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Diversity of entomopathogenic fungi in protected forest in the Eastern of Thailand

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Abstract: The entomopathogenic fungi or insect fungi are widespread in nature. They are a well-known as a rich source of bioactive compounds which prove to useful in medicinal and agricultural applications. There are about 400 species recorded from natural forests in Thailand. However, our knowledge of entomopathogenic fungi species diversity in the eastern of Thailand is limited. During June 2017-December 2018, we studied species diversity of entomopathogenic fungi in Khao Soi Dao Wildlife Sanctuary, Chanthaburi Province and Mu Ko Chang National Park, Trat Province. A total of 537 samples were classified to 3 families; Clavicipitaceae, Cordycipitaceae and Ophiocordycipitaceae in the order Hypocreales. These fungi were revealed into 43 species based on morphological character study. The infected insects were in 8 major orders including Coleoptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Neuroptera, Orthoptera and spider in Araneae order of class Arachnida. The most infected insects were in Hymenoptera order (44.87 percentage). The most abundant species was *Ophiocordyceps unilateralis*. In addition, *Ophiocordyceps* species found on coleoptera larvae and orthoptera in Mu Ko Chang National Park will be tentatively described as new species based on morphology and phylogenetic analysis.

Keywords: Entomopathogenic Fungi, Diversity, Taxonomy, Protected Forest, Eastern

Introduction

The term entomopathogenic refers to those microorganisms that are capable of attacking insects using them as hosts to develop part of their life cycle (Delgado and Murcia, 2011). Entomopathogenic fungi are fungi that parasitizes the wide range of insects and spiders. The entomopathogenic species are found in almost all ecosystems. The largest numbers of fungal species that are pathogenic to insects belong to the order (Molnar et al., 2010). For more than one thousand years, medicinal *Ophiocordyceps sinensis* has been known as a unique Tibet's prized parasitic fungus in the Qinghai-Tibetan Plateau for its mysterious life history (Lo et al., 2013). The genus *Beauveria* and *Metarhizium* (Cordycipitaceae) play an important role in controlling insect populations and have been increasingly utilized as biological control agents of insect pests throughout the world. Nowadays, the entomopathogenic fungi are becoming increasingly popular from medical and pharmacological researchers because these fungi are abundant source of useful natural products with various biological activities (Das et al., 2010). There are about 700 species of entomopathogenic fungi worldwide (Roy et al., 2006).

Thailand is rich in biodiversity located in the tropical areas (Luangsa-ard et al. 2010). At this present, more than 400 species of entomopathogenic species were reported from Thailand (Luangsa-ard et al. 2010). The fungus *Cordyceps gentilis* on a hornet from northern Thailand was the first species recorded by Petch (1932) which was later considered as *Cordyceps sphecocephala* by Hywel-Jones (1995).

The diversity of entomopathogenic fungi of the eastern part is poorly known and few collections have been made. The objectives of the present study were to explore diversity of entomopathogenic fungi in eastern Thailand. Specifically, Department of National Parks, Wildlife and Plant Conservation (DNP) and BIOTEC have contributed to the studying of diversity of entomopathogenic fungi in Khao Soi Dao Wildlife Sanctuary (KSD), Chanthaburi Province and Mu Ko Chang National Park (MKC), Trat Province, Thailand.

Materials and Methods

Collecting area

Entomopathogenic fungi specimens were collected in Khao Soi Dao Wildlife Sanctuary and Mu Ko Chang National Park during June 2017-December 2018.

Specimen collection and isolation

The undersides and upper sides of leaves, stems and leaf litter in the forest were examined for fungi growing on dead insects. Collected specimens were placed in plastic boxes and returned to the laboratory for further examination. Fresh materials were examined and isolated in pure culture from both the anamorph and

teleomorph using the technique described by Luangsa-ard et al. (2012). Strains were isolated from the infected specimens on potato dextrose agar (PDA). Cultures were incubated at 25°C for up to 10 weeks with high moisture provided by a moist cotton sheets. Slow growing isolates were daily checked. After incubation, fungal growth was transferred with a sterile needle to new PDA plates. Fungal culture were deposited in the Forest and Plant Conservation Research Office and the BIOTEC Culture Collection (BCC).

