

# How molecular phylogenetics helped change classification in Kingdom Fungi

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thedrinkbusiness.com

















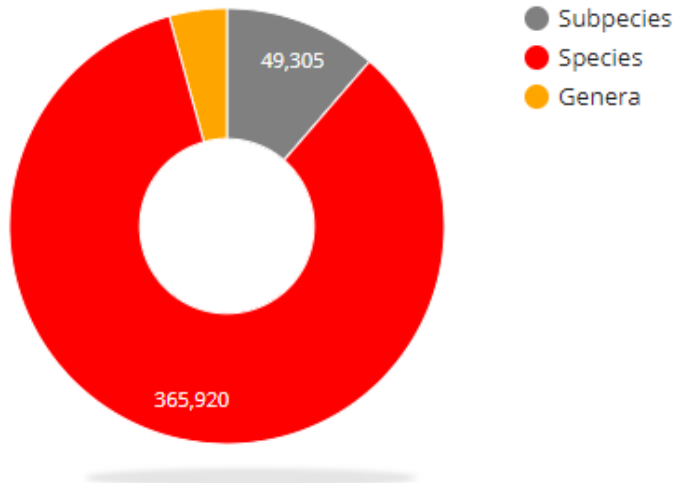


Alexander Fleming

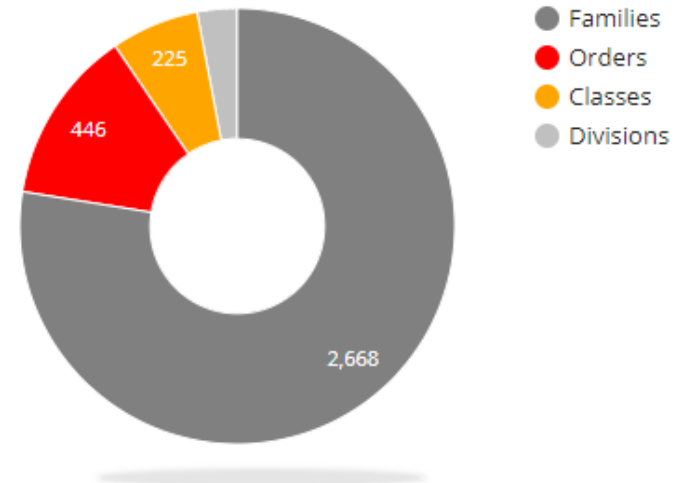


# Diversity of Fungi

Lower ranks



Higher ranks



Total number of major ranks described between 1689 and April 2017

**1.5 – 5.1 Million estimated number of species worldwide**

Source: MycoBank database





Texas A&M University

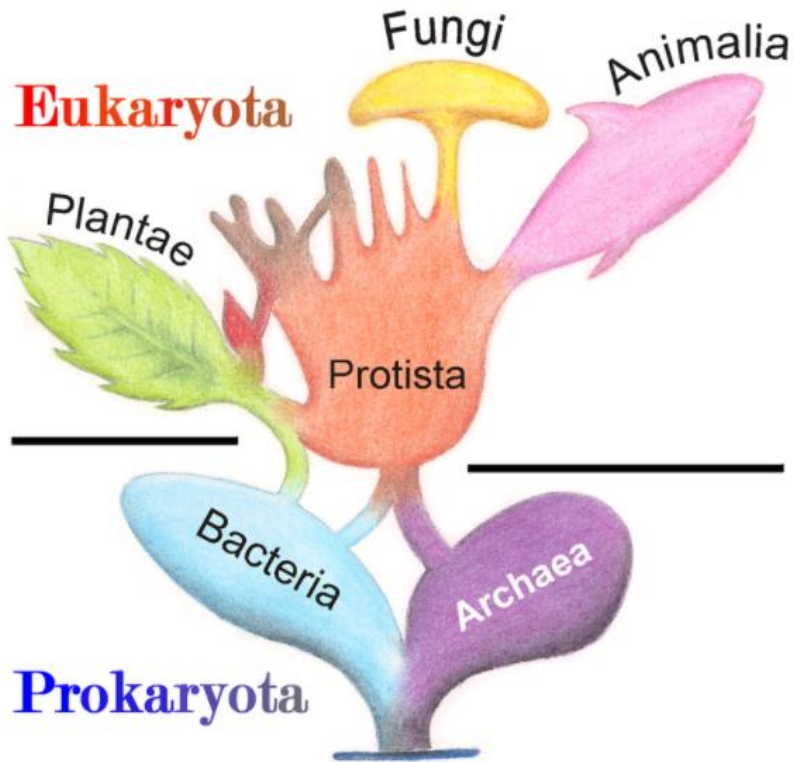
# What is a fungus?

- Fungi are non-motile (they don't move) heterotrophs (they get food and energy from other organisms).
- The defining characteristic of fungi is their mode of getting food: “extracellular digestion and absorption”. They secrete digestive enzymes into the environment, then absorb the nutrients released by those enzymes.
- Most fungi are decomposers: they live on decaying organisms.
- Some fungi are parasites: they extract food from living organisms.

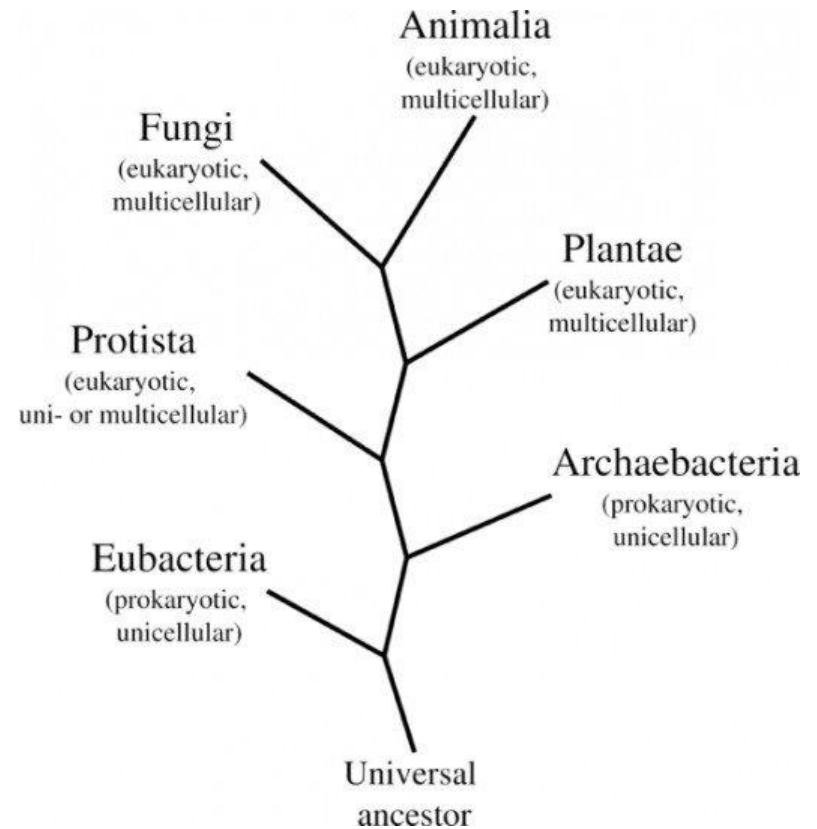


# Other Fungal Characteristics

- Fungi are **haploid** for most of their life cycle, becoming diploid only for the purpose of meiosis.
- Fungi are **non-vascular**: they have no internal pipes to distribute nutrients
- Fungi have a cell wall, like plants do, but it is composed of **chitin**, the same material that covers insects.
- Fungi reproduce by means of spores, which can be **sexual** (the products of **meiosis**) or **asexual** (the products of **mitosis**). Each group of fungi has a unique set of spores. Asexual reproduction is more common than sexual.
- Classically, the fungi were classified into the yeasts (which are unicellular) and the molds (which have a mat of fibers called hyphae as the main body of the organism).
- Recent DNA-based studies show that fungi are more similar to animals than to plants.



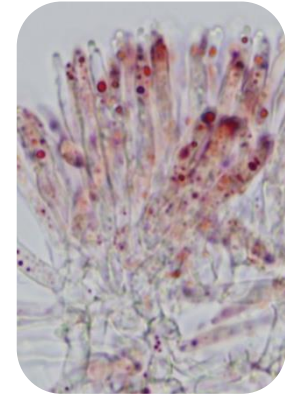
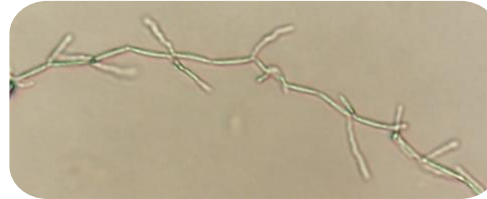
[https://en.wikipedia.org/wiki/Kingdom\\_\(biology\)#/media/File:Tree\\_of\\_Living\\_Organisms\\_2.png](https://en.wikipedia.org/wiki/Kingdom_(biology)#/media/File:Tree_of_Living_Organisms_2.png)



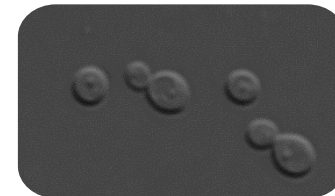
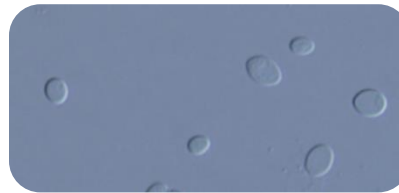


# Types of growth habits

- Filamentous – hyphal growth; multi-cellular; ascomycetes,
- basidiomycetes and zygomycetes



- Unicellular cells - budding yeasts (Saccharomycetales) and Archiascomycetes (*Schizosaccharomyces pombe*)



- ♣ There is some overlap between these two groups; because some fungi are dimorphic or even pleiomorphic, switching between different growth forms depending on environmental conditions or the stage of their life cycle.

# Anamorphs and teleomorphs

The importance of connecting  
life stages in fungi



# HOLOMORPH

- The whole fungus in all its morphs and life-cycle phases



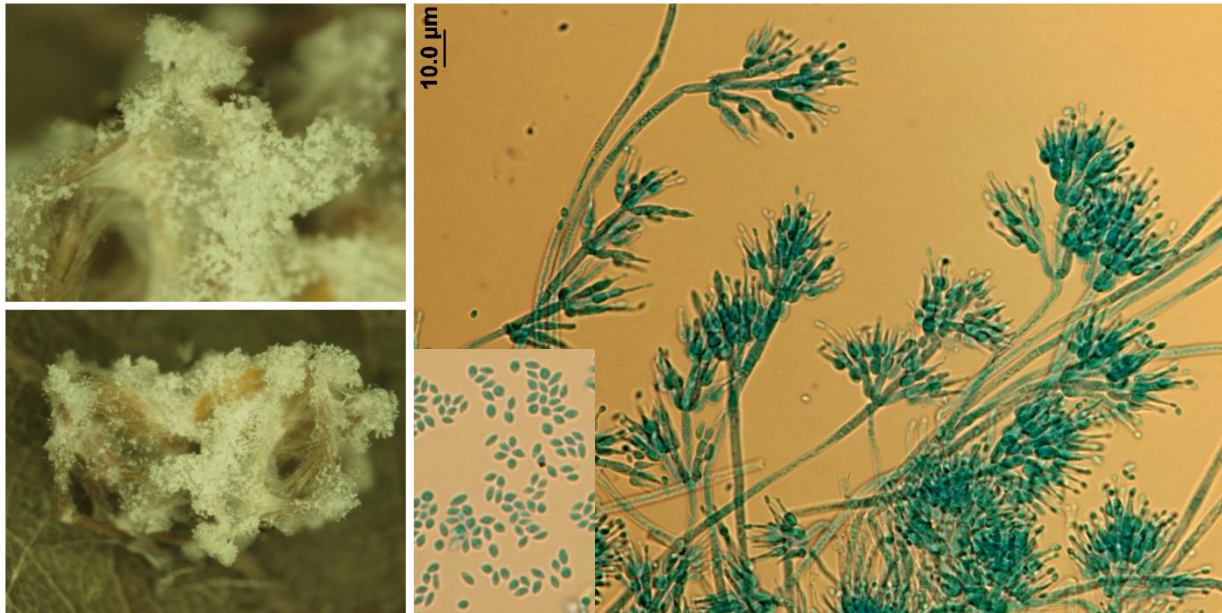
# HOLOMORPH

i.e. all manifestations of a genotype:  
in a fungus this frequently means one or more  
anamorphs plus a teleomorph.



# ANAMORPH

The **asexual** form or morph characterized by the presence or absence of **conidia**





# TELEOMORPH

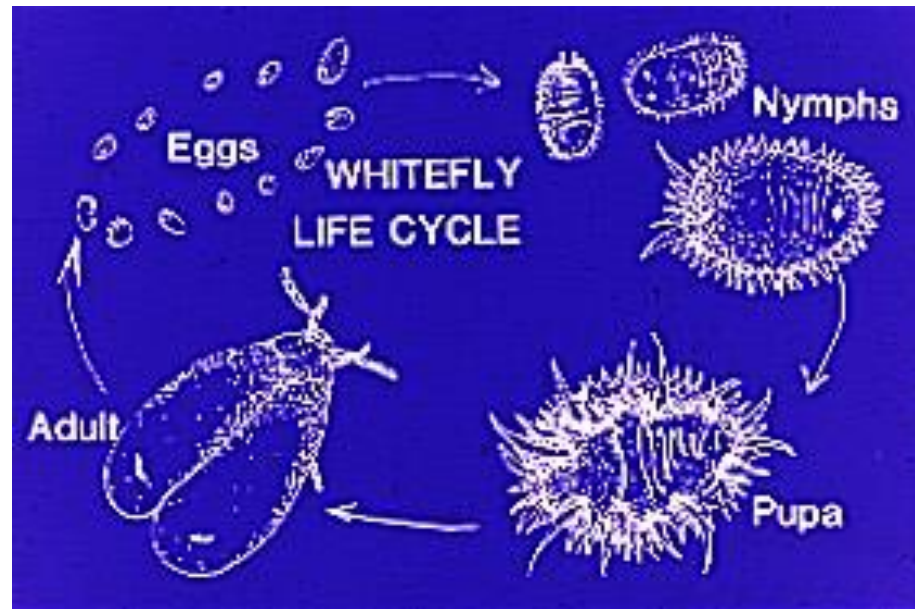
The **sexual** (perfect) form or morph characterized by the **presence of an ascoma** (fruiting body) in the Ascomycota

