

DIVERSITY AND MOLECULAR PHYLOGENY OF
ENTOMOPATHOGENIC FUNGI OF SABAH



INSTITUTE FOR TROPICAL BIOLOGY AND
CONSERVATION

UNIVERSITI MALAYSIA SABAH

2022

**DIVERSITY AND MOLECULAR PHYLOGENY OF
ENTOMOPATHOGENIC FUNGI OF SABAH**

MOHAMMAD HAFIZ SYUKRI BIN KASSIM



UMS

**THESIS SUBMITTED IN FULFILLMENT OF
THE REQUIREMENT FOR THE DEGREE OF
MASTERS IN SCIENCE**

**INSTITUTE FOR TROPICAL BIOLOGY AND
CONSERVATION
UNIVERSITI MALAYSIA SABAH
2022**

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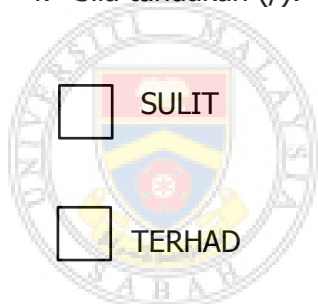
JUDUL: **KEPELBAGAIAN DAN FILOGENI MOLEKUL KULAT
ENTOMOPATOGENIK SABAH, MALAYSIA**

IJAZAH: **SARJANA SAINS**

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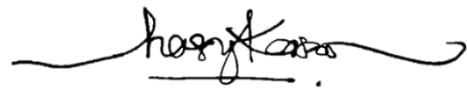
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DEGREE : **MASTERS IN SCIENCE**

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ACKNOWLEDGMENT

I would like to thank Allah s.w.t. for all the blessings He has given me, especially my health, my family, and my friends. He has given me the gift of strength, perseverance, and patience, without which I could not have achieved this aspiration.

I would like to express extend the deepest appreciation to my supervisor, Dr. Jaya Seelan Sathiya Seelan for giving me his belief and guidance during the period of this project. He continually and convincingly conveyed a spirit of adventure regarding research and an excitement in regard to teaching. I would like to thank my co-supervisor, Assoc. Prof. Dr. Mahadimenakbar Mohd. Dawood for his guidance and encouragement. Many thanks to the Director of Institute for Tropical Biology and Conservation (ITBC), Universiti Malaysia Sabah, Dr Monica Suleiman for her approval and valuable courage to finish this project. I appreciate all the ITBC staff and lab assistants in the fields, laboratories and other managements.

I thank the Ministry of Education Malaysia for their support through the Fundamental Research Grant Scheme (FRGS/1/2017/WAB13/UMS/02/2) and Universiti Malaysia Sabah the UMS Great Grant (GUG0218-2/2018). I am also grateful to Sabah Biodiversity Council, Sabah Foundation, Sabah Forestry Department and Sabah Wildlife Department for providing access to the sampling sites.

I thank Foo She Fui, Ibrahem Ghani Wasti and all labmate for their encouragement, cooperation and assistant in the laboratory and out of the laboratory. I also thank my Yayasan Sabah and "fungi club" best friends for their encouragement, moral supports and deeply support my passion in Mycology and Sciences. An appreciation also to my late undergraduate supervisor, Prof. Dr. Sepiah Muid as my role model in mycology. Finally, I express sincere appreciation to my family especially my parents, Kassim Bin Ali and Saibah Binti Isab, who have supported me financially and emotionally, and for being understanding and supportive all this while.

