata*, parasitic on a hemipteran nymph in northern Thailand". **Mycotaxon.** 97: 241-245.

Bainier, G. 1907. "Mycothéque de l'école de Pharmacie. XI. *Paecilomyces*, genre nouveau de Mucedinees". **Bulletin trimestriel de la Société mycologique de France.** 23: 26-27.

Bastola, D.R., Otu, H.H., Doukas, S.E., Sayood, K., Hinrich, S.H. and Iwen, P.C. 2004. "Utilization of the relative complexity measure to construct a phylogenetic tree for fungi". **Mycological Research.** 108: 117-125.

Benjamin, R.K., Blackwell, M., Chapela, I.H., Humber, R.A., Jones, K.G., Klepzig, K.A., Lichtwardt, R.W., Malloch, D., Noda, H., Roeper, R.A., Spatafora, J.W. and Weir, A. 2004. "Insects-and other arthropod-associated fungi". In: **Biodiversity of Fungi Inventory and Monitoring Methods.** (eds. G.M. Mueller, G.F. Bills and S. Mercedes). Foster. Elsevier Academic Press, USA: 395-434.

Berch, S.M., Allen, T.R. and Berbee, M.L. 2002. "Molecular detection, community structure and phylogeny of ericoid mycorrhizal fungi". **Plant and Soil.** 244: 55-66.

Bhattarai, N.K. 1989. "Traditional phytotherapy among the Sherpas of Helambu, central Nepal". **Journal of Ethnopharmacology.** 27: 45-54.

Bhattarai, N.K. 1992a. "Folk use of plants in veterinary medicine in central Nepal". **Fitoterapia.** 63: 497-506.

Bhattarai, N.K. 1992b. "Medical ethnobotany in the Karnali Zone, Nepal". **Economic Botany.** 46: 257-261.

Bhattarai, N.K. 1993. "Folk herbal medicines of Dolakha District, Nepal". **Fitoterapia.** 66: 387-395.

Bhattarai, N.K. 1994. "Folk herbal remedies for gynaecological complaints in central Nepal". **International Journal of Pharmacognosy.** 32: 13-26.

Bisset, J. and Widden, P. 1988. "A new species of *Beauveria* isolated from Scottish moorland soil". **Canadian Journal of Botany.** 66: 361-362.

Bissett, J. 1979. **Fungi Canadenses** No. 151-159.

Blackwell, M. and Jones, K. 1997. "Taxonomic diversity and interactions of insect-associated ascomycetes". **Biodiversity and Conservation.** 6: 689-699.

Blackwell, M. and Vega, F.E. 2005. "Introduction: Seven wonders of the insect-fungus world". In: **Insect-Fungal Associations Ecology and Evolution.** (eds. F.E. Vega and M. Blackwell). Oxford University Press: xiii-xvii.

Bok, J.W., Lermer, L., Chilton, J., Klingeman, H.G. and Towers, G.H. 1999. "Antitumor sterols from the mycelia of *Cordyceps sinensis*". **Phytochemistry.** 51:891-898.

Boudier, E. 1885. "Note sur un nouveau genre et quelques nouvelles espéces des Pyrénomycétes". **Revue Mycologique.** 7: 224-277.

Brady, B.L.K. 1979. ***Beauveria bassiana.*** CMI descriptions of pathogenic fungi and bacteria, No. 602, Commonwealth Mycological Institute: Kew, Surrey, England.

Brown, A.H.S. and Smith, G. 1957. "The genus *Paecilomyces* Bainier and its perfect stage *Byssochlamys* Westling". **Transactions of the British Mycological Society.** 40: 17-89.

Buenz, E. and Badley, A. 2004. "Impact of mitochondrial regulation of apoptosis on the pathogenesis of HIV-1 induced immunodeficiency". **Mitochondrion.** 4: 235-254.

Buenz, E.J., Bauer, B.A., Osmundson, T.W. and Motley, T.J. 2005. "The traditional Chinese medicine *Cordyceps sinensis* and its effects on apoptotic homeostasis". **Journal of Ethnopharmacology.** 96: 19-29.

Burges, H.D. 1981. "Strategy for the microbial control of pests in 1980 and beyond". In: **Microbial Control of Pests and Plant Diseases 1970-1980.** (ed. H.D. Burges). Academic Press, London and New York: 797-836.

Cai, L., Jeewon, R. and Hyde, K.D. 2005. "Phylogenetic evaluation and taxonomic revision of *Schizothecium* based on ribosomal DNA and protein coding genes". **Fungal Diversity.** 19: 1-21.

Cai, L., Jeewon, R. and Hyde, K.D. 2006. "Phylogenetic investigations of *Sordariaceae* based on multiple gene sequences and morphology". **Mycological Research.** 110: 137-150.

Carmichael, J.W., Kendrick, B., Conners, I.L. and Sigler, L. 1980. ***Genera of Hyphomycetes.*** University of Alberta Press, Edmonton: 1-386.

Carvalho, M.B.D., Aquino, M.D.L.N. and Oliveria, M.H.C.C.D. 1972. "Considerations on the biological control of *Aleurodicus cocois* (Curtis) (Homoptera: *Aleyrodidae*), Cashew white fly". **Anais Instituto Ciencias Biology.** 2: 25-30.

Chang, H.M. and But, P.P.H. 1987. **Pharmacology and Applications of Chinese Materia Medica.** Vol. 2. Singapore: World Scientific.

Chen, H.C., Yeh, S.F., Ong, G.T., Wu, S.H., Sun, C.M. and Chou, C.K. 1995. "The novel desmethyldestruxin B2, from *Metarhizium anisopliae*, that suppresses hepatitis B virus surface antigen production in human hepatoma cells". **Journal of Natural Products.** 58: 527-531.

Chen, Y.C., Huang, Y.L. and Huang, B.M. 2005. "*Cordyceps sinensis* mycelium activates PKA and PKC signal pathways to stimulate steroidogenesis in MA-10 mouse Leydig tumor cells". **The International Journal of Biochemistry and Cell Biology.** 37: 214-223.

Chen, Y.J., Shiao, M.S., Lee, S.S. and Wang, S.Y. 1997. "Effect of *Cordyceps sinensis* on the proliferation and differentiation of human leukemic U937 cells". **Life Sciences.** 60: 2349-2359.

Chen, Z. and Xu, S. 1989. "Preliminary studies on cultural characters and pharmacological function of *Paecilomyces tenuipes*". **Acta Mycologica Sinica.** 8: 214-220.

Chiou, W.F., Chang, P.C., Chou, C.J. and Chen, C.F. 2000. "Protein constituent contributes to the hypotensive and vasorelaxant activities of *Cordyceps sinensis*". **Life Sciences.** 66:1369-1376.

Choi, S.B., Park, C.H., Choi, M.K., Jun, D.W. and Park, S. 2004. "Improvement of insulin resistance and insulin secretion by water extracts or *Cordyceps militaris*, *Phellinus linteus*, and *Paecilomyces tenuipes* in 90% pancreatectomized rats". **Bioscience Biotechnology and Biochemistry.** 68: 2257-2264.

Choi, Y.W., Hyde, K.D. and Ho, W.W.H. 1999. "Single spore isolation of fungi". **Fungal Diversity.** 2: 29-38.

Cory, S. and Adams, J.M. 2002. "The Bcl2 family: regulators of the cellular life-or-death switch". **Nature Reviews Cancer.** 2: 647-656.

Cruickshank, R.H. and Pitt, J.I. 1980. "Identification of species in *Penicillium* subgenus *Penicillium* by enzyme electrophoresis". **Mycologia.**79: 614-620.

Cunningham, K.G., Hutchinson, S.A., Manson, W. and Spring, F.S. 1951. "Cordycepin, a metabolic product from cultures of *Cordyceps militaris* (Linn.) Link, Part 1. Isolation and characterization". **Journal of the Chemical Society.** : 2299-2300.

Cunnington, J.H., Takamatsu, S., Lawrie, A.C. and Pascoe, I.G. 2003. "Molecular identification of anamorphic powdery mildews (*Erysiphales*)". **Australasian Plant Pathology.** 32: 421-428.

Darwin, C. 1859. **On the Origin of Species by Means of Natural Selection.** J. Murray, London.

De Hoog, G.S. 1972. "The genera *Beauveria*, *Isaria*, *Tritirachium* and *Acrodontium* gen. nov.". **Studies in Mycology.** 1: 1-41.

De Hoog, G.S. 1974. "The genera *Blastobotrys*, *Sporothrix*, *Calcarisporium* and *Calcarisporiella* gen. nov.". **Studies in Mycology.** 7: 1-84.

Devi, P.S.V. 1994. "Conidia production of the entomopathogenic fungus *Nomuraea riley* and its evaluation for control of *Spodoptera litura* (Fab) on *Ricinus communis*". **Journal of Invertebrate Pathology.** 63: 145-150.

Disarapong, S. 2003. **Screening of Insect Fungus *Hypocrella scutata* producing Antimicrobial Substances.** M.Sc. Thesis, Prince of Songkla University, Thailand.

Druzhinina, I.S., Kopchinskiy, A.G., Komo, M., Bissett, J., Szakacs, G. and Kubicek, C.P. 2005. "An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*". **Fungal Genetics and Biology.** 42: 813-828.

Du Halde, P. 1736. **The general history of China.** Vol. 4. John Watts, London.

Ellis, M.B. 1971. **Dematiaceous Hyphomycetes.** Commonwealth Mycological Institute, Kew, Surrey, England.

Ellis, M.B. 1976. **More Dematiaceous Hyphomycetes.** Commonwealth Mycological Institute, Kew, Surrey, England.

Elton, C.S. 1973. "The structure of invertebrate populations inside neotropical rain forest". **Journal of Animal Ecology.** 42: 55-104.

Evans, H.C. 1982. "Entomogenous fungi in tropical forest ecosystems: an appraisal". **Ecological Entomology.** 7: 47-60.

Evans, H.C. 1988. "Coevolution of entomogenous fungi and their insect hosts". In: **Coevolution of Fungi with Plants and Animals.** (eds. K.A. Pirozynski and D.L. Hawksworth). Academic Press, New York: 149-171.

Evans, H.C. and Samson, R.A. 1982. "*Cordyceps* species and their anamorphs pathogenic on ants (*Formicidae*) in tropical forest ecosystems. I. The *Cephalotes* (*Myrmicinae*) complex". **Transactions of the British Mycological Society.** 79: 431-453.

Evans, H.C. and Samson, R.A. 1984. "*Cordyceps* species and their anamorphs pathogenic on ants (*Formicidae*) in tropical forest ecosystems. II: The *Camponotus* (*Formicinae*) complex". **Transactions of the British Mycological Society** 82: 127-150.

Fargues, J. and Remaudiere, G. 1977. "Consideration on the specificity of entomopathogenic fungi". **Mycopathologia.** 62: 31-37.

Fargues, J. and Maniania, N.K. 1992. "Variabilite' de la sensibilite' de *Spodoptera littoralis* [Lep.: Noctuidae] a` l'hyphomycete entomopathogene *Nomuraea rileyi*". **Entomophaga.** 37: 545-554.

Feng, M.G., Poprawski, T.J. and Khachatourians, G.G. 1994. "Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: Current status". **Biocontrol Science and Technology.** 4: 3-34.

Ferron, P., Fargues, J. and Riba, G. 1991. "Fungi as microbial insecticides against pest". In: **Handbook of Applied Mycology Vol. 2: Humans, Animals, and Insects** (eds. D.K. Arora, L. Ajello and K.G. Mukerji). Marcel Dekker, Inc., New York: 665-706.

Fukatsu, T., Sato, H. and Kuriyama, H. 1997. "Isolation, inoculation to insect host, and molecular phylogeny of an entomogenous fungus *Paecilomyces tenuipes*". **Journal of Invertebrate Pathology**. 70: 203-208.

Furuya, T., Hirotani, M. and Matsuzawa, M. 1983. "N6-(2-hydroxyethyl) adenosine, a biologically active compound from cultured mycelia of *Cordyceps* and *Isaria* species". **Phytochemistry**. 22: 2509-2512.

Gams, W. 1971. ***Cephalosporium-artige* Schimmelpilze (Hyphomycetes).** Gustav Fischer Verlag, Stuttgart.

Gams, W. and Samson, R.A. 1985. "Typification of *Aspergillus* and related teleomorph genera". In: **Advances in *Penicillium* and *Aspergillus* Systematics.** (eds. R.A. Samson and J.I. Pitt). Plenum Press New York-London: 23-31.

Geiser, D.M., Jiménez-Gasco, M.D.M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T.J., Zhang, N. and O'Donnell, K. 2004. "FUSARIUM-ID v. 1.0: A DNA sequence database for identifying *Fusarium*". **European Journal of Plant Pathology**. 110: 473-479.

Glare, T.R. and Inwood, A.J. 1998. "Morphological and genetic characterisation of *Beauveria* spp. from New Zealand". **Mycological Research**. 102: 250-256.

Glare, T.R. and Milner, R.J. 1991. "Ecology of entomopathogenic fungi". In: **Handbook of Applied Mycology, Vol.2, Humans, Animals and Insects.** (eds. D.K. Arora, L. Ajello and K.G. Mukerji). New York: Marcel Dekker: 547-612.

Glass, N.L. and Donaldson, G. 1995. "Development of primer sets designed for use with the PCR to amply conserved genes from filamentous ascomycetes". **Applied and Environmental Microbiology**. 61: 1323-1330.

Glenn, A.E., Bacon, C.W., Price, R. and Hanlin, R. 1996. "Molecular phylogeny of *Acremonium* and its taxonomic implications". **Mycologia**. 88: 369-383.

Goettel, M.S. and Inglis, G.D. 1997. "Fungi: Hyphomycetes". In: **Manuals of Techniques in Insect Pathology.** (ed. L.A. Lacey). Academic Press: 213-247.

Goettel, M.S., Inglis, G.D. and Wraight, S.P. 2000. "Fungi". In: **Field Manual of Techniques in Invertebrate Pathology: Application and Evaluation of Pathogens for Control of**

Insects and Other Invertebrate Pests. (eds. L.A. Lacey and H.K. Kaya). Kluwer Academic, Dordrecht: 255-282.

Goettel, M.S., Johnson, D.L. and Inglis, G.D. 1995. "The role of fungi in the biological control of grasshoppers". **Canadian Journal of Botany**. 73(Suppl. 1): S1-S75.

Goettel, M.S., Poprawski, T.J., Vandenberg, J.D., Li, Z. and Roberts, D.W. 1990. "Safety to nontarget invertebrates of fungal biocontrol agents". In: **Safety of Microbial Insecticides**. (eds. M. Laird, L.A. Lacey, and E.W. Davidson). CRC Press, Boca Raton, FL.: 209-231.

Gotelli, N.J. and Colwell, R.K. 2001. "Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness". **Ecology Letters**. 4: 379-391.

Gray, G.R. 1858. **Notices of Insects that are Known to Form the Basis of Fungoid Parasites.** London.

Green, S.J., Freeman, S., Hadar, Y. and Minz, D. 2004. "Molecular tools for isolate and community studies of Pyrenomycete fungi". **Mycologia**. 96: 439-451.

Guadet, J., Julien, J., Lafay, J.F. and Brygoo, Y. 1989. "Phylogeny of some *Fusarium* species, as determined by large-subunit rRNA sequence comparison". **Molecular Biology and Evolution**. 6: 227-242.

Haeckel, E. 1866. **Generelle Morphologie der Organismen: Allgemeine Grundzuge der organischen Formen-Wissenschaft, mechanisch begrundet durch die von Charles Darwin reformirte Descendenz-Theorie**. George Riemer, Berlin.

Hajek, A.E. and St. Leger, R.J. 1994. "Interaction between fungal pathogens and insect hosts". **Annual Review of Entomology**. 39: 293-322.

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W. and Hebert, P.D.N. 2006. "DNA barcodes distinguish species of tropical Lepidoptera". **Proceedings of the National Academy of Sciences the United States of America**. 103: 968-971.