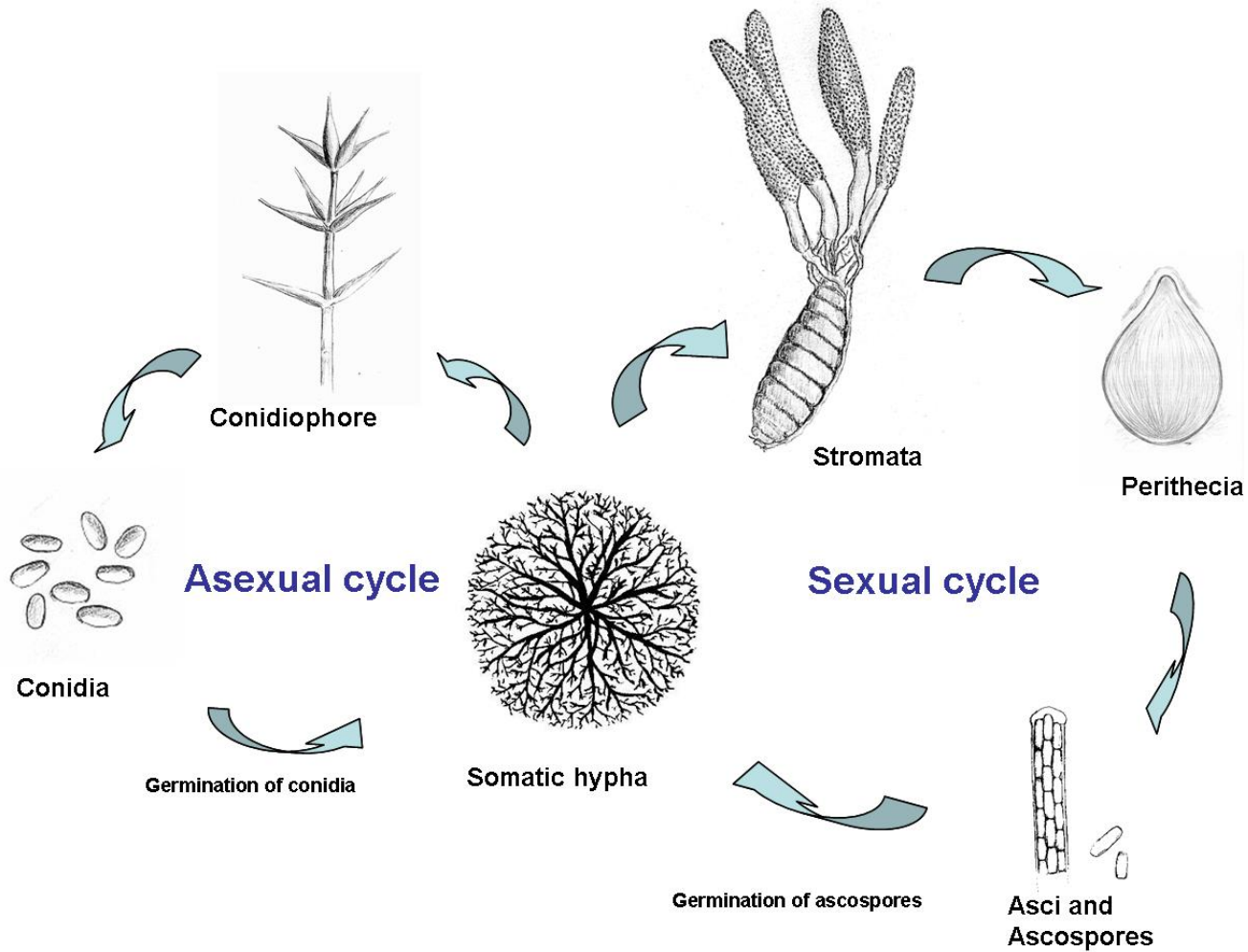


# A question of life-cycles

- The perfect and imperfect insect

- The perfect and imperfect fungus





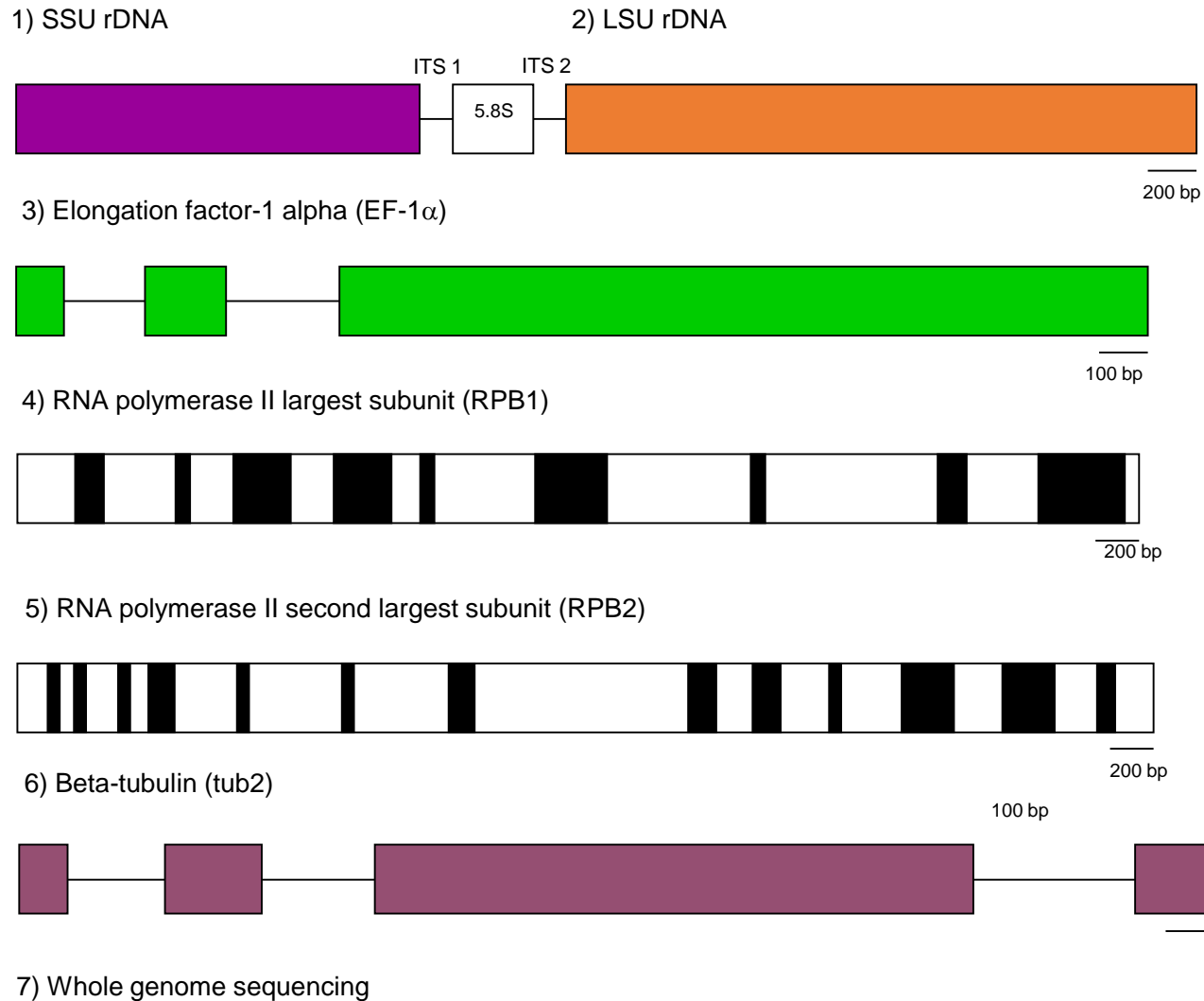
**Life cycle of an insect-pathogenic fungus**



One Fungus = One Name

**1F = 1N**

# Phylogenetic analysis of molecular sequence data



## Advantages of sequence data:

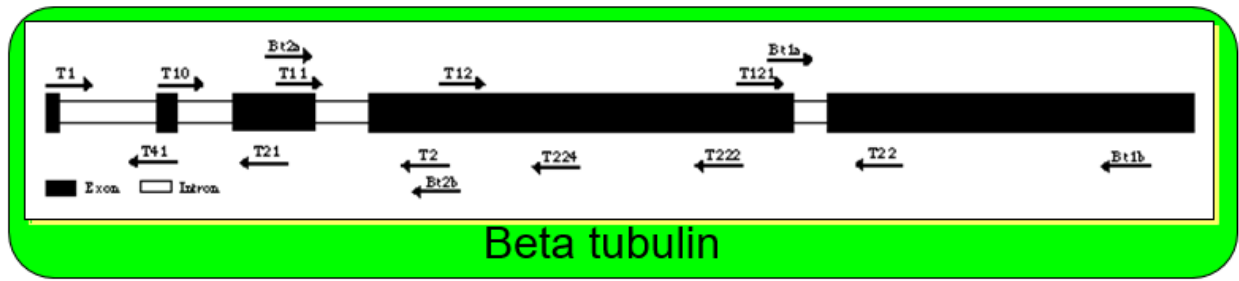
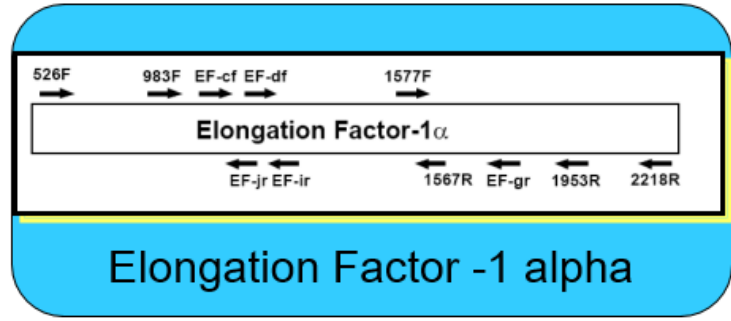
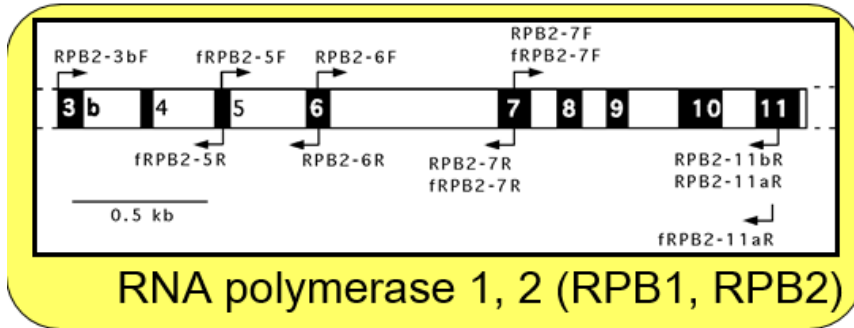
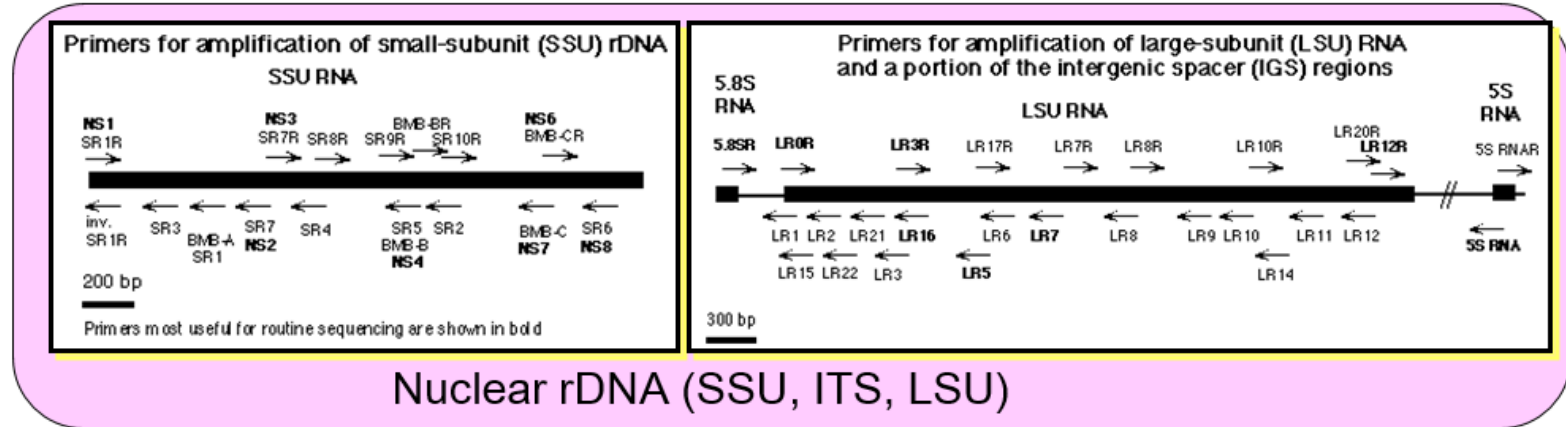
- no plasticity in the data unlike morphological characters
- High number of potential characters to be used for comparison
- use of molecular data for modeling patterns of nucleotide substitution
- Phylogenetic trees (estimates of evolutionary history) allows biologists to make predictions
- Phylogeny is also an integral part of interpreting any co-evolutionary relationships such as host and parasite
- Use of 'molecular clocks' to predict both past and future molecular divergence in genes



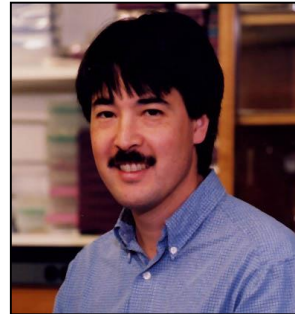
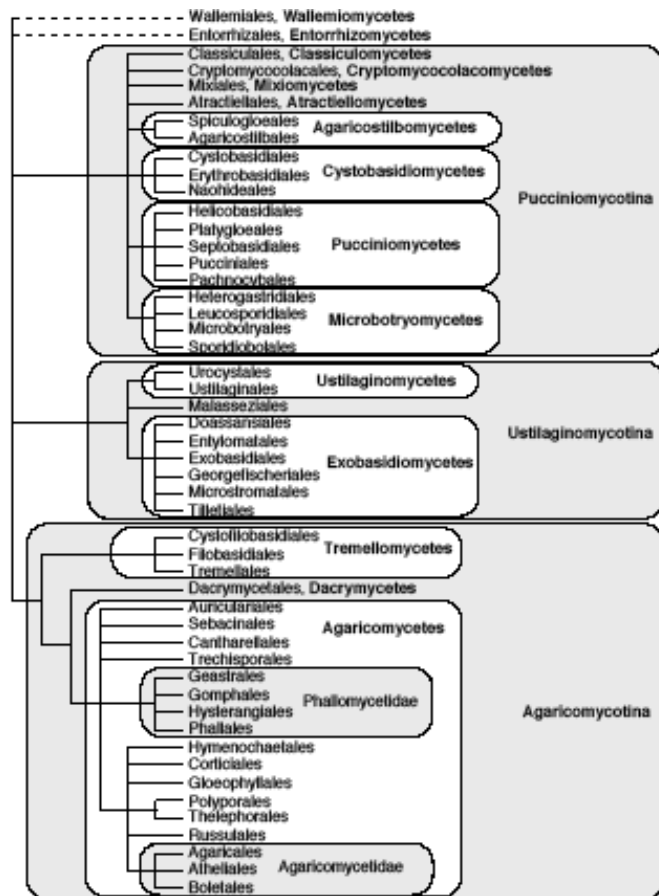
# Disadvantages

- difficulties of inferring positional homology
- incongruence between organismal and gene genealogies
- low likelihood of recovering the correct phylogeny given certain patterns in the timing of speciation events

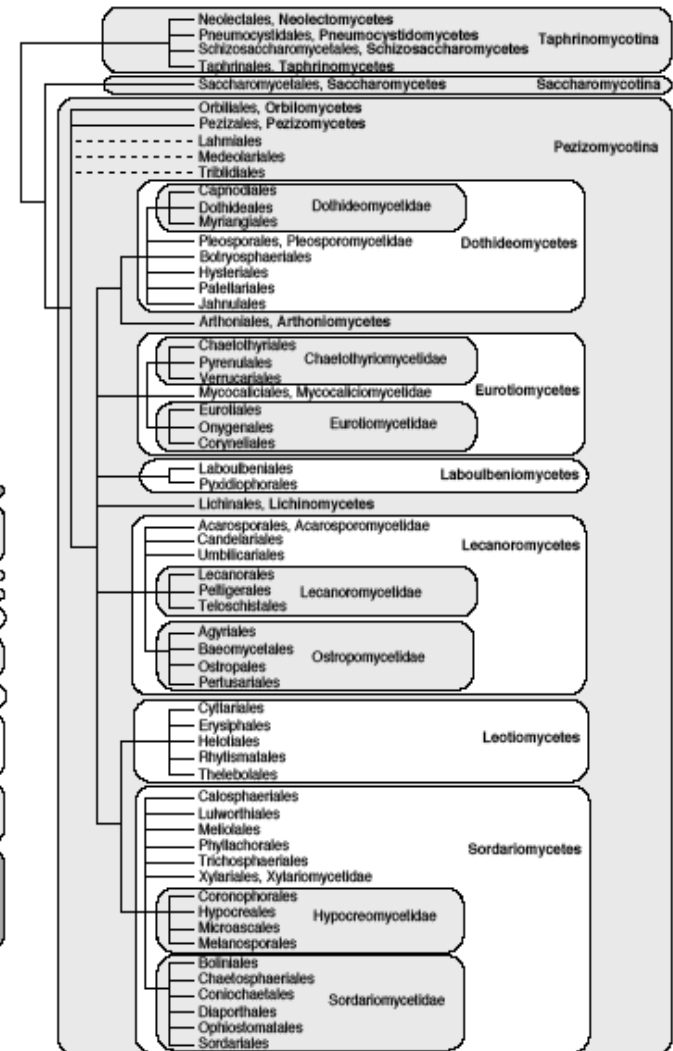
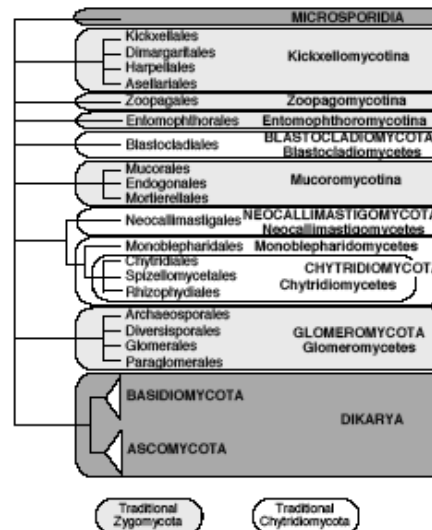
# Fungal Barcoding genes