Mohammad Hafiz Syukri Bin Kassim

23 March 2022

ABSTRACT

Entomopathogenic fungi or widely known as “Zombie Fungi” are group of parasitic fungi that infect wide range of insect host and then evolved to exploit by manipulating and kill the insects. This study was done to assess the diversity, to isolate, characterize and curate the parasitic fungi using molecular and morphological method and to infer the evolutionary relationship and phylogenetic classification of known lineage of entomopathogenic fungi which is the Ophiocordycipitaceae. The effects of elevation and disturbances were also studied. All samples were collected using opportunistic sampling and were conducted at 14 sampling sites around Sabah with different forest vegetations and habitats and the isolation of the fungi were done. All the collected fungi samples were identified based on their morphology by studying their size, colour, physical structures of ascoma and perithecia and their micro-morphology with the help of molecular analysis for further confirmation. A total of 93 specimens were collected in this study and representing nine genera and 14 species. The most dominant genera collected was *Ophiocorydyceps* sp. then followed by *Cordyceps* spp., *Isaria* spp., *Hirsutella* spp., *Aspergillus*, *Hevansia* and *Metarhizium* respectively. Most of the specimens were collected from under-leaf habitats (67%) and dominated by the genus *Ophiocordyceps*. Other habitats recorded were on the forest floor (19%), buried under soil (10%), on understory tree leaf (2%), and some collected biting on tree branches (2%). In this study, the most infected insect host is from the order of Hymenoptera (61%) and then followed by Hemiptera (11%), Lepidoptera (10%), Araneae (4%), Coleoptera (4%), Blattodea (2%), Orthoptera (1%), and 7% was unidentified host respectively. The diversity analysis showed different pattern where the agricultural area has the highest diversity in Simpson analysis (Simpson (1-D)=4.4984) and evenness index (Evenness (e^H/S)= 0.2602) while undisturbed forest is highest in Shannon analysis (Shannon (H)= 1.7839) than disturbed forests and agricultural area respectively. The elevational and disturbance effect on EPF were statistically significant (Elevation; $t=4.94$, $p= 0.001$; Disturbance; $t=-3.21$, $p=0.02$). The results suggests that the increase in elevations will increase the species abundances and from the undisturbed forests to disturbed forest and agricultural area, the species abundances were decreasing, respectively. 42 successful sequences (ITS: 21 and LSU: 21) were generated. The sequences were

identified into eleven species consist of *Ophiocordyceps unilateralis*, *Hirsutella rhossiliensis*, *Hirsutella citriformis*, *Isaria cicadae*, *Cordyceps javanica*, *Cordyceps cylindrica*, *Hevansia websteri*, *Purpureocillium lilacinum*, *Metarhizium granulamotis*, *Aspergillus oryzae* and *Beuveria brongniartii*. 58 total sequences of ITS and 49 total LSU sequences were collected from the Genbank with *Xylaria bambusicola* as an outgroup. Phylogenetic trees of ML and BI were generated. ML tree within the Ophiocordycipitaceae with four sequences (ITS:4 and LSU:4) were generated with addition of 27 Genbank sequences and *Nectria cinnabarina* as an outgroup. Three phylogenetic datasets were generated include ITS Dataset, nITS + LSU dataset and nITS + LSU dataset of *O. unilateralis* s.l. The Ophiocordycipitaceae ML tree were shown that a new species of *Ophiocordyceps* sp. nov. were recorded from Borneo. Favourable environment with elevations, cool atmosphere, relatively high humidity and high diversity of insects is a suitable environment that promotes the high diversity of entomopathogenic fungi recorded in Sabah. The classical method with addition of advance molecular analysis and technologies used in this study, offered rapid and more accurate species identification and alleviated the evolutionary relationships of this unique fungus in Sabah.

Keywords: Entomopathogenic fungi, insectcs, diversity, morphology, phylogeny, classification

