Biodiversity of invertebrate-pathogenic fungi in Thailand

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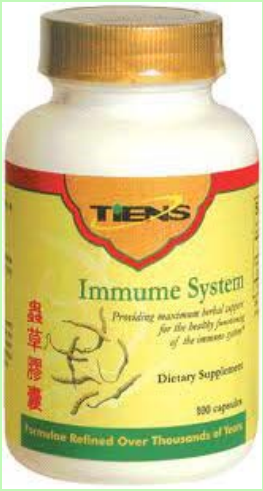
Ophiocordyceps sinensis

dong chong xia cao

‘winter-worm, summer-grass’



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Why study invertebrate-pathogenic fungi?

- Important component in traditional Chinese medicine
- Historic use in biological control
- Important sources of novel metabolites and enzymes

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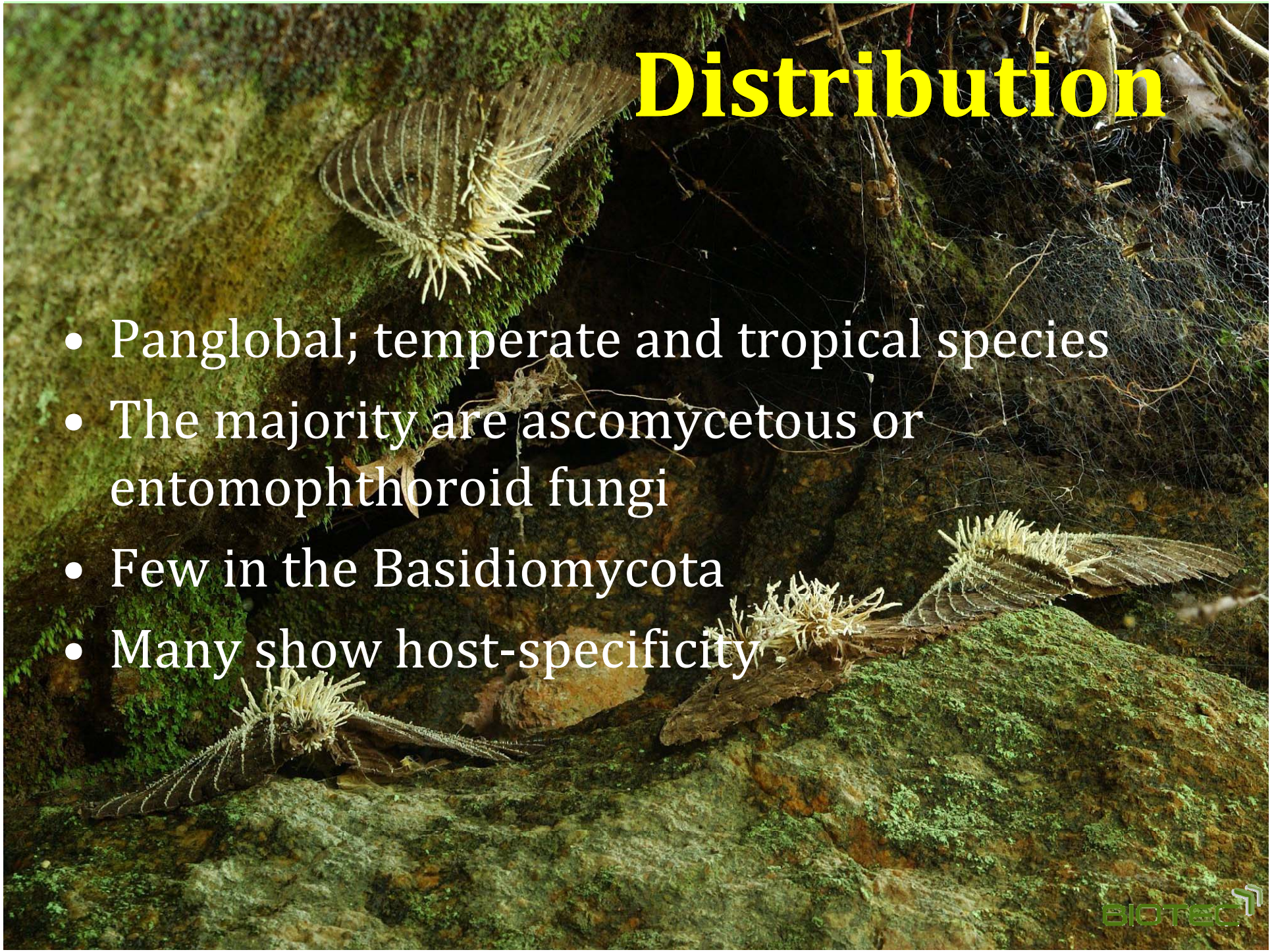
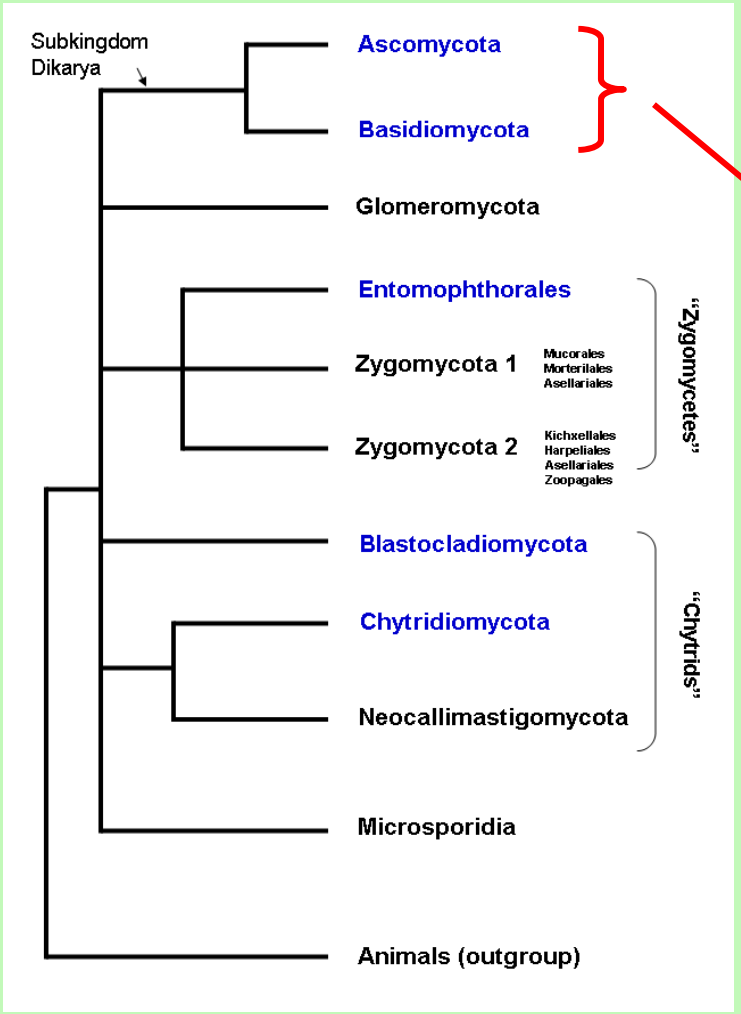
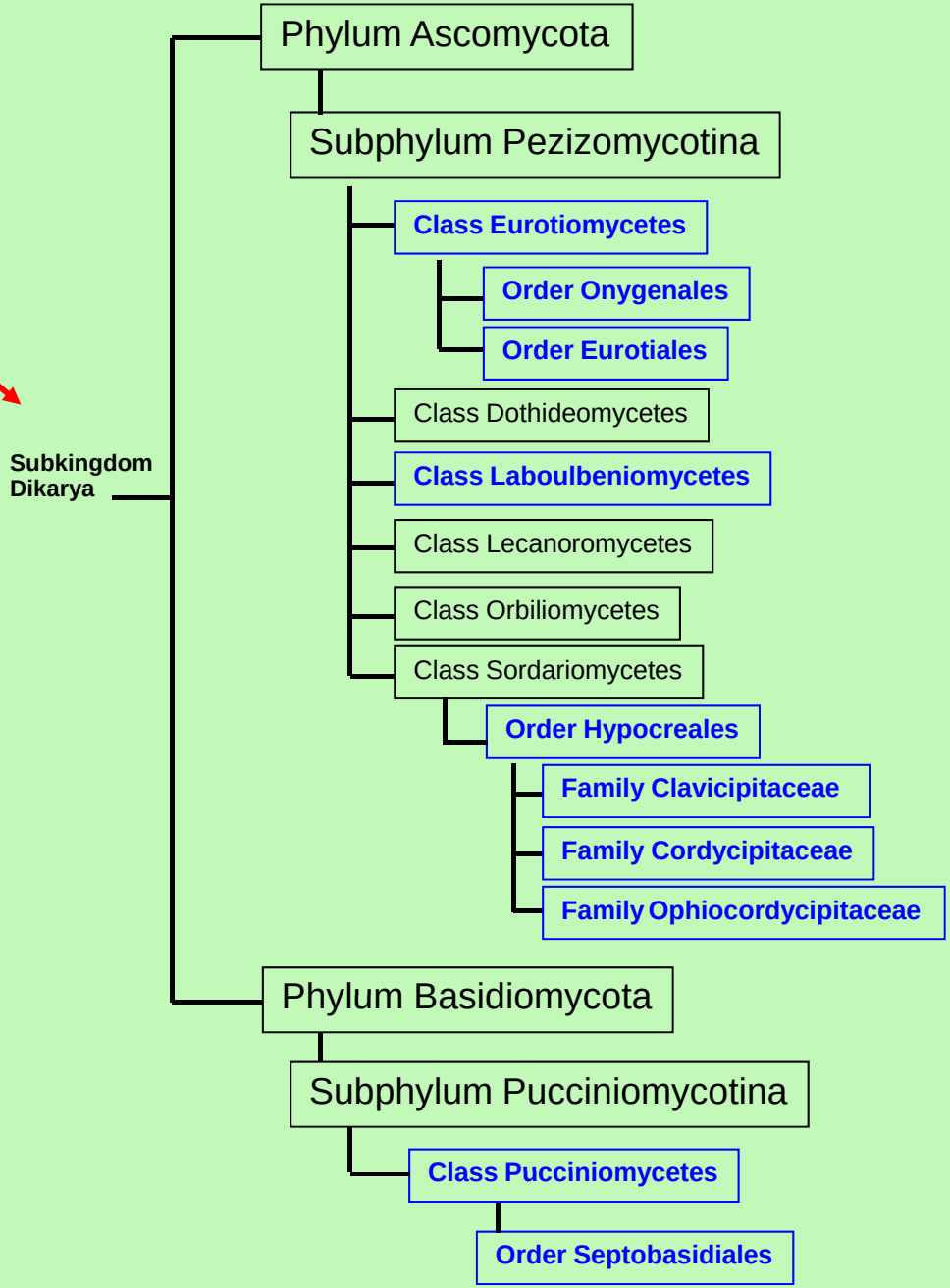