# Higher level classification of Kingdom Fungi



David Hibbett





**31 January 2007**

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## A Higher-Level Phylogenetic Classification of the Fungi

David S. HIBBETT, Manfred BINDER, Joseph F. BISCHOFF, Meredith BLACKWELL, Paul F. CANNON, Ove ERIKSSON, Sabine HUHNDORF, Timothy JAMES, Paul M. KIRK, Robert LÜCKING, Thorsten LUMBSCH, François LUTZON, P. Brandon MATHENY, David J. MCLAUGHLIN, Martha J. POWELL, Scott REDHEAD, Conrad L. SCHOCH, Joseph W. SPATAFORA, Joost A. STALPERS, Rytas VILGALYS, M. Catherine AIME, André APTROOT, Robert BAUER, Dominik BEGEROW, Gerald L. BENNY, Lisa A. CASTLEBURY, Pedro W. CROUS, Yu-Cheng DAI, Walter GAMS, David M. GEISER, Gareth W. GRIFFITH, David L. HAWKSWORTH, Valerie HOFSTETTER, Kentaro HOSAKA, Richard A. HUMBER, Kevin HYDE, Urmas KÕLJALG, Cletus P. KURTZMAN, Karl-Henrik LARSSON, Robert LICHTWARDT, Joyce LONGCORE, Andrew MILLER, Jean-Marc MONCALVO, Sharon MOZLEY STANDRIDGE, Franz OBERWINKLER, Erast PARMASTO, Jack D. ROGERS, Leif RYVARDEN, José Paulo SAMPAIO, Arthur SCHUESSLER, Junta SUGIYAMA, John W. TAYLOR, R. Greg THORN, Leif TIBELL, Wendy A. UNTEREINER, Christopher WALKER, Zheng WANG, Alex WEIR, Michael WEISS, Merlin WHITE, Katarina WINKA, Yi-Jian YAO, Ning ZHANG

# Entomopathogenic Fungi: Clavicipitaceae



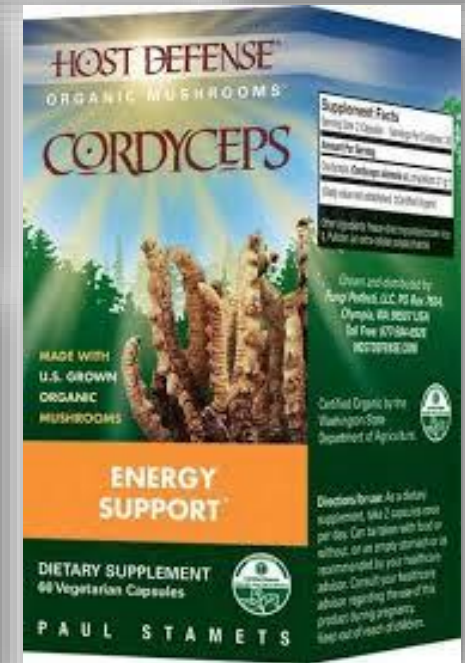
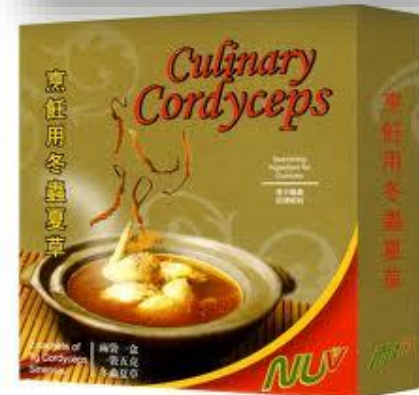
*Cordyceps militaris* – type species of *Cordyceps*



# Ophiocordyceps sinensis

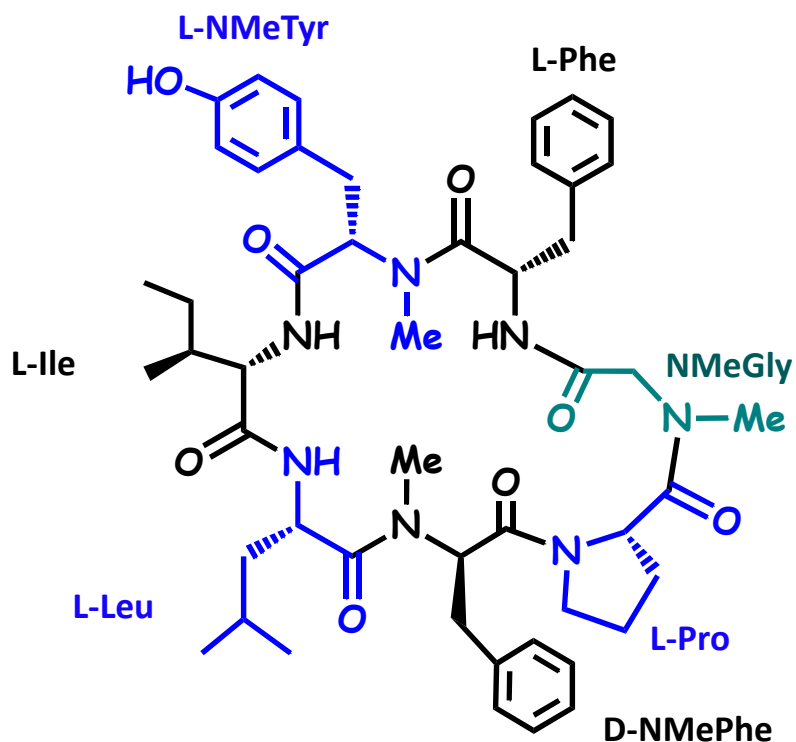
*dong chong xia cao*

'winter-worm, summer-grass'



## *Cordyceps* sp. BCC 1788

From Khao Soi Dao Wildlife Sanctuary, Chantaburi



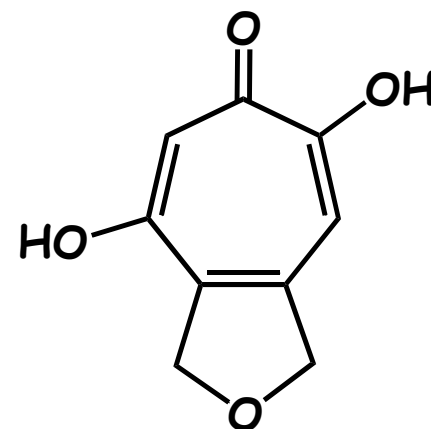
### Cordyheptapeptide A

	IC <sub>50</sub> (μM)
<i>P. falciparum</i>	5.35
cytotoxicity (Vero cell lines)	>50

Rukachaisirikul V., *J. Nat. Prod.*, 2006

## *Cordyceps* sp. BCC 1681

From Khao Soi Dao Wildlife Sanctuary, Chantaburi



IC<sub>50</sub> = 50% inhibitory concentration

### Cordytropolone

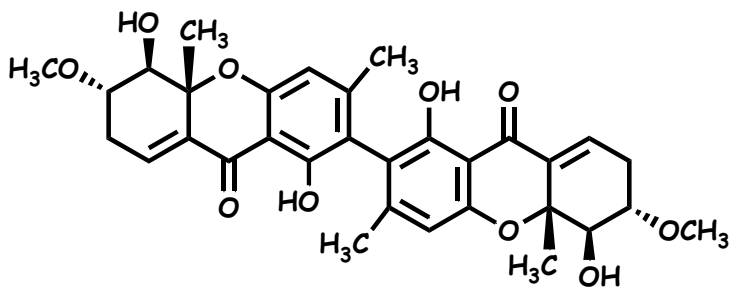
	IC <sub>50</sub> (μM)
	12.2
	>50

Seephonkai P., *J. Anti Biot.*, 2001



# Aschersonia sp. BCC 8401

from Khao Yai National Park



Ascherxanthone A



BCC 8401



	<i>P. falciparum</i> K1 (IC <sub>50</sub> , μg/mL)	Cytotoxicity (IC <sub>50</sub> , μg/mL)			
		Vero cells	KB cells	BC-1 cells	NCI-H178 cells
Ascherxanthone	0.20	0.8	1.7	1.7	0.16
Artemisinin	0.0011	>20	>20	>20	>20

Article

## Five Unprecedented Secondary Metabolites from the Spider Parasitic Fungus *Akanthomyces novoguineensis*

Soleiman E. Helaly <sup>1,2,†</sup>, Wilawan Kuephadungphan <sup>1,3,†</sup>, Souwalak Phongpaichit <sup>3,4</sup>, Janet Jennifer Luangsa-ard <sup>5</sup>, Vatcharin Rukachaisirikul <sup>6,7</sup> and Marc Stadler <sup>1,\*</sup>

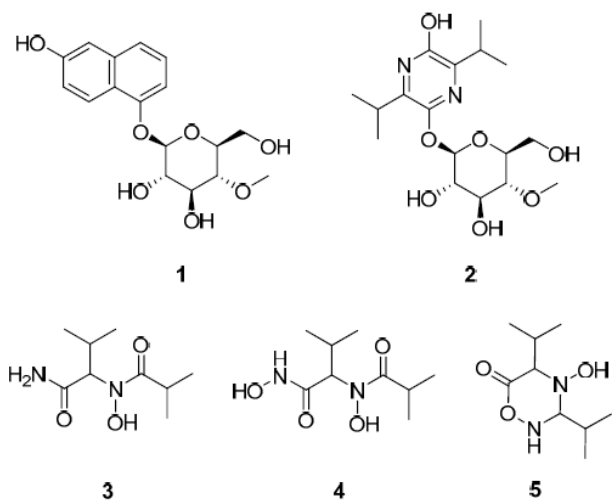


Figure 1. Structures of Compounds 1–5.



Article

## Akanthopyrones A–D, $\alpha$ -Pyrone Bearing a 4-*O*-Methyl- $\beta$ -D-glucopyranose Moiety from the Spider-Associated Ascomycete *Akanthomyces novoguineensis*

Wilawan Kuephadungphan <sup>1,2,†</sup>, Soleiman E. Helaly <sup>1,3,†</sup>, Charuwan Daengrot <sup>4</sup>, Souwalak Phongpaichit <sup>2,5</sup>, Janet Jennifer Luangsa-ard <sup>6</sup>, Vatcharin Rukachaisirikul <sup>4,7</sup> and Marc Stadler <sup>1,\*</sup>

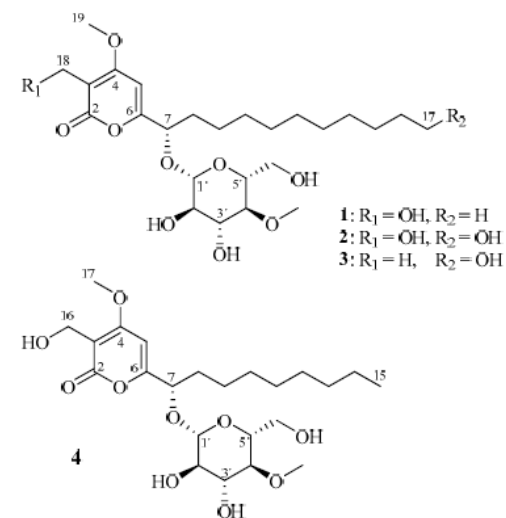


Figure 1. Structures of akanthopyrones A–D (1–4)

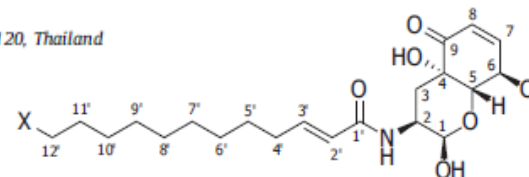
*Molecules* **2017**, *22*, 991; doi:10.3390/molecules22060991

*Molecules* **2017**, *22*, 1202;  
doi:10.3390/molecules22071202

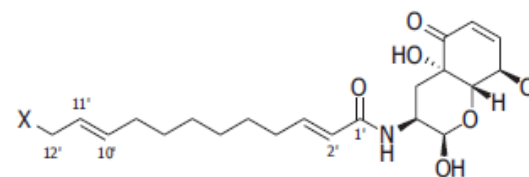
# Isariotins G–J from cultures of the *Lepidoptera* pathogenic fungus *Isaria tenuipes*

Taridaporn Bunyapaiboonsri, Seangaroon Yoiprommarat, Urarat Srisanoh, Wilunda Choowong, Kanoksri Tasanathai, Nigel L. Hywel-Jones, J. Jennifer Luangsa-ard, Masahiko Isaka\*

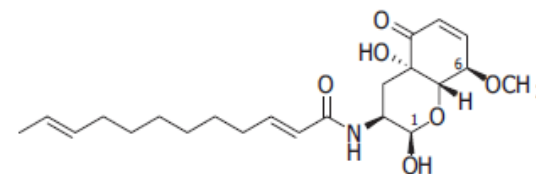
National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park, Phaholyothin Road, Klong Luang, Pathumthani 12120, Thailand



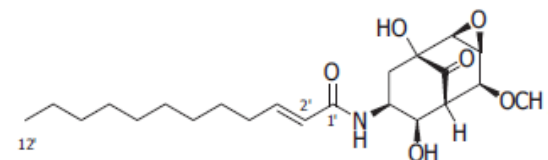
isariotin G (1):X =OH  
isariotin F (5):X =H



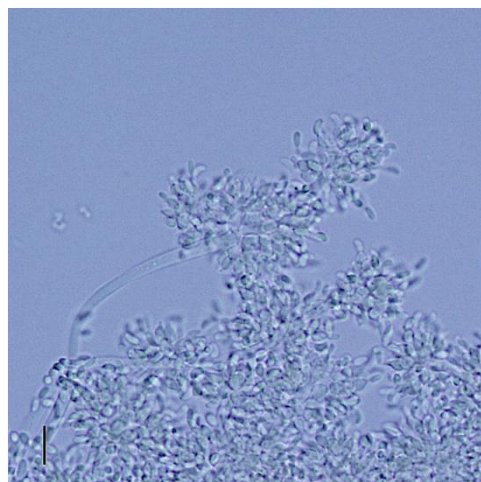
isariotin H (2):X =H  
isariotin I (3):X =OH