ABSTRAK

KEPELBAGAIAN DAN FILOGENI MOLEKUL KULAT ENTOMOPATOGENIK BORNEO

Kulat entomopatogenik (KEP) atau dikenali sebagai "Kulat Zombi" adalah kelompok kulat parasit yang menjangkiti pelbagai perumah serangga dan kemudian berkembang untuk mengeksploitasi dengan memanipulasi dan membunuh banyak populasi serangga. Matlamat utama kajian ini adalah untuk menilai kepelbagaian, mengkulturkan, mencirikan dan mengkaji kulat parasit dengan menggunakan sinergi kaedah molekul dan morfologi dan menyimpulkan hubungan evolusi dan klasifikasi keturunan kulat entomopatogenik yang menekankan kepada keluarga *Ophiocordycipitaceae*. Kesan ketinggian dan gangguan juga dikaji secara statistik. Sebanyak 93 KEP dengan sampel serangga telah dikumpulkan menggunakan pensampelan oportunistik dan dijalan di 14 tapak kajian di sekitar Sabah yang terdiri daripada jenis-jenis hutan dan mikrohabitat yang berlainan dan isolasi kulat tersebut juga dikumpulkan. Semua sampel KEP dan perumah serangga yang terkumpul telah dikenal pasti berdasarkan morfologi seperti saiz, warna, struktur fizikal ascoma dan perithecia dan mikro-morfologi mereka dan dibantu oleh analisis molecular untuk pengesahan selanjutnya. Sebanyak 93 spesies telah dikumpulkan dalam kajian ini mewakili sembilan genera dan 14 spesies. Genera yang paling dominan dikumpul ialah *Ophiocorydyceps* sp. kemudian diikuti oleh *Cordyceps* spp., *Isaria* spp., *Hirsutella* spp., *Aspergillus*, *Hevansia* dan *Metarhizium* mengikut urutan. Kebanyakan spesimen yang dikumpulkan ialah habitat di bawah daun (67%) yang didominasi oleh genus *Ophiocordyceps*. Lain-lain habitat yang direkodkan ialah di lantai hutan (19%), tertanam di bawah tanah (10%), di atas daun pokok lantai hutan (2%), dan ada yang menggigit pada ranting pokok (2%). Hasil kajian ini, perumah serangga yang paling banyak dijangkiti adalah dari Hymenoptera (61%) dan diikuti oleh Hemiptera (11%), Lepidoptera (10%), Araneae (4%), Blattodea (2%), Orthoptera (2%), dan 7% adalah perumah yang tidak dikenalpasti, mengikut urutan. Semua spesies yang dikenalpasti adalah di identifikasi berdasarkan morfologi dan analisis molecular. Analisis Kepelbagaian menunjukkan corak berbeza dimana kawasan agrikultur mempunyai kepelbagaian tertinggi dari Analisis Simpson (Simpson (1-D)=4.4984) dan Index Keseimbangan (Keseimbangan: e^H/S)= 0.2602) manakala hutan yang

tidak diganggu adalah tertinggi dari Analisis Shannon (Shannon (H)= 1.7839) berbanding hutan diganggu dan kawasan agrikultur, mengikut urutan. Kesan ketinggian dan gangguan terhadap KEP menunjukkan statistik yang ketara (Ketinggian; $t=4.94$, $p= 0.001$; Gangguan; $t=-3.21$, $p=0.02$). Hasil tersebut menunjukkan peningkatan ketinggian akan meningkatkan kelimpahan spesies dan daripada hutan yang tidak diganggu ke hutan diganggu dan kawasan agrikultur, kelimpahan spesies akan berkurangan, mengikut urutan. 42 susunan jujukan (ITS: 21 dan LSU: 21) telah dihasilkan. Susunan jujukan tersebut telah dikenalpasti kepada sebelas spesies iaitu *Ophiocordyceps unilateralis*, *Hirsutella rhossiliensis*, *Hirsutella citriformis*, *Isaria cicadae*, *Cordyceps javanica*, *Cordyceps cylindrica*, *Hevansia websteri*, *Purpureocillium lilacinum*, *Metarhizium granulomotis*, *Aspergillus oryzae* and *Beuveria brongniartii*. Sejumlah 58 susunan jujukan ITS dan 49 susunan jujukan LSU telah dikumpulkan daripada Genbank dengan *Xylaria bambusicola* sebagai kumpulan luaran (Outgroup) dan pokok filogenetik ML dan BI telah dihasilkan. Pokok ML dalam lingkungan *Ophiocordycipitaceae* dengan empat urutan gen (ITS:4 dan LSU:4) telah dihasilkan dengan penambahan 27 susunan Genbank dan *Nectria cinnabarina* sebagai kumpulan luaran (Outgroup). Tiga set data telah dihasilkan termasuk set data ITS, set data nITS + LSU dan set data nITS + LSU *O. unilateralis* s.l. Pokok ML *Ophiocordycipitaceae* menunjukkan satu spesies baru *Ophiocordyceps* sp. nov. telah direkodkan in Borneo. Persekitaran yang baik dengan ketinggian yang baik, suasana sejuk, kelembapan yang tinggi dan kepelbagaian serangga yang tinggi adalah persekitaran yang sesuai yang mempromosikan kepelbagaian tinggi fungi entomopatogenik yang direkodkan di Sabah. Kaedah klasik dengan penambahan analisis sedia ada dan teknologi molekul terkini yang digunakan dalam kajian ini, menawarkan pengenalan spesies yang cepat dan tepat dan menambahbaik hubungan evolusi kulat unik ini di Sabah.