Distribution

- Panglobal; temperate and tropical species
- The majority are ascomycetous or entomophthoroid fungi
- Few in the Basidiomycota
- Many show host-specificity


Subkingdom
Dikarya
Ascomycota
Basidiomycota
Glomeromycota
Entomophthorales
Zygomycota 1
Mucorales
Morterilales
Asellariales
Zygomycota 2
Kichxellales
Harpeliales
Asellariales
Zoopagales
"Zygomycetes"
Blastocladiomycota
Chytridiomycota
Neocallimastigomycota
"Chytrids"
Microsporidia
Animals (outgroup)


Subkingdom
Dikarya
Phylum Ascomycota
Subphylum Pezizomycotina
Class Eurotiomycetes
Order Onygenales
Order Eurotiales
Class Dothideomycetes
Class Laboulbeniomycetes
Class Lecanoromycetes
Class Orbiliomycetes
Class Sordariomycetes
Order Hypocreales
Family Clavicipitaceae
Family Cordycipitaceae
Family Ophiocordycipitaceae
Phylum Basidiomycota
Subphylum Pucciniomycotina
Class Pucciniomycetes
Order Septobasidiales

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Oldest record of insect-fungi on a scale insect

Molecular Phylogenetics and Evolution xxx (2008) xxx–xxx

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journal homepage: www.elsevier.com/locate/ympev

The oldest fossil evidence of animal parasitism by fungi supports a Cretaceous diversification of fungal–arthropod symbioses

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[b] Department of Zoology, Oregon State University, Corvallis, OR 97331, USA