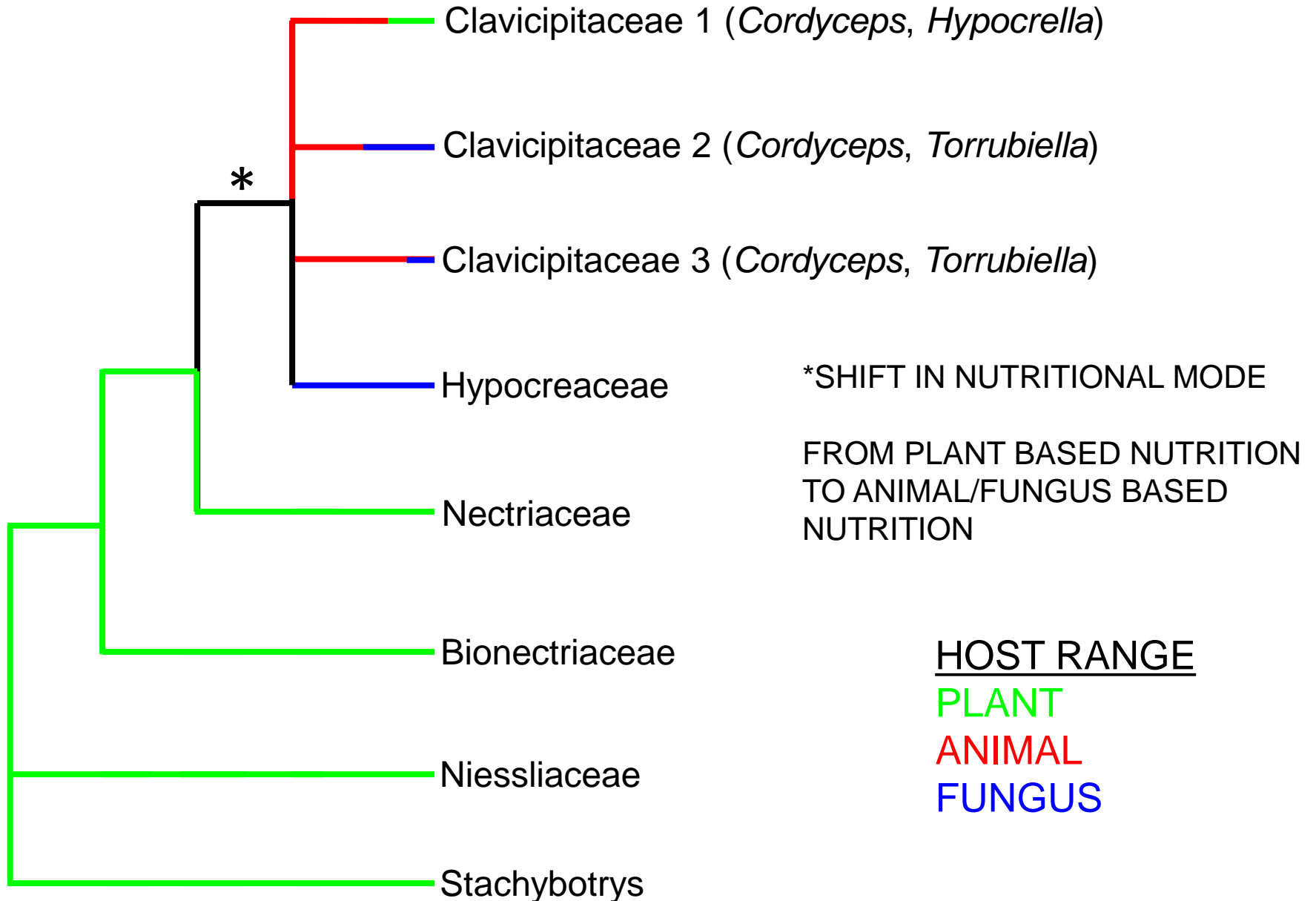
isariotin J (4)



TK-57-164A (6)



# Phylogeny of the Hypocreales





# Clavicipitaceae 1 - Clavicipitaceae s.s.



on *Smilax* seeds



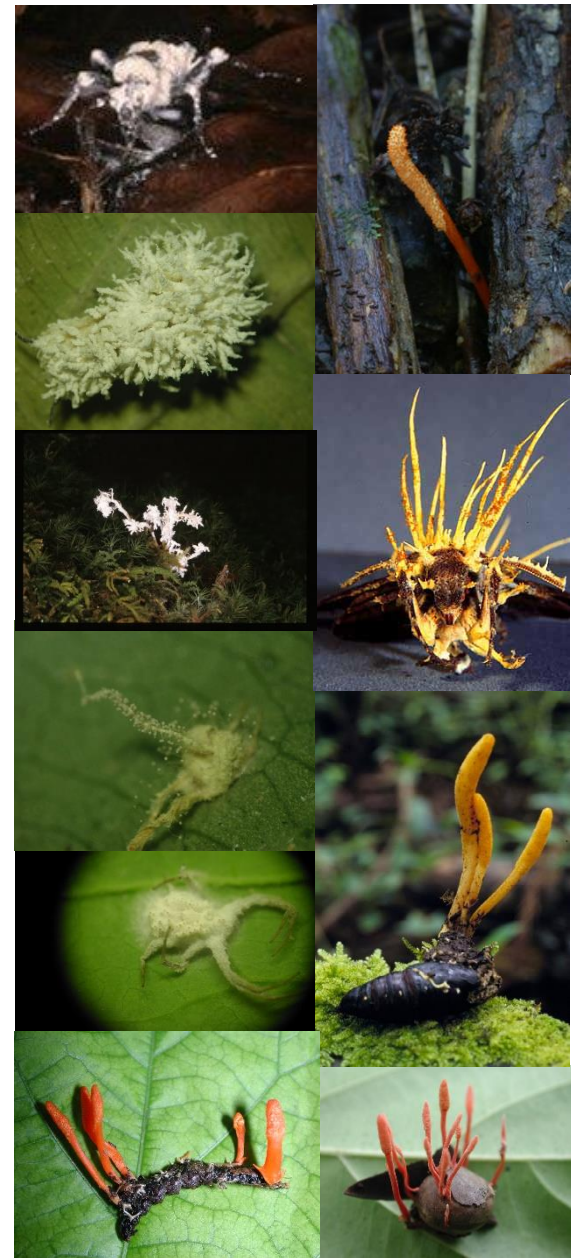
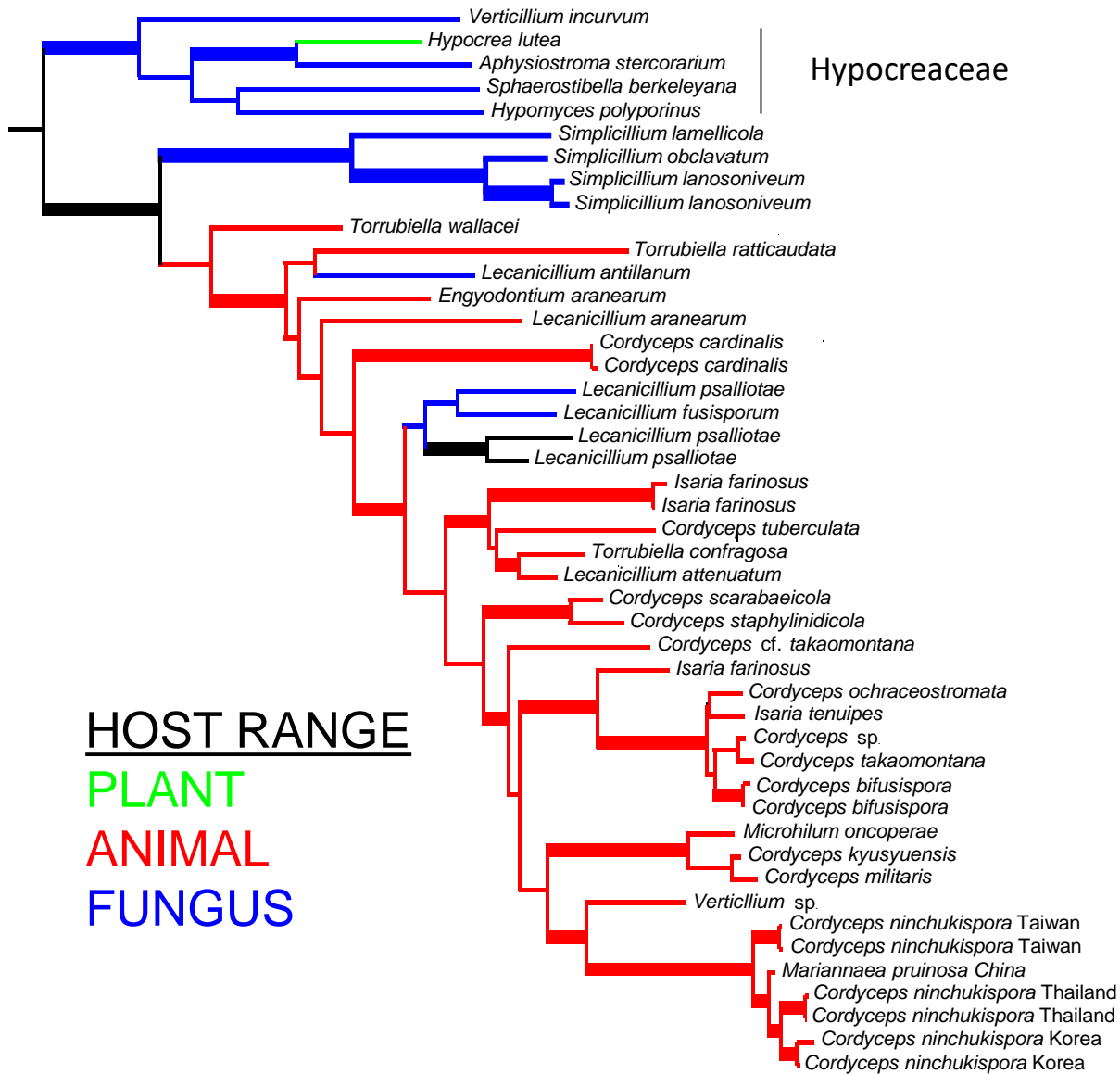
*Hypocrella* on scale insects



*Cordyceps*  
*Metarhizium*, *Pochonia*, *Paecilomyces*

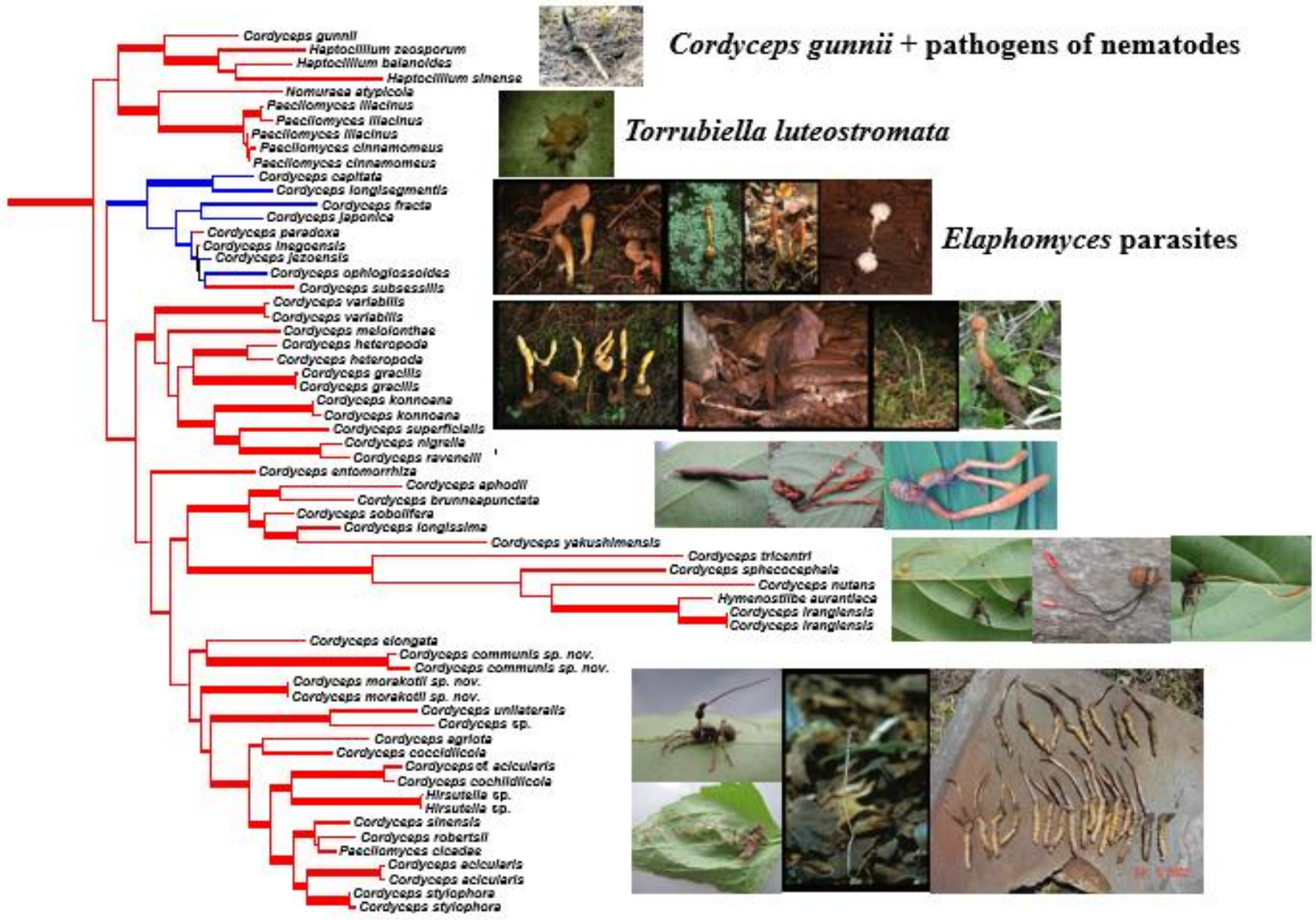


# Clavicipitaceae 2 - "Cordyceps" clade





### Clavicipitaceae 3 - "Ophiocordyceps clade"



# Evolution of host affiliation

- individual species have narrow host range
- closely related species are not necessarily on closely related hosts
- host jumping is common
  - 4-5 onto fungi
  - 1-2 onto plants
  - 1-2 onto animals
- 3 clades of Clavicipitaceae seem to display some level of niche specialization



## “Cordyceps” clade

## Niche specialization?



- attacks host in leaf litter, moss
- usually not deeply buried in soil or wood
- stromata brightly colored, fleshy



## Clavicipitaceae clade



- often attack hosts directly associated with plants
- stromata pallid to green to brightly colored



## “Ophiocordyceps” clade



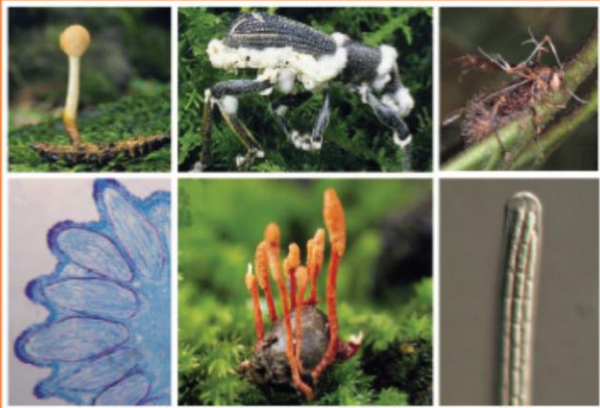
- attack hosts buried in soil or decaying wood
- except species that attack adults
- stromata darkly pigmented, often with sterile apex



Studies in Mycology 57 (2007)

## Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi

Gi-Ho Sung, Nigel L. Hywel-Jones, Jae-Mo Sung, J. Jennifer Luangsa-ard,  
Bhushan Shrestha and Joseph W. Spatafora

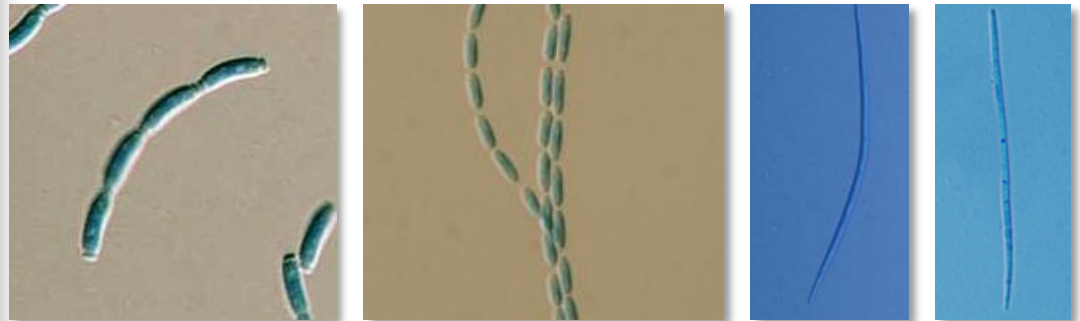


Centraalbureau voor Schimmelcultures,  
Utrecht, The Netherlands  
An institute of the Royal Netherlands Academy of Arts and Sciences