Hall, T.A. 1999. "BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT". **Nucleic Acids Symposium Series**. 41: 95-98.

Hansen, K., Læssøe, T. and Pfister, D.H. 2001. "Phylogenetics of the *Pezizaceae*, with an emphasis on Peziza". **Mycologia**. 93: 958-990.

Hawksworth, D.L. 1991. "The fungal dimension of biodiversity: magnitude, significance, and conservation". **Mycological Research**. 95: 641-655.

Hawksworth, D.L. and Rossman, A.Y. 1997. "Where are all the undescribed fungi?". **Phytopathology.** 87: 888-891.

Hawksworth, D.L., Sutton, B.C. and Ainsworth, G.C. 1983. **Ainsworth and Bisby's Dictionary of the Fungi.** 7th edn. Commonwealth Mycological Institute: Kew, U.K.

Hayek, L.A.C. and Buzas, M.A. 1997. **Surveying Natural Populations.** Columbia University Press.

Hebert, P.D.N. and Gregory, T.R. 2005. "The promise of DNA barcoding for taxonomy". **Systematic Biology**. 54: 852-859.

Hebert, P.D.N., Cywinska, A., Ball, S.L. and deWaard, J.R. 2002. "Biological identifications through DNA barcodes". **Proceedings of the Royal Society of London Series.** B 270: 313-321.

Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H. and Hallwachs, W. 2004a. "Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*". **Proceedings of the National Academy of Sciences of the United States of the America.** 101: 14812-14817.

Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T.S. and Francis, C.M. 2004b. "Identification of birds through DNA barcodes". **PLoS Biology.** 2: e312.

Hegedus, D.D. and Khachatourians, G.G. 1996. "Identification and differentiation of the entomopathogenic fungus *Beauveria bassiana* using polymerase chain reaction and single-strand conformation polymorphism analysis". **Journal of Invertebrate Pathology.** 67: 289-299.

Hibbett, D.S. 1992. "Ribosomal RNA and fungal systematics". **Transactions of the Mycological Society of Japan.** 33: 533-556.

Hidalgo, A. and Ffrench-Constant, C. 2003. "The control of cell number during central nervous system development in flies and mice". **Mechanisms of Development.** 120: 1311-1325.

Holst-Jensen, A. and Schumacher, T. 1997. "Molecular phylogeny and evolution of *Monilinia* (*Sclerotiniaceae*) based on coding and noncoding rDNA sequences". **American Journal of Botany.** 84: 686-701.

Hsu, C.C., Huang, Y.L., Tsai, S.J., Sheu, C.C. and Huang, B.M. 2003a. "*In vivo* and *in vitro* stimulatory effects of *Cordyceps sinensis* on testosterone production in mouse Leydig cells". **Life Sciences.** 73: 2127-2136.

Hsu, C.C., Tsai, S.J., Huang, Y.L. and Huang, B.M. 2003b. "Regulatory mechanism of *Cordyceps sinensis* mycelium on mouse Leydig cell steroidogenesis". **FEBS Letters.** 543: 140-143.

http://www.cbs.knaw.nl/databases/ index.htm

Huang, B., Li, C.R., Li, Z.G. and Fan, M.Z. 2002. "Molecular identification of the teleomorph of *Beauveria bassiana*". **Mycotaxon.** 81: 229-236.

Huang, B.M., Chuang, Y.M., Chen, C.F. and Leu, S.F. 2000. "Effects of extracted *Cordyceps sinensis* on steroidogenesis in MA-10 mouse Leydig tumor cell". **Biological and Pharmaceutical Bulletin.** 23: 1532-1535.

Huang, B.M., Hsiao, K.Y., Chuang, P.C., Wu, M.H., Pan, H.A. and Tsai, S.J. 2004a. "Upregulation of steroidogenic enzymes and ovarian 17β-estradiol in human granulose-lutein cells by *Cordyceps sinensis* mycelium". **Biology of Reproduction.** 70: 1358-1364.

Huang, B.M., Hsu, C.C., Tsai, S.J., Sheu, C.C. and Leu, S.F. 2001b. "Effect of *Cordyceps sinensis* on testosterone production in normal mouse Leydig cells". **Life Sciences.** 69: 2593-2602.

Huang, B.M., Ju, S.Y., Wu, C.S., Chuang, W.J., Sheu, C.C. and Leu, S.F. 2001a. "*Cordyceps sinensis* and its fractions stimulate MA-10 mouse Leydig tumor cell steroidogeneiss". **Journal of Andrology.** 22: 831-837.

Huang, Y.L., Leu, S.F., Liu, B.C., Sheu, C.C. and Huang, B.M. 2004b. "*In vivo* stimulatory effect of *Cordyceps sinensis* mycelium and its fractions on mouse testosterone production". **Life Sciences.** 75: 1051-1062.

Huelsenbeck, J.P. and Ronquist, F. 2001. "MrBAYES: Bayesian inference of phylogenetic trees". **Bioinformatics.** 17: 754-755.

Hyde, K.D. 2001. "Where are the missing fungi? Does Hong Kong have any answers?". **Mycological Research.** 105: 1514-1518.

Hywel-Jones, N.L. 1993. "A systematic survey of insect fungi from natural, tropical forest in Thailand". Abstract. In: **Aspects of Tropical Mycology** (eds. S. Isaac, J.C. Frankland, R. Watling, and A.J.S. Whalley). Cambridge University Press, Cambridge: 295-296.

Hywel-Jones, N.L. 2001. "The biological diversity of invertebrate pathogenic fungi". In: **Biodiversity of Tropical Microfungi.** (ed. K.D. Hyde). Hong Kong University Press, Hong Kong: 107-118.

Index Fungorum. 2007. **An online database currently coordinated and supported by CABI Bioscience, CBS and Landcare Research,** available at www.speciesfungorum.org

Inglis, G.D., Goettel, M.S., Butt, T.M. and Strasser, H. 2001. "Use of hyphomycetous fungi for managing insect pests". In: **Fungi as Biocontrol Agents.** (eds. T.M. Butt, C. Jackson and N. Magan). CABI Publishing, Wallingford, UK: 23-69.

Isaka, M., Tanticharoen, M. and Thebtaranonth, Y. 2000. "Cordyanhydrides A and B. two unique anhydrides from the insect pathogenic fungus *Cordyceps pseudomilitaris* BCC 1620". **Tetrahedron Letters.** 41: 1657-1660.

Ito, Y. and Hirano, T. 1997. "The determination of the partial 18S ribosomal DNA sequences of *Cordyceps* species". **Letters in Applied Microbiology.** 25: 239-242.

Jacobs, K.A. and Rehner, S.A. 1998. "Comparison of Cultural and Morphological Characters and ITS Sequences in Anamorphs of *Botryosphaeria* and Related Taxa". **Mycologia.** 90: 601-610.

Jaturapat, A., Isaka, M., Kamchonwongpaisan, S., Kirtikara, K., Tanticharoen, M. and Thebtaranonth, Y. 2001. "Bioxanthracenes from the insect pathogenic fungus *Cordyceps pseudomilitairs* BCC 1620". **Journal of Antibiotics.** 54: 29-35.

Jeewon, R., Liew, E.C.Y. and Hyde, K.D. 2002. "Phylogenetic relationships of *Pestalotiopsis* and allied genera inferred from ribosomal DNA sequences and morphological characters". **Molecular Phylogenetics and Evolution.** 25: 378-392.

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

Jeewon, R., Liew, E.C.Y. and Hyde, K.D. 2004. **Molecular systematics of the *Amphisphaeriaceae* based on cladistic analyses of partial LSU rDNA gene sequences.** Cambridge University Press: 1392-1402.

Johns, D.G. and Adamson, R.H. 1976. "Enhancement of the biological activity of cordycepin (3-deoxyadenosine) by the adenosine deaminase inhibitor 2-deoxycoformycin". **Biochemical Pharmacology.** 25, 1441-1444.

Jones, E.B.G. and Hyde, K.D. 2004. "Introduction to Thai fungal diversity". In: **Thai Fungal Diversity.** (eds. E.B.G. Jones, M. Tantichareon, and K.D. Hyde). BIOTEC, Thailand: 7-35.

Jones, E.B.G. 2004. "Fungi on arthropods, crustaceans and fish". In: **Thai Fungal Diversity.** (eds. E.B.G. Jones, M. Tantichareon and K.D. Hyde). BIOTEC, Thailand: 227-239.

Keller, S. 1987. "Arthropod-pathogenic *Entomophthorales* of Switzerland I. *Conidiobolus*, *Entomophaga* and *Entomophthora*". **Sydowia.** 40: 122-167.

Keller, S. 1991. "Arthropod-pathogenic *Entomophthorales* of Switzerland II. *Erynia*, *Eryniopsis*, *Neozygites* and *Entomophthora*". **Sydowia.** 43: 39-122.

Keller, S. 1993. "Taxonomic considerations on some species of *Erynia* (*Zygomycetes*: *Entomophthorales*) attacking flies (Diptera)". **Sydowia.** 45: 252-263.

Keller, S. and Eilenberg, J. 1993. "Two new species of *Entomophthoraceae* (Zygomycetes: *Entomophthorales*) linking the genera *Entomophaga* and *Eryniopsis*". **Sydowia.** 45: 264-274.

Keller, S., Keller, E., Schweizer, C., Auden, J.A.L. and Smith, A. 1989. "Two large field trials to control the cockchafer *Melolontha melolontha* L. with the fungus *Beauveria brongniartii* (Sacc.) Petch". **British Crop Protection Council Monograph.** 43: 183-190.

Keller, S., Schweizer, C., Keller, E. and Brenner, H. 1997. "Control of white grubs (*Melolontha melolontha* L) by treating adults with the fungus *Beauveria brongniartii*". **Biocontrol Science and Technology.** 7: 105-116.

Kendrick, B. and DiCosmo, F. 1979. "Anamorph-Teleomorph connections in ascomycetes". In: **The Whole Fungus Vol. 1.** (ed. W.B. Kendrick). National Museums of Canada, Ottawa: 283-410.

Khan, H.K., Jayaraj, S. and Gopalan, M. 1993. "Muscardine fungi for the biological control of agroforestry termite *Odontotermes obesus* (Rambur)". **Insect Science and Its Application.** 14: 529-535.

Kim, J.R., Yeon, S.H., Kim, H.S., Ahn, Y.J. 2002. "Larvicidal activity against *Plutella xylostella* of cordycepin from the fruiting body of *Cordyceps militaris*". **Pest Management Science.** 58: 713-717.

Kirk, P.M., Cannon, P.F., David, J.C. and Stalpers, J.A. 2001. **Ainsworth and Bisby's Dictionary of the Fungi.** 9th edn. Commonwealth Mycological Institute: Kew, U.K.

Kirkpatrick, L.A. and Feeney, B.C. 2006. **A simple guide to SPSS for Windows for version 12.0 & 13.0.** Thomas Wadsworth, Thomson corporation, Belmont, USA.

Kittakoop, P., Punya, J., Kongsaeree, P., Lertwerawat, Y., Jintasirikul, A., Tanticharoen, M. and Thebtaranonth, Y. 1999. "Bioactive naphthoquinones from *Cordyceps unilateralis*". **Phytochemistry**. 52: 453-457.

Klich, M.A. and Pitt, J.I. 1988. **A laboratory guide to the common *Aspergillus* species and their teleomorphs.** CSIRO Division of Food Processing, North Ryde, N.S.W.

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

Kobayasi, Y. 1981. "Revision of the genus *Cordyceps* and its allies 2". **Bulletin of the National Science Museum, Tokyo.** Series B. (Botany) 7: 123-129.

Kobayasi, Y. 1982. "Keys to the taxa of the genera *Cordyceps* and *Torrubiella*". **Transaction of the Mycological Society of Japan**. 23: 329-364.

Kobayasi, Y. and Shimizu, D. 1976. **Some species of *Cordyceps* and its allies on spiders.** Kew Bulletin 31: 557-566.

Kobayasi, Y. and Shimizu, D. 1983. "*Cordyceps* species from Japan 6". **Bulletin of the National Science Museum,Tokyo**. Series B. (Botany) 9: 1-211.

Kodama, E.N., McCaffrey, R.P., Yusa, K. and Mitsuya, H. 2000. "Antileukemic activity and mechanism of action of cordycepin against terminal deoxynucleotidyl transferase-positive (TdT+) leukemic cells". **Biochemical Pharmacology**. 59: 273-281.

Kodsueb, R., Lumyong, S., Ho, W.H., Hyde, K.D., McKenzie, E.H.C. and Jeewon, R. 2007. "Morphological and molecular characterization of *Aquaticheirospora* and phylogenetics of *Massarinaceae* (*Pleosporales*)". **Botanical Journal of the Linnean Society**. 155: 283-296.

Kõljalg, U., Larsson, K.H., Abarenkov, K., Nilsson, R.H., Alexander, I.J., Eberhardt, U. and Erland, S. and Ursing, B.M. 2005. "UNITE: A database providing web-based methods for the molecular identification of ectomycorrhizal fungi". **New Phytologist**. 166: 1063-1068.

Kress, W.J., Wurdack, K.J., Zimmer, E.A., Weigt, L.A. and Janzen, D.H. 2005. "Use of DNA barcodes to identify flowering plants". **Proceedings of the National Academy of Sciences the United States of America**. 102: 8369-8374.

Krijnen, P.A., Nijmeijer, R., Meijer, C.J., Visser, C.A., Hack, C.E. and Niessen, H.W. 2002. "Apoptosis in myocardial ischaemia and infarction". **Journal of Clinical Pathology**. 55, 801-811.

Kucharczak, J., Simmons, M., Fan, Y. and Gelinas, C. 2003. "To be, or not to be: NF-kappaB is the answer—role of Rel/NF-kappaB in the regulation of apoptosis". **Oncogene**. 22: 8961-8982.

Kuo, Y.C., Lin, C.Y., Tsai, W.J., Wu, C.L., Chen, C.F. and Shiao, M.S. 1994. "Growth inhibitors against tumor cells in *Cordyceps sinensis* other than cordycepin and polysaccharides". **Cancer Investigation**. 12: 611-615.

Kuo, Y.C., Tsai, W.J., Shiao, M.S., Chen, C.F. and Lin, C.Y. 1994. "*Cordyceps sinensis* as an immunomodulatory agent". **American Journal of Chinese Medicine**. 24: 111-125.

Kuraishi, H., Aoki, M., Itoh, M. and Katayama, Y. 1991. "Distribution of ubiquinones in *Penicillium* and related genera". **Mycological Research**. 95: 705-711.

Kurtzman, C.P. and Robnett, C.J. 1998. "Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences". **Antonie van Leeuwenhoek**. 73: 331-371.

Lacap, D.C., Liew, E.C.Y. and Hyde, K.D. 2003. "An evaluation of the fungal 'morphotype' concept based on ribosomal DNA sequences". **Fungal Diversity**. 12: 53-66.

Lacey, L.A. and Brooks, W.M. 1997. "Initial handling and diagnosis of diseased insects". In: **Manuals of Techniques in Insect Pathology.** (ed. L.A. Lacey). Academic Press: 1-15.

Lacey, L.A., Fransen, J.J., and Carruthers, R. 1996. "Global distribution of naturally occurring fungi of *Bemisia*, their biologies and use as biological control agents". In: **Bemisia 1995: Taxonomy, Biology, Damage, and Management**. (eds. D. Gerling and R. Mayer), Intercept, Andover: 401-433.

Lacey, L.A., Frutos, R. Kaya, H.K. and Vail, P. 2001. "Insect Pathogens as Biological Control Agents: Do They Have a Future?". **Biological Control**. 21: 230-248.

Lamarck, J.B. 1809. **Philosophie Zoologique, ou Exposition des Considerations Relatives à l'histoire Naturelle des Animaux.** Dentu, Paris.