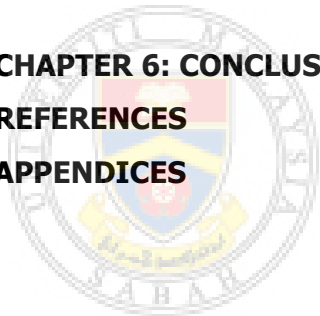
Kata kunci: kulat entomopathogenik, serangga, kepelbagaian, morfologi, klasifikasi filogeni

LIST OF CONTENTS

	Page
TITLE	i
DECLARATION	ii
CERTIFICATION	iii
ACKNOWLEDGEMENT	iv
ABSTRACT	v
<i>ABSTRAK</i>	vii
LIST OF CONTENTS	ix
LIST OF TABLES	xi
LIST OF FIGURES	xii
LIST OF SYMBOLS	xx
LIST OF ABBREVIATIONS	xxi
LIST OF APPENDICES	xxii
CHAPTER 1: INTRODUCTION	1
1.1 Background of Study	1
1.2 Problem Statement	3
1.3 Research Objectives	4
CHAPTER 2: LITERATURE REVIEW	6
2.1 Fungal Pathogenicity	6
2.2 Entomopathogenic Fungi	6
2.2.1 Geographical and ecological distribution of entomopathogenic fungi	7
2.2.2 Mode and mechanisms of action of entomopathogenic fungi	8
2.3 Classification of Entomopathogenic Fungi	12
2.3.1 Ascomycota	12
2.4 Importance of Entomopathogenic Fungi	13
2.5 Entomopathogenic Fungi as Benign Fungal Biological Control Agents (BCAs)	15

2.6	Occurences of Tropical versus Temperate Entomopathogenic Fungi	18
2.7	Arthropods-Fungi Interactions	20
	2.7.1 Hemiptera	20
	2.7.2 Diptera	20
	2.7.3 Lepidoptera	21
	2.7.4 Coleoptera	21
	2.7.5 Hymenoptera	22
2.8	Host Specificity and Cryptic Species in Entomopathogenic Fungi	23
2.9	Sampling and Isolation Techniques	28
2.10	Identification technologies of Entomopathogenic Fungi	29
2.11	Conservation Value of Entomopathogenic Fungi	30
CHAPTER 3: METHODOLOGY		26
3.1	Sampling Sites	26
3.2	Sample Collection	38
3.3	Isolation of Entomopathogenic Fungi	38
3.4	Morphological Identification	39
3.5	Molecular Identification	40
	3.5.1 Genomic DNA Isolation	40
	3.5.2 Polymerase Chain Reaction (PCR) for DNA Amplification	41
	3.5.3 Gel Electrophoresis	43
	3.5.4 DNA Sequencing	43
	3.5.5 Basic Local Alingment Search Tool (BLASTn)	43
	3.5.6 Phylogenetic Analysis	44
3.6	Statistical Analysis	45
CHAPTER 4: RESULTS		47
4.1	Distribution of Entomopathogenic Fungi in Sabah	47
4.2	Elevetional and Disturbance Effect of Entomopathgoenic Fungi	53
4.3	Morphology of Entomopathogenic Fungi from Sabah	54

4.4	Genomic DNA and Phylogenetic Tree of Entomopathogenic Fungi of Sabah	108
4.5	Entomopathogenic Fungi Single Gene Phylogeny	112
4.6	ITS Phylogeny of Entomopathogenic Fungi	115
4.7	ITS + LSU Phylogeny of Entomopathogenic Fungi Cave Fungi in Sabah	118
4.8	ITS + LSU Phylogeny <i>Ophiocordyceps unilateralis</i> complex of Sabah	121
CHAPTER 5: DISCUSSION		123
5.1	Distribution of Entomopathogenic Fungi in Sabah	123
5.2	Morphology of Entomopathogenic Fungi of Sabah	130
5.3	Phylogenetic Relationship of Entomopathogenic Fungi of Sabah	141
CHAPTER 6: CONCLUSION		148
REFERENCES		151
APPENDICES		164



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LIST OF TABLES

	Page
Table 2.1 : Secondary metabolites of entomopathogenic fungi based on fungi species	13
Table 2.2 : Entomopathogenic fungi used in Biological Control Agents (BCA)	16
Table 3.1 : List of sampling site correspond with forest types and coordinates. Sampling site highlighted in bold.	28
Table 3.2 : Details of primer sets used in amplification of PCR amplicons	42
Table 4.1 : Entomopathogenic fungi (EPF) taxa collected from Sabah, Malaysia. The number of occurrences for each EPF taxa in each forest type is shown.	51
Table 4.2 : Diversity index scores based on species abundance counts of each taxon collected from the different forest type.	51
Table 4.3 : Comparison of the morphological characters between Bornean <i>Ophiocordyceps</i> species and closely related species	57
Table 4.4 : Phylogenetic dataset used in this study	98