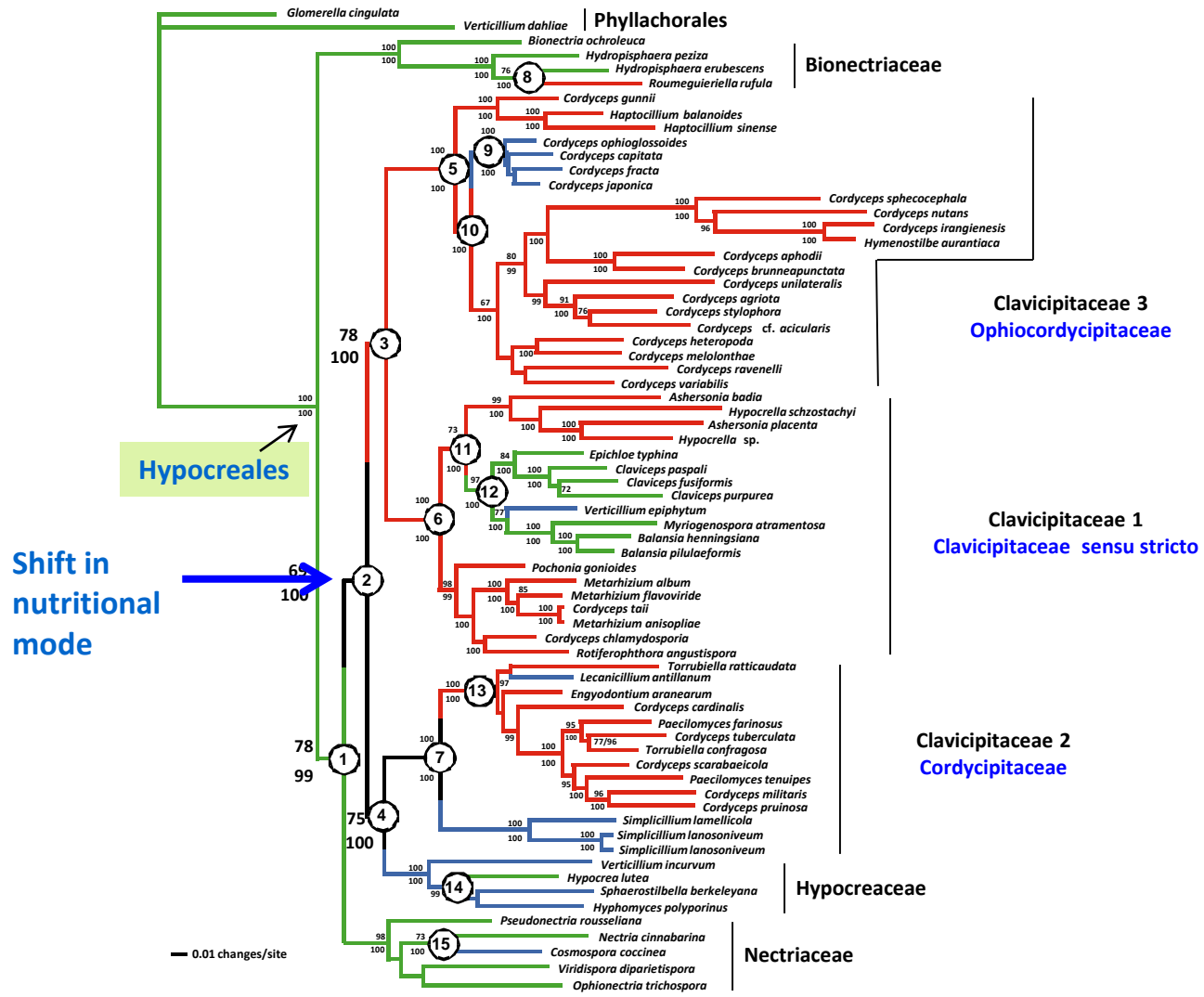
To refine the classification of *Cordyceps* and the *Clavicipitaceae*, the phylogenetic relationships of 162 taxa were estimated based on analyses consisting of five to seven loci, including the nuclear ribosomal small and large subunits (*nrSSU* and *nrLSU*), the elongation factor 1- $\alpha$  (*tef1*), the largest and the second largest subunits of RNA polymerase II (*rpb1* and *rpb2*),  $\beta$ -tubulin (*tub*), and mitochondrial ATP6 (*atp6*).

Synapomorphy of entomopathogenic fungi:

- Filiform ascospores

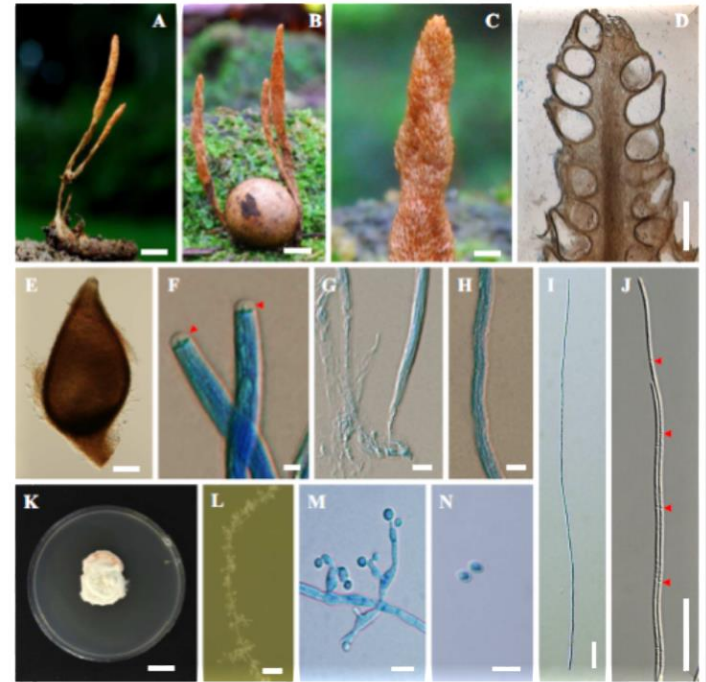
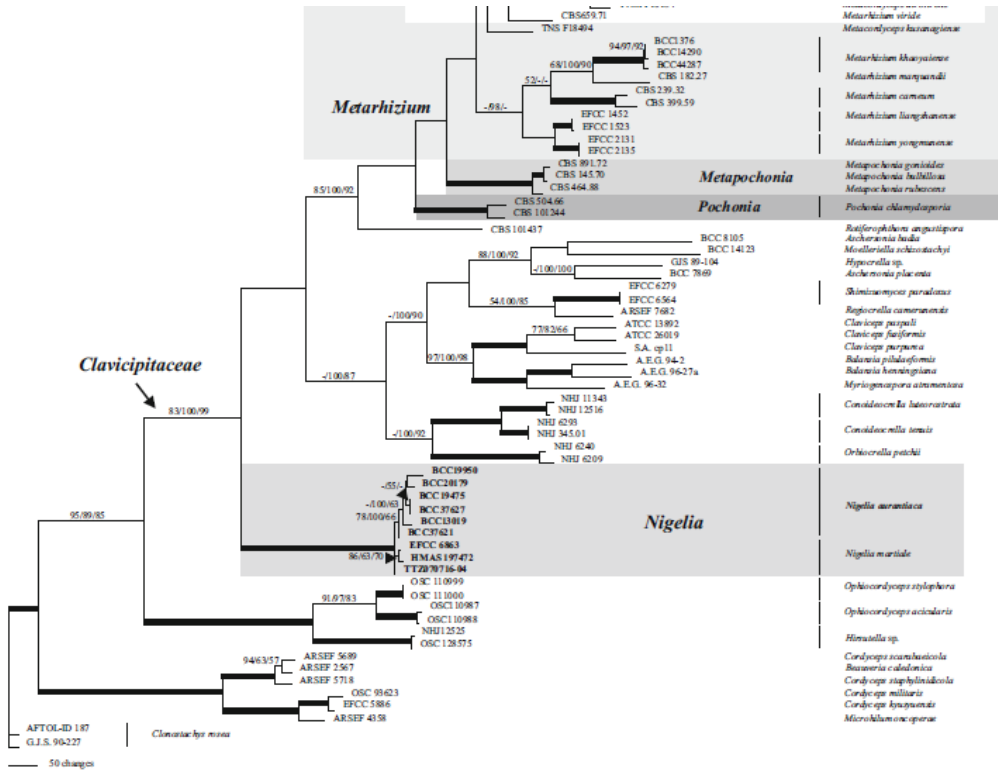








# Entomopathogenic fungi: recognizing new genera



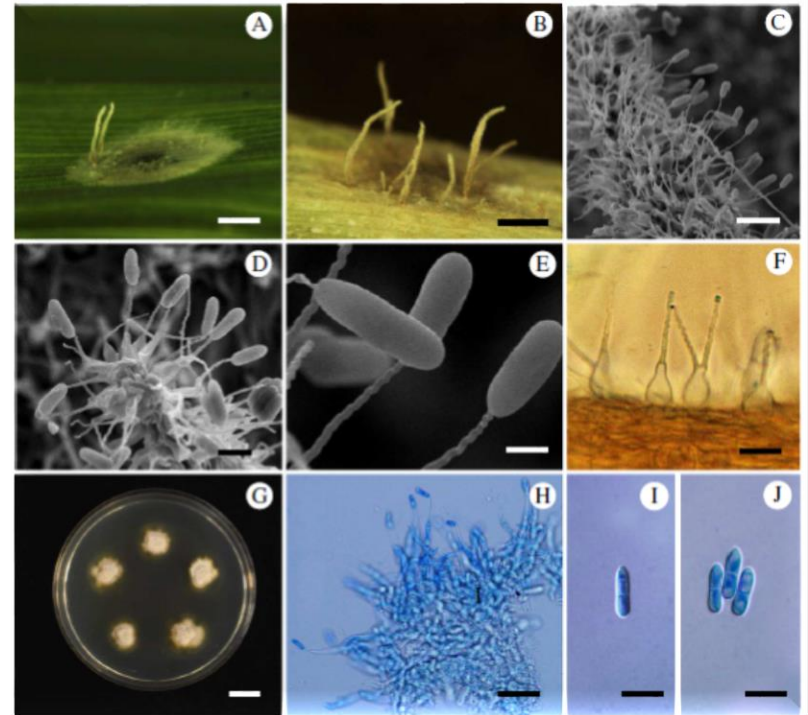
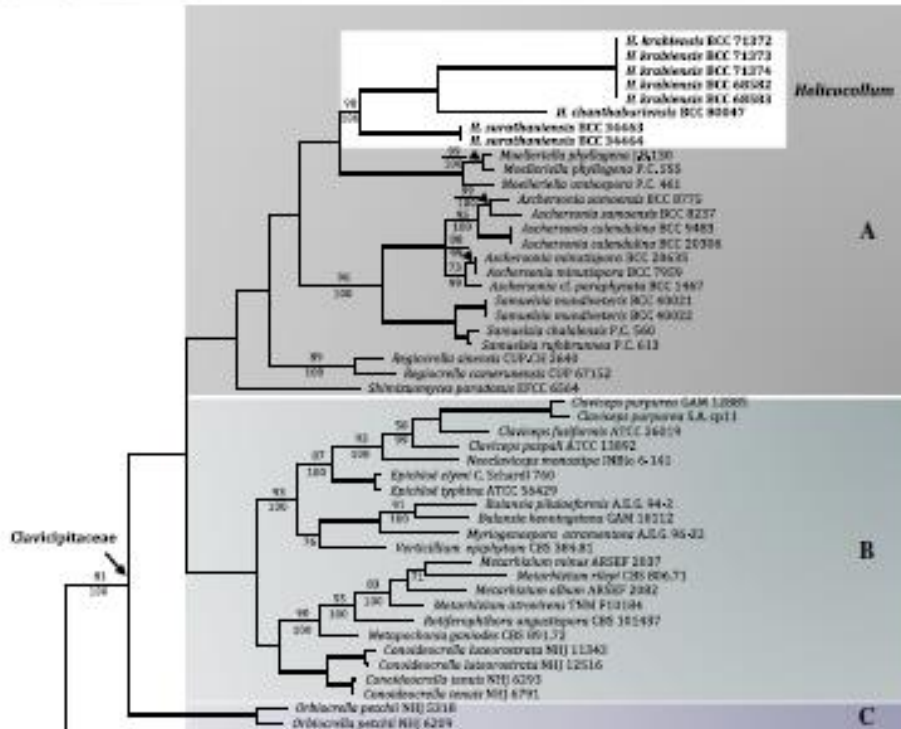
SSU, LSU, *tef*, *rpb1*, *rpb2*

*Nigelia*

# Entomopathogenic fungi: recognizing new genera

Mycol Progress (2017) 1:64–104

425



LSU, *tef*, *rpb1*

*Helicocollum*

# *Metarhizium* Sorokin 1879

Type species: *Metarhizium anisopliae* (Metsch.) Sorokin

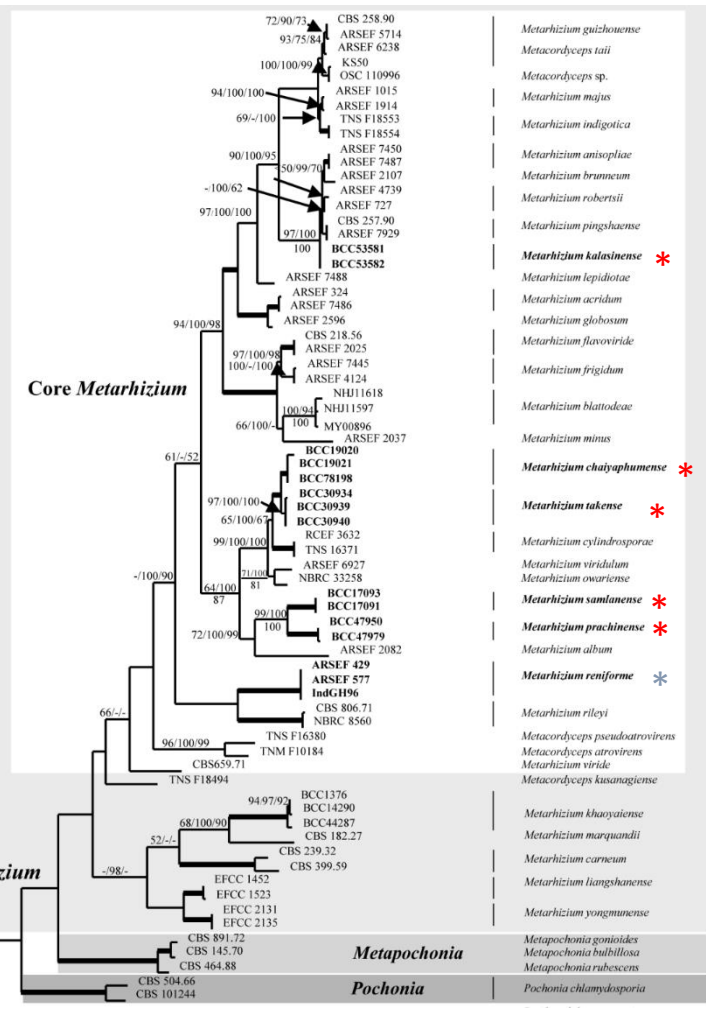
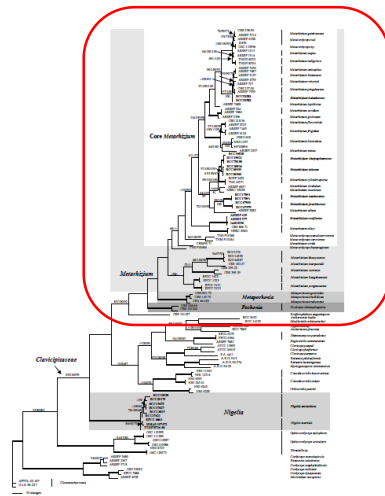
Conidiophores aggregate, mostly mononematous, sometimes synematus when parasitic on arthropods in soil; conidiogenous cells phialidic, cylindrical to clavate, producing conidia in long basipetal chains; conidia on celled, hyaline or slightly pale greenish, smooth, ovoid to cylindrical, aggregate in prismatic columns.