Li, Z.Z. 1988. "A list of insect hosts of *Beauveria bassiana*". In: **Study and Application of Entomogenous Fungi in China** (eds. Y.W. Li, Z.Z. Li, Z.Q. Liang, J.W. Wu, Z.K. Wu and Q.F. Xi). Academic Periodical Press, Beijing, PRC: 241-255.

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

Liang, Z.Q. 1985. "Isolation and determination of conidial stage for *Cordyceps gunnii*". **Acta Mycologica Sinica**. 4: 162-166.

Liang, Z.Q. 1991. "Anamorphs of *Cordyceps* and their determination". **Southwest China Journal of Agricultural Sciences**. 4: 1-8.

Liang, Z.Q. 1997. "Biodiversity of the genus *Cordyceps* and its anamorphs". **Fungal Science**. 12: 51-57.

Liang, Z.Q. and Liu, A.Y. 1991. "A new species of *Cordyceps* and its anamorph, *Metarhizium tait*". **Acta Mycologica Sinica**. 10: 257-262.

Liang, Z.Q., Han, Y.F., Chu, H.L. and Liu, A.Y. 2005. "Studies on the genus *Paecilomyces* in China. I". **Fungal Diversity**. 20:83-101.

Lin, L.J., Shiao, M.S. and Wang, Z.N. 1996. "Active fractions of *Cordyceps sinensis* and method of isolation thereof'. US. **Patent No.** 5:582-828.

Liu, Z., Liang, Z., Liu, A., Yao, Y., Hyde, K.D. and Yu, Z. 2002. "Molecular evidence for teleomorph-anamorph connections in *Cordyceps* based on ITS-5.8 S rDNA sequences". **Mycological Research**. 106: 1100-1108.

Liu, Z.Y., Yao, Y.J., Liang, Z.Q., Liu, A.Y., Pegler, D.N. and Chase, M.W. 2001. "Molecular evidence for the anamorph-teleomorph connection in *Cordyceps sinensis*". "Mycological Research 105: 827-832".

LoBuglio, K.F. and Taylor, J.W. 1993. "Phylogenetic analysis of two ribosomal DNA regions indicates multiple independent loss of a sexual *Penicillium* species in subgenus *Biverticillium*". **Mycologia**. 85: 592-604.

LoBuglio, K.F., Pitt, J.I. and Taylor, J.W. 1993. "Phylogenetic analysis of two ribosomal DNA regions indicates multiply independent losses of a sexual *Talaromyces* state among asexual *Penicillium* species in subgenus *Biverticillium*". **Mycologia**. 85: 592-604.

Luangsa-Ard, J.J., Hywel-Jones, N.L. and Samson, R.A. 2004. "The polyphyletic nature of *Paecilomyces* sensu lato based on 18S-generated rDNA phylogeny". **Mycologia**. 96: 773-780.

Luangsa-Ard, J.J., Hywel-Jones, N.L., Manoch, L. and 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. and Hywel-Jones, N.L. 2007. **Atlas of invertebrate-pathogenic fungi of Thailand.** BIOTEC, NSTDA, Thailand.

Madelin, M.F. 1966. "Fungal parasites of insects". **Annual Review of Entomology.** 11: 423-448.

Mains, E.B. 1951. "Entomogenous species of *Hirsutella*, *Tilachlidium* and *Synnematium*". **Mycologia.** 43: 691-717.

Mains, E.B. 1954. "Species of *Cordyceps* on spiders". **Bulletin of the Torrey Botanical Club.** 81: 492-500.

Mains, E.B. 1957. "Species of *Cordyceps* parasitic on *Elaphomyces*". **Bulletin of the Torrey Botanical Club.** 84: 243-251.

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: 46-58.

Mao, X.B. and Zhong, J.J. 2004. "Hyperproduction of Cordycepin by two-stage dissolved oxygen control in submerged cultivation of medicinal mushroom *Cordyceps militaris* in bioreactors". **Biotechnology Progress.** 20: 1408-1413.

Massee, G. 1895. "A revision of the genus *Cordyceps*". **Annals of Botany.** 9: 1-44.

Mayr, E. 1983. **The Growth of Biological Thought: Diversity, Evolution and Inheritance.** Harvard University Press, Cambridge, Massachusetts.

McCoy, C.W., Samson, R.A., and Boucias, D.G. 1988. "Entomogenous fungi". In: **Handbook of Natural Pesticides, Vol. V: "Microbial Insecticides, Part A: Entomogenous Protozoa and Fungi".** (eds. C.M. Ignoffo and N B. Mandava). CRC Press, Boca Raton, Florida, USA:151-236.

Metchnikoff, E. 1879. **Diseases of the larva of the grain weevil. Insects harmful to agriculture [series]; Issue III, the grain weevil.** Published by the Commission attached to the Odessa Zemstvo Office for the investigation of the problem of insects harmful to agriculture. Odessa.

Miller, A.N. and Huhndorf, S.M. 2004. "A natural classification of *Lasiosphaeria* based on nuclear LSU rDNA sequences". **Mycological Research.** 108: 26-34.

Milner, R.J. 1997. "Prospects for biopesticides for aphid control". **Entomophaga.** 42: 227-239.

Milner, R.J. and Prior, C. 1994. "Susceptibility of Australian plague locust, *Chortoicetes terminifera*, and wingless grasshopper, *Phaulacridium vittatum*, to the fungi *Metarhizium* spp.". **Biological Control.** 4: 132-137.

Moritz, C. and Hillis, D.M. 1996. "Molecular Systematics: Context and Controversies". In: **Molecular Systematics.** (eds. D.M. Hillis, C. Mortiz and K.B. Mable). Sinauer Associates, Inc., Sunderland, Massachusetts, USA: 1-10.

Moss, S.T. 1979. "Commensalism of the *Trichomycetes*". In: **Insect-Fungus Symbioses.** (ed. L.R. Batra). Allenheld Osmum, N.J.: 175-227.

Mugnai, L., Bridge, P.D. and Evans, H.C. 1989. "A chemotaxonomic evaluation of the genus *Beauveria*". **Mycological Research.** 92: 199-209.

Muller, W.E.G., Seihard, G., Beyer, R., Breter, H.J., Maidhof, A. and Zahn, R.K. 1977. "Effects of cordycepin on nucleic acid metabolism in L5178Y cells and on nucleic acid-synthesizing enzyme systems". **Cancer Research.** 37: 3824-3833.

Namba, T. 1993. **The Encylopedia of Wakan-Yaku (Traditional Sino-Japanese Medicine) with Color Pictures.** Hoikusha. Osaka, Japan.

Nan, J.X., Park, E.J., Yang, B.K., Song, C.H., Ko, G. and Sohn, D.H. 2001. "Antifibrotic effect of extracellular biopolymer from submerged mycelial cultures of *Cordyceps militaris* on liver fibrosis induced by bile duct ligation and scission in rats". **Archives of Pharmacol Research.** 24: 327-332.

Nei, M. 1996. "Phylogenetic analysis in molecular evolutionary genetics". **Annual Review of Genetics.** 30: 371-403.

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

Nilanonta, C., Isaka, M., Chanphen, R., Thong-orn, N., Tanticharoen, M. and Thebtaranonth, Y. 2003. "Unusual enniatins by the insect pathogenic fungus *Verticillium hemipterigenum*: isolation and studies on precursor-directed biosynthesis". **Tetrahedron.** 59: 1015-1020.

Nilanonta, C., Isaka, M., Kittakoop, P., Palittapongpaisran, P., Kamchonwongpaisan, S., Pittayakhajonwut, D., Tanticharoen, M. and Thebtaranonth, Y. 2000. "Antomycobacterial and antiplasmodial cyclodepsipeptides from the insect pathogenic fungus *Paecilomyces tenuipes* BCC 1614". **Planta Medica.** 66: 756-758.

Nisbet L.J. and Porter, N. 1989. "The impact of pharmacology and molecular biology on the exploitation of microbial prducts". In: **Symposium for Society of General Microbiology.**

Vol. 44. (eds. S. Baumberg, I. Hunter and M. Rhodes). Cambridge University Press, Cambridge, UK: 309-342.

Nylander, J.A.A. 2004. **MrModeltest v2. Program distributed by the author.** http://www.ebc.uu.se/systzoo/staff/nylander.html. Evolutionary Biology Centre, Uppsala University.

O'Donnell, K. and Cigelnik, E. 1997. "Two Divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous". **Molecular Phylogenetics and Evolution.** 7: 103-116.

Obornik, M., Jirku, M. and Dolezel, D. 2001. "Phylogeny of mitosporic entomopathogenic fungi: is the genus *Paecilomyces* polyphyletic?". **Canadian Journal of Microbiology.** 47: 813-819.

O'Donnell, K.L., Common, R.S. and Imshaug, H.A. 1977. "A new species of *Torrubiella* on a spider from the Falkland Islands". **Mycologia.** 69: 618-622.

Odum, E.P. 1971. **Fundamentals of Ecology.** 3rd edn. WB Saunders, Philadelphia, PA.

Ohmori, T., Tamura, K., Tsuru, S. and Nomoto, K. 1986. "Antitumor activity of protein bound polysaccharide from *Cordyceps ophioglossoides* in mice". **Japan Journal of Cancer Research.** 77: 1256-1263.

Pacioni, G. and Frizzi, G. 1978. "*Paecilomyces farinosus*, the conidial state of *Cordyceps memorabilis*". **Canadian Journal of Botany.** 56: 391-394.

Page, R.D.M. 1996. "Treview: An application to display phylogenetic trees on personal computers". **Computer Applications in the Biosciences.** 12: 357-358.

Pathan, A.A.K., Devi, K.U., Vogel, H. and Reineke, A. 2007. "Analysis of differential gene expression in the generalist entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin grown on diferent insect cuticular extracts and synthetic medium through cDNA-AFLPs". **Fungal Genetics and Biology.** 44: 1231-1241.

Pegler, D.N., Yao, Y.J. and Li, Y. 1994. "The Chinese 'caterpillar fungus'". **Mycologist.** 8: 3-5.

Petch, T. 1921. "Studies in entomogenous fungi. II. The genera of *Hypocrella* and *Aschersonia*". **Annals of the Royal Botanic Gardens Peradeniya.** 7: 167-278.

Petch, T. 1923. "Studies in the entomogenous fungi III. *Torrubiella*". **Transaction of the British Mycological Society.** 9: 108-128.

Petch, T. 1924. "Studies in entomogenous fungi IV. Some Ceylon *Cordyceps*". **Transactions of the British Mycological Society.** 10:28-45.

Petch, T. 1925. "Entomogenous fungi and their use in controlling insect pests". **Bulletin of the Department of Agriculture Ceylon.** 7: 1-40.

Petch, T. 1931. "New species of fungi collected during the Whitby foray". **The Naturalist.** 1931: 101-103.

Petch, T. 1931. "Notes on entomogenous fungi 1-21". **Transaction of the British Mycological Society.** 16: 55-75.

Petch, T. 1932a. "Notes on entomogenous fungi 22-48". **Transaction of the British Mycological Society.** 16: 209-245.

Petch, T. 1932b. "Notes on entomogenous fungi. 101-134". **Transactions of the British Mycological Society.** 21: 34-67.

Petch, T. 1932c. "Notes on entomogenous fungi. 181-200". **Transactions of the British Mycological Society.** 27: 81-93.

Petch, T. 1933. "Notes on entomogenous fungi 49-75". **Transaction of the British Mycological Society.** 18: 48-75.

Petch, T. 1935. "Notes on entomogenous fungi 76-100". **Transaction of the British Mycological Society.** 19: 161-194.

Petch, T. 1937. "Notes on entomogenous fungi 101-134". **Transaction of the British Mycological Society.** 21: 34-67.

Petch, T. 1939. "Notes on entomogenous fungi 135-160". **Transaction of the British Mycological Society.** 23: 127-148.

Petch, T. 1942. "Notes on entomogenous fungi 161-180". **Transaction of the British Mycological Society.** 25: 250-265.

Petch, T. 1944. "Notes on entomogenous fungi 181-200". **Transaction of the British Mycological Society.** 27: 81-93.

Petch, T. 1948. "A revised list of British entomogenous fungi". **Transactions of the British Mycological Society.** 31: 286-304.

Petch, T. 1948. "A revised list of British entomogenous fungi". **Transaction of the British Mycological Society.** 31: 286-304.

Peterson, S.W. 1993. "Molecular genetic assessment of relatedness of *Penicillium* subgenus *Penicillium*". In: **The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation**

in Fungal Systematics. (eds. D.R. Reynolds and J.W. Taylor). Wallingford: CAB International: 121-128.

Photita, W., Taylor, P.W.J., Ford, R., Hyde, K.D. and Lumyong, S. 2005. "Morphological and molecular characterization of *Colletotrichum* species from herbaceous plants in Thailand". **Fungal Diversity.** 18: 117-133.

Poprawski, T.J., Riba, G., Jones, W.A. and Aioun, A. 1988. "Variation in isoesterase profiles of geographic populations of *Beauveria bassiana* (*Dueteromycotina*: *Hyphomycetes*) isolates from *Sitona* weevils (Coleoptera: *Curculionidae*". **Environmental Entomology.** 17: 275-279.

Rannala, B. and Yang, Z. 1996. "Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference". **Journal of Molecular Evolution.** 43: 304-311.

Raper, K.B. and Fennell, D.I. 1965. **The Genus *Aspergillus*.** Williams & Wilkins, Baltimore.

Réaumur, M. de. 1726. "Remarques sur la plante appellée a la Chine Hia Tsao Tom Tchom, ou plante ver.". **Mem. Acad. Roy. Sci.**: 302-5.

Reed, C.F. and Farr, D.F. 1993. **Index to Saccardo's Sylloge Fungorum Volumes I-XXVI IN XXIX 1882-1972.** Reed Herbarium, Darlington, Maryland.

Rehner, S.A. 2005. "Phylogenetics of the insect pathogenic genus *Beauveria*". In: **Insect-fungal associations ecology and evolution.** (eds. F.E. Vega and M. Blackwell). Oxford University Press, Inc., New York, USA: 3-27.

Rehner, S.A. and Buckley, E. 2005. "A *Beauveria* phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs". **Mycologia.** 97: 84-98.

Rehner, S.A. and Samuels, G.J. 1995. "Molecular systematics of the *Hypocreales*: a teleomorph gene phylogeny and the status of their anamorphs". **Canadian Journal of Botany.** 73: S816-S823.

Riba, G., Couteaudier, Y., Maurer, P. and Neuve'glise, C. 1994. "Molecular methods offer a new challenge for fungal bioinsecticides". In: **Proceeding of VIth International Colloquium on Invertebrate Pathology and Microbial Control.** Montpellier, France, 28 Aug-2 Sept 1994: 16-22.

Roberts, D.W. and Hajek, A.E. 1992. "Entomopathogenic fungi as bioinsecticides". In: **Frontiers of Industrial Mycology**. (ed. G.F. Leatham). New York, Chaman and Hall: 144-159.

Roffey, J. 1968. "The occurrence of the fungus *Entomophthora grylli* Fresenius on locusts and grasshoppers in Thailand". **Journal of Invertebrate Pathology**. 11: 237-241.

Rogers, J.S. and Swofford, D.L. 1998. "A fast method for approximating maximum likelihoods of phylogenetic trees from nucleotide sequences". **Systematic Biology**. 47: 77-89.

Rojas, T., Pons, N. and Arnal, E. 1998. "*Cladosporium herbarum* on whiteflies (Homoptera: *Aleyrodidae*) in Venezuela". **Boletin Entomology Venezuela, Series Monog**. 13: 57-65.

Rossman, A.Y. 1994. "A strategy for an all-taxa inventory of fungal biodiversity". In: **Biodiversity and Terrestrial Ecosystems.** (eds. C.I. Peng and C.H. Chou). Institute of Botany, Academia Sinica Monograph Series 14: 169-194.

Saccardo, P.A. 1882-1931. **Sylloge Fungorum Omnium Cognitorum**. 25 Volumes. Pavia, Italy.

