BRT R_250002

Final report (Phase I)

(September 2006 – August 2007)

การศึกษาความสัมพันธ์ของราไซลาเรียที่เจริญบนดินปลวกในประเทศไทย

(A study on the termite-associated *Xylaria* in Thailand)

Investigators: Mr. Prasert Srikitikulchai

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Acknowledgement

"This work was supported by the TRF/BIOTEC Special Program for Biodiversity Research and Training grant BRT R_250002"

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Final report (Phase I) : September 2006 – August 2007

Investigators: **Mr. Prasert Srikitikulchai**
Dr. Janet Jennifer Divinagracia Luangsa-ard

Summary of Report:

The main purpose of the project is to collect and isolate axenic fungal cultures of Xylariaceous fungi. Another objective is to study the phylogenetic relationships of Xylariaceous fungi in Thailand, especially those *Xylaria* species associated with termite nests and wood decay xylariaceous fungi. One hundred and sixty four pure cultures were isolated from various sites of Thailand. These cultures were identified to 9 genera (*Annulohypoxylon, Biscogniauxia, Daldinia, Hypoxylon, Kretzschmaria, Nemania, Sarcoxylon, Whalleya* and *Xylaria*), 45 species. The total genomic DNA of seventy strains comprising eight genera in the Xylariales was extracted. These genera are: *Annulohypoxylon*, *Biscogniauxia*, *Daldinia*, *Hypoxylon*, *Kretzscharia*, *Nemania*, *Whalleya* and *Xylaria*. Thirty-six strains comprising twenty-two *Xylaria*, six *Hypoxylon*, three *Nemania*, two *Biscogniauxia*, one *Daldinia*, *Annulohypoxylon* and *Kretzschmeria* were included in the analysis. Seventeen *Xylaria* strains were collected from termite nests. The species identified from these nests were *Xylaria escharoidea*, *Xylaria* cf. *piperiformis*, *Xylaria* cf. *nigripes* and six other unidentified *Xylaria* species. *Ophiocordyceps sphecocephala* was used as the outgroup. The phylogenetic tree gave interesting results of the seventeen species collected on termite nests. Three clades (clade A, B and C) were formed independently, with clade A forming a basal clade. This indicates that those species on termite nests were ancestral and gave rise to other species that have later on adapted to different substrates (dicotyledonous and monocotyledonous plants). Clades B and C were later derived from those species collected on wood indicating the switch on hosts again. It seems that these fungi are capable of host jumping and host switching. The relationships and identities of *Xylaria* cf. *nigripes*, *Xylaria* cf. *escharoidea* and *Xylaria* cf. *piperiformis* should be clarified as well as they are phylogenetically closely related but fall into several distinctly related clades. Based on this analysis, it is evident that the genus *Xylaria* is not monophyletic

and the distinguishing feature used for delimiting *Xylaria* from other genera in the Xylariaceae does not give a good phylogenetic signal to infer relationships.

Objective:

1. To collect and isolate
    - Termite-associated Xylariaceae
    - Other Xylariaceae
2. To study the morphological/taxonomic relationships between termite-associated and saprophytic/endophytic *Xylaria* spp. from natural forests and habitats in Thailand
3. To study the phylogenetic relationships of termite-associated and saprophytic/endophytic *Xylaria* spp. using LSU and ITS rDNA sequences.
4. To deposit collections in the BIOTEC Bangkok Herbarium (BBH) and BIOTEC Culture Collection (BCC)
5. To produce database of past and current records of Thai Xylariaceae
6. To disseminate information gathered in the international science community in the forms of presentation at scientific meetings and publications

Introduction

The Xylariaceae is a large family of Ascomycota with more than 40 genera and 1,200 species (Kirk *et al.*, 2001; T. Læssøe pers. com.). Most are wood degraders (including woody bamboos and palm fronds), although some also occur on animal dung, fruits and seeds, leaves and herbaceous stems. They are particularly common in the tropics (Rogers, 1979; Whalley, 1996). While most Xylariaceae are saprotrophs, some also occur as endophytes of various plants (Van der Gucht, 1994; Bayman *et al.*, 1998; Mekkamol, 1998). However, it is not known if these are precursors of saprophytes, with a ready access to a nutrient rich source or maybe even dead ends for spores. Many Xylariaceae initially produce an anamorphic stage but later in the season a teleomorph

with perithecia is often formed. A few can survive as sclerotia until favorable conditions re-appear.