**Parasitic on insect or saprobic in soil**



# Recognizing new species in *Metarhizium*

Our findings:



Mycol Progress (2017) 16:369–391  
DOI 10.1007/s11557-017-1277-1

ORIGINAL ARTICLE



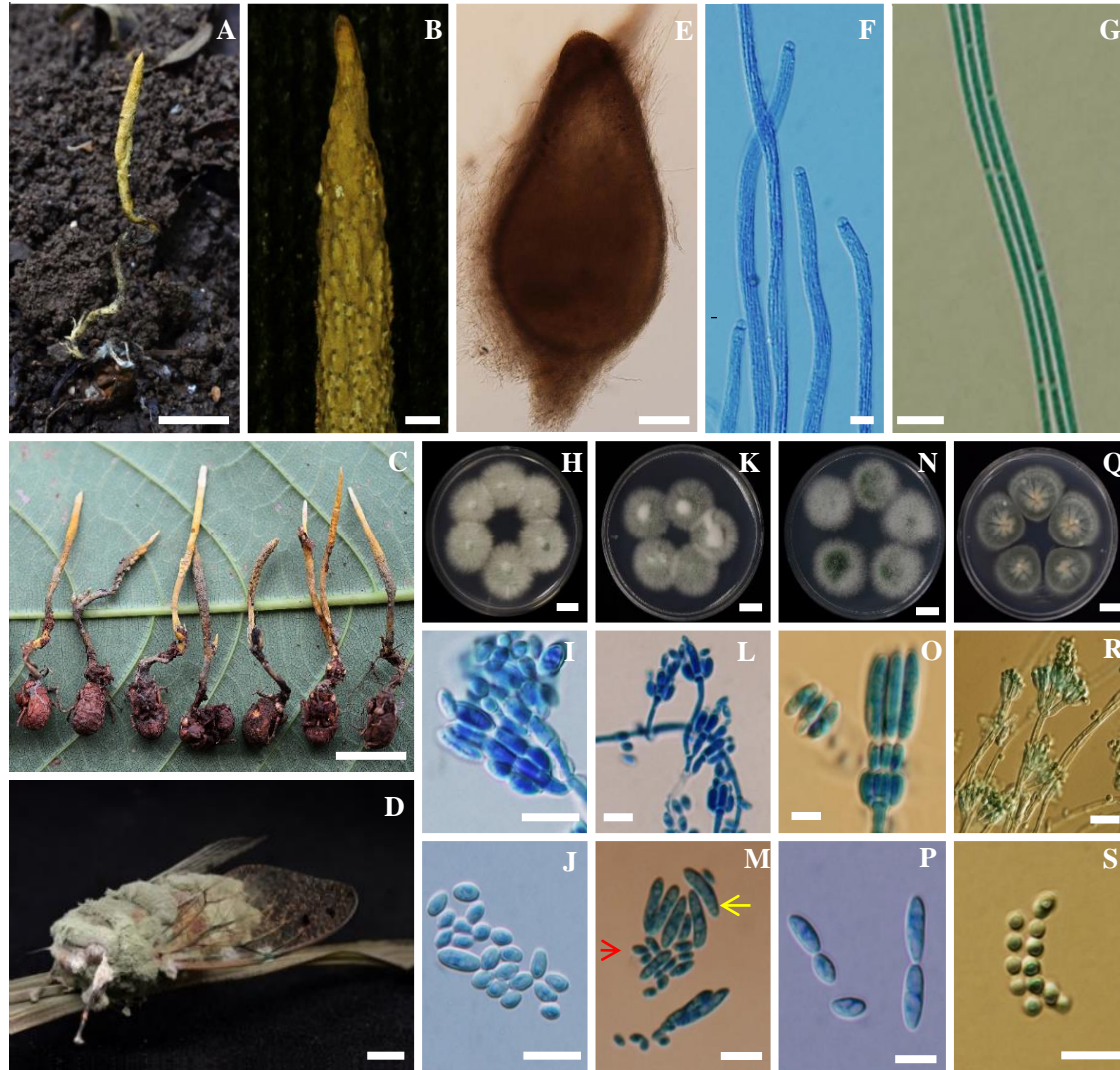
DGfM

## Clavicipitaceous entomopathogens: new species in *Metarhizium* and a new genus *Nigelia*

J. Jennifer Luangsa-ard<sup>1</sup> · Suchada Mongkolsamrit<sup>1</sup> · Donnaya Thanakittipattana<sup>1</sup> · Artit Khonsanit<sup>1</sup> · Kanoksri Tسانathai<sup>1</sup> · Wasana Noisripoon<sup>1</sup> · Richard A. Humber<sup>2</sup>