Saccardo, P.A. 1972. **Sylloge Fungorum**: volume 26 (Index of fungi).

Samson R.A. and Evans H.C. 1974. "Notes on entomogenous fungi from Ghana. II. The genus *Akanthomyces*". **Acta Botanica Neerlandica**. 23: 28-35.

Samson, R.A. 1974. "*Paecilomyces* and some allied hyphomycetes". **Studies in Mycology.** 6: 1-119.

Samson, R.A. and Evans, H.C. 1973. "Notes on entomogenous fungi from Ghana. I. The genera *Gibellula* and *Pseudogibellula*". **Acta Botanica Neerlandica**. 22: 522-528.

Samson, R.A. and Evans, H.C. 1975. "Notes on entomogenous fungi from Ghana. III. The genus *Hymenostilbe*". **Proceedings, Koninklijke Nederlandse Akadamie van Wetenschappen, Series.** C 78: 73-80.

Samson, R.A. and Evans, H.C. 1982. "Two new *Beauveria* spp. From South America". **Journal of Invertebrate Pathology**. 39:93-97.

Samson, R.A. and Evans, H.C. 1992. "New species of *Gibellula* on spiders (Araneida) from South America". **Mycologia**. 84: 300-314.

Samson, R.A., Evans, H.C. and Latgé, J.P. 1988. **Atlas of Entomopathogenic Fungi.** Springer-Verlag, Berlin, Heidelberg. New York.

Samson, R.A., Seifert, K.A., Kuijpers, A.F.A., Houbraken, J.A.M.P. and Frisvad, J.C. 2004. "Phylogenetic analysis of *Penicillium* subgenus *Penicillium* using partial Beta-tubulin sequences". **Studies in Mycology**. 49: 175-200.

Samson, R.A., van Reenen-Hoekstra, E.S. and Evans, H.C. 1989. "New species of *Torrubiella* (Ascomycotina: *Clavicipitales*) on insects from Ghana". **Studies in Mycology**. 31: 123-132.

Saunders, G.W. 2005. "Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications". **Philosophical Transactions of the Royal Society of London Series**. B 360: 1879-1888.

Schumacher, T. 1982. "Ascomycetes from Northern Thailand". **Nordic Journal of Botany**. 2: 257-263.

Schumacher, T. and Holst-Jensen, A. 1997. "A synopsis of the genus *Sclerotinia* (= *Verpatinia*) (Ascomycotina: *Helotiales*: *Sclerotiniaceae*)". **Mycoscience**. 38: 55-69.

Seephonkai, P., Isaka, M., Kittakoop, P., Boonudomlap, U. and Thebtaranonth, Y. 2004. A novel ascochlorin from the insect pathogenic fungus *Verticillium hemipterigenum* BCC 2370". **Journal of Antibiotics**. 57: 10-16.

Seifert, K.A. 1985. "A Monograph of *Stilbella* and some allied *Hyphomycetes*". **Studies in Mycology**. 27: 1-235.

Seifert, K.A., Samson, R.A., deWaard, J.R., Houbraken, J., Lévesque, C.A., Moncalvo, J., Louis-Seize, G. and Hebert, P.D.N. 2007. "Prospects for fungus identification using CO1 DNA barcodes, with *Penicillium* as a test case". **Proceedings of the National Academy of Sciences of the United States of America**. 104: 3901-3906.

Shahed, A.R., Kim, S.I. and Shoskes, D.A. 2001. "Down-regulation of apoptotic and inflammatory genes by *Cordyceps sinensis* extract in rat kidney following ischemia/reperfusion". **Transplantation Proceedings**. 33: 2986-2987.

Shearer, C.A., Raja, H.A. and Schmit, J.P. 2007. **Freshwater *Ascomycetes* and their anamorphs** - website available online at http://www.life.uiuc.edu/fungi/

Shenoy, B.D., Jeewon, R. and Hyde, K.D. 2007. "Impact of DNA sequence-data on the taxonomy of anamorphic fungi". **Fungal Diversity**. 26: 1-54.

Shenoy, B.D., Jeewon, R., Wu, W.P., Bhat, D.J. and Hyde, K.D. 2006. "Ribosomal and RPB2 DNA sequence analyses suggest that *Sporidesmium* and morphologically similar genera are polyphyletic". **Mycological Research**. 110: 916-928.

Shiao, M.S., Lin, L.J., Lien, C.Y., Tzean, S.S. and Lee, K.R. 1989. "Natural products in *Cordyceps*". **Proceedings of the National Science Council.** Republic of China 13: 382-387.

Shimazu, M., Mitsuhashi, W. and Hashimoto, H. 1988. "*Cordyceps brongniartii* sp. nov., the teleomorph of *Beauveria brongniartii*". **Transactions of the Mycological Society of Japan.** 29: 323-330.

Shimizu, D. 1994. **Color iconography of vegetable wasps and plant worms.** Seibundo Shinko-sha, Tokyo.

Shin, K.H., Lim, S.S., Lee, S., Lee, Y.S., Jung, S.H. and Cho, S.Y. 2003. "Anti-tumor and immuno-stimulating activites of the the fruiting bodies of *Paecilomyces japonica*, a new type of *Cordyceps* spp. ". **Phytotherapy Research.** 17: 830-833.

Sivanesan, A. 1984. **The Bitunicate Ascomycetes and their Anamorphs.** Strauss and Cramer, Vaduz, Germany.

Spatafora, J.W., Sung, G.H., Sung, J.M., Hywel-Jones, N.L. and White, J.F. Jr. 2007. "Phylogenetic evidence for an animal pathogen origin for ergot and the grass endophytes". **Molecular Ecology.** 16: 1701-1711.

St. Leger, R.J. and Joshi, L. 1997. "The application of molecular techniques to insect pathology with empahsis on entomopathogenic fungi". In: **Manual of Techniques in Insect Pathology.** (ed. L.A. Lacey). Academic Press, London: 367-394.

St. Leger, R.J. and Roberts, D.W. 1997. "Engineering improved mycoinsecticides". **Trends in Biotechnology.** 15: 83-85.

Steinhaus, E.A. 1956. "Microbial control—the emergence of an idea, a brief history of insect pathology through the nineteenth century". **Hilgardia.** 26: 107-160.

Steinhaus, E.A. 1975. **Disease in a Minor Cord.** Ohio State Univ. Press, Columbus, OH.

Steinkraus, D.C. and Whitfield, J.B. 1994. "Chinese caterpillar fungus and world record runners". **American Entomologist.** 40: 235-239.

Steinkraus, D.C. and Hollingsworth, R.G. 1994. "Predicting fungal epizootics on cotton aphids". **Arkansas Farm Research.** 46: 10-11.

Steinkraus, D.C., Hollingsworth, R.G. and Slaymaker, P.H. 1995. "Prevalence of *Neozygites fresenii* (*Entomophthorales*: *Neozygitaceae*) on cotton aphids (Homoptera: A*phididae*) in Arkansas cotton". **Environmental Entomology.** 24: 465-474.

Steinkraus, D.C., Kring, T.J. and Tugwell, N.P. 1991. "*Neozygites fresenii* in *Aphis gossypii* on cotton". **Southwestern Entomologist.** 16: 118-123.

Stensrud, O., Hywel-Jones, N.L. and Schumacher, T. 2005. "Towards a phylogenetic classification of *Cordyceps*: ITS nrDNA sequence data confirm divergent lineages and paraphyly". **Mycological Research.** 109: 41-56.

Subramanian, C.V. 1983. **Hyphomycetes: Taxonomy and Biology.** Academic Press, London.

Sugiyama, J. 1987. **Pleomorphic Fungi: The Diversity and Its Taxonomic Implications.** Kodansha Ltd., Tokyo.

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

Sung, G.H., Sung, J.M., Hywel-Jones, N.L. and Spatafora, J.W. 2007. "A multi-gene phylogeny of *Clavicipitaceae* (*Ascomycota*, Fungi): Identification of localized incongruence using a combinational bootstrap approach". **Molecular Phylogenetics and Evolution.** 44: 1204-1223.

Sutton, B.C. and Hennebert, G.L. 1994. "Interconnections amongst anamorphs and their possible contribution to ascomycete systematics". In: **Ascomycete Systematics: Problems and Perspectives in the Nineties.** (ed. D.L. Hawksworth). Plenum Press, New York: 77-100.

Swofford, D.L. 2002. **PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods) Version 4.0b10.** Sinauer Associates, Sunderland, Massachusetts USA.

Tang, W. and Eisenbrand, G. 1992. **Chinese Drugs of Plant Origin.** Springer-Verlag.

Tanticharoen, M. 2003. **Bioresources initiatives: Thailand.** Paper presented at the Asia Pacific Natural Products Conference: Regulations, Developments and Drug Discovery held in Bangkok on September 29-30, 2003.

Tateno, Y., Nei, M. and Tajima, F. 1982. "Accuracy of estimated phylogenetic trees from molecular data. I. Distantly related species". **Journal of Molecular Evolution.** 18: 387-404.

Taylor, J.W. 1993. "A contemporary view of the holomorph: nucleic acid sequence and computer databases are changing fungal classification". In: **The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics.** (eds. D.R. Reynolds and J.W. Taylor). Wallingford: CAB International: 3-13.

Taylor, J.W. 1995. "Making the Deuteromycota redundant: a practical integration of mitosporic and meiosporic fungi". **Canadian Journal of Botany**. 73: S754-S759.

The Anamorph-Teleomorph database. 2007. **An online database maintained by Centraalbureau voor Schimmelcultures (CBS).** Available at www.cbs.knaw.nl/databases.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. 1997. "The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools". **Nucleic Acids Research**. 25: 4876-4882.

Thungrabeab, M., Blaeser, P. and Sengonca, C. 2006. "Possibilities for biocontrol of the onion thrips *Thrips tabaci* Lindeman (Thys., Thripidae) using different entomopathogenic fungi from Thailand. MITT". **Deutsche Gesellschaft für Allgemeine und Angewandte Entomologie**. 15: 299-304.

Townsend, R.J., Glare, T.R. and Willoughby, B.E. 1995. "The fungi *Beauveria* spp. causes grass grub population collapse in some Waikato pastures". **Proceedings of the 48th New Zealand Plant Protection Conference.** 48: 237-241.

Tubaki, K. 1981. ***Hyphomycetes* - their perfect-imperfect connexions**. J. Cramer, Vaduz.

Tzean, S.S., Hsieh, L.S. and Wu, W.J. 1997. **Atlas of Entomopathogenic Fungi from Taiwan.** Council of Agriculture, Executive Yuan, Taiwan.

Vaux, D.L. and Korsmeyer, S.J. 1999. **Cell death in development.** Cell 96: 245-254.

Vuillemin, P. 1912. "*Beauveria*, nouveau genre de Verticilliacées". **Bulletin de la societe Botanique de France** 29: 34-40.

Wang, S.Y. and Shiao, M.S. 2000. "Pharmacological functions of Chinese medicinal fungus *Cordyceps sinensis* and related species". **Journal of Food and Drug Analysis**. 8: 248-257.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. and Hebert, P.D.N. 2005. "Barcoding Australia's fish species". **Philosophical Transactions of the Royal Society of London.** B 360: 1847-1857.

Watts, P., Kittakoop, P., Veeranondha, S., Wanaitith, S., Thongwichian, R., Saisaha, P., Intama, S. and Hywel-Jones, N.L. 2003. "Cytotoxicity against insect cells of entomopathogenic fungi of the genera *Hypocrella* (anamorph *Aschersonia*): possible agents for biological control". **Mycological Research.** 107: 581-586.

White, T.J., Bruns, T., Lee, S. and Taylor, J. 1990. "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics". In: **PCR Protocols: A Guide to Methods**

and Applications (eds. M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White). Academic Press San Diego: 315-322.

Won, S.Y. and Park, E.H. 2005. "Anti-inflammatory and related pharmacological activities of cultured mycelia and fruiting bodies of *Cordyceps militaris*". **Journal of Ethnopharmacology.** 96: 555-561.

Wraight, S., Ramos, M., Williams, J., Avery, P., Academy, L.E.E., Jaronski, S. and Vandenberg, J. 2003. "Comparative virulence and host specificity of *Beauveria bassiana* isolates assayed against lepidopteran pests of vegetable crops". **Society for Invertebrate Pathology Annual Meeting Proceedings**: 36.

Wu, Z.L., Wang, X.X. and Cheng, W.Y. 2000. "Inhibitory effect of *Cordyceps sinensis* and *Cordyceps militaris* on human glomerular mesangial cell proliferation induced by native LDL". **Cell Biochemistry and Function.** 18: 93-97.

www.mushtech.org **Entomopathogenic Fungal Culture Collection.**

Yamada, H., Kawaguchi, N., Ohmori, T., Takeshita, Y., Taneya, S. and Yamazaki, T. 1984. "Structure and antitumor activity of alkali-soluble polysaccharide from *Cordyceps ophioglossoides*". **Carbohydrate Research.** 125: 107-115.

Yoo, H.S., Shin, J.W., Cho, J.H., Son, C.G., Lee, Y.W., Park, S.Y. and Cho, C.K. 2004. "Effects of *Cordyceps militaris* extract on angiogenesis and tumor growth". **Pharmacologica Sinica.** 25: 657-665.

Yu, R., Wang, L., Zhang, H., Zhou, C. and Zhao, Y. 2004. "Isolation, purification and identification of polysaccharides from cultured *Cordyceps militaris*". Fitoterapia 75: 662-666.

Zare, R. and Gams, W. 2001. "A revision of *Verticillium* sect. Prostrata. IV. The genera *Lecanicillium* and *Simplicillium* gen. nov.". **Nova Hedwigia.** 73: 1-50.

Zhang, S., Zhang, D., Zhu, T. and Chen, X. 1991. "A pharmacological analysis of the amino acid components of *Cordiceps sinensis*". **Acta Pharmacologica Sinica.** 26: 326-330.

Zhaxybayeva, O. and Gogarten, J.P. 2002. "Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses". **Genomics.** 3: 1-15.

Zheng, Z., Lee, J.E. and Yenari, M.A. 2003. "Stroke: molecular mechanisms and potential targets for treatment". **Current Molecular Medicine.** 3: 361-372.

Zhu, J.S., Halpern, G.M. and Jones, K. 1988a. "The scientific rediscovery of an ancient Chinese herbal medicine: *Cordyceps sinensis*: part I". **The Journal of Alternative and Complementary Medicine.** 4:289-303.

Zhu, J.S., Halpern, G.M. and Jones, K. 1988b. "The scientific rediscovery of a precious ancient Chinese herbal regimen: *Cordyceps sinensis*: part II". **The Journal of Alternative and Complementary Medicine.** 4:429-457.

Zimmermann, G. 1993. "The entomopathogenic fungus *Metarhizium anisopliae* and its potential as a biocontrol agent". **Pesticide Science.** 37: 375-379.

APPENDICES

Publications pertaining to thesis

Aung, O.M., Kang, J.C., Liang, Z.Q., Soytong, K. and Hyde, K.D. (2006). *Cordyceps mrciensis* sp nov from a spider in Thailand. Mycotaxon 97:235-240.

Aung, O.M., Kang, J.C., Liang, Z.Q., Soytong, K. and Hyde, K.D. (2006). A new entomopathogenic species, *Hymenostilbe furcata,* parasitic on a hemipteran nymph in northern Thailand. Mycotaxon 97:241-245.