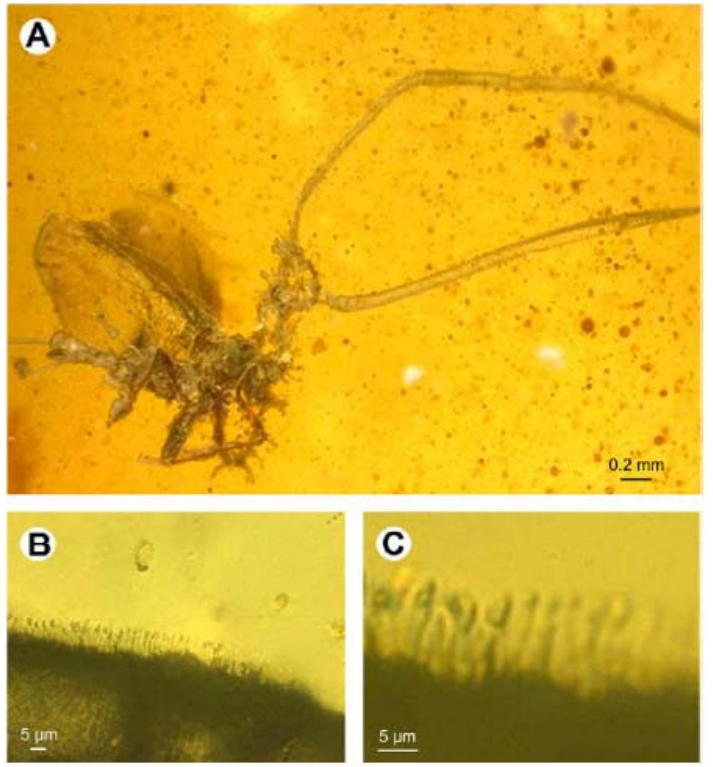

Fig. 1. Photographs of the holotype of *P. coccophagus gen. et sp. nov.* that shows the oldest evidence of fungal parasitism of animal. (A) Synnemata arising from a male scale insect (Albicoccidae) in Burmese amber. (B) Conidiogenous cells that are distributed in a hymenium-like layer. (C) Conida and conidiogeneous cells.

Paleoophiocordyceps coccophagus

Early Cretaceous period
(100 – 110 mya)

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There is good evidence that the Himalayan people and Romans were aware of insect fungi over 2000 years ago.

Japanese sericulturists knew of insect diseases of silkworms over 1000 years ago

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Early work
Saw *Beauveria bassiana*
as a **BAD** guy
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Beauveria bassiana for biocontrol

Rice field at Phak Hai district, Ayutthaya

After field infected with brown planthopper

B. bassiana used as biocontrol a in rice field

Cassava field at Sikhio district, Nakhon Ratchasima

After field infected with pink mealy bug

B. bassiana used as biocontrol a in Cassava field

Insects

- have the most dominant diversity on the planet
- they have adapted to every major habitat except the sea
- There are 41 taxonomic orders known

To Put Things into Perspective

The vast majority of insects are either beneficial or harmless to humans

<1% of known insect species are considered pests.

The Pestiferous Few

• destroy our crops	- brown plant hoppers - scale insects, - mealy bugs, - aphids
• eat the food from our table	- ants, flies
• eat the table	- termites
• eat us	- mosquitoes