LIST OF FIGURES

- | | Page |
|---|------|
| Figure 2.1 : Illustration of different habitat of EPF in the forest. A. Under leaf. B. Biting on tree branches. C. Under leaf litter on the forest floor. D. On small shrub on forest understory. E. Buried on soil. Arrow showing the teleomorph (A,B,C and E) and anamorph (D) of the EPF. | 7 |
| Figure 2.2 : Mechanism of infection of entomopathogenic fungi (<i>Ascomycota – Ophiocordyceps unilateralis</i>). A. Ants leave their nest and walking on the forest floor; B. The ants infected by the <i>Ophiocordyceps</i> spores in the “killing zone” (red arrows); C. After infected by the fungi spores, the infected ant will leave their nest and looking for a suitable places for the fungi to grow usually in area with optimum temperature and humidity, perform “death bite” (Arrow in C) on the edge of under the leaf, and die. D. After two to eight weeks, once the fungi consumed the ant’s internal part the stroma will erupt (Short arrow in C) and once matured it will release spores. E. From 24 to 72 hours after being shot, the spores will germinate and form a secondary spore called the capilliconidiospore. | 10 |
| Figure 3.1 : UMS Hill. Left: Lowland forest vegetation in UMS Hill covered with bushes, shrubs and small tree. Right: Disturbed area of the UMS Hill with an open land because of human activities. | 29 |
| Figure 3.2 : Imbak Canyon Conservation Area (ICCA). Left: Mixed dipterocarp forest vegetation in Sg. Kangkawat Research Station, ICCA with remarkable presence of small tree and shrubs with the medium and big dipterocarp trees. Right: | 30 |

Highland forest vegetation in ICCA covered with small tree and shrubs with a trail.

- Figure 3.3 : Sungai Rawog conservation area. Left: Kapur merah forest stand with mixed dipterocarp trees. Right: Forest floor vegetation of mixed dipterocarp forest with dominated by small trees and shrubs. 31
- Figure 3.4 : Kinabalu rocker range parks. Left: Montane forest vegetation in Kinabalu Park Headquarters in Kundasang. Right: Forest vegetation in Sayap Substations with the present of shrubs, lianas and climbers. 32
- Figure 3.5 : Crocker range parks. Left: Highland elevated vegetation in Mahua Substation with absence of shrubs, lianas and climbers. Right: Understory vegetation of the sampling area covered with small tree and leaf litter. 33
- Figure 3.6 : Tawau Hills Park. Left: Vegetation in one of the trails (Sulphur Spring Trail) in Tawau Hills Park covered with shrubs and small vegetation. Right: Understory vegetation include small trees, shrubs, herbs and grasses with small stream nearby. 34
- Figure 3.7 : Keruak Hill, Sukau. Left: Vegetation in one of the trails in Kerauk Hill with opening and covered with shrubs and small vegetations. Right: The entrance of the caves in the area known as the Keruak Caves. 35
- Figure 3.8 : Madai agricultural vegetation. Left: Salak tree (*Salacca zalacca*) planted as the main crop in the sampling area. Right: Rambutan tree (*Nephelium lappaceum*) and Langsat tree (*Lansium domesticum*). 36

- Figure 3.9 : Map of Sabah with all indicated study sites divided into 5 devision. A. Kinabalu Parks: I) Serinsim Substation, II) Sayap Substation, III) Poring Substation and IV) Kinabalu Park. B. West Coast Region: I) UMS, II) Gaya Island. III) Inobong Substation and IV) Mahua Substation. C. Sandakan Region: I) Gomantong Cave and II) Sukau. D. Interior Division. I) ICCA, II) Sg. Kangkawat and III) Sg. Rawog. E. Tawau Region: I) Madai and II) Tawau Hill Park 37
- Figure 3.10 : Inoculation points. A. Two points inoculation. B. Three points inoculation. 39
- Figure 4.1 : Bubble map of the distribution of entomopathogenic fungi in Sabah from 2017-2020. The size of bubble indicates the weightage of the total abundance of specimens collected. 48
- Figure 4.2 : Order of insect host infected by entomopathogenic fungi 49
- Figure 4.3 : Habitat of entomopathogenic fungi in Sabah 50
- Figure 4.4 : Macrofeatures of *Ophiocordyceps unilateralis*. A. The ascome of *O. unilateralis* arises from the back of the head. B. Fertile region of lateral cushions of the ascoma (black arrow) and brown mycelium covering the outer body and attach to the leaf (white arrow) C. *O. unilateralis* on ant host of *Dinomyrmex gigas* D. *O. unilateralis* on ant host of *Polyrhachis* sp. ant, the ants was identified based on its spine (white arrows). Scale bar of A – 10 mm, Scale bar of B – 20 mm, Scale bar of C – 40 mm, Scale bar of D – 25 mm. 55
- Figure 4.5 : Macrofeatures of *Ophiocordyceps unilateralis*. A. Section through stroma of the fungi. The *O. unilateralis* perithecia are in the upper portion (white arrows) and abundant of 56