Previous work done in Thailand clearly has shown the richness of this particular group of fungi. Thiernhjrun (1997) has shown that the Xylariaceae is a highly diverse and species-rich family within Thailand with 80 fully identified species and a further 60 species awaiting identification and description. Forty-six strains found in the course of a 2 field study in February 2006 belong to 41 species within 9 genera, several of which clearly belong to undescribed taxa. Other studies have demonstrated that the family is equally interesting from a secondary metabolite point of view, and it is important to safely deposit this diversity before it becomes extinct in nature.

Several baseline monographs have been published over the last 10 years and it is now timely to take this approach a step further (especially for Thailand) by doing a thorough regional inventory and in depth studies (including sequencing) of a particular group, the termite associated *Xylaria* species, that would seem to have both a very interesting evolutionary biology and phylogeny.

Early studies of these species were highly inaccurate and it became known as a fact that these species lacked germination slits on their ascospores, a feature found in almost all xylariaceous fungi. We now know that all species involved with termitaria have germination slits on their spores, either in the form of a straight slit on the more flattened side of the spore or as a short oval opening. The name *Pseudoxylaria* has been introduced for these fungi (originally only for *X. nigripes*), partly based on the erroneous assumption of the lack of germ slits and also based on the rather soft and non-carbonized nature of the stromata (Boedijn, 1959). Dennis (1961) added *X. tanganyikaensis* and *X. furcata* but treated *Pseudoxylaria* at the subgeneric level. Both Petch (1906, 1913) and von Höhnel (1908) made extensive collections of *Xylaria* spp. on termite nests and provided interesting observations on their biology, but their failure to circumscribe the taxa involved has devalidated their observations. Sands (1969) and Heim (1977) summarized observations and data of *Xylaria* on termite nests, considering them to be saprotrophs. Batra and Batra (1979) studied the interactions of *Odontotermes obesus* with *Xylaria* species and the mushroom genus *Termitomyces*. They in contrast to Sands and Heim considered both *Termitomyces* and *Xylaria* to be mutualistic symbionts

with the termite. Rogers *et al.* (2005) studied the relevant types of the *Xylaria* names known or thought to be associated with termite nests. The following species were recognized: *Xylaria arenicola, X. brasiliensis, X. escharoidea, X. furcata, X. nigripes, X. piperiformis* and *X. rhizomorpha*. *Xylaria tanganyikaensis* and *X. readeri* were also considered likely candidates, although the latter species only occurs in Australia, where the termites are gut digesters and not fungus growers. They described a new variety, *X. furcata var. hirsuta* from Africa, and discuss an unnamed Asian *Xylaria* that probably represents a new species.

The use of polymerase chain reaction (PCR) for amplifying target regions that are suitable for inferring relationships and identification of organisms is widely used today. Sequencing of some regions within the ribosomal DNA has proven to be a valuable tool in the study of molecular evolution, population biology and molecular taxonomy. In fungi, the use of the internal transcribed spacer region of the rDNA (ITS regions) has proven to be good targets for inferring intra- and interspecific relationships and diversity (Bruns *et al.*, 1991). These untranscribed regions show a high degree of variability that can be used to discriminate fungi at the generic and species level. The large subunit of the ribosomal DNA has long been used to infer relationships at the generic and family level. Universal primers will be used to amplify the ITS1-5.8S-ITS2 rDNA and LSU rDNA (White *et al.*, 1990).

In this project, the phylogenetic relationships among 100 isolates of termite and wood *Xylaria* spp. will be explored based on comparison of the ITS and LSU sequences.

Materials and Methods

4.1 Sites survey and specimen collecting

Ten trips to the 13 collecting sites were surveyed for the collection of *Xylaria* spp. that are associated with termite mounds in natural forests and also other xylariaceous fungi in Thailand. Xylariaceous fungi were collected in different regions of Thailand to reflect different forest/habitat ranges from north to south (Fig. 1) for isolation and identification. A total of 5-10 stromata of all different morphology were collected from each collecting site. Specimens were kept in the BIOTEC Bangkok Herbarium (BBH)

while all successful isolates were transferred and preserved in BIOTEC Culture Collection (BCC).

1. Doi Inthanon National Park (trip 1)
2. Phu Suan Sai National Park (Na Haew) (trip 2)
3. Phu Phan National Park (trip 2)
4. Nam Nao National Park (trip 3)
5. Thung Yai Naresuan Wildlife Sanctuary (trip 4)
6. Khao Yai National Park (trip 5)
7. Phu Chong-Nayoi National Park (trip 6)
8. Kaeng Krachan National Park (trip 7)
9. Mu Ko Chang National Park (trip 8)
10. Khao Sok National Park (trip 9)
11. Khao Luang National Park (trip 9)
12. Khao Pu-Khao Ya National Park (trip 9)
13. Hala-Bala Wildlife Sanctuary (trip 10)