Coleoptera











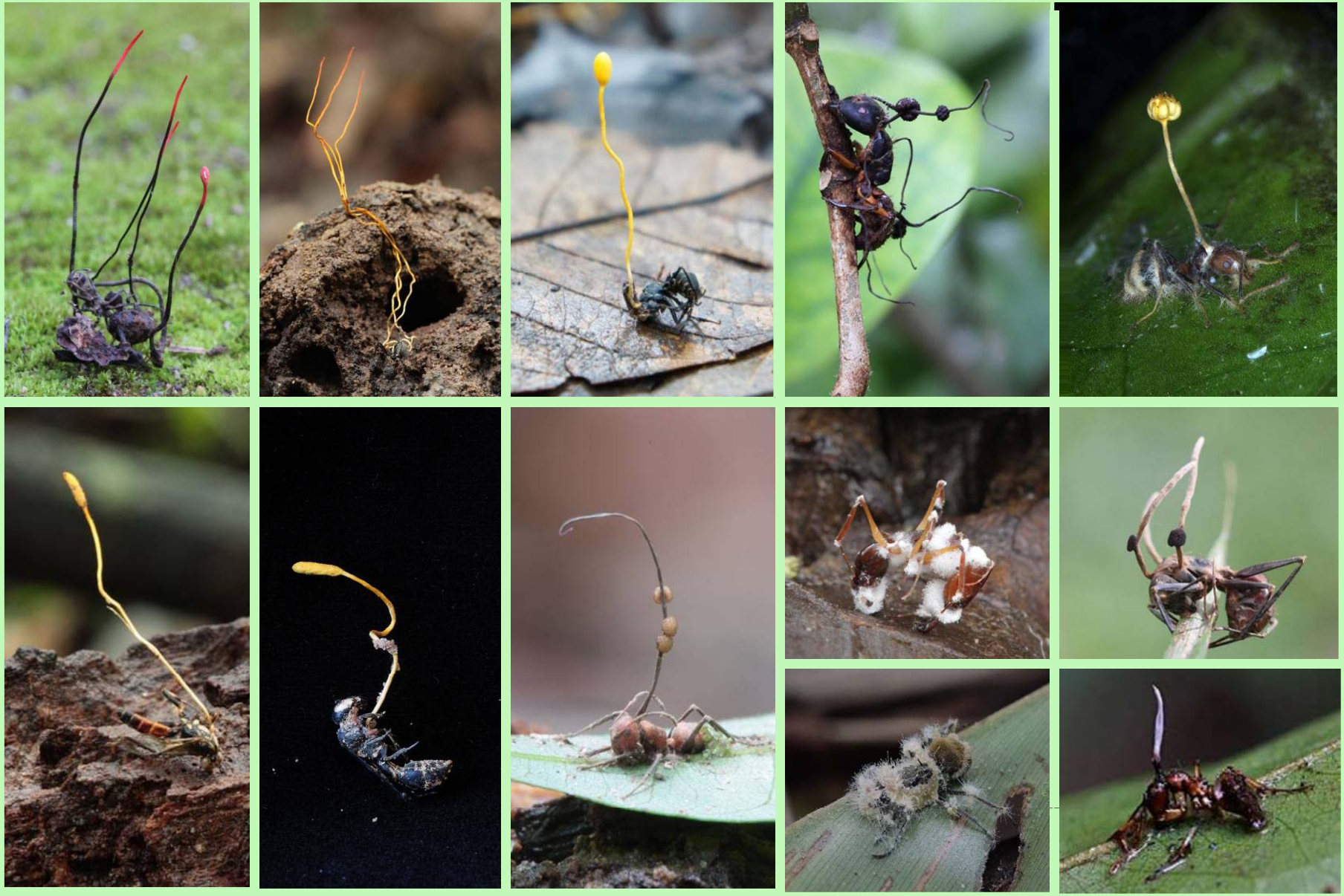
Dermaptera

Diptera

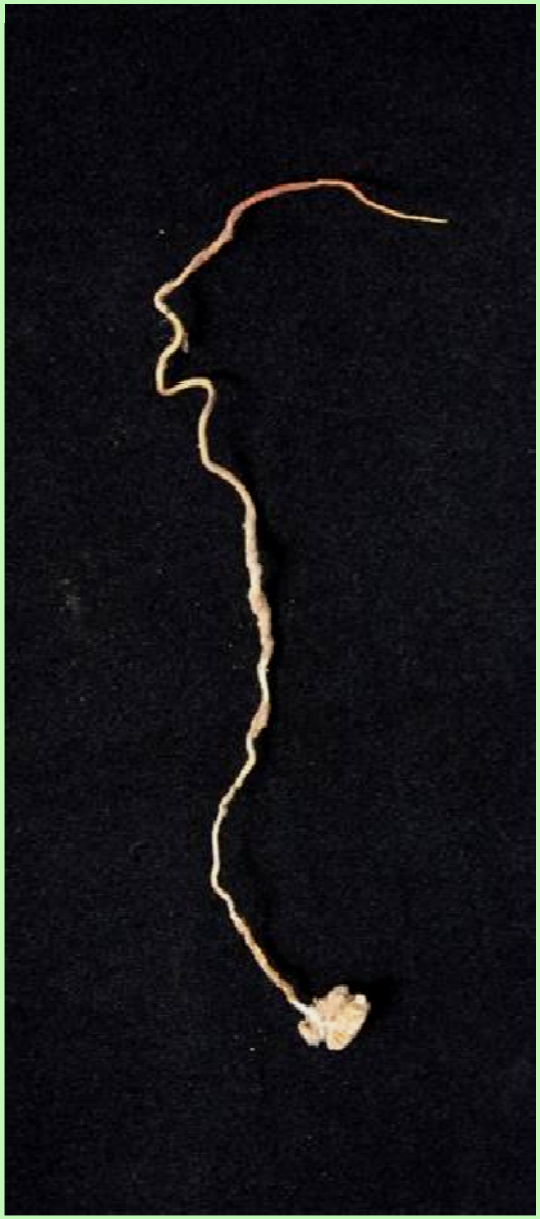



Hemiptera

Hymenoptera



Isoptera



Lepidoptera











Odonata

Orthoptera



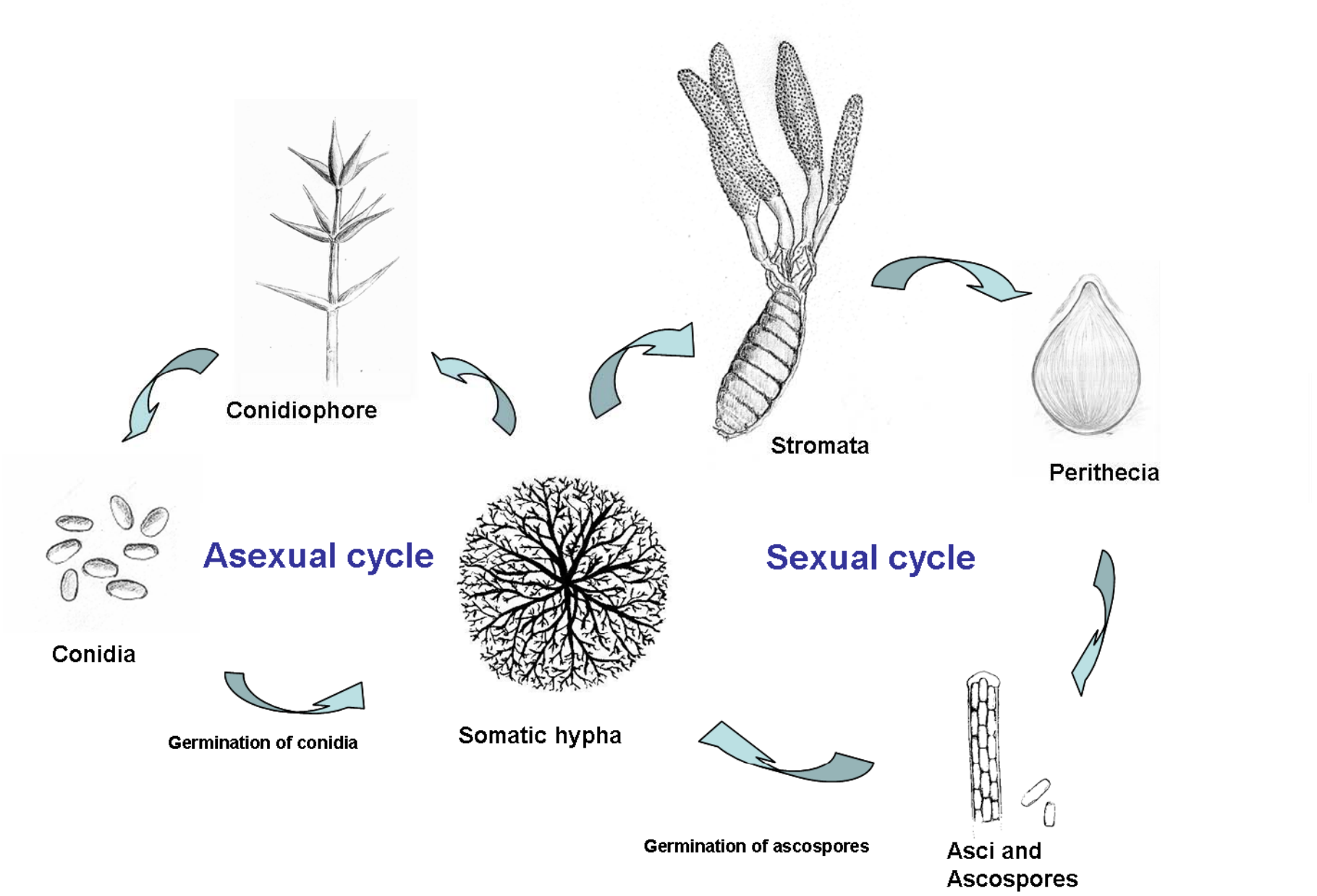





The Honorary Insects - Araneida: mainly Thomisidae and Salticidae

Anamorphs and teleomorphs

The importance of connecting
life stages in fungi



Life cycle of an invertebrate-pathogenic fungus

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Host specificity

- **General opportunistic pathogens**

  Metarhizium, Beauveria, Isaria

- **Fastidious pathogens**

  Most *Ophiocordyceps* and their anamorphs

BIODIVERSITY OF FUNGI

- Ca. 414,682 species currently known.
- 17,669 genera.
- 1.5 - 5.1 million species of fungi suspected.