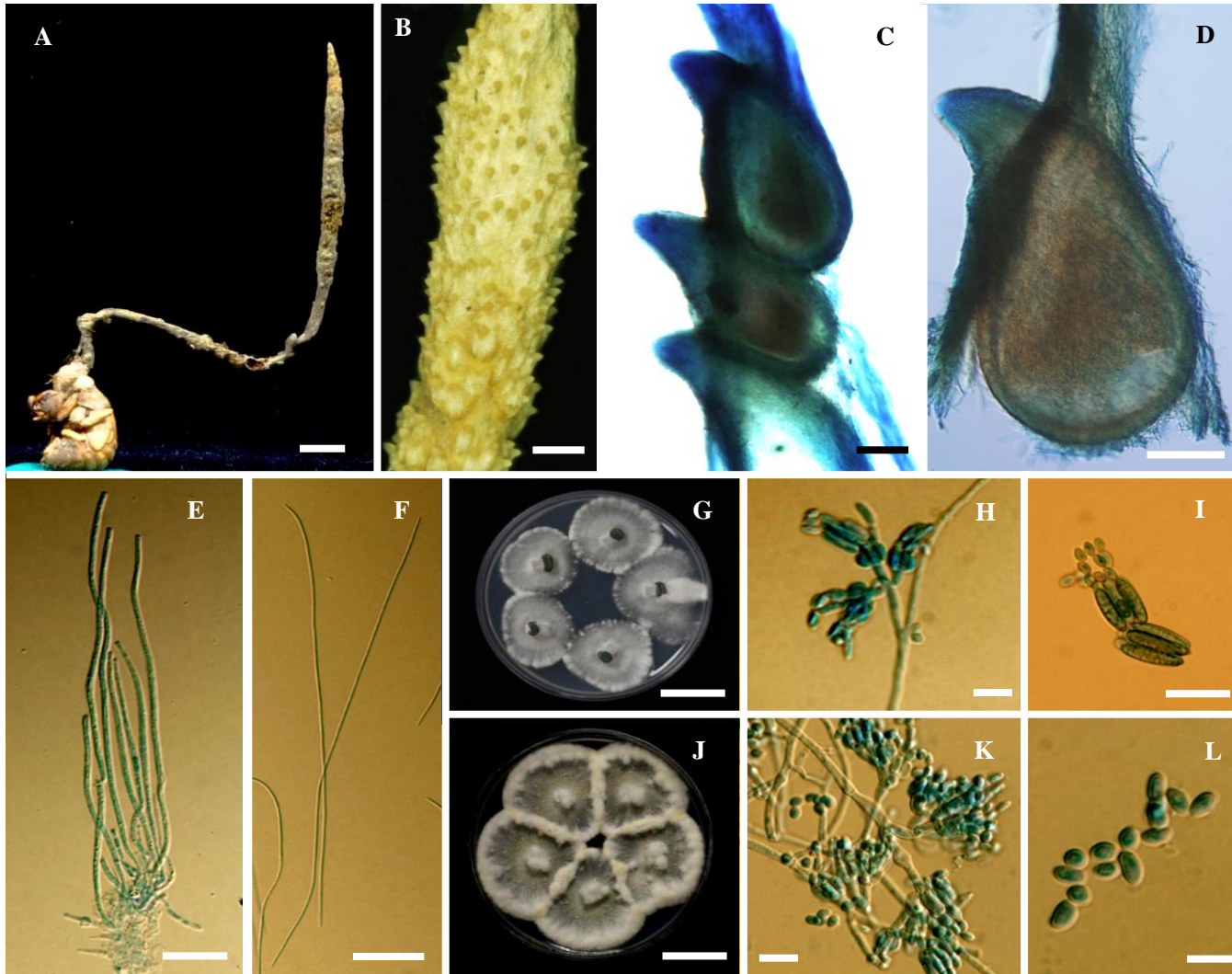


# New Species in *Metarhizium*



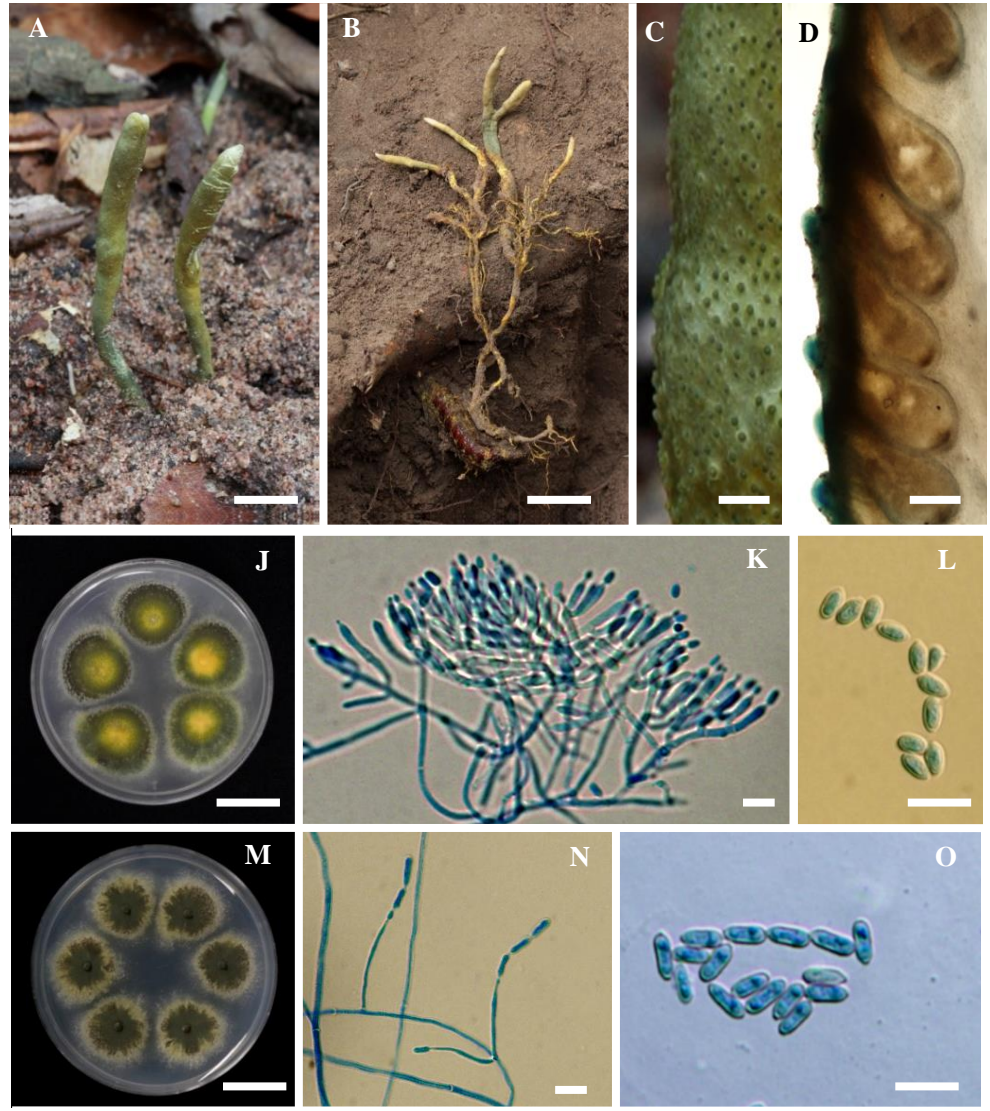
*Metarhizium chaiyaphumense*

# New Species in *Metarhizium*



*Metarhizium takense*

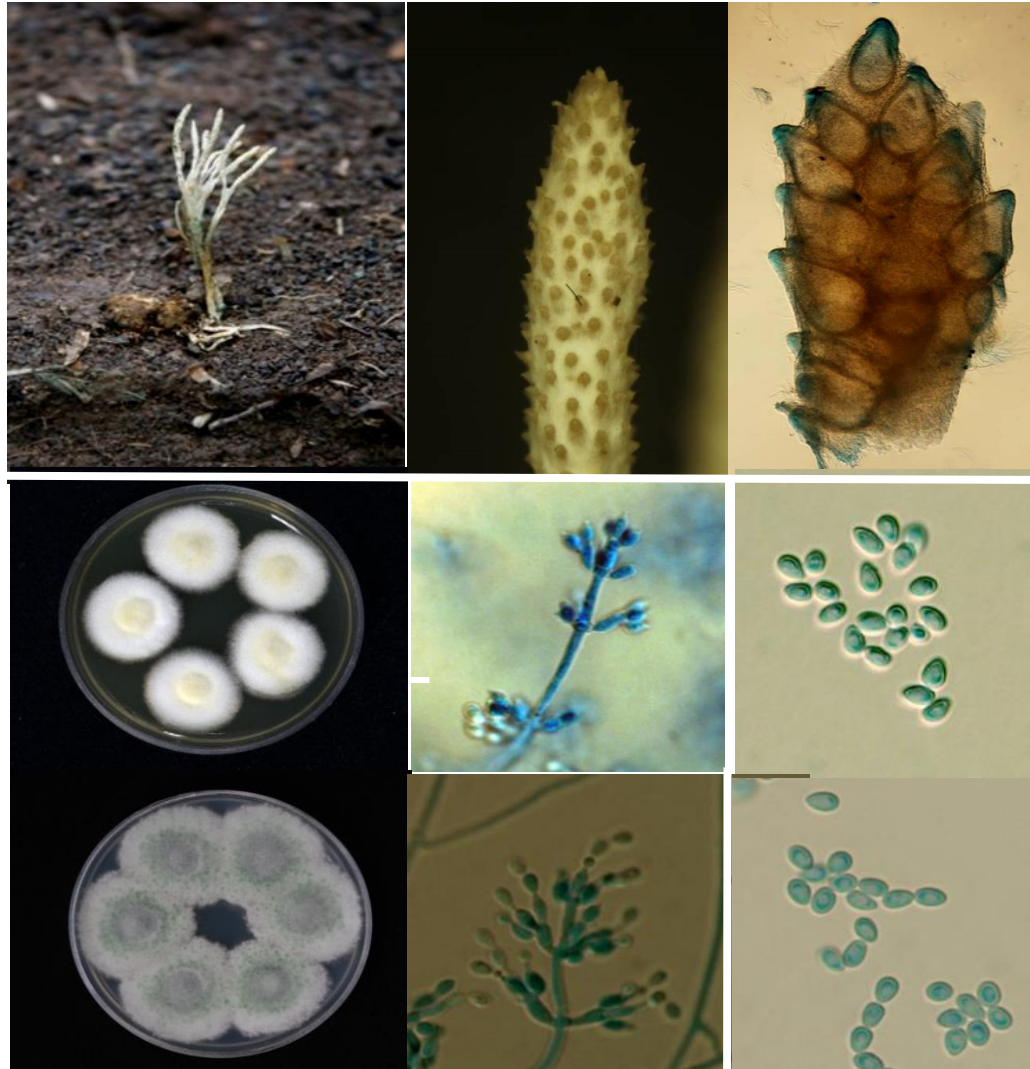
# New Species in *Metarhizium*



*Metarhizium kalasinense*



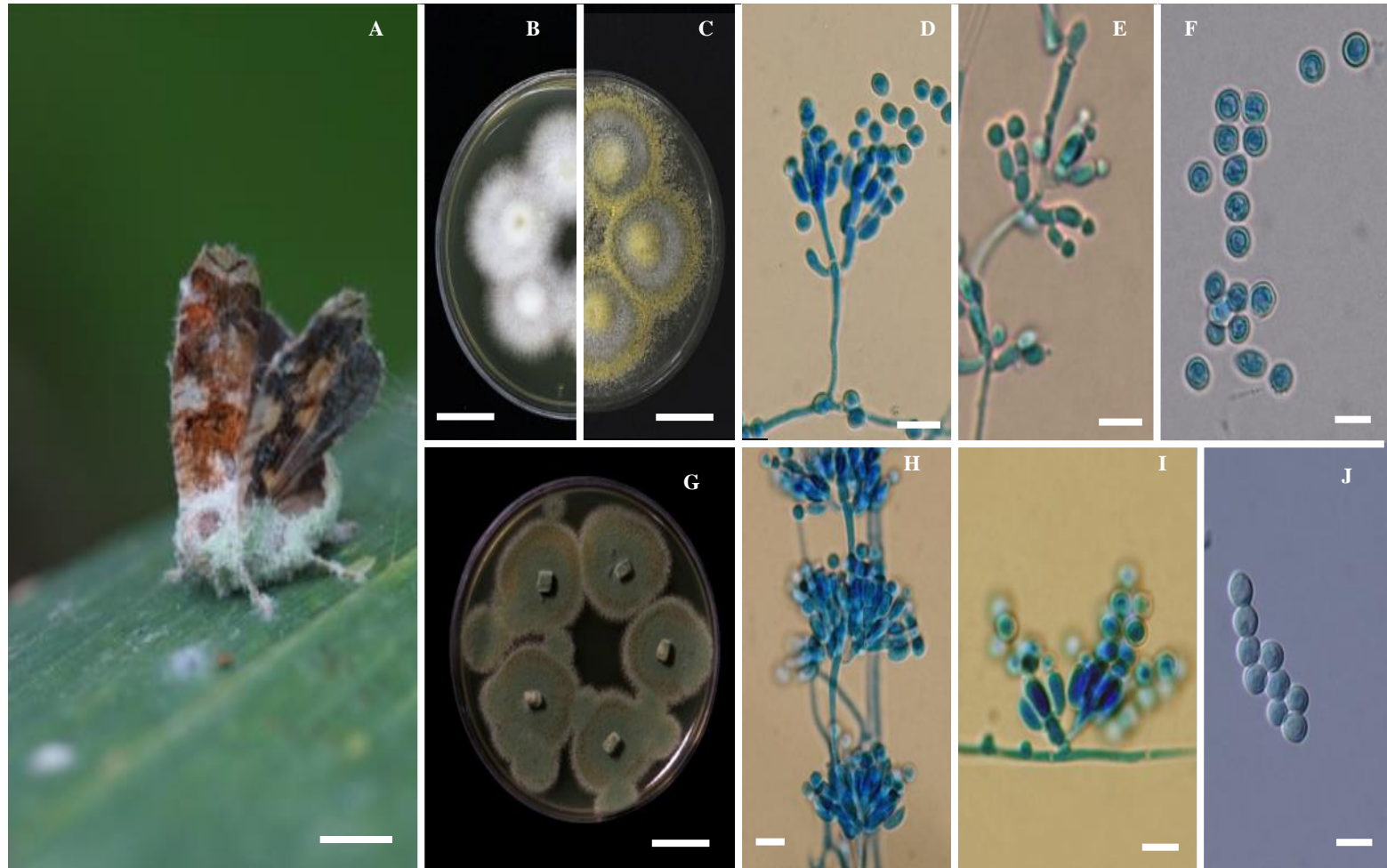
# New Species in *Metarhizium*



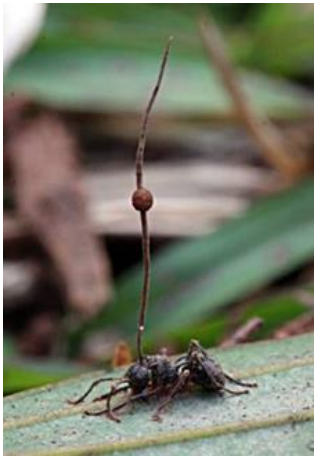
*Metarhizium prachinense*



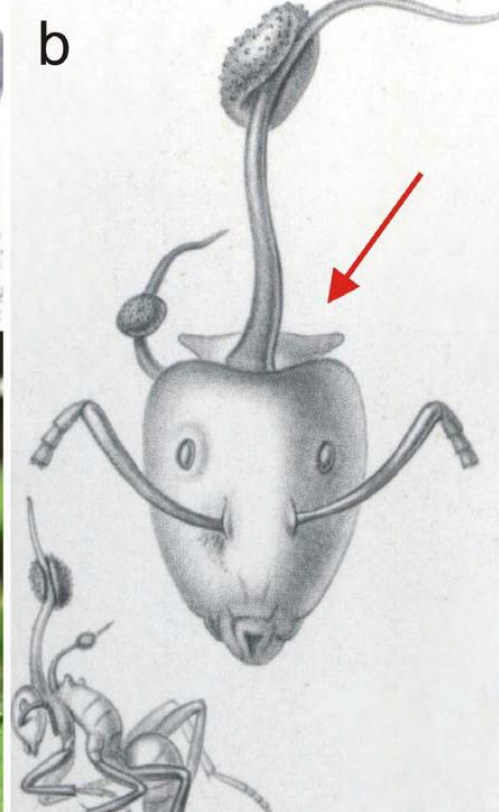
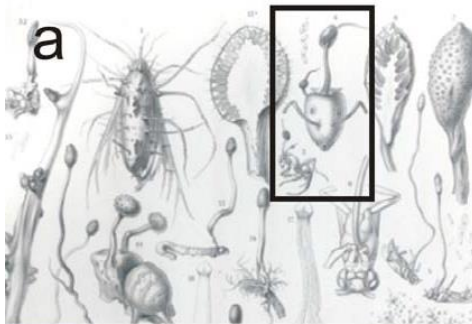
# New Species in *Metarhizium*



*Metarhizium samlanense*



## Zombie Ant *Ophiocordyceps unilateralis*



*O. unilateralis* is characterized by lateral ascomatal cushion developing on the stroma growing from the back of the ants' heads (Tulasne & Tulasne, 1865).



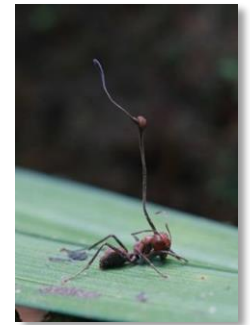
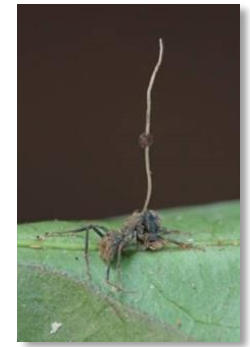
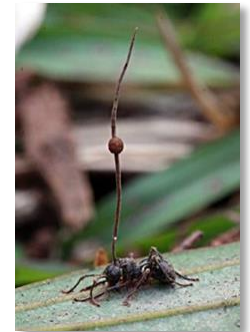
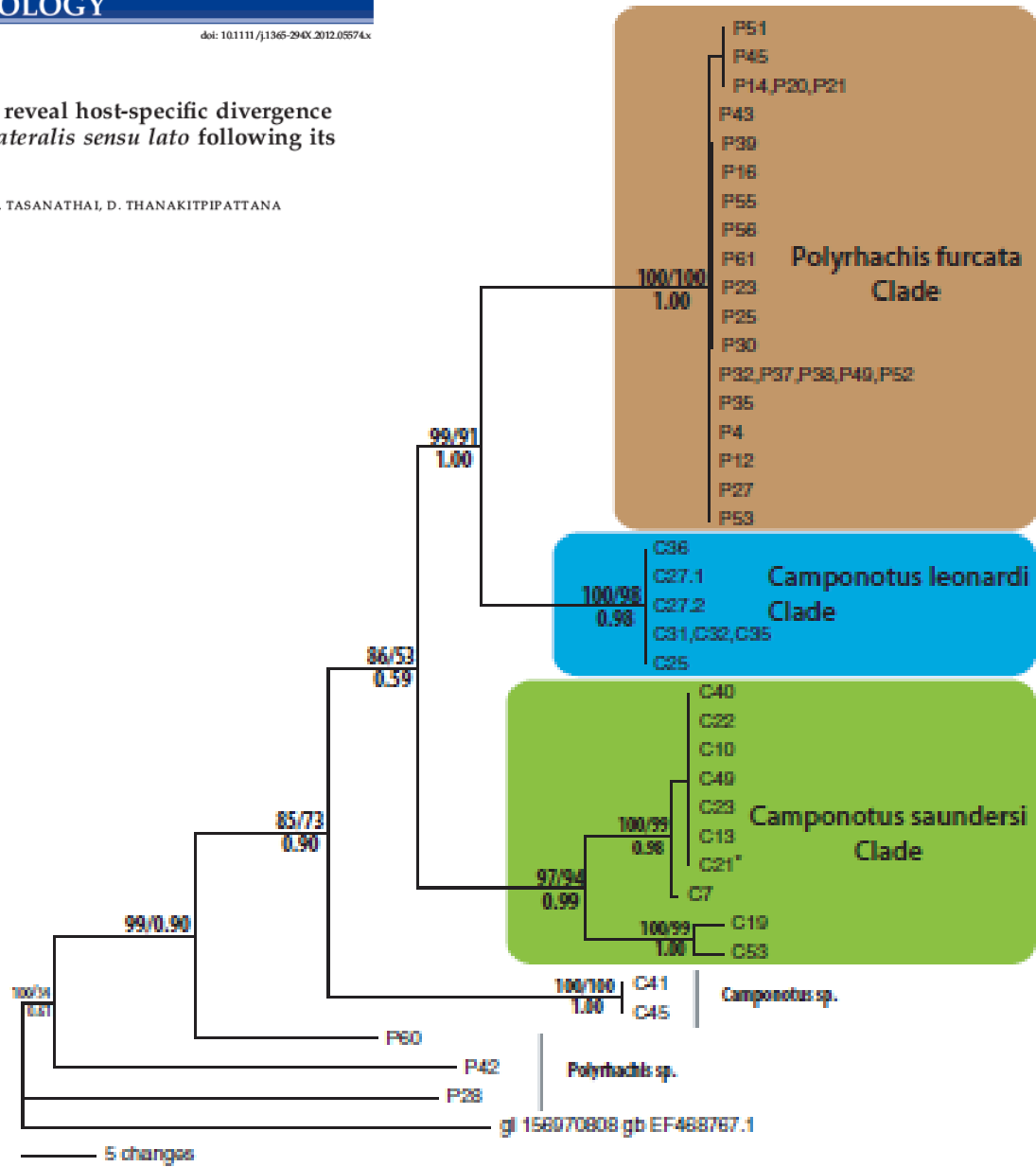
All another specimens suiting this description and found on formicine ants are identified as *O. unilateralis* !

Distribution:

South and North America, Japan, South-East Asia

**Molecular phylogenies reveal host-specific divergence of *Ophiocordyceps unilateralis sensu lato* following its host ants**

N. KOBMOO, S. MONGKOLSAMRIT, K. TASANATHAI, D. THANAKITPIPATTANA and J. J. LUANGSA-ARD



# Marine Fungi

- Early phase of marine mycology: Sutherland (1915)- seaweed fungi

**Obligate marine fungi** = fungi that grow & sporulate in marine / estuarine habitats

Jones, et al. 2015 listed 1,112 spp. (in 472 genera):

- 805 Ascomycota \*\*
- 21 Basidiomycota
- 26 Chytridiomycota
- 3 Zygomycota
- 1 Blastocladiomycota
- 43 asexual morphs
- 213 marine yeasts

**\*\* 188 spp. reported for Thailand.**





# Habitats and substrata

## Ecological functions:

1. Saprobies / Decomposer on dead organic materials (esp. woody substrata) \*\*
2. Parasites on plants, algae, sponges and animals
3. Symbionts in lichenoid associations with algae