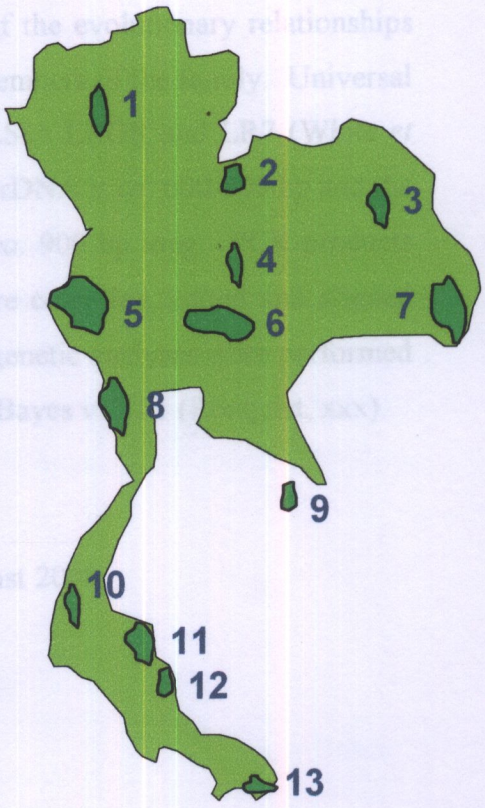

Figure 1. Proposed collecting sites for specimen

4.2 Isolation and identification

Cultures were initiated by removing a portion of stromata surface with a sterilized razorblade. The stromata contents were picked up with a fine needle and placed in Petri dishes containing Potato Dextrose Agar (PDA). Typical characteristics of the isolates were described and photographs of the colony appearance on media were taken for comparative studies. The field collected stromata are likewise described and after isolation heat dried and stored in air tight containers before deposition in the BIOTEC herbarium.

4.3 Database of Xylariaceous fungi in Thailand

A database was constructed to hold all associated data (from this and previous studies) on Thai xylariaceous fungi, including taxa, collections, cultures, pictures, sequences etc. The MIMS database program at BIOTEC was used alongside FileMaker Pro.

4.4 Molecular studies

The herbarium materials and cultures from this project provided the material for molecular phylogenetics work to test the hypotheses of the evolutionary relationships between the termite-associated Xylariaceae and other members of the family. Universal primers for the ITS rDNA: ITS5/ITS1 and ITS4 and LSU: LROR and LR7 (White *et al.*, 1990) were used in this study. The ITS1-5.8S-ITS2 rDNA is *ca.* 600-800 bp and the partial sequence of the ribosomal large unit (LSU) is *ca.* 900 bp long. PCR products were sent to Macrogen, Inc., Korea and sequences were compiled, edited and aligned using the program BioEdit v. 6.0.7 (Hall, 2004). Phylogenetic analyses were performed using the program PAUP*b10 (Swofford, 2002) and MrBayes v. 3.01 (Ronquist, xxx).

Duration: Two-year period (September 2006 - August 2008)

Phase I (September 2006 to August 2007)

Phase II (September 2007 to August 2008)

RESULTS

4.1 Sites survey and specimen collecting

Sites of collection: Nine trips were selected for sample collection in table 1: Doi Inthanon National Park (Chiang Mai), Chatchanat Farm and Phu Luang Wildlife Sanctuary (Loei), Khao Yai National Park and Khao Sam Lan National Park (Nakhon Ratchasima and Saraburi), Thung Salang Luang National Park, Namtok Chattrakan National Park and Phu Hin Rong Kla National Park (Phitsanulok), Khao Nan National Park and Khao Luang Wildlife Sanctuary (Nakhon Si Thammarat), Khao Ban Tad Wildlife Sanctuary (Phatthalung), Kaeng Krachan National Park (Phetchaburee), Khao Yai National Park (Nakhon Ratchasima) and Khlong Lan National Park (Kamphaengphet).

Table 1. Collection sites

Collecting Sites	Collecting Dates
Doi Inthanon National Park	5-7 Sep. 2006
Ampure Phu Ruea and Phu Luang Wildlife Sanctuary	26-27 Sep. 2006
Khao Yai National Park and Khao Sam Lan National Park	7-8 Nov. 2006
Thung Salang Luang National Park, Namtok Chattrakan National Park and Phu Hin Rong Kla National Park	13-16 Nov. 2006
Khao Nan National Park and Khao Luang National Park	20-21 Feb. 2007
Khao Ban Tad Wildlife Sanctuary	16-20 Mar. 2007
Kaeng Krachan National Park	25-29 Jun. 2007
Khao Yai National Park	9-13 Jul. 2007
Khlong Lan National Park	22-24 Aug. 2007