thread-like ascospores of the fungi (black arrow) coming out of the perithecia. B. Section of the ascoma showing the arrangement of the peridium (white arrow) and the apical peridium (black arrow). C. Ascus cap, characteristic of the Clavicipitales (arrow). D. Long, cylindrical asci containing the filiform asexual spores or ascospores of the fungi. Scale bar of A – 500µm, Scale bar of B - 25µm, Scale bar of C - 10µ, Scale bar of D - 25µm.

Figure 4.6 : Macrofeatures of *Ophiocordyceps myrmecophila*. A. The ascome of *O. myrmecophila* arising from the leaf litter in the field. B. *Camponotus* sp. ant with stromata arising from the dorsal pronotum with a characteristic yellowish coloration, orange yellowish when dry. C. Lateral, fertile cushion (ascoma). D. Close up of the dead ant's head showing the arising of the stromata of the fungi from the dorsal pronotum. Scale bar of A – 10mm, Scale bar of B – 20mm, Scale bar of C – 5mm, Scale bar of D – 2.5mm.

Figure 4.7 : Macrofeatures of *Ophiocordyceps myrmecophila*. A. Section through stroma of the fungi. The *O. myrmecophila* perithecia (arrows). B. Long, cylindrical asci containing the filiform asexual spores or ascospores of the fungi and ascus cap, characteristic of the Clavicipitales (arrow). C. Section of the ascoma showing the arrangement of the peridium (arrow) and the apical peridium (white arrow). D. Section showing flask-shaped perithecia (short arrow) containing abundant of thread-like asci (arrow). Scale bar of A – 500µm, Scale bar of B – 5µm, Scale bar of C – 100µm, Scale bar of D – 100µm.

Figure 4.8 : Macrofeatures of *Hirsutella rhossiliensis*. A. The *H. rhossiliensis* infect a wasp with a stromata arising from the wing part. B. Upper view showing a stroma arise from the

wing part of the wasp. C. The wasp perform death bite and brown mycelium covered the mandible and attached it to the leaf (black arrow) and aerial brown mycelium (white arrow). D. Close up of the lateral, fertile cushion, ascoma of the fungi. Scale bar of A – 25mm, Scale bar of B – 10mm, Scale bar of C – 10mm, Scale bar of D – 10mm.

Figure 4.9 : Microfeatures of *Hirsutella rhossiliensis*. A. Section through stroma of the fungi with the abundant of thread-like ascospores of the fungi (black arrow) coming out of the perithecia B. Section showing flask-shaped perithecia (white arrow) containing abundant of thread-like asci (black arrow). C. Long, cylindrical asci containing the filiform asexual spores or ascospores of the fungi and ascus cap, characteristic of the Clavicipitales (arrow). D. Long, cylindrical asci containing the filiform asexual spores or ascospores of the fungi. Scale bar of A – 500µm, Scale bar of B – 100µm, Scale bar of C – 5µm, Scale bar of D - 25µm.

Figure 4.10 : Macro and micro-features of *Hirsutella citriformis*. A. The *H. citriformis* infect a wasp with a brown mycelium and a long synnemata covering the host. B. Close up view showing synnemata arise from the abdomen of the wasp (white arrow) and brown mycelium covering the host body (black arrow). C. Phialides arise as lateral cells from the outer hyphae of the synnema (black arrow). D. Microscopic view of the phialides and close up view of the anamorph structure of the fungi. Scale bar of A – 2mm, Scale bar of B – 0.5mm, Scale bar of C – 0.5mm, Scale bar of D - 500µm.

