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

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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

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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 entomolo-

gists 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, conidigenous 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: Geysir 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	
<i>Acremonium charticola</i>									1			0.35
<i>A. crassum</i>					1							0.35
<i>Aschersonia</i> sp.					1							0.35
<i>Aspergillus</i> sp.	1	1					1					1.06
<i>Beauveria bassiana</i>		3			1	1		1				2.12
<i>B. brongniartii</i>		7	1	2					1	2	1	4.95
<i>Cladosporium</i> sp.								1				0.35
<i>Cordyceps militaris</i>								12				4.24
<i>C. militaris</i> f. <i>sphaerocephala</i>								1				0.35
<i>C. nelumboides</i>	1											0.35
<i>Cordyceps</i> sp.								1				0.35
<i>Hymenostilbe furcata</i>				1								0.35
<i>Hypocrella</i> sp.					2							0.71
<i>Isaria cicadae</i>					3							1.06
<i>I. farinosus</i>								2				0.71
<i>I. fumosoroseus</i>				37								13.07
<i>I. tenuipes</i>								15				5.3
<i>Ophiocordyceps crinalis</i>								1				0.35
<i>O. dipterigena</i>			1									0.35
<i>O. elongata</i>								1				0.35
<i>O. filiformis</i>								1				0.35
<i>O. longissima</i>					1							0.35
<i>O. mrciensis</i>	1											0.35
<i>O. myrmecophila</i>						64						22.61
<i>O. nutans</i>				11								3.89
<i>O. oxycephala</i>						11						3.89
<i>O. pseudolloydii</i>						9						3.18
<i>O. sphecocephala</i>						1						0.35
<i>O. unilateralis</i>						39						13.78
<i>Paecilomyces marquandii</i>		1		19							1	7.42
<i>Sporothrix insectorum</i>						12						4.24
<i>Stilbella buquetii</i>						3						1.06
<i>Torrubiella hemipterigena</i>				1								0.35
<i>Verticillium</i> sp.		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
<i>Cordyceps</i> and <i>Ophiocordyceps</i>	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 (Burgess, 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
<i>Cordyceps</i> and <i>Ophiocordyceps</i>	12	9	1
Other taxa	9	16	4
Species richness (<i>S</i>)	21	25	5
Individual numbers	142	80	61
Shannon index (<i>H'</i>)	2.08	2.73	1.03
Simpson index (<i>I-D</i>)	0.80	0.90	0.56
Evenness (<i>E_H</i>)	0.38	0.61	0.56

Table 5. Similarity indices of fungal taxa between different collection sites.

Hosts	Sørensen's index (<i>S'</i>)	
	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, Orthopter, 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 Lepi-

doptera, *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

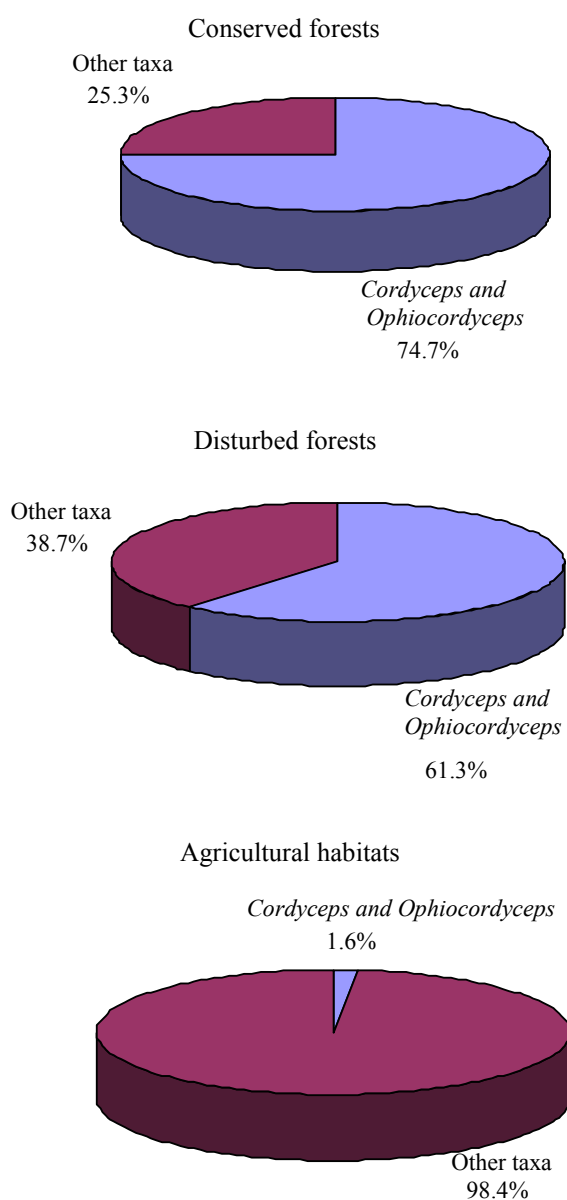


Fig. 2. Percentage of fungal records in different habitats.

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.

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Spore productivity in six entomopathogenic fungal strains isolated from insect cadavers at four locations in Chiang Mai province was evaluated in five cereal grains: white-rice, wheat, rye, corn and sorghum. According to sequence analysis of the internal transcribed spacer regions of these isolates

1 Microbiology Division, Department of Biology Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand. Entomopathogenic fungi are ubiquitous in tropical rainforests and feature a high level of diversity. This group of fungi not only has important ecological value but also medicinal value. Nevertheless, they are often ignored, and many unknown species have yet to be discovered and described. The present study aims to contribute to the taxonomical and phylogenetic understanding of the genus *Paraisaria* by describing three new species collected from Guizhou and Yunnan Provinces in China and Krabi Province in Thailand.

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