4.2 Isolation and identification

Sample collection and isolation: Xylariaceous samples were collected form different sites in Thailand. These were isolated into axenic culture and deposited in the BIOTEC culture Collection (BCC). The list of 164 pure cultures show in Appendix I. One hundred and sixty four cultures were identified to 9 genera and 45 species. *Xylaria* is the most dominant genus, with 100 isolates of which were found. The second is

Hypoxylon, with 41 isolates found. Khao Yai National Park (covering the provinces Nakhon Ratchasima, Saraburi, Prachinburi and Nakhon Sawan?) showed the highest diversity in this report. Thirteen isolations of *Xylaria* spp. associated with termite nests were found from Bangkok, Loei, Nakhon Ratchasima, Nakhon Si Thammarat, Petchaburee, Phetchabun and Tak. These were identified as *Xylaria escharoidea*, *Xylaria nigripes* and *Xylaria* sp.

Table 2. Diversity of fungi at selected sites

Genus	Province												Grand Total
	A	B	C	D	E	F	G	H	I	J	K	L	
Annulohypoxylon										1			1
Biscogniauxia										5			5
Daldinia			1			2							3
Hypoxylon		5		1	3	8	4	2		17	1		41
Kretzschmaria						1	4						5
Nemania					4					3			7
Sarcoxylon					1								1
Whalleya					1								1
Xylaria	1	12	7	12	23	9	15	8	5	7		1	100
Grand Total	1	17	8	13	32	20	23	10	5	33	1	1	164

Remarks: A = Bangkok B = Chiang Mai C = Kamphaeng Phet
D = Loei E = Nakhon Ratchasima F = Nakhon Si Thammarat
G = Petchaburee H = Phatthalung I = Phetchabun
J = Phitsanulok K = Saraburi L = Tak

4.3 Database of Xylariaceous fungi in Thailand

Microbial data recorded in the MIMS database program include the genus, species (if identified), isolate number (original code) source and date of isolation, isolator, site of collection and other related information. Data on the cultures isolated are recorded both in the database computer program and on a hard copy. The data of cultures recorded are shown in Appendix 1.

4.4 Molecular studies

DNA extraction

To prepare the strains for DNA extraction plugs were first grown on potato dextrose agar (PDA) plates as starter cultures for one week. Mycelia of pure cultures was grown on potato dextrose broth for 1-2 weeks, harvested and lyophilized. Total genomic DNA was extracted using 50-100 mg of lyophilized mycelia. The mycelia was ground and placed in a sterile 1.5 ml reaction tube. 700 µl of extraction buffer (NaCl 0.7 M; Tris-HCl 50mM pH 8.0; EDTA 2mM pH 8.0) that was preheated at 65°C was added to the powder. The suspension was thoroughly mixed and put in a 65°C water bath with opened lids for 10 minutes and further incubated for 1 hour with closed lids. After the suspension has cooled down 500 µl of chloroform/isoamyl alcohol (24:1 v/v) was added. The suspension was mixed by inversion until an emulsion was obtained. After a 20 spin down at 12000 rpm, the aqueous phase was slowly pipetted out and transferred to a new sterile tube. A 10% CTAB solution was added at one tenth of the volume of the aqueous phase and mixed. The supernatant was removed and transferred on to a new tube after a spin-down of 20 min. 700 µl of precipitation buffer (CTAB 1%; Tris-HCL 50 mM pH 8.0; EDTA 10 mM pH 8.0) was then added to the supernatant, left at room temperature for 5-10 min and centrifuged. The aqueous phase was discarded and 300 µl of TEHS buffer (NaCl 1M; TrisHCL 10 mM pH 8.0; EDTA 1 mM pH 8.0) was added to the pellet. Cold absolute ethanol (2.5 volumes) was added and centrifuged for 1 min at 12000 rpm. The DNA was resuspended in TE buffer, treated with ribonuclease A and precipitated again with absolute ethanol (2.5 volumes) and resuspended in TE buffer.

PCR and Sequencing

PCR amplification was done in 50 µl volume consisting of 1x PCR buffer, 200 µM of each of the four dNTPs, 2.5 mM $MgCl_2$, 1U Taq DNA polymerase (Promega, Madison, Wisconsin) and 0.5 µM of each primer. Amplification of the ITS regions was done using the primers ITS4 and ITS5 (White *et al.* 1990). Amplifications were performed using MJ Research DNA Engine ALD1244 thermal cycler following the procedure described in Luangsa-ard *et al.* (2005).