Figure 4.11 : Macrofeatures of *Isaria cicadae*. A. The *I. cicadae* emerge from the soil with its distinct cockscomb-like or broccoli-like branching synnemata (white arrow). B. The fungi with its

cicada host C. Close up images of the synnemata shows the cockscomb-like shape. D. Close up images of the cicada with the fungi synnemata emerge from the back part of the insects (white arrow). Scale bar of A – 25mm, Scale bar of B – 20mm, Scale bar of C – 10mm, Scale bar of D – 20mm.

Figure 4.12 : Microfeatures of *Isaria cicadae*. A. The microscope view of the *I. cicadae* synnemata shows the powdery and floccose near the apex (white arrow) and the branching of the synnemata (black arrow). B. The close up view of the apex of the synnemata shows the powdery and floccose apex. Scale bar of A - 500µm, Scale bar of B – 200µm. 68

Figure 4.13 : Macrofeatures of *Cordyceps javanica*. A. The host, spider covered by the mycelium of the *C. javanica*. B. Abdominal part of the spider covered with the pinkish colour spores of the fungi C. Close up images of the leg part of the spider shows the fungi covering the body part of the host (white arrow). D. Close up images of the mandible of the spider shows the white mycelium (black arrow) and the the fungi phialides (white arrow). Scale bar of A – 1cm, Scale bar of B – 1cm, Scale bar of C - 500µm, Scale bar of D - 500µm. 70

Figure 4.14 : Microfeatures of *Cordyceps javanica*. A. The fungi phialide in whorl of two and three (arrow). B. Smooth-walled, fusiform and hyaline conidia. Scale bar of A - 25µm, Scale bar of B - 10µm. 71

Figure 4.15 : Macrofeatures of *Cordyceps cylindrica*. A. Anamorph of *C. cylindrica* covered the spider host body. B. *C. cylindrica* infect the spider host with its natural habitat of on leaf C. Close up images of the leg part of the spider covered with the brownish-purple conidia of the fungi (white arrow). 73

Scale bar of A – 2cm, Scale bar of B – 5cm, Scale bar of C – 1cm.

- Figure 4.16 : Microfeatures of anamorph of *Cordyceps javanica*. A. The fungi phialide in whorl (arrow). B. Smooth-walled, fusiform and hyaline conidia. Scale bar of A - 20µm, Scale bar of B - 20µm. 74
- Figure 4.17 : Macrofeatures of *Hevansia websteri*. A. *H. websteri* covered the body part of the moth and white mycelia hold the cadaver on the leaf (white arrow) B. White synnemata of the fungi covered the abdomen of the host C. The ellipsoid phialides of the fungi. D. Apex of the fungi synnemata. Scale bar of A – 1cm, Scale bar of B – 500mm, Scale bar of C - 20µm, Scale bar of D - 200µm. 76
- Figure 4.18 : Macro and microfeatures of *Purpureocillium* sp. A. *Purpureocillium* white synnemata covered the body part of the whitefly. B. Close up view of the synnemata. C. The verticillate phialides of the fungi. D. Ellipsoidal conidia of the fungi with some is still in connected in chain. Scale bar of A – 1cm, Scale bar of B – 2mm, Scale bar of C - 10µm, Scale bar of D - 20µm. 78
- Figure 4.19 : Macrofeatures of *Metarhizium granulomatis*. A. Distinct green conidia of *Metarhizium* covered the whole body of the cadaver. B. *M. granulomatis* covered the insect host, cockroach. C. White mycelium of the fungi covered the leg part of the cadaver (white arrow). Scale bar of A – 2mm, Scale bar of B – 5mm, Scale bar of C – 2mm. 80
- Figure 4.20 : Microfeatures of *Metarhizium granulomatis*. A. Germinating 81

conidium of the fungi. B. Smooth-walled, fusiform and hyaline conidia. Scale bar of A – 20 μm , Scale bar of B – 20 μm .