Morphological study

Fruiting bodies were examined using free hand sections under a stereo-microscope (Olympus SZX16). Water-mounted slides were prepared for a microscope study and photographed under a compound microscope (Olympus CX41). Morphological characteristics of the fungus were examined using classical mycological techniques based on growth rate and macroscopic and microscopic characteristics. All specimens were dried at 50ºC using food dehydrator. The IF numbers refer to herbarium material and to cultures deposited at the Forest herbarium (mushroom section), DNP and the BCC, Thailand. In this study, clearly distinguishable morphological samples will be studied molecular taxonomy according to Luangsa-ard et al. (2018). However, the data of molecular taxonomy will not be showed and discussed in this paper.

Result & Discussion

During this study, we have collected infected insects 397 and 140 samples from KSD and MKC, respectively. A total of 537 entomopathogenic fungi were collected and then identification on the basis of morphological characters at Forest Microbiology Laboratory, DNP. The specimens were mostly founded during rainy season (May to July). All samples were identified 15 genera belonging to 3 families; Clavicipitaceae, Cordycipitaceae and Ophiocordycipitaceae in the order Hypocreales. These fungi were revealed into 43 species as shown in table 1. Currently, the strains of entomopathogenic fungi are listed in the following orders: Hypocreales (various genera), Onygenales (Ascosphaera genus), Entomophthorales, and Neozygitales (Entomophthoromycota) (Mora et al., 2017). Fungal species were varied on collections made over different site. Among these species, some were reported as biocontrol agents, some with medicinal properties. *Ophiocordyceps unilateralis* was the most abundant entomopathogenic fungi species of affixed to the underside of a leaves. Kobmoo et al. (2015) mentioned that *O. unilateralis* is an ubiquitous pathogen of ants and in addition the species had hidden phylogenetic diversity associated with host specificity. The occurrence of *B. bassiana* and *M. anisopliae* were zero number in KSD comparing with both were common in MKC.

The host insects were in 8 major orders including Coleoptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Neuroptera, Orthoptera and spider in Araneae of class Arachnida. In fact, the hosts are spread among 20 of the 31 orders of insects, in all developmental stages: eggs, larvae, pupae, nymphs, and adults (Araújo and Hughes, 2016). In this study, the highest frequency of infection insects were in Hymenoptera order (44.87 percentage). Some fungal species were highly host specific, whereas others had broad host. The genera *Aschersonia*, *Hypocrella* and *Moelleriella* were specific to scale insects. The *Gibellula* specimens were regularly collected on spider hosts that indicate its specific presence. Ants were found to be infected by *Ophiocordyceps irangiensis*/*myrmecophila* on the ground or underneath the leaf litter. A thin single yellow synnema of the *O. irangiensis*/ *myrmecophila* arising from the host was produced. In this case, the hosts were found on the ground, or underneath the leaf litter. In this study, we found that there were 19 species with hosts in the order Hemiptera. This has clearly shown that Hemiptera is wide host range. Faria and Wraight (2007) reported approximately 60% of insect diseases are caused by pathogenic fungi.

The isolation of these fungal species in culture was successful in some fungal species. However, the isolation of *O. unilateralis* was needed a complicated method and culture medium according by Wongsa et al. (2005).

Furthermore, two *Ophiocordyceps* species will be described as new species based on morphology and phylogenetic analysis. The *Ophiocordyceps* sp. 1 resemble *Ophiocordyceps bruneipunctata*, buried in the forest soil, were found parasitizing coleoptera larvae collected from dry evergreen forest at Mu Ko Chang National Park. The fungus produced pale pink stroma on morphological character. The *Ophiocordyceps* sp. 2 was collected from rotting log. Hosts were immature orthopteran. Their morphological character and phylogenetic study clearly distinct from genera *Ophiocordyceps*. The surveys of entomopathogenic fungi in Thailand have a very long history with BIOTEC staff. Their several field studies have reported the new species of entomopathogenic fungi from natural forests (Luangsa-ard et al., 2017; Luangsa-ard et al., 2018; Khonsanit et al., 2019; Kuephadungphan et al., 2019). These findings encourage further research on the fungus in protected forests including national parks and wildlife sanctuaries in Thailand.