Amplicon purification

PCR Products were purified using a QIAquick PCR Purification Kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's instructions. Purified PCR products were sent to Macrogen Inc., Korea for sequencing. Forward and reverse primers ITS5 and ITS4, respectively, were used for the sequencing reactions (White *et al.* 1990).

Results

PCR sequencing yielded *ca.* 550 bp on ITS rDNA. Thirty-six strains comprising twenty-two *Xylaria*, six *Hypoxylon*, three *Nemania*, two *Biscogniauxia*, one *Daldinia*, *Annulohypoxylon* and *Kretzschmeria* were included in the analysis. Seventeen *Xylaria* strains were collected from termite nests. The species identified from these nests were *Xylaria escharoidea*, *Xylaria* cf. *piperiformis*, *Xylaria* cf. *nigripes* and six other unidentified *Xylaria* species. *Ophiocordyceps sphecocephala* was used as the outgroup. Figure 1 shows the results of ITS rDNA analysis. The phylogenetic tree gave interesting results of the seventeen species collected on termite nests. Three clades were formed independently, with clade A forming a basal clade. This indicates that those species on termite nests were ancestral and gave rise to other species that have later on adapted to different substrates (dicotyledonous and monocotyledonous plants). From Clade A arose several clades that are found both on soil and on a plant substrate. The split to these clades is supported by 100 % posterior probability. Clade G comprising only with *Hypoxylon comedens* seem to be the first derived clade to adapt to a different substate other than soil. This clade is with 100 % posterior probability. After clade G there is a split into *Daldinia eschscholzii* and the clades B and C (both on termite nests), D and E (both on plant substrate). The support in these clades is a posterior probability of 74%. Clades B and C were later derived from those species collected on wood indicating the switch on hosts again. It seems that these fungi are capable of host jumping and host switching. Clade B is comprised of all unidentified *Xylaria* species. It must be the goal of a future project to identify all the species that have merely been labeled as 'sp." as they form a well-supported clade. The relationships and identities of *Xylaria cf. nigripes*, *Xylaria cf. escharoidea* and *Xylaria cf. piperiformis* should be clarified as well as they are phylogenetically closely related but fall into several distinctly related clades. Based on this analysis, it is evident that the genus *Xylaria* is not monophyletic and the distinguishing feature used for delimiting *Xylaria* from other genera in the Xylariaceae does not give a good phylogenetic signal to infer relationships. This is also true for

several other genera like *Biscogniauxia*, *Nemania*, and *Hypoxylon*. Although it is beyond the scope of this project to look into the natural classification of these genera, it must be a goal of future studies to investigate the relationships of species assigned to these genera if they comprise good species or not.

4.5 Future work

This project was proposed for a two-year study, with the first year to collect and isolate axenic culture of Xylariaceous fungi and extract some DNA for ITS rDNA sequencing. The phylogenetic tree gave interesting results of *Xylaria* spp. associated with termite nests. The next step (phase 2), we plan to continue the isolation of more *Xylaria* species associated with termite nests to increase the number of axenic cultures of this group. It is also planned, in addition to the ITS and LSU rDNA of some selected interesting strains of *Xylaria* spp. associated with termite nests, to sequence the β-tubulin and α-actin genes to infer the infrageneric and interspecies phylogenetic relationships.

Table 3. List of Xylariaceous fungi used to molecular study

Original code	Name	Substrate
XY00080	*Xylaria cubensis*	Wood
XY00089	*Xylaria globosa*	Wood
XY00096	*Xylaria fockei*	Wood
XY00111	*Hypoxylon fendleri*	Wood
XY00180	*Kretzschmaria pavimentosa*	Wood
XY00191	*Biscogniauxia uniapiculata*	Wood
XY00196	*Xylaria* cf. *nigripes*	Soil (termite nest)
XY00212	*Hypoxylon comedens*	Wood
XY00215	*Daldinia eschscholzii*	Wood
XY00217	*Annulohypoxylon* cf. *stygium*	Wood
XY00222	*Nemania* cf. *bipapillata*	Wood
XY00290	*Hypoxylon comedens*	Wood
XY00292	*Xylaria* sp.	Wood
XY00310	*Xylaria* cf. *piperifomis*	Soil (termite nest)
XY00333	*Xylaria* sp.	Soil (termite nest)