Diverse habitats

Fungi
World = 80,000
(Expected No. = 1,500,000)
Thailand = ca. 6,000

Other microbes
World = 84,000
(Expected No. = 1,600,000)
Thailand = 300
(Expected No. = 100,000)

Plants
World = 250,000
Thailand = 12,000

Mammals
World = 4,260
Thailand = 282

Birds
World = 9,934
Thailand = 982

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Thailand could therefore have about 150,000 species of fungi

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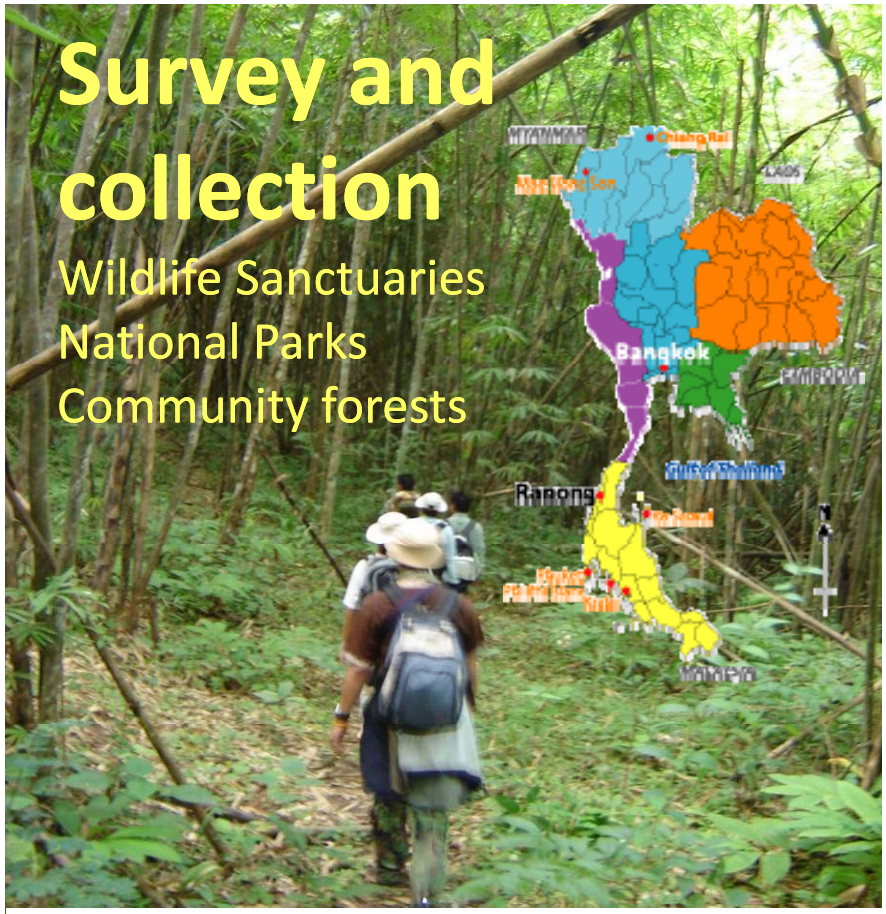
Survey and collection

Wildlife Sanctuaries
National Parks
Community forests



- Where to look
- When to look
- How to look

UNDISTURBED FOREST ECOSYSTEMS

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AGRICULTURAL ECOSYSTEMS

- **low species diversity**
- **high numbers of individuals**

Aschersonia marginata and *A. placenta*
on guava/citrus pests

Hypocrealean Entomopathogenic Fungi Common In Agricultural Ecosystems

- *Metarhizium anisopliae* – wide host range
- *Beauveria bassiana* – wide host range
- *Nomuraea rileyi* – exclusive to Lepidoptera
- *Hirsutella citriformis* – exclusive to hoppers
- *Hirsutella thompsonii* – exclusive to mites

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Species Found In Undisturbed Forest Ecosystems In Thailand

- *Beauveria bassiana* – rare on Coleoptera
- *Metarhizium anisopliae* – rare on Coleoptera or Hemiptera
- *Nomuraea rileyi* – seen once
- *Hirsutella citriformis* – seen once
- *Hirsutella thompsonii* – not looked for

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Fungi exist in a variety of habitats benign and extreme

- Tropical forests: +20 to 35°C
- Dry deserts: +45 to 50°C
- Hot springs: +60 to 80°C
- Antarctic wastes: -40 to -60°C

Invertebrate-Pathogenic Fungi

- 15-30°C in general
- Temperatures above 35°C rapidly prove lethal
- A few species can tolerate -10°C to 15°C

When to look

SEASONS IN THAILAND

Rainy - May to October

20-25/30-35°C

Cool dry - November to February

15-20/25-30°C

Hot dry - March to April

30-35/35-40°C

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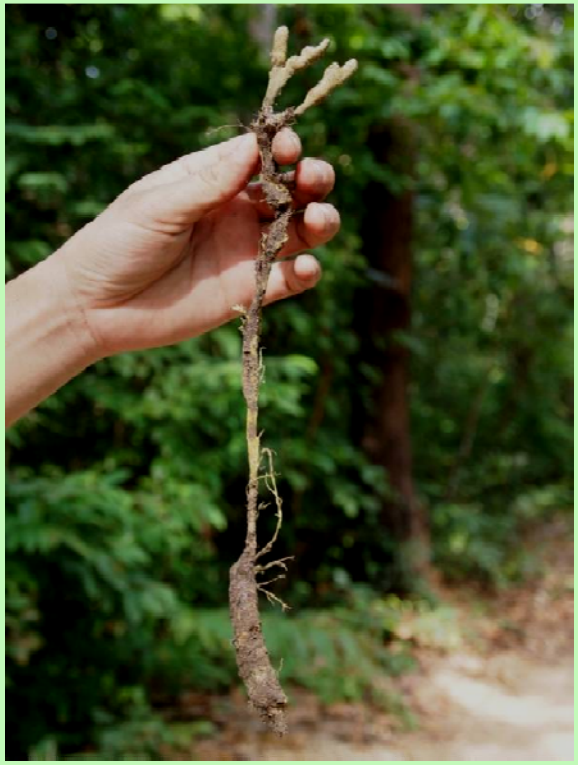
Looking close to rivers
high humidity