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

MYCOTAXON

Volume 97, pp. 235–240 *July–September 2006*

Cordyceps mrciensis sp. nov. from a spider in Thailand

OHNMAR MYO AUNG[1, 2], JI CHUAN KANG[3*],
ZONG QI LIANG[4], KASEM SOYTONG[2] & KEVIN DAVID HYDE[5]

**Jichuank@yahoo.co.uk*

[1] Biocontrol Research Unit and Mycology Section
Department of Plant Pest Management, Faculty of Agricultural Technology
King Mongkut's Institute of Technology Ladkrabang(KMITL)
Bangkok 10520, Thailand

[2]Mushroom Research Foundation
128 Moo3, Bahn Pha Daeng, T. Pa Pae, A. Mae Taeng
Chiang Mai 50150, Thailand

[3]The Research Center of Biochemistry, Guizhou University
Guiyang 550025, Guizhou Province, PR China

[4]College of Biological Technology, Guizhou University
Guiyang 550025, Guizhou Province, PR China

[5]Centre for Research in Fungal Diversity
Department of Ecology & Biodiversity, The University of Hong Kong
Pokfulam Road, Hong Kong, PR China

Abstract—A fungus associated with a spider collected from the Mushroom Research Centre, Chiang Mai, Thailand was found to represent a new species of the genus *Cordyceps*. It is described as *C. mrciensis* sp. nov. *C. mrciensis* differs from other species occurring on spiders in that the stromata have a fertile part with a distinctive sterile appendage, superficial perithecia and ascospores that do not break into secondary partspores.

Key words—entomogenous fungi

Introduction

Cordyceps is a morphologically and ecologically well-defined group of parasites on arthropods (insects, spiders and mites) and hypogeous fungi (Kobayasi 1941, 1982, Mains 1954, 1957, Kobayasi & Shimizu 1960, 1977, Evans 1982, Zhang et al. 2004, Stensrud et al. 2005). This genus is one of the two most important genera of invertebrate pathogens (Hywel-Jones 2001) and is cosmopolitan in

* *Corresponding author*

distribution (Hawksworth et al. 1995). Kirk et al. (2001) suggested that there are 100 *Cordyceps* species, although 280 species were listed by Kobayasi (1982). According to Index Fungorum (www. Indexfungorum. org), more than 500 epithets are assigned to *Cordyceps*, however, many are known to be taxonomic synonyms.

In Thailand, 26 species of *Cordyceps* have been identified, including four species on spiders (Hywel-Jones 2001). Kobayasi (1962) recorded five *Cordyceps* species parasitizing spiders (Arachnida) worldwide. Mains (1954) listed eight species of *Cordyceps* known to parasitize spiders.

While collecting entomogenous fungi in northern Thailand forests, a new *Cordyceps* species was found parasitizing a spider. This species is distinct from all other *Cordyceps* species and represents a novel taxon.

Materials and methods

Collections were made at the Mushroom Research Centre (MRC) in northern Thailand. Soil, litter, herbs, and trees, including the under sides of leaves were examined and dead and infected insects were collected. Specimens were stored in plastic containers and transported on the same day to the laboratory for identification. The holotype is now deposited in the Thai Mycological Association Herbarium (TMAH).

Taxonomic description

Cordyceps mrciensis Aung, J.C. Kang, Z.Q. Liang, Soytong & K.D. Hyde sp. nov. [MB 510252] FIGURES 1 & 2

Stromata e abdomine hospitis oriunda, ramosa, filiformia, 5-12 mm longa. Pars fertilis nigrescens. Appendix apicalis filiformis 4 mm longa. Perithecia superficialia, elongata vel ellipsoidea, 210-375 × 150-180 µm. Asci 135-306 × 9-15 µm, capitibus 5.4-8.4 µm in diam. Ascosporae 185-435 × 3-5 µm, multiseptatae, cellulis 3.6-21 µm longis, non-separabilis.

Etymology: mrciensis = refers to the Mushroom Research Centre (MRC), the locality where the specimen was found.

Holotype: Thailand, Chiang Mai, Mae Taeng, T. Pa Pae, Bahn Pha Daeng, 128 Moo 3, Mushroom Research Centre, from spider (Arachnida) attached to a rotten bamboo culm, 17 September 2005, Ohnmar Myo Aung TMAH 0001. The holotype is deposited in Thai Mycological Association Herbarium (TMAH).

Stomata arising from abdomen of infected spider, filiform, 5-12 mm long, light brown, branching. **Fertile part** black, with a 4 mm long sterile appendage. **Perithecia** superficial, elongate to ellipsoid, 210-375 × 150-180 µm, some with a short neck, about 120 × 30 µm. **Asci** filiform, 8-spored, 135-305 × 9-15 µm; caps of asci 4.2-6.6 µm high, 5.4-8.4 µm wide. **Ascospores** filiform, 185-435 × 3-5 µm, not breaking into secondary ascospores, septate at 3.6-21 µm intervals.

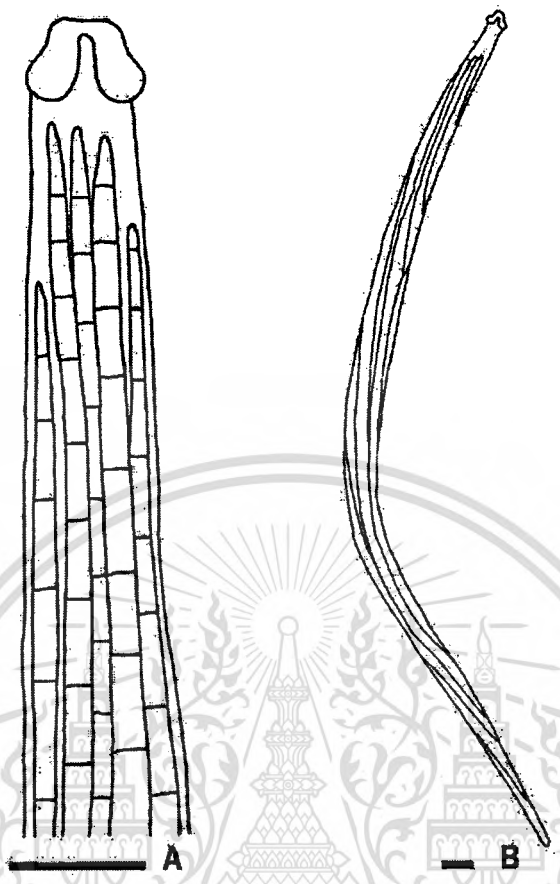


Fig 1. *Cordyceps mrciensis A. Upper part of* an ascus with mature ascospores. B. An ascus with filiform ascospores. Bars = 5 µm.

Discussion

Cordyceps mrciensis was associated with a single infected spider, attached to a rotten bamboo culm, collected at Mushroom Research Centre, Chiang Mai, Thailand.

Most *Cordyceps* species are believed to be specific to various arthropod groups, such as spiders with the degree of specificity differing from species to species (Nikoh & Fukatsu 2000). Therefore, our discussion will be based only on *Cordyceps* species associated with spiders (Arachnida).

According to Mains (1954) only eight species of *Cordyceps* have been recorded in association with spiders. *Cordyceps mrciensis* can be distinguished from these known species in having stromata with a fertile part and a stipe that continues as a distinctive sterile appendage, superficial perithecia and ascospores that do not break into partspores. There are only two species, *C. thaxteri* Mains and *C. engleriana* Henn., that have superficial perithecia. In *C. thaxteri* the perithecia are scattered, free, narrowly ovoid, and large (960-1200 x 300-360 µm, Mains,

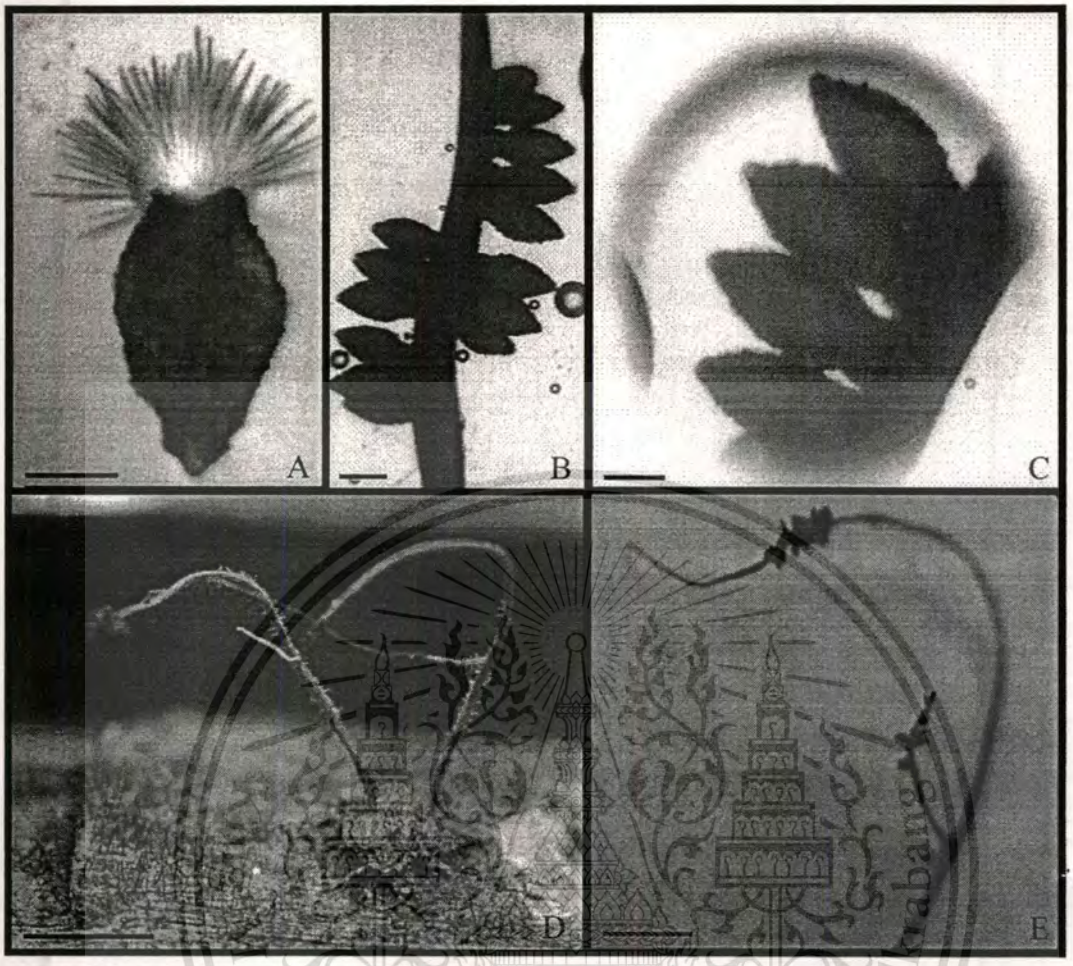


Fig 2. *Cordyceps mrciensis* (from holotype). A. A perithecium and asci. B. Superficial perithecia. C. Perithecia. D. Small spider bearing two stromata with superficial perithecia E. Appendage. Bars: A & C = 100 µm, B = 200 µm, D & E = 2.5 mm.

1954). The perithecia of *C. engleriana* are also superficial, but crowded at the apex of the stromata and ovoid or flask-shaped (Mains 1954). *Cordyceps mrciensis* also has superficial perithecia but they are elongate to ellipsoid, small, 210-375 × 150-180 µm and some have short necks. The ascospores of *C. thaxteri* and *C. engleriana* break into partspores, whereas those of *C. mrciensis* do not. *Cordyceps caloceroides* Berk. & M.A. Curtis and *C. grenadensis* Mains, also associated with spiders, posses ascospores that do not break into secondary partspores. *Cordyceps caloceroides* has immersed perithecia with slightly protruding ostioles, while *C. grenadensis* has partly imbedded, ovoid perithecia. The perithecia of *C. mrciensis* are entirely superficial and somewhat scattered on the stipe.

Besides the above characters, the distinctive fertile part of the stroma with a distinctive sterile appendage is sufficient to distinguish *C. mrciensis* from the known *Cordyceps* species from spiders (Table 1).

Table 1. Comparison of the characteristics of *Cordyceps* species associated with spiders

Species	Stroma	Perithecia (range in µm)	Ascospores (range in µm)	Reference
C. arachneicola	Cylindric, 50 × 2 mm	Completely embedded ellipsoid	-	Kobayasi 1941. Tokyo Bun. Daig. 5 no. 84: 123-125
C. caloceroides	Bright red, furcate, nearly 5 in long, ≤ 1 line thick	Immersed, prominent ostioles, ovoid, 215-250 × 100-150	Not breaking into partspores	Berk. & M.A. Curtis 1868. Jour. Linn. Soc. Bot. 10: 375
C. cylindrica	Cylindric, capitate, twisted-rounded apex 15 × 1.5-2.0 mm	Entirely embedded to the surface or at right angles to the surface 850-1200 × 220-270	-	Petch 1937. Trans British Myc. Soc. 21: 46
C. engleriana	Many, 15 × 0.25 mm	Superficial, crowded, free, ovoid or flask shaped, 600 × 300	Breaking into 22-25 × 1.5-2 µm cylindric fragments	Henn. 1897. Engler Bot. Jahrb. 23: 538
C. grenadensis	2, ovoid, cylindric 10-12 mm	Partly embedded, ovoid, 336-360 × 156-216	Not breaking into partspores	Mains 1954, Bull. Torrey Bot. Club 81: 492–500
C. ignota	Simple, branched, slender, 60 × 0.5-1.5 mm	Slightly embedded, very crowded, ovoid 100-140 × 60-75	-	Marchion. 1945. Physis 20: 17
C. mrciensis	2, branching, filiform, 5-12 mm	Superficial, elongate, ellipsoid, some with short neck, 210-375 × 150-180, 4 mm sterile appendage	Not breaking into partspores	sp. nov.
C. singeri	Clavate, subcapitate, 3-12 mm	Embedded, ovoid, 325-550 × 200-500	Breaking into one-cell segments- 3-4 × 0.7-1	Mains 1954, Bull. Torrey Bot. Club 81: 492–500
C. thaxteri	Subcylindric, 1.5-2.5 × 0.1-0.2 mm	Superficial, few, narrowly ovoid, 960-1200 × 300-360	Breaking into one-cell segments	Mains 1939. Jour. Elisha Mitchell Soc. 55: 120

Acknowledgements

We are grateful to Drs E.H.C. McKenzie and Wei Min Zhang for kindly reviewing the manuscript. The first author would like to acknowledge Lam M. D. for his kind assistance in collecting specimens and sharing experiences in the laboratory. She would like to extend her special thanks to Dr Edward Grand, Zhao Ruilin, Huyen T.T., Hanh T.M.T. and Iman Hidayat for their kind support in many ways during the field work.

Literature Cited

Evans HC. 1982. Entomogenous fungi in tropical forest ecosystems: an appraisal. Ecological Entomology 7: 47-60.

Hywel-Jones NL. 2001. The biological diversity of invertebrate pathogenic fungi. In "Biodiversity of Tropical Microfungi"ed. K.D. Hyde. Hong Kong University Press, Hong Kong. Pp. 107-120.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Ainsworth & Bisby's Dictionary of the Fungi. 9th edn. Commonwealth Mycological Institute: Kew, U.K. 655 pp.

Kobayasi Y. 1941. The genus *Cordyceps* and its allies. Tokyo Bunrika Daigaku, Science Report 5(84): 53-260.

Kobayasi Y. 1982. Keys to the taxa of the genera *Cordyceps* and *Torrubiella*. Transactions of the Mycological Society of Japan. 23: 329-364.

Kobayasi Y, Shimizu D. 1960. Monographic studies of *Cordyceps* 1. Group parasitic on *Elaphomyces*. Bulletin of the National Science Museum, Tokyo 5: 69-85.

Kobayasi Y, Shimizu, D. 1977. Some species of *Cordyceps* and its allies on spiders. Kew Bulletin 31: 557-566.

Mains EB. 1954. Species of *Cordyceps* on spiders. Bulletin of the Torrey Botanical Club 81: 492-500.

Mains EB. 1957. Species of *Cordyceps* parasitic on *Elaphomyces*. Bulletin of the Torrey Botanical Club 84: 243-251.

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

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.

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

Zhang WM, Li TH, Chen YQ, Qu LH. 2004. *Cordyceps campsosterna*, a new pathogen of *Campsosternus auratus*. Fungal Diversity 17: 239-242.