XY00359	*Xylaria* cf. *piperifomis*	Soil (termite nest)
XY00360	*Xylaria* cf. *piperifomis*	Soil (termite nest)
XY00402	*Hypoxylon comedens*	Wood
XY00448	*Xylaria escharoidea*	Soil (termite nest)
XY00449	*Xylaria escharoidea*	Soil (termite nest)
XY00466	*Xylaria escharoidea*	Soil (termite nest)
XY00469	*Xylaria ianthino-velutina*	Wood
XY00471	*Xylaria escharoidea*	Soil (termite nest)
XY00483	*Biscogniauxia citriforme* var. *macrospora*	Wood
XY00493	*Xylaria* cf. *nigripes*	Soil (termite nest)
XY00498	*Xylaria* sp.	Soil (termite nest)
XY00507	*Nemania* sp.	Wood
XY00508	*Nemania diffusa*	Wood
XY00534	*Hypoxylon comedens*	Wood
XY00535	*Hypoxylon comedens*	Wood
KHW0706001*	*Xylaria* sp.	Soil (termite nest)
MKS0705001*	*Xylaria* sp.	Soil (termite nest)
NN0706003*	*Xylaria* sp.	Soil (termite nest)
NN0706007*	*Xylaria* sp.	Soil (termite nest)
PJN0706001*	*Xylaria* sp.	Soil (termite nest)
PSS0706001*	*Xylaria* sp.	Soil (termite nest)
NHJ13125**	*Ophiocordyceps sphecocephala*	Insect

* DNA extract form fruiting body

** Used as the outgroup

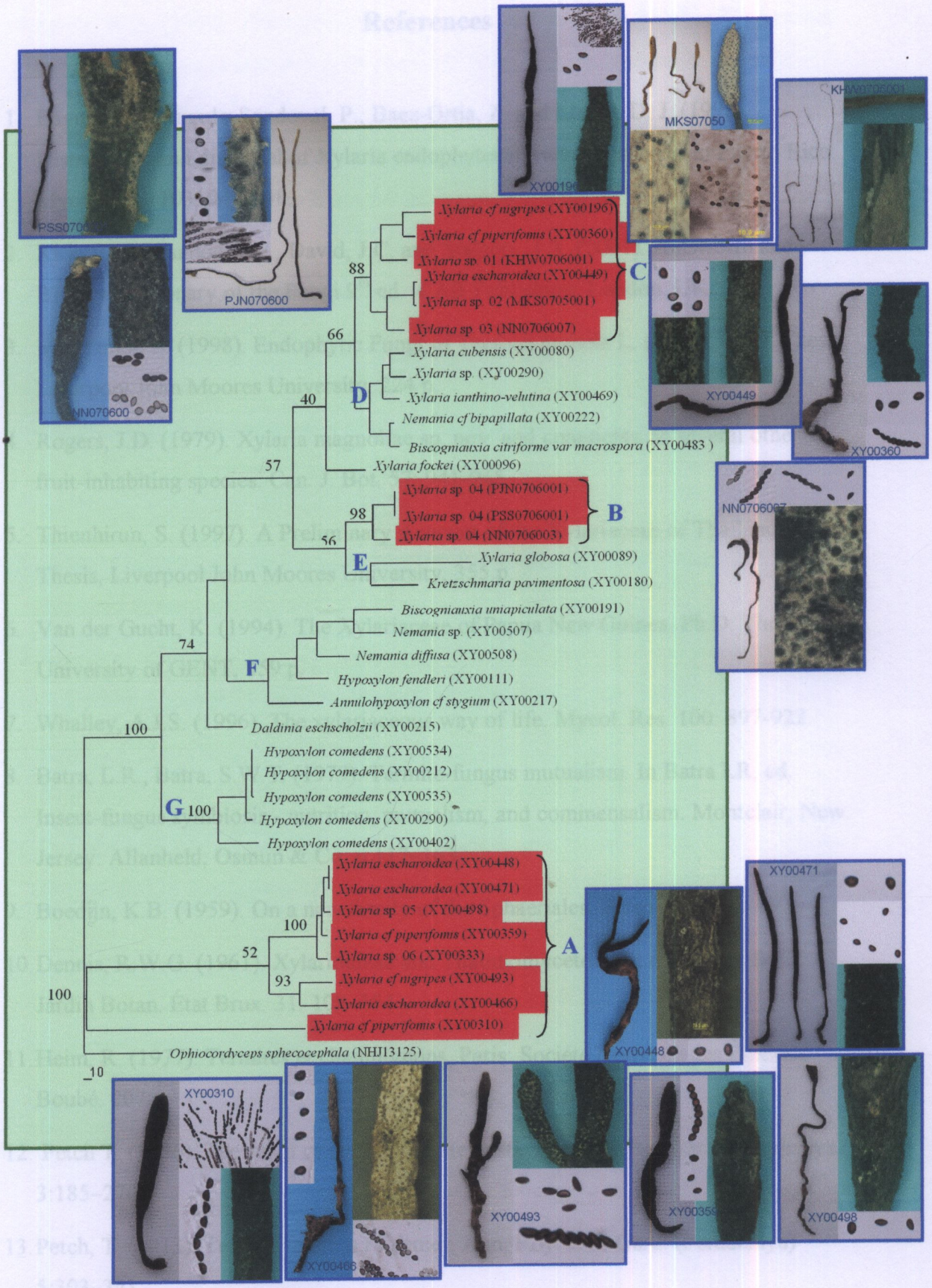

Figure 2. Phylogenetic tree of 36 Xylariaceous fungi, consisting 3 clades (A, B and C) of the termite-associated Xylaria. *Ophiocordyceps sphecocephala* was used as the outgroup.