Conclusion

Entomopathogenic fungi were mainly found in order Hypocreales (Clavicipitaceae, Cordycipitaceae and Ophiocordycipitaceae). Fungus diversity was 43 species from both protected forests in the eastern part of Thailand. Fungal common to both study sites was *Ophiocordyceps irangiensis*/ *myrmecophila.* Two species of *Ophiocordyceps* proved to be undescribed and will be proposed as new species after confirmed with molecular results. At this

present, studying biodiversity need to explore the potential economic benefit from entomopathogenic fungi concurrently. Thus, there is an important to contribute the collaboration with many research groups including biotechnologists, pharmacologists, analytical chemists, medical scientists etc. (Shrestha et al., 2016).

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Table 1. Diversity of entomopathogenic fungi in KSD, Khao Soi Dao Wildlife Sanctuary and MKC, Mu Ko Chang National Park in eastern, Thailand (June 2017 to December 2018).

Genus/species	Host/Insect Order	KSD	MKC
Family Clavicipitaceae			
Aschersonia badia	Scale insect/Hemiptera	0	1
Aschersonia confluens	Scale insect/Hemiptera	2	8
Aschersonia luteola	Scale insect/Hemiptera	0	3
Aschersonia marginata	Scale insect/Hemiptera	1	1
Aschersonia placenta	Scale insect/Hemiptera	6	1
Aschersonia sp.	Scale insect/Hemiptera	2	1
Aschersonia oxystoma	Scale insect/Hemiptera	0	3
Conoideocrella luteorostrata	Scale insect/Hemiptera	2	7
Conoideocrella tenuis	Scale insect/Hemiptera	3	0
Hypocrella badia	Scale insect/Hemiptera	1	0
Hypocrella calendulina	Scale insect/Hemiptera	5	0
Hypocrella discoidea	Scale insect/Hemiptera	2	6
Hypocrella sp.	Scale insect/Hemiptera	1	0
Hypocrella luteola	Scale insect/Hemiptera	0	1
Metarhizium anisopliae	Coleopteran/Coleoptera	0	1
Moelleriella mollii	Scale insect/Hemiptera	3	7
Moelleriella raciborskii	Scale insect/Hemiptera	8	4
Family Cordycipitaceae			
Akanthomyces pistillariiformis	Moth/Lepidoptera	0	1
Akanthomyces sp.	Moth/Lepidoptera	0	1
Beauveria bassiana	Coleopteran/Coleoptera	0	21
Beauveria sp.	Coleopteran/Coleoptera	0	5
Cordyceps javanica	Spider/Araneae	1	0
Cordyceps sp.	Spider/Araneae	1	0
Cordyceps tenuipes	Lepidopteran pupae/Lepidoptera	1	2
Cordyceps tuberculata	Moth/Lepidoptera	0	3
Engyodontium sp.	Scale insect/Hemiptera	0	1
	Spider/Araneae	0	1
Gibellula pulcha	Spider/Araneae	1	2
Gibellula sp.1	Spider/Araneae	1	3
Gibellula sp.2	Spider/Araneae	8	0
Isaria sp.	Spider/Araneae	0	1
Torrubiella hemipterigenum	Leafhopper/ Hemiptera	1	3

Table 1. (continued)

Genus/species	Host/Insect Order	KSD	MKC
Family Ophiocordycipitaceae			
Ophiocordyceps irangiensis/myrmecophila	Ant/Hymenoptera	52	29
Ophiocordyceps longissima	Cicada/Hemiptera	3	7
Ophiocordyceps sporangifera	Elateridae larvae/Coleoptera	0	1
Ophiocordyceps sp. 1	Coleopteran larvae/Coleoptera	0	28
Ophiocordyceps sp. 2	Orthopteran/Orthoptera	0	7
Ophiocordyceps sphecocephala	Wasp/Hymenoptera	0	3
Ophiocordyceps unilateralis	Ant/Hymenoptera	141	15
Ophiocordyceps cf. *communis*	Termite/Isoptera	51	0
Ophiocordyceps nutan	Stink bug/Hemiptera	2	0
Ophiocordyceps houaynhangensis	Lepidopteran/Lepidoptera	49	0
Polycephalomyces nipponicus	Neuroptera	10	0
Purpureocillium takamizusanensis	Stink bug/Hemiptera	1	0
Total		397	140

Figure 1. a) *Ophiocoryceps* sp. 1 in habitat showing pale pink stroma emerging from coleopteran larva underneath the forest ground b) *Ophiocoryceps* sp. 2 with mushroom-like head on orthopteran.