Buried in the ground



Insect fungi are also found under leaves

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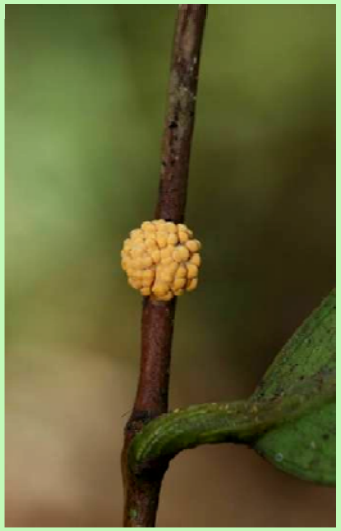
On stems, branches and fallen logs

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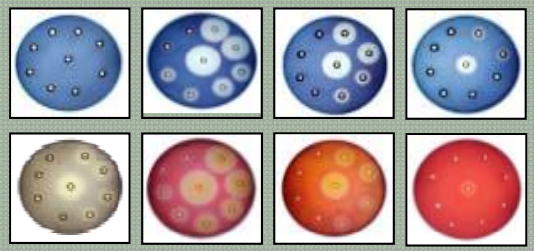
Searching the Leaf Litter

Microbial resource research at BIOTEC


Collection and Identification
(Microbial Interaction & Biodiversity Laboratories)

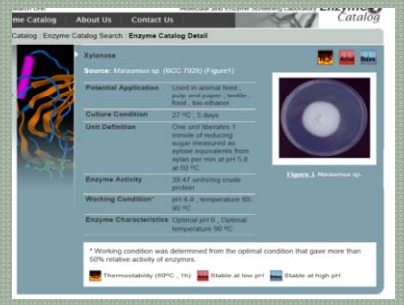

Preservation
(BIOTEC Bangkok Herbarium (BBH) & BIOTEC Culture Collection, BCC)


Cultivation
(Fermentation Technology Laboratory)



Identification of enzymes for industrial application
(Enzyme Technology Laboratory)


Characterization and structure elucidation
(Bioresources Research Laboratory)

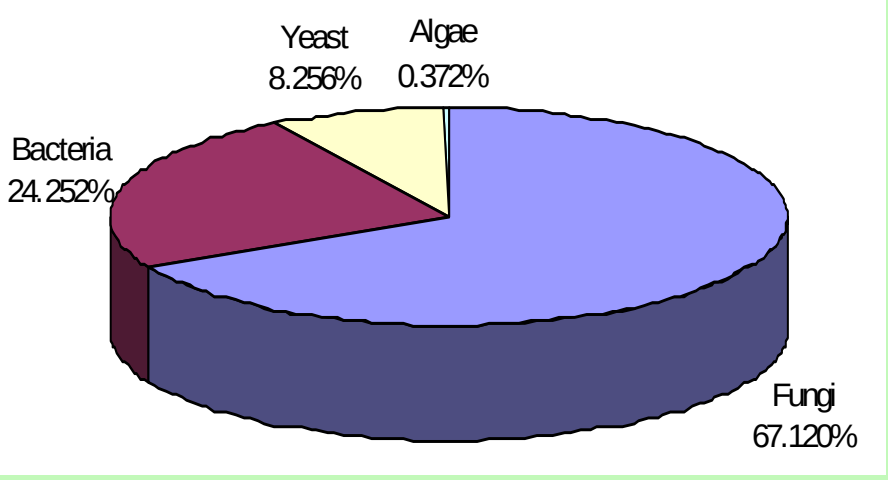



Screening for active compounds
(Bioassay laboratory)