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Appendix I

List of Xylariaceae cultures

No.	Original code	Name	Substrate	Collecting date	Isolating date	Area	Site	Province
1	Xy 00402	*Hypoxylon comedens*	Wood	5 Sep. 2006	5 Sep. 2006	Pun Churee Study Trail	Doi Inthanon National Park	Chiang Mai
2	Xy 00403	*Hypoxylon comedens*	Wood	5 Sep. 2006	5 Sep. 2006	Pun Churee Study Trail	Doi Inthanon National Park	Chiang Mai
3	Xy 00404	*Xylaria* sp.	Wood	5 Sep. 2006	5 Sep. 2006	Pun Churee Study Trail	Doi Inthanon National Park	Chiang Mai
4	Xy 00406	*Xylaria* sp.	Wood	5 Sep. 2006	5 Sep. 2006	Pun Churee Study Trail	Doi Inthanon National Park	Chiang Mai
5	Xy 00407	*Xylaria* sp.	Wood	5 Sep. 2006	5 Sep. 2006	Pun Churee Study Trail	Doi Inthanon National Park	Chiang Mai
6	Xy 00409	*Xylaria* sp.	Wood	5 Sep. 2006	5 Sep. 2006	Pun Churee Study Trail	Doi Inthanon National Park	Chiang Mai
7	Xy 00411	*Xylaria* sp.	Wood	5 Sep. 2006	5 Sep. 2006	Pun Churee Study Trail	Doi Inthanon National Park	Chiang Mai
8	Xy 00413	*Xylaria* sp.	Seed	5 Sep. 2006	6 Sep. 2006	Pun Churee Study Trail	Doi Inthanon National Park	Chiang Mai
9	Xy 00415	*Xylaria* sp.	Seed	5 Sep. 2006	6 Sep. 2006	Pun Churee Study Trail	Doi Inthanon National Park	Chiang Mai
10	Xy 00417	*Xylaria* sp.	Seed	5 Sep. 2006	6 Sep. 2006	Pun Churee Study Trail	Doi Inthanon National Park	Chiang Mai

List of Xylariaceae cultures (continued)

11	Xy 00419	*Hypoxylon* sp.	Wood	7 Sep. 2006	7 Sep. 2006	Doi Hua Suea	Doi Inthanon National Park	Chiang Mai
12	Xy 00421	*Xylaria* sp.	Wood	6 Sep. 2006	6 Sep. 2006	Phadogsiaw Waterfall km. 30	Doi Inthanon National Park	Chiang Mai
13	Xy 00423	*Xylaria* sp.	Seed	6 Sep. 2006	6 Sep. 2006	Phadogsiaw Waterfall km. 30	Doi Inthanon National Park	Chiang Mai
14	Xy 00452	*Xylaria* sp.	Wood	5 Sep. 2006	5 Sep. 2006	Pun Churee Study Trail	Doi Inthanon National Park	Chiang Mai
15	Xy 00453	*Xylaria* sp.	Bamboo	6 Sep. 2006	6 Sep. 2006	Phadogsiaw Waterfall km. 30	Doi Inthanon National Park	Chiang Mai
16	Xy 00455	*Xylaria grammica*	Wood	13 Nov. 2006	13 Nov. 2006	Thungsalang Luang Nature Trail	Thungsalang Luang National Park	Phitsanulok
17	Xy 00456	*Hypoxylon* cf. *crocopeplum*	Wood	13 Nov. 2006	13 Nov. 2006	Thungsalang Luang Nature Trail	Thungsalang Luang National Park	Phitsanulok
18	Xy 00457	*Hypoxylon* sp.	Wood	13 Nov. 2006	13 Nov. 2006	Thungsalang Luang Nature Trail	Thungsalang Luang National Park	Phitsanulok
19	Xy 00458	*Hypoxylon* sp.	Wood	13 Nov. 2006	13 Nov. 2006	Thungsalang Luang Nature Trail	Thungsalang Luang National Park	Phitsanulok
20	Xy 00459	*Hypoxylon* cf. *nitens*	Wood	13 Nov. 2006	13 Nov. 2006	Thungsalang Luang Nature Trail	Thungsalang Luang National Park	Phitsanulok
21	Xy 00460	*Hypoxylon stygium*	Wood	13 Nov. 2006	13 Nov. 2006	Thungsalang Luang Nature Trail	Thungsalang Luang National Park	Phitsanulok
22	Xy 00461	*Hypoxylon stygium*	Wood	13 Nov. 2006	13 Nov. 2006	Thungsalang Luang Nature Trail	Thungsalang Luang National Park	Phitsanulok