Figure 4.21 : Macrofeatures of *Aspergillus oryzae*. A. The bees cadaver covered by *A. oryzae* fungi. B. Close up images of the phialides of the fungi. C. Front view of the culture, yellow conidia were clearly seen. D. Reverse view of the culture shows the white mycelium. Scale bar of A – 0.5cm; Scale bar of B – 2mm; Scale bar of C – 5cm; Scale bar of D – 5cm. 84

Figure 4.22 : Microfeatures of *Aspergillus oryzae*. A. Phialides of the *A. oryzae* covering the entire surface of the vesicle B. (Sub)spherical conidia of the fungi (white arrow). C. Phialides of the fungi covering the upper three to fourth surface of the vesicle. D. Conidiogenous cells of *A. oryzae* (white arrow). Scale bar of A - 200 μm ; Scale bar of B – 20 μm ; Scale bar of D – 50 μm ; Scale bar of D – 50 μm . 85

Figure 4.23 : Macrofeatures of *Ophiocordyceps nutans*. A. Stroma of the fungi emerge from the body part of the shield bug with a brown ascoma B. The stroma emerge from side of the abdomen of the cadaver (white arrow). C. Stipitate with globose to elongated fertile ascoma. Scale bar of A – 25mm, Scale bar of B – 25mm, Scale bar of C – 10mm. 88

Figure 4.24 : Microfeatures of *Ophiocordyceps nutans*. A. Section through stroma of the fungi. B. Section of the ascoma showing the arrangement of the peridium (white arrow) and the flask shape perithecia containing abundant of thread-like asci (black arrow). C. Long, cylindrical asci containing the filiform asexual spores or ascospores. D. Ascus cap of the fungi, one 89

of the distinct characteristics of the Clavicipitales (arrow). Scale bar of A - 500µm, Scale bar of B - 300µm, Scale bar of C - 20µm, Scale bar of D - 5µm.

Figure 4.25 : Micro and macrofeatures of *Beauveria brongniartii*. A. 91
Cadaver of long-legged spider on a tree leaf. B.
Conidiogenous cells arising from the insect body of the
insects (white arrow). C. Globose, hyaline, thin-walled and
smooth conidia. (black arrow). D. Ellipsoidal, hyaline, and
smooth conidia (black arrow). Scale bar of A – 10mm, Scale
bar of B – 2mm, Scale bar of C - 5µm, Scale bar of D - 5µm.

Figure 4.26 : The amplicons for ITS (A) and LSU (B) separated on 1% 93
(w/v) agarose gel. Lanes B1 to B11 are amplicons of various
samples, DNA ladder is the 1 kb ladder, and negative and
positive control indicated with -ve and +ve respectively. A.
ITS 1 and ITS 4. B. LROR and LR5.

Figure 4.27 : A portion of the assembled ITS sequences alignment 94
between Genbank sequences of entomopathogenic fungi of
Sabah in this study shown in MEGA 5.2. The same color
within each vertical column indicated the similarities.

Figure 4.28 : A portion of the assembled LSU sequences alignment 94
between Genbank sequences of entomopathogenic fungi of
Sabah in this study. The same color within each vertical
column indicated the similarities.

Figure 4.29 : A portion of the assembled concatenated ITS and LSU 95
sequences alignment between Genbank sequences of
entomopathogenic fungi of Sabah in this study. The same
color within each vertical column indicated the similarities.

- Figure 4.30 : A portion of the assembled concatenated of ITS and LSU sequences alignment between Genbank sequences of Ophiocordycipitaceae within the *unilateralis* clade in this study. The same color within each vertical column indicated the similarities. 95
- Figure 4.31 : Single gene phylogeny of Entomopathogenic fungi species based on Maximum Likelihood (ML) analysis. Support values are obtained from ML bootstrap values ($BS \geq 70$) and Bayesian posterior probability ($PP \geq 0.90$). Sequences from this study are indicated in bold and orders are indicated on the right. A. ITS phylogeny. B. LSU phylogeny. 97
- Figure 4.32 : Maximum Likelihood (ML) analysis of ITS gene phylogeny of representative entomopathogenic fungi DNA from this study (5% similarity cutoff) and GenBank sequences ($n = 65$). Support values are obtained from ML bootstrap values ($BS \geq 70$) and Bayesian posterior probability ($PP \geq 0.90$). Major clade indicated in different colors. Sequences from this study are indicated in bold and orders are indicated on the right. 101
- Figure 4.33 : Fungi-Host relationship with Maximum Likelihood (ML) analysis of concatenated phylogeny (ITS + LSU) of representative entomopathogenic fungi DNA and their hosts from this study (5% similarity cutoff) and GenBank sequences ($n = 51$). Different host indicated in different colors. Sequences from this study are indicated in bold and orders are indicated on the right. 104
- Figure 4.34 : Maximum Likelihood (ML) analysis of concatenated phylogeny (ITS and LSU) of representative *Ophiocordyceps* 106