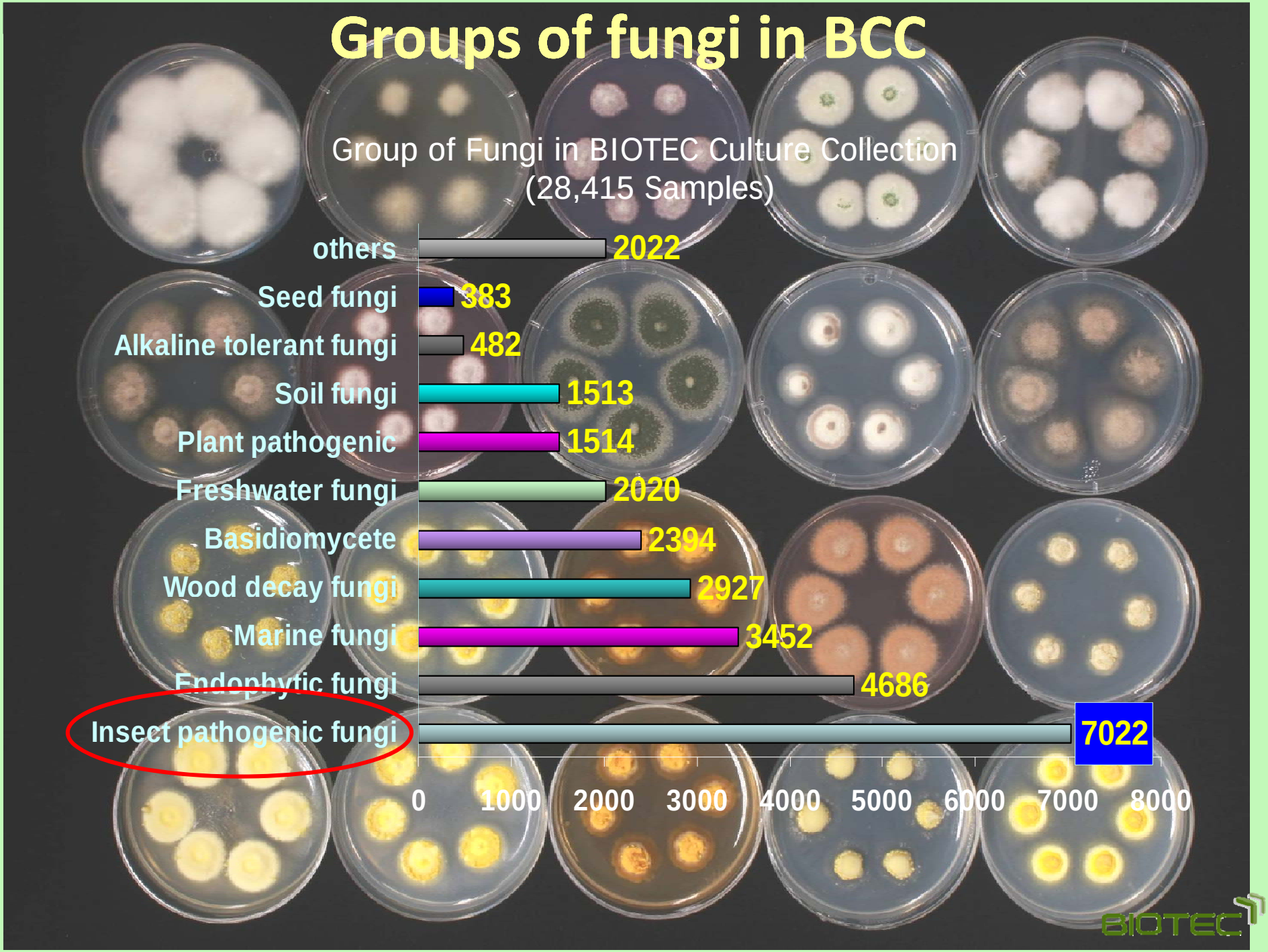
BIOTEC Culture Collection

Status of microorganisms 2013

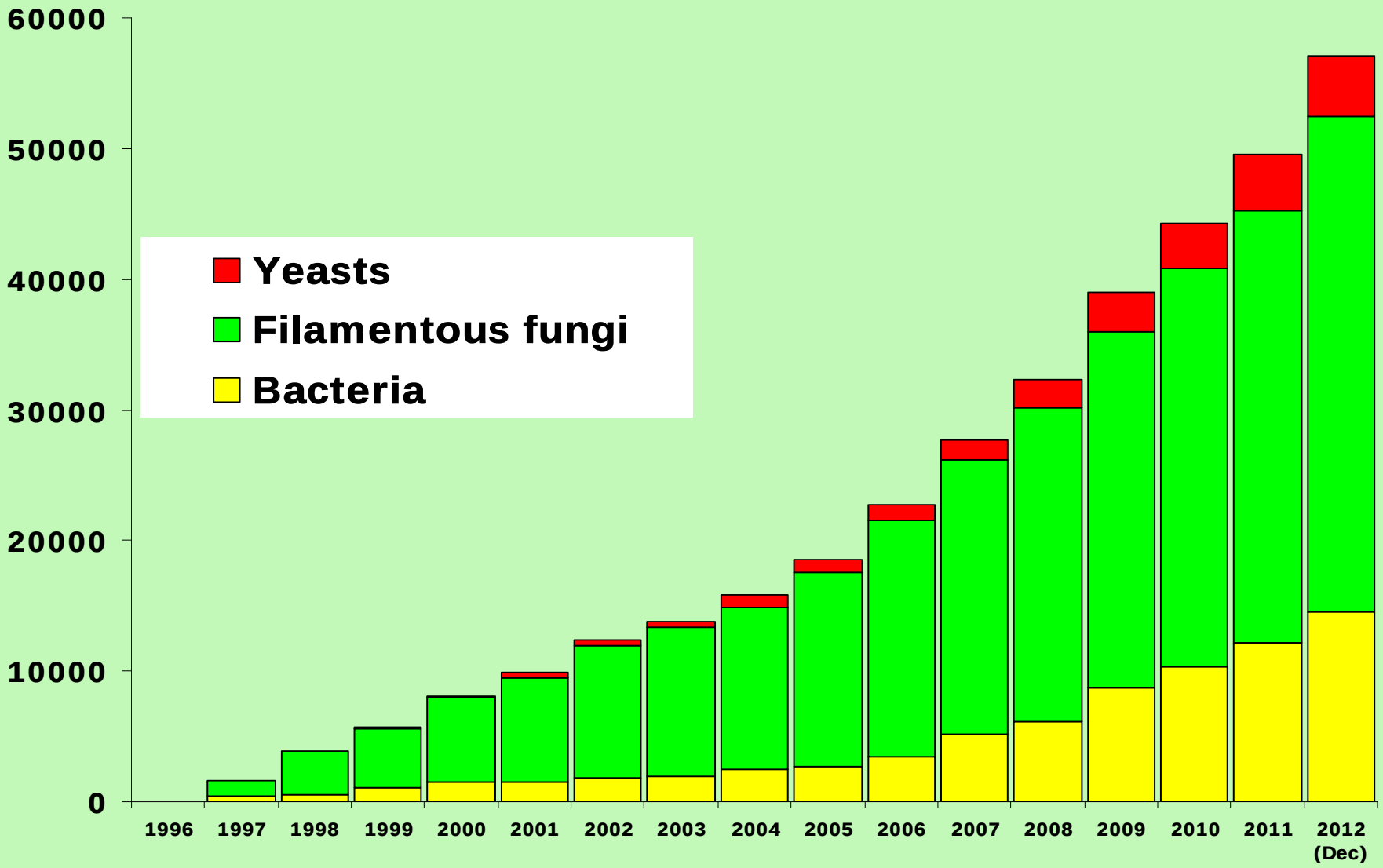
Microorganism	Strain	Genera	Species
Fungi	38,012	767	1,158
Bacteria	14,562	155	424
Yeast	4,621	62	314
Algae	208	37	74
Total	57,403	1,021	1,970



Groups of fungi in BCC



The number of microorganisms at BCC (57,079 samples)