MYCOTAXON

Volume 97, pp. 241–245 *July–September 2006*

A new entomopathogenic species, *Hymenostilbe furcata*, parasitic on a hemipteran nymph in northern Thailand

Ohnmar Myo Aung[1, 2], Ji Chuan Kang[3*], Zong Qi Liang[4], Kasem Soytong[2] & Kevin David Hyde[5]

Jichuank@yahoo.co.uk

[1] *Biocontrol Research Unit and Mycology Section*
Department of Plant Pest Management, Faculty of Agricultural Technology
King Mongkut's Institute of Technology Ladkrabang(KMITL)
Bangkok 10520, Thailand

[2]*Mushroom Research Foundation, 128 Moo3, Bahn Pha Daeng, T. Pa Pae*
A. Mae Taeng, Chiang Mai 50150, Thailand

[3]*The Research Center of Biochemistry, Guizhou University*
Guiyang 550025, Guizhou Province, PR China

[4]*College of Biological Technology, Guizhou University*
Guiyang 550025, Guizhou Province, PR China

[5]*Centre for Research in Fungal Diversity: Ecology & Biodiversity*
The University of Hong Kong, Pokfulam Road, Hong Kong, PR China

Abstract—*Hymenostilbe furcata* sp. nov., parasitic on a hemipteran nymph in a northern Thailand forest is described and illustrated. Its morphology is compared with that of other species with forked denticles.

Key words—hyphomycete genus

Introduction

The entomopathogenic hyphomycete genus *Hymenostilbe* was introduced by Petch (1931) to accommodate *H. muscarium* Petch, a species parasitic on dipteran insects. It was described as having cylindrical synnemata covered by a hymenium-like layer of conidiogenous cells (Samson &Evans 1975). It was later found to be the anamorph of *Cordyceps forquignonii* Quél. (Petch 1948). *Hymenostilbe* species can be distinguished from *Akanthomyces* species, also parasitic on insects and spiders, as the conidia of *Akanthomyces* form in chains on phialides, while those of *Hymenostilbe* are solitary, polyblastic and form on a denticle (Petch 1932c, Mains 1950, Samson & Evans 1975). *Akanthomyces*

*Corresponding author

and *Hymenostilbe* produce synnemata that are more or less cylindric and often are somewhat attenuated towards the apex. In *Hymenostilbe* the synnemata are composed of more or less parallel, longitudinal hyphae, usually forming a compact bundle. The longitudinal hyphae produce conidiogenous cells at their ends, especially in the upper portions of the synnemata. Most of the conidiogenous cells, however, are produced either as lateral cells or frequently as terminal cells of short lateral branches produced along the entire length of the outer hyphae of the synnemata. This results in a hymenial layer that covers the surface of the synnemata. In most species there is abundant production of conidiogenous cells resulting in a compact hymenial layer. In some species the conidiogenous cells are scattered and well separated from each other (Mains 1950).

Samson & Evans (1975) reviewed *Hymenostilbe* accepting nine species and excluding 11 doubtful species. *Hymenostilbe* species parasitize arachnids and dipteran, orthopteran and hymenopteran insects. *Hymenostilbe longispora* Samson & H.C. Evans is commonly found on several ant species of the subfamilies Ponerinae and Myrmicinae. *H. ghanensis* Samson & H.C. Evans was collected on a spider. Several species of *Hymenostilbe* have been associated with a *Cordyceps* teleomorph. For instance, *H. dipterigena* Petch is the anamorph of *Cordyceps dipterigena* Berk. & Broome (Petch 1932a), *H. nutans* Samson & H.C. Evans is the anamorph of *C. nutans* Pat. and *H. fragilis* Petch is the anamorph of *C. uleana* Henn. (Petch 1932b). Three species of *Hymenostilbe* have been recorded in Thailand; *H. ventricosa* Hywel-Jones was rarely found as an entomopathogen of cockroach nymphs (Hywel-Jones 1995), while *H. aurantiaca* Hywel-Jones was found on formicine ants in the same location as *C.* cf. *myrmecophila* Ces. (Hywel-Jones 1996).

Based on the previous records and distinctive morphological characteristics, the fungus described in this paper is accommodated in *Hymenostilbe* as a new species.

Materials and methods

A general survey of entomopathogenic fungi was carried out in northern Thailand forests from May to October 2005. The collection sites included in this survey were Mushroom Research Centre (MRC), Doi Suthep National Park, Mokfa Waterfall and Toung Jaw Village, Chiang Mai. Soils, litter, herbaceous plants, and tree leaves were examined for dead insects, which were collected and transported the same day to the laboratory in plastic containers for identification and isolation. Conidial isolations were made on potato dextrose agar (PDA). The holotype is deposited in Thai Mycological Association Herbarium (TMAH).

Taxonomic description

Hymenostilbe furcata Aung, J.C. Kang, Z.Q. Liang, Soytong & K.D. Hyde **sp. nov.**

[MB 510253] FIGURES 1-2

Synnemata multiplicata, oriunda corpa, alba, cylindrica, 10-14 mm longa, 94-120 μm crassa. Cellulae conidiogenae 5-18 × 3.5-6.5 μm, polyblasticae, clavata vel cylindricae, sursum denticulis furcatis 0.6-2.4 μm longis dense obtectae. Conidia solitaria, levia, hyalina, fusiformis, 8.5-15 μm longa, 3- 4.5 μm crassa.

Etymology: The species name refers to the forked sterigmata-like projections from the conidiogenous cells.

Holotype: Thailand, Chiang Mai, Mae Taeng, T. Pa Pae, Bahn Pha Daeng, 128 Moo 3, Mushroom Research Centre, from hemipteran nymph (Hemiptera) attached to the underside of a leaf in forest, 25 June 2005, Ohnmar Myo Aung TMAH 0002.

Synnemata slender, 10-14 mm long, 94-120 μm wide, arising from head and thorax of insect, cylindrical, white; central core of parallel hyphae composed of cells 3-55 × 2.5-4 μm; covered by an outer hymenium-like layer of conidiogenous cells with basal cells 7.5-20 × 2.5-5 μm. Conidiogenous cells 5-18 × 3.5-6.5 μm, polyblastic, clavate or cylindrical, apically with 2-7 furcellate denticles, 0.6-2.4 μm. Conidia 8.5-15 × 3- 4.5 μm, solitary, smooth, hyaline, fusiform.

Unfortunately, attempts to culture *H. furcata* on agar were unsuccessful.

Discussion

Species in the entomopathogenic genus *Hymenostilbe* are rarely encountered in the tropics (Hywel-Jones 1995). *Hymenostilbe furcata* was collected only once on a hemipteran nymph in the rain forests in Thailand. It can be separated from *H. sulphurea* and *H. nutans*, which also occur on hemipteran insects, by the creamy white synnemata and the two to seven, forked denticles on the conidiogenous cells. *Hymenostilbe sulphurea* Samson & H.C. Evans has sulphur-yellow synnemata and subglobose to ellipsoidal, rough-walled conidia, while *H. furcata* has smooth, fusiform conidia. *Hymenostilbe nutans* has fusoid conidia but they are smaller than those of *H. furcata* (6-10 x 3.2-4 μm vs. 8.5-15 x 3-4.5 μm). The conidiogenous cells of *H. furcata* are clavate or cylindrical while those of *H. nutans* are cylindrical, apically pointed and the denticles are crowded at the apex. The conidiogenous cells of *H. furcata* are 5-18 μm long × 3.5-6.5 μm wide, whereas those of *H. nutans* are 15-24 μm long × 4.5-6.5 μm wide. Those of *H. sulphurea* are cylindrical to clavate, 15-25 x 5-6.5 μm and the denticles are crowded at the apex (Samson & Evans 1975).

244

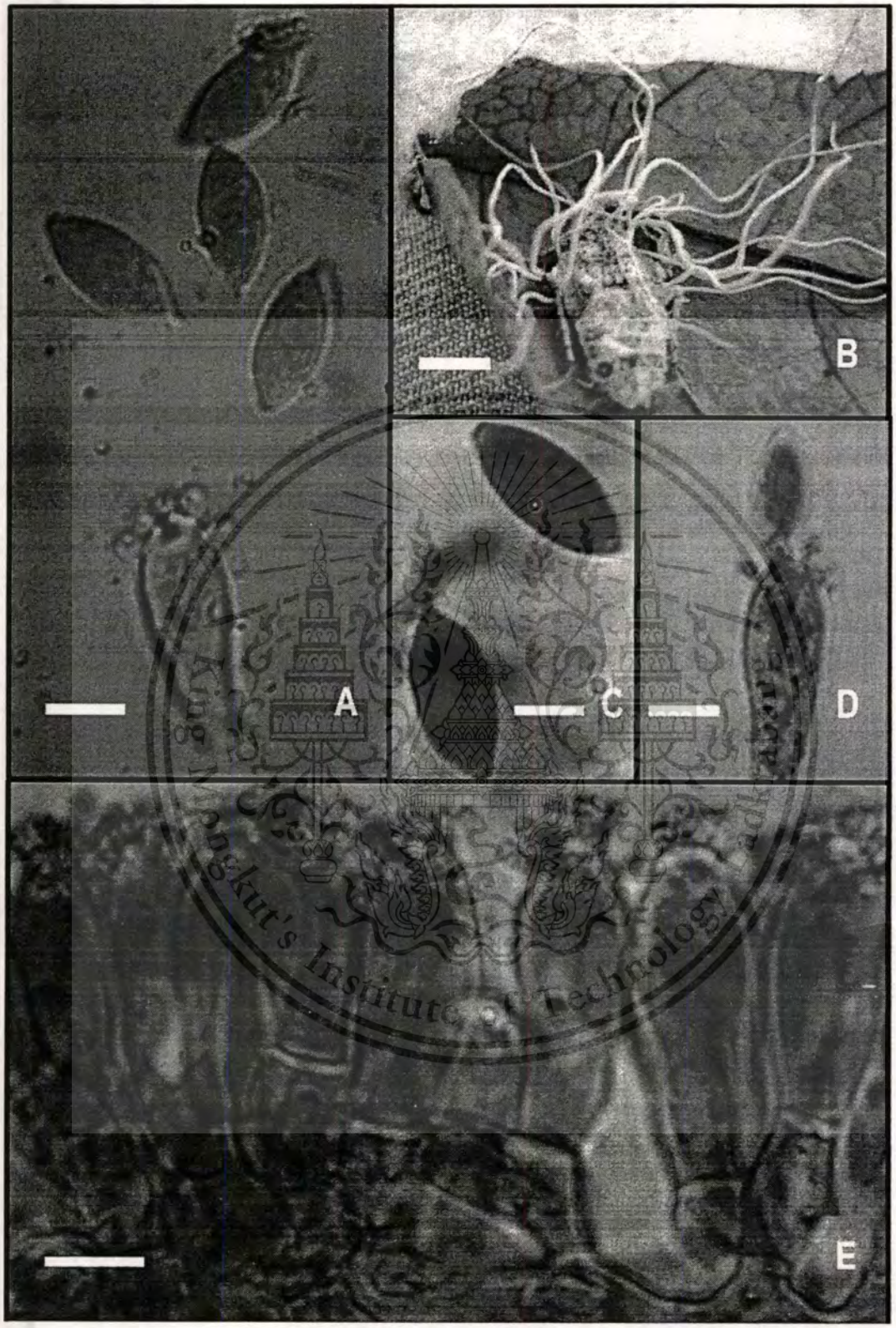


Fig.1: *Hymenostilbe furcata* (from holotype). A. Detached conidia. B. Infected hemipteran insect with synnemata. C. Conidia D. Conidiogenous cell with forked denticles and conidium. E. Conidiogenous cells forming a hymenium-like layer. Scale bars: A, C, D & E = 5 μm, B = 5 mm.

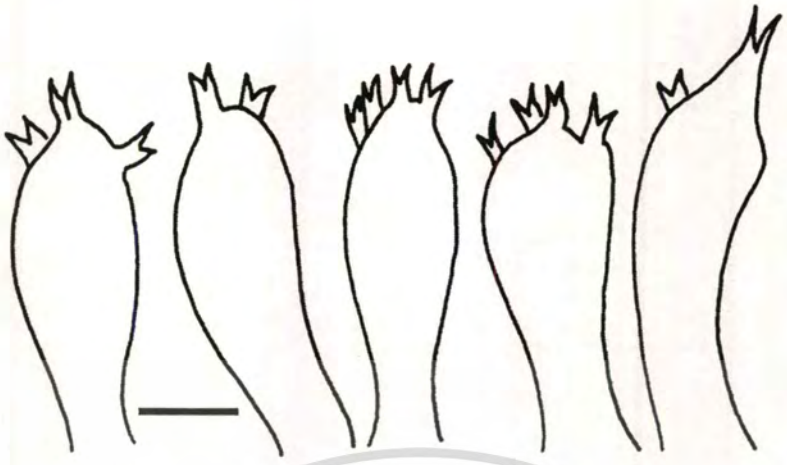


Fig. 2: Conidiogenous cells of *Hymenostilbe furcata* (from holotype). Scale bar = 5 µm.

Acknowledgements

We are grateful to Dr. E.H.C. McKenzie and Dr. Wei Min Zhang for presubmittal review of the manuscript.

Literature cited

Hywel-Jones NL. 1995. *Hymenostilbe ventricosa* sp. nov. a pathogen of cockroaches in Thailand. Mycological Research 99, 1201-1204.

Hywel-Jones NL. 1996. *Cordyceps myrmecophila*-like fungi infecting ants in the leaf litter of tropical forest in Thailand. Mycological Research 100, 613-619.

Mains EB. 1950. Entomogenous species of *Akanthomyces, Hymenostilbe* and *Insecticola* in North America. Mycologia 42, 566-589.

Petch T. 1931. New species of fungi collected during the Whitby foray. The Naturalist 1931, 101-103.

Petch T. 1932a. Notes on entomogenous fungi. 22-48. Transactions of the British Mycological Society 16, 209-245.

Petch T. 1932b. Notes on entomogenous fungi. 101-134. Transactions of the British Mycological Society 21, 34-67.

Petch T. 1932c. Notes on entomogenous fungi. 181-200. Transactions of the British Mycological Society 27, 81-93.

Petch T. 1948. A revised list of British entomogenous fungi. Transactions of the British Mycological Society 31, 286-304.

Samson RA, Evans HC. 1975. Notes on entomogenous fungi from Ghana. 3. The genus *Hymenostilbe*. Proceedings, Koninklijke Nederlandse Akadamie van Wetenschappen, Series C 78, 73-80.

Fungal Diversity

Diversity of entomopathogenic fungi in rainforests of Chiang Mai Province, Thailand

Aung, O.M.[1,2,3], Soytong, K.[2] and Hyde, K.D.[1,4,5*]

[1]International Tropical Mycological Research and Development Instituite, Kunming University of Science and Technology, Kunming, Yunnan, P.R. China
[2]Biocontrol Research Unit and Mycology Section, Department of Plant Pest Management, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok 10520, Thailand
[3]Mushroom Research Foundation, 128 Moo3, Bahn Pha Daeng, T. Pa Pae, A. Mae Taeng, Chiang Mai 50150, Thailand
[4]School of Science, Mae Fah Luang University, Tasud, Chiang Rai 57100, Thailand
[5]International Fungal Research & Development Centre, The Research Institute of Resource Insects, Chinese Academy of Forestry, Balongsi, Kunming 650224, PR China

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

A survey of entomopathogenic fungi was carried out in both conserved and disturbed rainforests and agricultural habitats in Chiang Mai Province. Dead insects, other arthropods, and soil samples were collected from 2005 to 2006 during the rainy seasons. Thirty-four entomogenous taxa belonging to 15 genera were encountered. Entomopathogenic fungi were dominated by *Ophiocordyceps myrmecophila* (22.6%) and *O. unilateralis* (13.8%) on ants. Species diversity on Homoptera was highest, followed by Lepidoptera and Hymenoptera. Highest species diversity was found in disturbed rainforests, followed by conserved rainforests and agricultural habitats. *Cordyceps* and *Ophiocordyceps* species contributed 74.7% of total taxa in conserved rainforests, 61.3% in disturbed forests but only 1.6% in agricultural habitats.