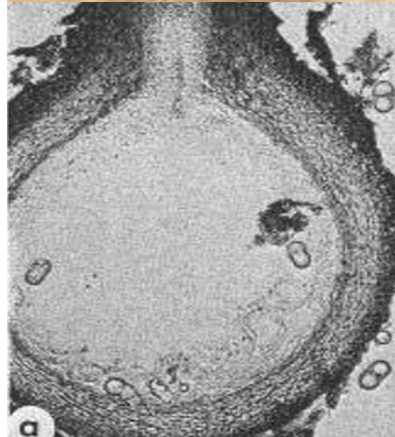




# Halosphaeriaceae: a largest family of marine ascomycetes with 166 species in 63 genera

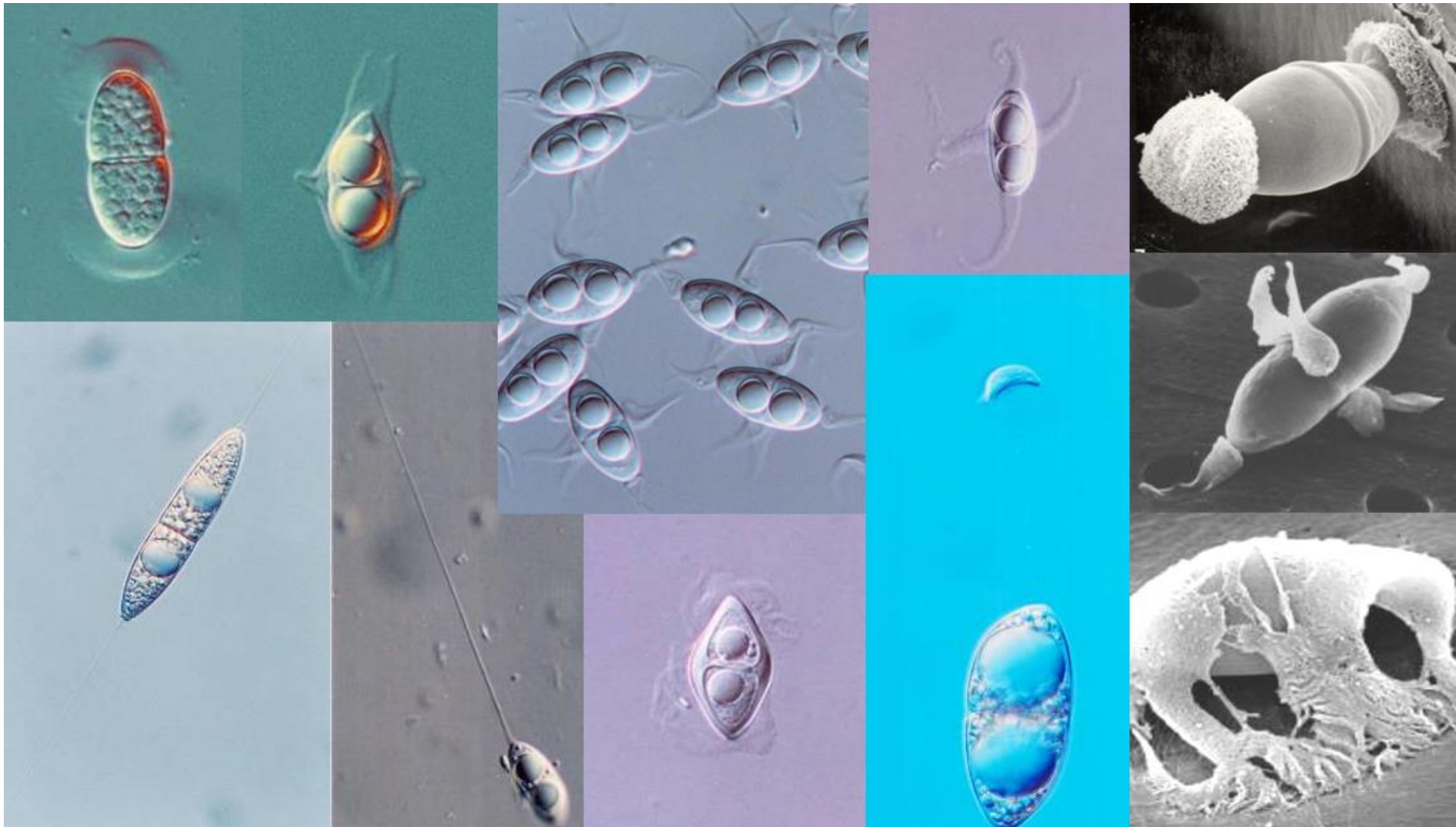


Ascomata



Deliquescing asci,  
facilitate liberation

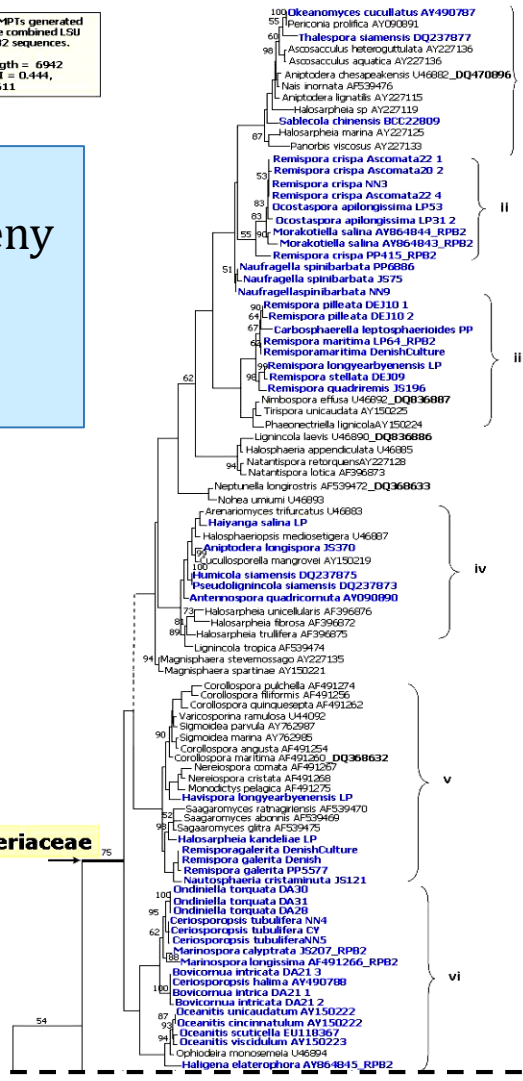
# Appendaged ascospores



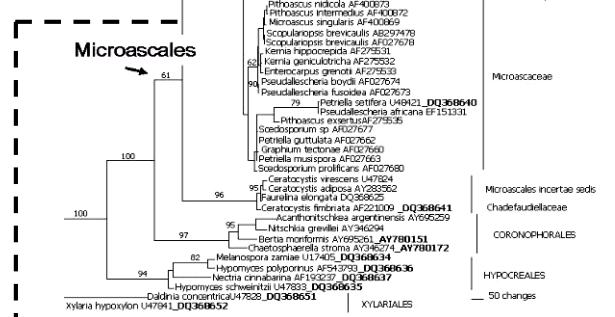


1 of 18 MPis generated from the combined LSU and rPB2 sequences.  
Tree length = 6942 steps, CI = 0.444, RI = 0.611

To elucidate taxonomy, classification and phylogeny of selected fungi using multiple gene analysis approach



• Diversity & speciation of the Halosphaeriaceae is prolific



# Classification of environmental sequences

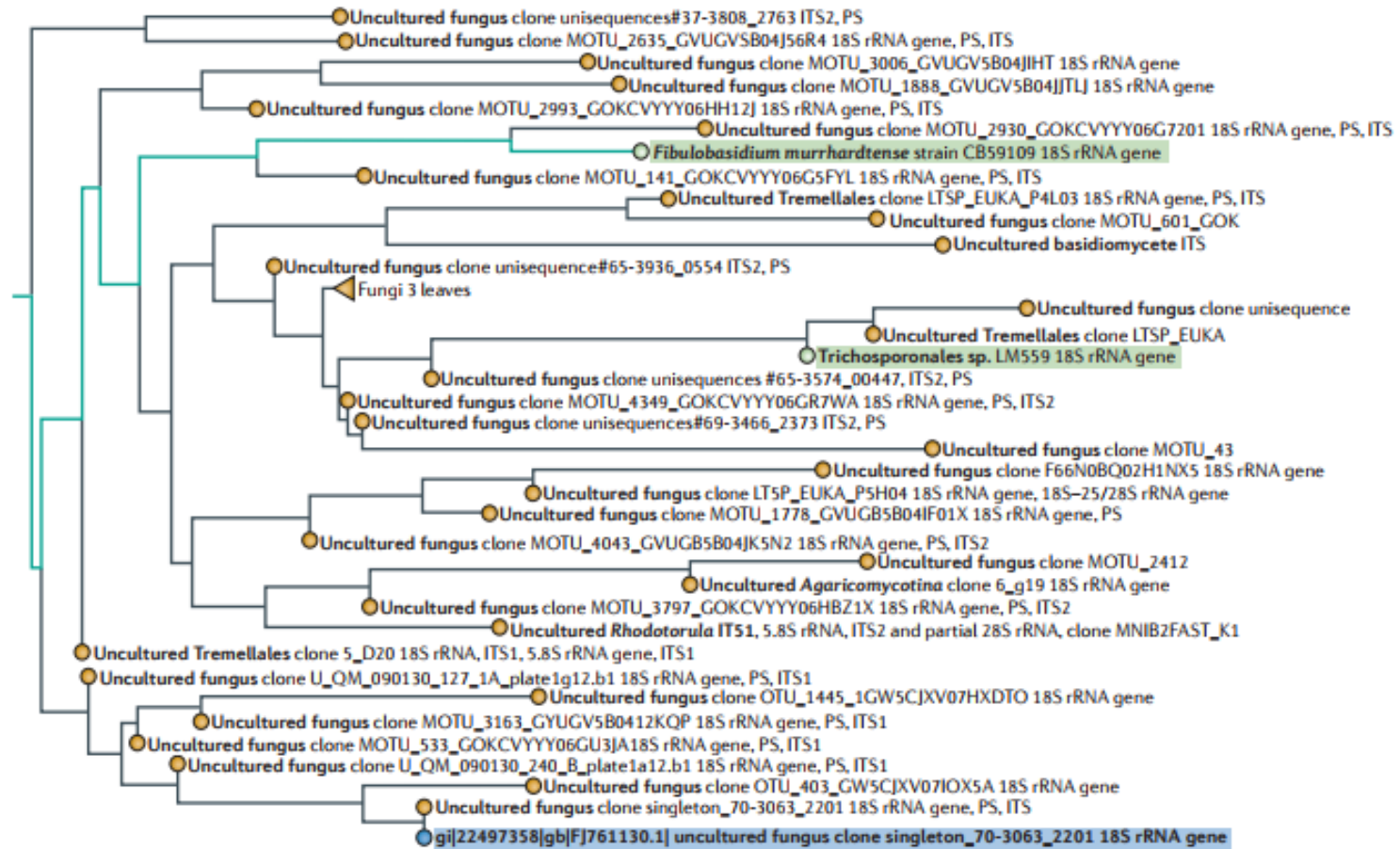
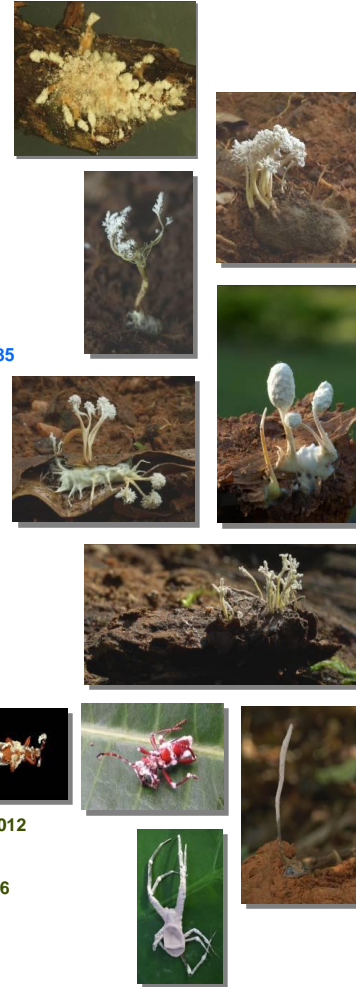
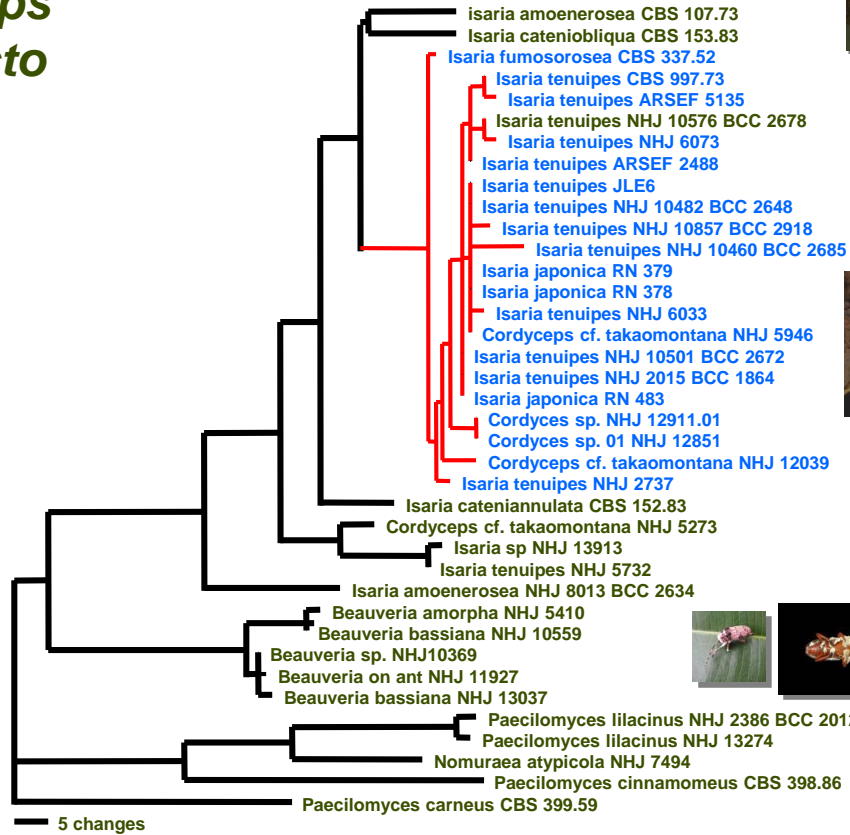


Figure 2 | **Unnamed diversity.** A demonstration of the problem posed by unnamed fungi that are known only from environmental DNA sequences. When a new environmental sequence (the bottom-most operational taxonomic unit, *gi|22497358*; blue box) was used in a BLAST search of the GenBank database and the result displayed using the BLAST

distance tree tool, only two of the 35 most closely related sequences were from cultured organisms (green boxes), and only one was named (*Fibulobasidium murrhardtense*). Without names, the information content of this tree leaves much to be desired. ITS, internal transcribed spacer; PS, partial sequence.

# Beauvericin- production in *Cordyceps* *sensu stricto*



Luangsa-ard, J.J., Berkaew, P., Ridkaew, R., Hywel-Jones, N.L. and Isaka, M. 2009. A Beauvericin Hotspot in the genus *Isaria*. *Mycological Research* **113**: 1389-1395.

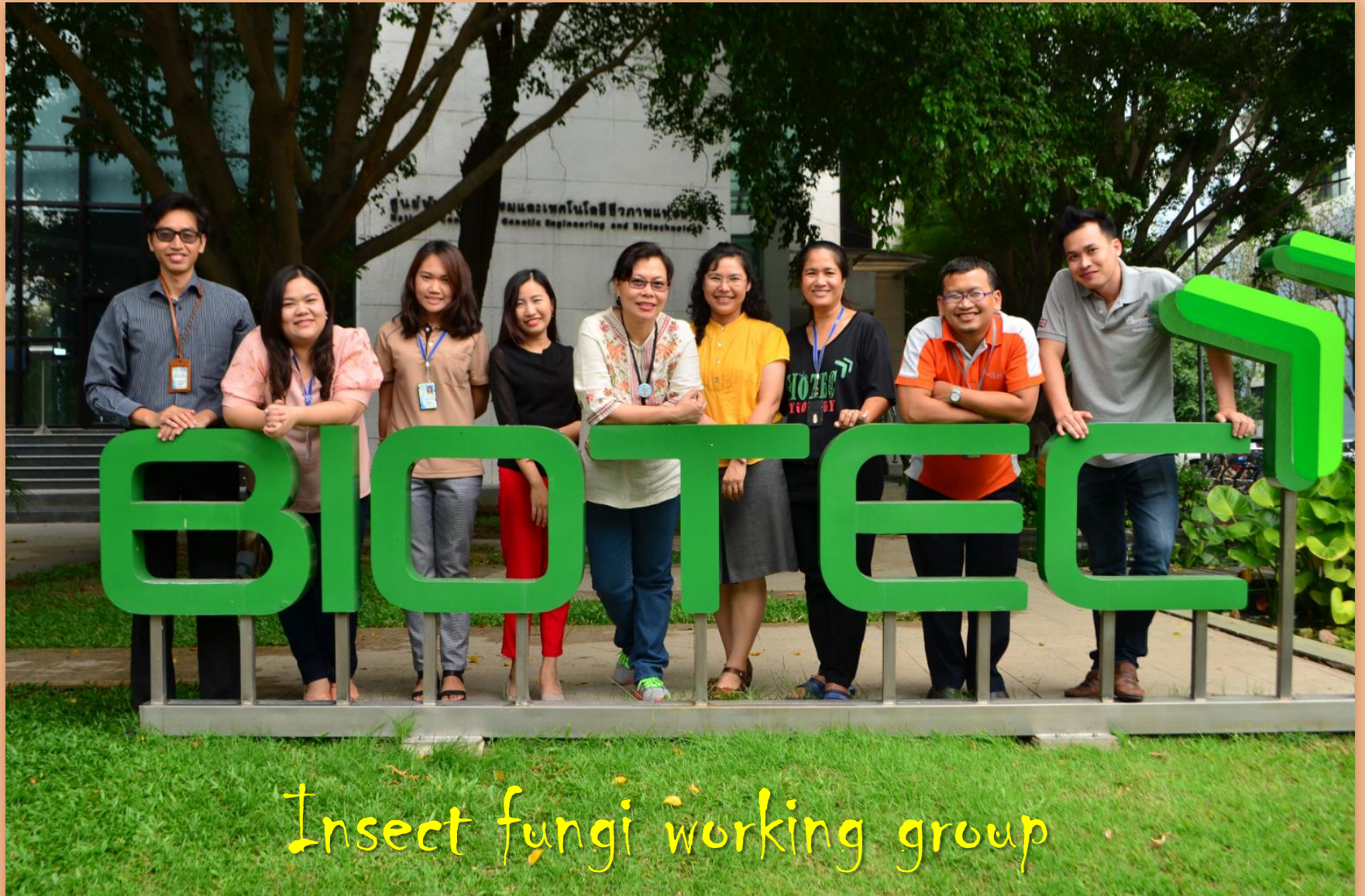
# Summary

Molecular phylogenetics helps us understand about the evolutionary relationships of organisms by recognizing monophyletic clades

Phylogenetic relationships gives us insight about host and parasite interactions

Knowing these relationships allow scientists to make biological predictions





*Insect fungi working group*



**Thank you for your attention!**



Shanghai Entomological Museum



**San Juan, Puerto Rico**

*Mycological Discoveries for a Better World*

July 16-21, 2018

Puerto Rico Convention Center

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