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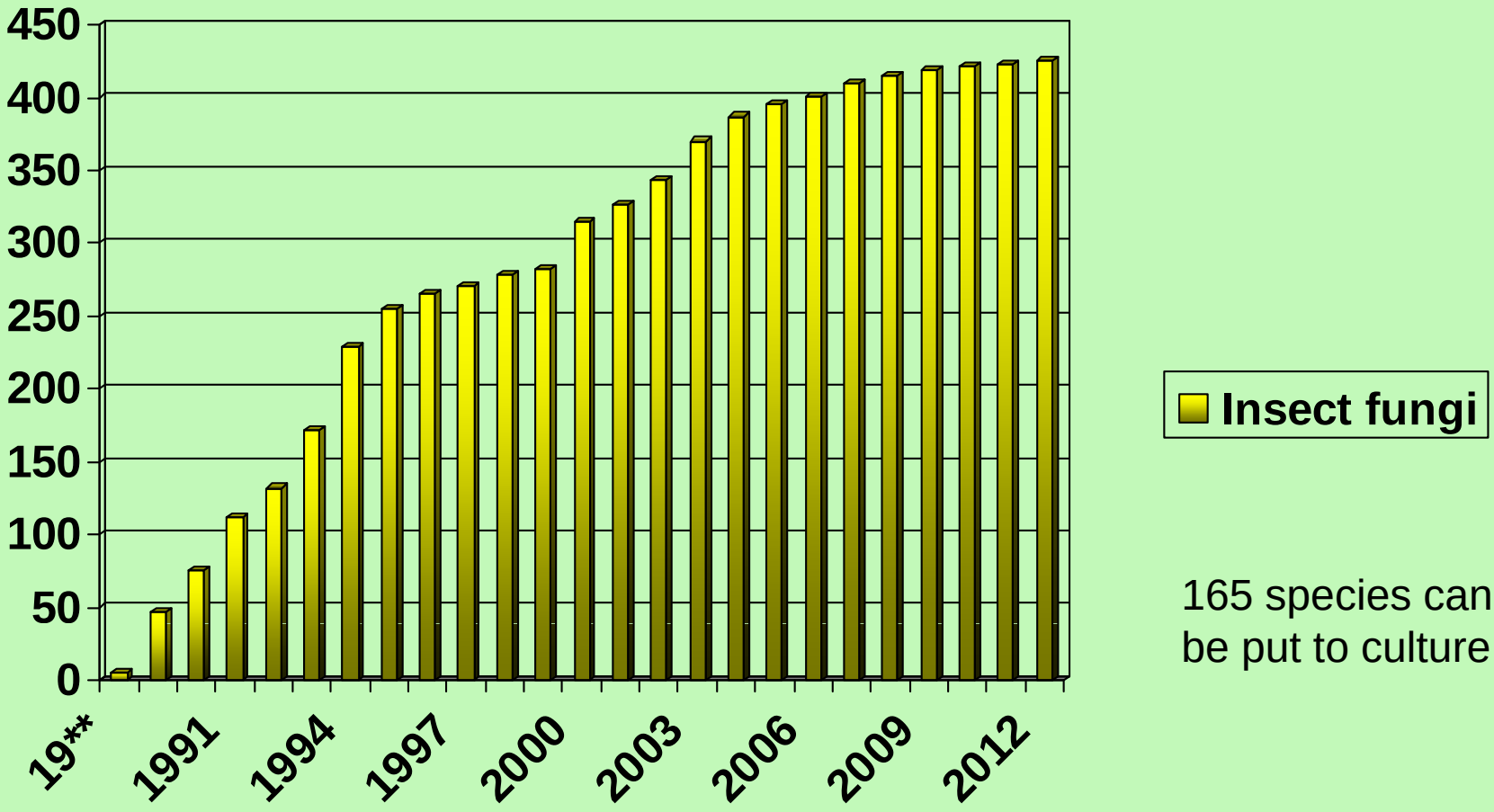
Numbers of Insect Fungi in Thailand



165 species can be put to culture

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Acc. Chem. Res. **2005**, *38*, 813−823

Bioactive Substances from Insect Pathogenic Fungi

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Received March 7, 2005

ABSTRACT

Insect pathogenic fungi have opened up a relatively untapped area of natural product research which, unfortunately, has not received much attention to date. Found in wild abundance in wet tropical Thailand, the insect fungi are shown to contribute not only as controllers of insect populations but also as rich sources of structurally novel biologically active substances.

FIGURE 3. Excavation revealing the larval host of *Cordyceps sinensis*.

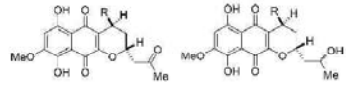
FIGURE 4. *Cordyceps sinensis* stroma on Lepidoptera larvae.

FIGURE 5. *Cordyceps unilateralis* on an ant.

derivatives, **1**−**6**. Interestingly, these naphthoquinones exhibited antimalarial activity with IC_{50} values of 2.5−10.1 μg/mL (Table 1). The above naphthoquinones show a

FIGURE 6. *Cordyceps nipponica* on ant lions.

deep red color under acidic conditions but intense purple in basic environments; such color characteristics are attractive to the pigment industry. Production of naphthoquinones by *C. unilateralis*, after optimization of fermentation conditions, can attain yields up to 3 g/L of culture broth.[28]



1, R = OH
2, R = H
3, R = OMe

4, R = OH
5, R = H

6

Cordyceps nipponica was originally described from cicadas in Japan and is found infecting both cicadas and ant lions (*Neuroptera*) in Thailand. Two *N*-hydroxy-2-pyridones, cordypyridones A (**7**) and B (**8**), and two tricyclic *N*-methoxy-2-pyridones, cordypyridones C (**9**) and D (**10**), were isolated from *Cordyceps nipponica* BCC 1389 (collected from Khao Yai National Park, central Thailand, Figure 6).[29] Cordypyridone A (**7**) is identical to 8-methyl-pyridoxatin, previously isolated from an unidentified fungus OS-F61800,[30] while its atropisomer, cordypyridone B (**8**), was shown to be a metabolite of BCC 1389. A careful study indicated that interconversion between compounds **7** and **8** occurred upon heating the solution, and the absolute configuration of cordypyridone **7** (and hence its atropisomer, **8**) was later determined using chemical means. Epoxidation of compound **11** (1-*O*-methyl derivative of **7**) and subsequent cyclization gave the major product **12**, which is the 14-hydroxy derivative of cordypyridone C (**9**). X-ray analysis of **13**, the *p*-bromobenzolate derivative of **12**, revealed the proposed absolute configuration. Cordypyridones A (**7**) and B (**8**) exhibited potent antimalarial activity with respective IC_{50}

- Phomalactone – common in various family and genera
- Oosporein - common in various family and genera
- Beauvericin - 7 strains, Coleopterans and lepidopterans
- Cordytropolone – only from *Cordyceps*

Acknowledgements

Insect-fungi working group
BIOTEC
CPMO

Thank you for your attention