Key words: biodiversity, host specificity, insect order, species diversity

Article Information
Received 9 June 2007
Accepted 4 November 2007
Published online 31 May 2008
*Corresponding author: K.D. Hyde; e-mail: kdhyde1@gmail.com

Introduction

Tropical rainforests are characterized by high richness in entomopathogenic mycotaxa (Evans, 1982) and this is especially true if rainforests are not disturbed. The genus *Cordyceps* and its many proven or suspected anamorphs (e.g. *Hirsutella*, *Hymenostilbe*, *Nomuraea*, *Paecilomyces*, *Verticillium*) are particularly well represented in such habitats. *Cordyceps* species are however, rare in depleted rainforests and in agricultural land bordering these habitats (Samson *et al.*, 1988). In contrast, non-specialized insect pathogens such as *Beauveria* and *Metarhizium* are poorly represented in forest habitats. These fungal genera are commonly encountered by agricultural entomologists and have potential as biological control agents (Madelin, 1966; Samson *et al.*, 1988).

Comprehensive studies of entomopathogenic fungi have been carried out in Thailand (Hywel-Jones, 2001; Jones and Hyde, 2004). Although the morphology and phylogeny of entomopathogenic fungi have been studied, the relationships between these fungi and arthropods has received little attention. For this reason, a general survey of entomopathogenic fungi was carried out in several rainforests in Chiang Mai Province, in the northern region of Thailand. The Province, which is characterized by high richness in flora and fauna, has several national parks and rainforests. To document the diversity of entomopathogenic fungi on different hosts, eleven collection sites were chosen

as study areas. This survey mainly focused on relationships between entomopathogenic fungi from different arthropod hosts. The purpose of the present survey was: 1) to list the specific taxa in rainforest habitats, 2) to compare the diversity of entomopathogenic fungi in different arthropod hosts and different habitats and 3) to add to our knowledge of these fungi in Thailand.

Materials and methods

Special attention was directed towards different arthropod hosts. In addition, soil close to dead insects was sampled and the data was incorporated. The collection sites were visited at 15-day intervals during the rainy seasons from June 2005 to October 2006. Soil, litter, herbaceous plants, and tree leaves were examined for dead insects and other arthropods, which were collected and transported the same day to the laboratory in plastic containers. The samples were examined and microscopic measurements made of the fungi. Details of methods used can be found in Lacey and Brooks (1997). Soil sampling and isolation methodology used in this study follow Goettel and Inglis (1997). Single spore isolations were made according to Choi *et al.* (1999). Subcultures were made onto Czapek agar, potato dextrose agar, and Sabouraud dextrose agar according to Brown and Smith (1957) and Samson (1974). After incubation at 25-26°C for 14 days, the colony characters, conidiogenous structures, and other biological features were recorded. Identification of species of entomopathogenic fungi follow Samson *et al.* (1988), Kobayasi (1981, 1982), Kobayasi and Shimizu (1983), Luangsa-Ard *et al.* (2005, 2007) and Sung *et al.* (2007).

Study areas

The survey was carried out in both conserved and disturbed rainforests. In order to compare different non-forest habitats were included in this study.

Collection sites

Conserved rainforests

1: Doi Inthanon National Park, at 25 km marker on Highway 1009, North 18° 32.54' East 98° 33.51'.
2: Doi Suthep-Pui National Park, North 18° 48.62' East 98° 54.6'.
3: Mokfa Waterfall, located near 18 km marker on Highway 1095.
4: New Waterfall, located near 36 km marker on Highway 1095.
5: Geyser Pong Dueb Hot Spring.
6: Mae Sae National Park, Located near 50 km marker on Highway 1095.

Disturbed rainforests

7: Mushroom Research Centre (MRC), Bahn Pha Deng, North 19° 07.123′ East 98° 44.009'.
8: Pha Daeng Village.
9: Tung Joaw Village, North 19° 8.07' East 98° 38.9'.

Agricultural habitat

10: Mae Ma Lei Village, Mango orchard, Located near 20 km marker on Highway 1095.
11: Mae Lod Village, Coffee plantation.

Data analysis

The frequency of occurrence for each species was calculated by the following formula.

$$\text{Occurrence frequency of taxon A} = \frac{\text{Occurrence of taxon A}}{\text{Total number of all species}} \times 100$$

The Shannon diversity and Simpson diversity indices were applied to evaluate the diversities of entomogenous fungi on different orders of host arthropods and in different study sites (Hayek and Buzas, 1997). Evenness indices were estimated to establish the closeness of equability of species present (Gotelli and Colwell, 2001). Index of similarity was calculated using Sørensen's formula to determine the similarity in species occurrences (Odum, 1971). The similarity values range from 0 to 1 (1 meaning very similar, 0 indicating no similarity) by using the following formula.

$$S' = 2C/(A+B)$$

Where S′ is the degree of similarity, A and B are the number of species at host/sites A and B, respectively and C is the number of species common to both collections.

Results

Biodiversity of entomopathogenic fungi

Thirty-four entomopathogenic taxa belonging to 15 genera were encountered during

Table 1. Fungal taxa found on different insect orders and soil.

Taxa	N_i (individual no. of i^{th} species)											f
	AR	CO	DI	HE	HO	HY	IS	LP	OR	SO	UI	
Acremonium charticola									1			0.35
A. crassum					1							0.35
Aschersonia sp.					1							0.35
Aspergillus sp.	1	1					1					1.06
Beauveria bassiana		3			1	1		1				2.12
B. brongniartii		7	1	2					1	2	1	4.95
Cladosporium sp.								1				0.35
Cordyceps militaris								12				4.24
C. militaris. f. *sphaerocephala*								1				0.35
C. nelumboides	1											0.35
Cordyceps sp.								1				0.35
Hymenostilbe furcata				1								0.35
Hypocrella sp.					2							0.71
Isaria cicadae					3							1.06
I. farinosus								2				0.71
I. fumosoroseus				37								13.07
I. tenuipes								15				5.3
Ophiocordyceps crinalis								1				0.35
O. dipterigena			1									0.35
O. elongata								1				0.35
O. filiformis								1				0.35
O. longissima					1							0.35
O. mrciensis	1											0.35
O. myrmecophila						64						22.61
O. nutans				11								3.89
O. oxycephala						11						3.89
O. pseudolloydii						9						3.18
O. sphecocephala						1						0.35
O. unilateralis						39						13.78
Paecilomyces marquandii		1		19							1	7.42
Sporothrix insectorum						12						4.24
Stilbella buquetii						3						1.06
Torrubiella hemipterigena				1								0.35
Verticillium sp.		1				1		1				1.06

AR: Archnida, CO: Coleoptera, DI: Diptera, HE: Hemiptera, HO: Homoptera, HY: Hymenoptera, IS: Isoptera, LP: Lepidoptera, OR: Orthoptera, SO: Soil, UI: Unidentified insect, f = Occurrence frequency.

Table 2. Summary of species diversity on different insect orders and soil.

	AR	CO	DI	HE	HO	HY	IS	LP	OR	SO	UI
Cordyceps and *Ophiocordyceps*	2	0	1	1	1	5	0	6	0	0	0
Other taxa	1	5	1	5	5	4	1	5	2	2	2
Species richness (S)	3	5	2	6	6	9	1	11	2	1	2
Individual numbers	3	13	2	71	9	141	1	37	2	2	2
Shannon index (H')	1.10	1.26	0.69	1.20	1.68	1.49	0	1.67	0.69	0	0.69
Simpson index (1-D)	0.67	0.64	0.50	0.63	0.79	0.70	0	0.72	0.50	0	0.50
Evenness (E_H)	1	0.71	1	0.55	0.89	0.49	1	0.48	1	1	1

AR: Archnida, CO: Coleoptera, DI: Diptera, HE: Hemiptera, HO: Homoptera, HY: Hymenoptera, IS: Isoptera, LP: Lepidoptera, OR: Orthoptera, SO: Soil, UI: Unidentified insect.

this study (Table 1). These were identified from 301 arthropod cadavers and two soil samples. The most common taxa were *Ophiocordyceps myrmecophila* and *O. unilateralis* (on Hymenoptera), *Isaria fumosoroseus* (Hemiptera), *Paecilomyces marquandii* (Coleoptera), and *I. tenuipes* (Lepidoptera). During this survey, two species (*Ophiocordyceps mrciensis* and *Hymenostilbe furcata*) were described as new species (Aung *et al.*, 2006a, b).

Species diversity and similarities between hosts

Species diversity from different hosts, using the Shannon- and Simpson indices, gave similar results (Table 2). The species diversity index for Homoptera was the highest, followed by Lepidoptera and Hymenoptera. The species richness (S) for Lepidoptera was highest, followed by Hymenoptera, Homoptera, Hemiptera, Coleoptera, Arachnida, Diptera, Orthoptera, Isoptera and soil (Table 2).

Similarity indices of fungal taxa between different hosts (Table 3) showed values between Coleoptera and Hymenoptera or between Coleoptera and Lepidoptera were higher than those between Hymenoptera and Lepidoptera, between Lepidoptera and Homoptera, between Homoptera and Hymenoptera, or between Homoptera and Coleoptera.

Table 3. Similarity indices of fungal taxa between different hosts

Hosts	Sørensen's index (S')		
	Lepidoptera	Hymenoptera	Coleoptera
Homoptera	0.12	0.1	0.18
Lepidoptera		0.2	**0.25**
Hymenoptera			**0.29**

Species diversity and similarities between different collection sites

The highest species diversity was found in disturbed rainforests, followed by conserved rainforests and agricultural habitats (Table 4). The highest individual number of fungi was found in conserved rainforests (142 individual records), followed by disturbed forest (80 individual records) and agricultural habitats (61 individual records). The greatest species richness was recorded in disturbed rainforests (25 taxa), followed by conserved rainforests (21 taxa) and agricultural habitats (5 taxa). *Cordyceps* and *Ophiocordyceps* species were the most abundant in conserved forest (12 taxa), followed by disturbed forest (9 taxa), and agricultural habitats (1 taxon) (Fig 1).

The similarity index of the fungal taxa between the conserved rainforests and the disturbed rainforests was higher than that between the disturbed rainforests and the agricultural habitats, or the conserved rainforests and the agricultural habitats (Table 5).

Discussion

Entomopathogenic fungi are mainly found amongst the Zygomycetes (*Entomophthorales*) and Ascomycetes (*Clavicipitales*, *Hypocreales* and hyphomycetous anamorphs) (Evans, 1988). The *Entomophthorales* are commonly reported as pathogens of forest pests in temperate forest habitats (Burges, 1981), but are rare in tropical forests (Evans, 1982).

Occurrence frequency of entomopathogenic fungi

The occurrence frequencies of entomopathogenic fungi on arthropods in study areas were dominated by *Ophiocordyceps myrmecophila* (22.6%) and *O. unilateralis* (13.8%) on ants. This is not surprising as ants are the dominant arthropods in lowland tropical rainforests (Elton, 1973) and, therefore, are the most affected quantitatively by entomopathogenic fungi (Samson *et al.*, 1988). Ants are infected at the adult stage, and the hard exoskeleton is not colonized by the fungus, thus sufficient salient taxonomic features are present to enable host identification at the generic or species level.

High occurrence frequencies can also be found in *Isaria fumosoroseus* (13%), *Paecilomyces marquandii* (7.4%), and *I. tenuipes* (5.3%). *Paecilomyces* incorporates many species (Liang *et al.*, 2005), but only three species were found in this study. Although *I. fumosoroseus*, a geographically widespread species, is reported as a pathogen of many insects (Lepidoptera, Coleoptera, Diptera and Homoptera) (Obornik *et al.*, 2001), the fungus was found only on Hemiptera in this study.

Table 4. Summary of species diversity in different habitats.

	Conserved forests	Disturbed forests	Agricultural Habitats
Cordycep and *Ophiocordyceps*	12	9	1
Other taxa	9	16	4
Species richness (*S*)	21	25	5
Individual numbers	142	80	61
Shannon index (*H'*)	2.08	2.73	1.03
Simpson index (*1-D*)	0.80	0.90	0.56
Evenness (E_H)	0.38	0.61	0.56

Table 5. Similarity indices of fungal taxa between different collection sites.

Habitats	Sørensen's index (S')	
	DF	AH
CF	0.48	0.23
DF		0.33

Paecilomyces marquandii was the most frequently encountered pathogen on Hemiptera while *I. tenuipes* was recorded only from Lepidoptera pupa in this study. This finding is in agreement with Tzean *et.al.* (1997) that *Paecilomyces* species were recorded for different infected hosts of Lepidoptera, Coleoptera, Homoptera, Hymenoptera, Diptera Hemiptera, Orthoptera, or even Arachnida, the Lepidoptera however appears to occur on preferred hosts. Fukatsu *et al.*(1997) have also reported that *P. tenuipes* (sometimes referred to as *Isaria japonica* or other synonyms) parasitizes various Lepidoptera in larva and pupal stages.

Fungal diversity and similarities between hosts

Most entomopathogenic fungi have relatively broad host ranges, but apparently reoccur on some hosts (Tzean *et al.*, 1997); they are well represented on plant sucking homopterans in tropical rainforests (Petch, 1925) and coccids and whiteflies with ascomycete infections are also prominent in tropical rainforests (Mains, 1958; Evans, 1982; Samson *et al.*, 1988). Based on our findings, the species diversity on Homoptera was highest, followed by Lepidoptera and Hymenoptera, a result that is consistent with a previous report of invertebrate pathogenic fungi in Thailand. Jones (2004) pointed out that the most common host for invertebrate pathogenic fungi in Thailand was Homoptera, followed by Lepidoptera, *Araneae*, Coleoptera and Hymenoptera. The species diversity value of Lepidoptera in our study, however, was lower than for Homoptera but its species richness was highest among the hosts.

Fungal diversity and similarities between collection sites

Observations of entomopathogenic fungi in tropical rainforests in both Africa and South America reported that richness of entomopathogenic fungi decreases as rainforests are exploited; whether this is due to the disappearance of the specific hosts or the loss of optimum conditions for infection, or a combination of both, is unknown (Samson *et al.*, 1988). In our study the highest diversity of entomopathogenic fungi was found in disturbed forests while the conserved forests and agricultural habitats had low diversity values. This finding does not support Samson *et al* (1988). This is perhaps due to the fact that the disturbed rainforest comprises both forest habitats and agricultural habitats and both types of specialized and generalist entomopathogens are well presented in this environment.

Cordyceps species are usually found in undisturbed habitats where there is clean air, high humidity, and adequate shading by overhanging trees to help retain soil moisture levels (www.mushtech.org). A similar finding was observed in our study. *Cordyceps* and *Ophiocordyceps* species contributed 74.7% of total taxa in conserved rainforests, with 61.3% in disturbed forest and only 1.6% in agricultural habitats.

The similarity indices among different collection sites show that the similarity between conserved forest and disturbed forest was high. The two environments had 11 fungal

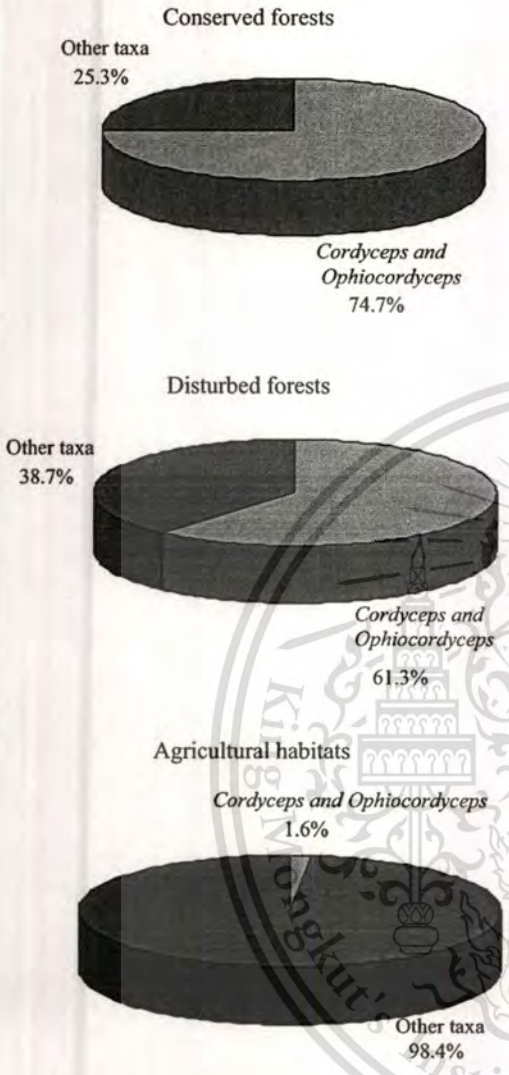


Fig. 2. Percentage of fungal records in different habitats.

species in common. Only three taxa in both conserved forests and agricultural habitats while 5 taxa were common to both disturbed forest and agricultural habitats. Three taxa, *Beauveria bassiana*, *Ophiocordyceps pseudolloydii* and *Isaria tenuipes* were found in all collection sites. *Beauveria bassiana* is one of the most widely recognized and encountered of all entomopathogenic fungi due to its cosmopolitan distribution, easy recognition, and frequent appearance nature (Rehner, 2005). Generally, *Beauveria* and *Metarhizium* species are rarely encountered on insects in tropical rainforests, although *Beauveria* can be found colonizing insect remains in the soil (Evans, 1988). In the present study, *B. brongniartii* was isolated from the soil underneath dead insects. *Metarhizium* was not found in the current study.

Host specificity

Many entomopathogenic fungi are thought to be host specific. *Cordyceps* species most frequently attack Lepidoptera, Hymenoptera, Coleoptera and Orthoptera, and several life cycle stages of a particular host may be infected, but not necessarily by the same species of fungi (Benjamin *et al.*, 2004). In our study, Hymenoptera, Lepidoptera, Hemiptera and Arachnida were mostly infected by *Cordyceps* and *Ophiocordyceps* species. Some *Cordyceps* species are obligately parasitic on ants and are important pathogenic fungi in tropical ecosystems (Evans and Samson, 1982). Disease appears to be maintained at a constant or enzootic level partly by the activities of the infected hosts (Evans and Samson, 1982, 1984). Infected ants escape from their normal ant trails and nests, radically modify their behavioral patterns to find selected niches. After infection with *Cordyceps*, ground-dwelling ponerine ants go up to vegetation and die in exposed positions, grasping the substratum with legs and mandibles (Evans, 1988). Three *Ophiocordyceps* species were recorded in our study. Only *O. pseudolloydii* infection on dolichoderine ants was found at every collection site. The most abundant species, *O. myrmecophila* and *O. unilateralis*, infected formicine ants in the rainforests habitats. *Ophiocordyceps myrmecophila* was found both in conserved and disturbed rainforests while *O. unilateralis* was found only in conserved rainforests. This result is strongly indicative that there is a high degree of specificity within these associations, as a single *Cordyceps* species is typically confined to a single genus or tribe of ants (Samson *et al.*, 1988). Host identification in the majority of cases however is rudimentary and thus the specific insect-fungal association has not been determined. The complete life cycles of many of the tropical forest *Cordyceps* species still require elucidation (Samson and Evans, 1973; Evans and Samson, 1982).

Based on our findings, a number of entomopathogenic fungi are found to be associated with different hosts including soils. The data obtained in this study also reveal the general conclusion of diversity and complexity of fungus-host associations, diversity and similarity of fungal taxa among different hosts and habitats. To add our knowledge of entomopathogenic fungi, mycologists and entomologists must cooperate in broad research relating to studies of natural ecosystems.

Acknowledgements

OMA gratefully acknowledges the Mushroom Research Foundation for funding and is also grateful to Aung Swe for statistical and technical assistance during this study. Dr. Eric H.C. McKenzie is thanked for his valuable comments on the manuscript.

References

Aung, O.M., Kang, J.C., Liang, Z.Q., Soytong, K. and Hyde, K.D. (2006a). *Cordyceps mrciensis* sp. nov. from a spider in Thailand. Mycotaxon 97: 235-240.

Aung, O.M., Kang, J.C., Liang, Z.Q., Soytong, K. and Hyde, K.D. (2006b). A new entomopathogenic species, *Hymenostilbe furcata*, parasitic on a hemipteran nymph in northern Thailand. Mycotaxon 97: 241-245.

Benjamin, R.K., Blackwell, M., Chapela, I.H., Humber, R.A., Jones, K.G., Klepzig, K.A., Lichtwardt, R.W., Malloch, D., Noda, H., Roeper, R.A., Spatafora, J.W. and Weir, A. (2004). Insects-and other arthropod-associated fungi. In: *Biodiversity of Fungi Inventory and Monitoring Methods* (eds. G.M. Mueller, G.F. Bills and S. Mercedes). Foster.Elsevier Academic Press, USA: 395-434.

Brown, A.H.S. and Smith, G. (1957). The genus *Paecilomyces* Bainier and its perfect stage *Byssochlamys* Westling. Transactions of the British Mycological Society 40: 17-89.

Burges, H.D. (1981). Strategy for the microbial control of pests in 1980 and beyond. In: *Microbial Control of Pests and Plant Diseases 1970-1980* (ed. H.D. Burges). Academic Press, London and New York: 797-836.

Choi, Y.W., Hyde, K.D. and Ho, W.W.H. (1999). Single spore isolation of fungi. Fungal Diversity 2: 29-38.

Elton, C.S. (1973). The structure of invertebrate populations inside neotropical rain forest. Journal of Animal Ecology 42: 55-104.

Evans, H.C. (1982). Entomogenous fungi in tropical forest ecosystems: an appraisal. Ecological Entomology 7: 47-60.

Evans, H.C. (1988). Coevolution of entomogenous fungi and their insect hosts. In: *Coevolution of Fungi with Plants and Animals* (eds. K.A. Pirozynski and D.L. Hawksworth). Academic Press, New York: 149-171.

Evans, H.C. and Samson, R.A. (1982). *Cordyceps* species and their anamorphs pathogenic on ants (*Formicidae*) in tropical forest ecosystems. I. The *Cephalotes* (*Myrmicinae*) complex. Transactions of the British Mycological Society 79: 431-453.

Evans, H.C. and Samson, R.A. (1984). *Cordyceps* species and their anamorphs pathogenic on ants (*Formicidae*) in tropical forest ecosystems. II: The *Camponotus* (*Formicinae*) complex. Transactions of the British Mycological Society 82: 127-150.

Fukatsu, T., Sato, H. and Kuriyama, H. (1997). Isolation, inoculation to insect host, and molecular phylogeny of an entomogenous fungus *Paecilomyces tenuipes*. Journal of Invertebrate Pathology 70: 203-208.

Goettel, M.S. and Inglis, G.D. (1997). Fungi: Hyphomycetes. In: *Manuals of Techniques in Insect Pathology* (ed. L. Lacey). Academic Press: 213-247.

Gotelli, N.J. and Colwell, R.K. (2001). Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters 4: 379-391.

Hayek, L.A.C. and Buzas, M.A. (1997). *Surveying Natural Populations*. Columbia University Press.

Hywel-Jones, N.L. (2001). The biological diversity of invertebrate pathogenic fungi. In: *Biodiversity of Tropical Microfungi* (ed. K.D. Hyde). Hong Kong University Press, Hong Kong: 107-119.

Jones, E.B.G. (2004). Fungi on arthropods, crustaceans and fish. In: *Thai Fungal Diversity* (eds. E.B.G. Jones, M. Tantichareon and K.D. Hyde). BIOTEC, Thailand: 227-239.

Jones, E.B.G. and Hyde, K.D. (2004). Introduction to Thai fungal diversity. In: *Thai Fungal Diversity* (eds. E.B.G. Jones, M. Tantichareon and K.D. Hyde). BIOTEC, Thailand: 7-35.

Kobayasi, Y. (1981). Revision of the genus *Cordyceps* and its allies 2. Bulletin of the National Science Museum, Tokyo, Ser. B. (Bot.) series B 7: 123-129.

Kobayasi, Y. (1982). Keys to the taxa of the genera *Cordyceps* and *Torrubiella*. Transaction of the Mycological Society of Japan 23: 329-364.

Kobayasi, Y. and Shimizu, D. (1983). *Cordyceps* species from Japan 6. Bulletin of the National Science Museum, Tokyo, Ser. B. (Bot.) 9: 1-211.

Lacey, L.A. and Brooks, W.M. (1997). Initial handling and diagnosis of diseased insects. In: *Manuals of Techniques in Insect Pathology* (ed. L. Lacey). Academic Press: 1-15.

Liang, Z.Q., Han, Y.F., Chu, H.L. and Liu, A.Y. (2005). Studies on the genus *Paecilomyces* in China I. Fungal Diversity 20: 83-101.

Luangsa-Ard, J.J., Hywel-Jones, N.L., Manoch, L. and 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. and Hywel-Jones, N.L. (2007). *Atlas of invertebrate-pathogenic fungi of Thailand*. BIOTEC, NSTDA, Thailand.

Madelin, M.F. (1966). Fungal parasites of insects. Annual Review of Entomology 11: 423-448.

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

Obornik, M., Jirku, M. and Dolezel, D. (2001). Phylogeny of mitosporic entomopathogenic fungi: is the genus *Paecilomyces* polyphyletic? Canadian Journal of Microbiology 47: 813-819.

Odum, E.P. (1971). *Fundamentals of ecology*. 3rd edn. WB Saunders, Philadelphia, PA.

Petch, T. (1925). Entomogenous fungi and their use in controlling insect pests. Bulletin of the Department of Agriculture Ceylon 7: 1-40.

Rehner, S.A. (2005). Phylogenetics of the insect pathogenic genus *Beauveria*. In: *Insect-fungal Associations Ecology and Evolution* (eds. F.E. Vega and M. Blackwell). Oxford University Press, Inc., New York, USA: 3-27.

Samson, R.A. (1974). *Paecilomyces* and some allied hyphomycetes. Studies in Mycology 6: 1-119.

Samson, R.A. and Evans, H.C. (1973). Notes on entomogenous fungi from Ghana. I. The genera *Gibellula* and *Pseudogibellula*. Acta Botanica Neerlandica 22: 522-528.

Samson, R.A., Evans, H.C. and Latgé, J.P. (1988). *Atlas of Entomopathogenic Fungi*. Springer-Verlag, Berlin, Heidelberg. New York. 1-187.

Sung, G.H., Hywel-Jones, N.L., Sung, J.M., Luangsa-Ard, J.J., Shrestha, B. and Spatafora, J.W. (2007). Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. Studies in Mycology 57: 5-59.

Tzean, S.S., Hsieh, L.S. and Wu, W.J. (1997). *Atlas of Entomopathogenic Fungi from Taiwan*. 1st edn. Council of Agriculture, Executive Yuan, Taiwan.

www.mushtech.org. Entomopathogenic Fungal Culture Collection. (26 December 2007).

AUTHOR'S BIOGRAPHY

Name: Ohnmar Myo Aung

Date of Birth: 3 April 1963

Nationality: Myanmar

Address: Myanma Cotton and Sericulture Enterprise, Ministry of Agriculture and Irrigation, Myanmar.

Education Background:

2005 to date	PhD candidate, Biocontrol Research Unit and Mycology Section, Department of Plant Pest Management, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang(KMITL), Bangkok 10520, Thailand. Supervisors: Dr. Kasem Soytong and Dr. Kevin D. Hyde
2003	M. Agr. Sc. (Entomology), Yezin Agricultural University, Yezin, Myanmar
1992	Dip. in Accountancy (DA)
1985	B.Ag., Institute of Agriculture, Yezin, Myanmar

Specialization: Insect pathology and its molecular phylogeny.

Work experiences:

1997 to 2005	Head of the Plant Protection Section, Extension Division. Mynama Cotton & Sericulture Enterprise, Ministry of Agriculture and Irrigation. Duties involved planning, monitoring and evaluating of the projects relating to cotton production, extension and cotton pest management.
1994-1997	Assistant Manager in Plant Protection Division of the Myanma Agriculture Service.
1991-1994	State Plant Protection Officer of the Plant Protection Team in Mon State. Implementing plant protection activities and research works.
1985-1991	Assistant Supervisor, Assisting project Director on Plant Protection and Rodent Control Project implemented by the technical aid of (GTZ) from Federal Republic of Germany.

Conferences, Presentations and Trainings:

1) The Asian Mycology Congress and Xth International Marine and Freshwater Mycology Symposium, Penang, Malaysia. 2-6 December, 2007.

   - Oral Presentation "Ecological and phylogenetic studies of entomopathogenic genera *Beauveria*, *Cordyceps* and *Paecilomyces* from Chiang Mai Province, Thailand".

2) The International Conference on Integration of Science and Technology for Sustainable Development (ICIST) "Biological Diversity, Food and Agricultural Technology", Bangkok, Thailand. 26-27 April, 2007.

   - Oral Presentation "New and interesting species of entomogenous fungi in northern Thailand".

3) The Annual Meeting of Thai Mycological Association (TMA) and Mycology Conference in Thailand, Bangkok, Thailand. 28-29 October, 2006.

   - Oral Presentation "Biodiversity and Molecular Phylogeny of Entomopathogenic Fungi in Chiang Mai Province, Thailand".

4) The IV Mycological Taxonomy Workshop in Mushroom Research Centre, Chiang Mai, Thailand, 10-19 July 2006.

   - Instructor in Insect Fungi.

5) The III Mycological Taxonomy Workshop in Mushroom Research Centre, Chiang Mai, Thailand, 28 August-4 September 2005.

   - Participant.

6) COSTAB Training Course, joint sponsored by Ministry of Agriculture and Irrigation and FAO. 12-17 January, 2004.

7) The Multivariate-Analyses Course,Yezin Agricultural University, Yezin, Myanmar, 10-26 March, 2003.

8) Training course on "Crop Protection in the Tropics with Emphasis on IPM" joint sponsored by Yezin Agricultural University (Myanmar) and Georg-August-University (Germany), Yezin, Myanmar, 7-26 June, 2002.

Publications:

International

1) **Aung, O.M.**, Soytong, K. and Hyde, K.D. (2008). Diversity of entomopathogenic fungi in rainforests of Chiang Mai Province, Thailand. Fungal Diversity 30: 15-22. (SCI).

2) **Aung, O.M.**, Kang, J.C., Liang, Z.Q., Soytong, K. and Hyde, K.D. (2006). *Cordyceps mrciensis* sp. nov. from a spider in Thailand. Mycotaxon 97: 235-240. (SCI).

3) **Aung, O.M.**, Kang, J.C., Liang, Z.Q., Soytong, K. and Hyde, K.D. (2006). A new entomopathogenic species, *Hymenostilbe furcata*, parasitic on a hemipteran nymph in northern Thailand. Mycotaxon 97: 241-245. (SCI).

4) **Ohnmar Myo Aung** (2003) Studies on Insect Pests and Natural Enemies in Cotton Agroecosystem (M.Agr.Sc. Thesis).

5) **Ohnmar Myo Aung**, Myint Thaung and Aung Kyi (2004) Changes of arthropod populations on different varieties of cotton at different locations. Journal of Agricultural, Forestry, Livestock and Fishery Sciences (Special Issue for Agricultural Sciences No.3).

Myanmar

1) Htay Unt, Aung Swe and **Ohnmar Myo Aung** (1991) Stored Product Pests in Myanmar (Myanmar Handbook).

2) **Ohnmar Myo Aung** (1999) Chemical Pesticides: Currently Used in Cotton Crop Protection (Myanmar Handbook).