List of Xylariaceae cultures (continued)

23	Xy 00462	*Hypoxylon* sp.	Wood	13 Nov. 2006	13 Nov. 2006	Thungsalang Luang Nature Trail	Thungsalang Luang National Park	Phitsanulok
24	Xy 00464	*Xylaria* cf. *bambusicola*	Bamboo	26 Sep. 2006	26 Sep. 2006	Chatchanat Farm	Ampure Phu Ruea	Loei
25	Xy 00465	*Xylaria* cf. *bambusicola*	Bamboo	26 Sep. 2006	26 Sep. 2006	Chatchanat Farm	Ampure Phu Ruea	Loei
26	Xy 00466	*Xylaria escharoidea*	Soil (termite nest)	27 Sep. 2006	27 Sep. 2006	Chatchanat Farm	Ampure Phu Ruea	Loei
27	Xy 00467	*Xylaria allantoidea*	Wood	14 Nov. 2006	14 Nov. 2006	Chattrakan Waterfall	Namtok Chattrakan National Park	Phitsanulok
28	Xy 00468	*Hypoxylon haematostroma*	Wood	14 Nov. 2006	14 Nov. 2006	Chattrakan Waterfall	Namtok Chattrakan National Park	Phitsanulok
29	Xy 00469	*Xylaria ianthino-velutina*	Wood	14 Nov. 2006	14 Nov. 2006	Chattrakan Waterfall	Namtok Chattrakan National Park	Phitsanulok
30	Xy 00471	*Xylaria escharoidea*	Soil (termite nest)	7 Nov. 2006	8 Nov. 2006	Gibbon Trail	Khao Yai National Park	Nakhon Ratchasima
31	Xy 00472	*Hypoxylon* sp.	Wood	15 Nov. 2006	15 Nov. 2006	Lan Hin Pum	Phu Hin Rong Kla National Park	Phitsanulok
32	Xy 00473	*Biscogniauxia* sp.	Wood	15 Nov. 2006	15 Nov. 2006	Lan Hin Pum	Phu Hin Rong Kla National Park	Phitsanulok
33	Xy 00474	*Xylaria allantoidea*	Wood	15 Nov. 2006	15 Nov. 2006	Phu Hin Rong Kla Km.35	Phu Hin Rong Kla National Park	Phitsanulok
34	Xy 00475	*Hypoxylon* sp.	Wood	15 Nov. 2006	15 Nov. 2006	Phu Hin Rong Kla Km.35	Phu Hin Rong Kla National Park	Phitsanulok

List of Xylariaceae cultures (continued)

35	Xy 00476	*Xylaria obovata*	Wood	16 Nov. 2006	16 Nov. 2006	School of Military Politic	Phu Hin Rong Kla National Park	Phitsanulok
36	Xy 00480	*Hypoxylon* sp.	Wood	16 Nov. 2006	16 Nov. 2006	School of Military Politic	Phu Hin Rong Kla National Park	Phitsanulok
37	Xy 00483	*Biscogniauxia citriforme var.macrospora*	Wood	16 Nov. 2006	16 Nov. 2006	School of Military Politic	Phu Hin Rong Kla National Park	Phitsanulok
38	Xy 00484	*Annulohypoxylon* cf. *bovei var. microspora*	Wood	16 Nov. 2006	16 Nov. 2006	School of Military Politic	Phu Hin Rong Kla National Park	Phitsanulok
39	Xy 00485	*Biscogniauxia* sp.	Wood	16 Nov. 2006	16 Nov. 2006	School of Military Politic	Phu Hin Rong Kla National Park	Phitsanulok
40	Xy 00486	*Biscogniauxia* sp.	Wood	16 Nov. 2006	16 Nov. 2006	School of Military Politic	Phu Hin Rong Kla National Park	Phitsanulok
41	Xy 00487	*Hypoxylon* sp.	Wood	15 Nov. 2006	15 Nov. 2006	Saphan Morana	Phu Hin Rong Kla National Park	Phitsanulok
42	Xy 00488	*Hypoxylon* sp.	Wood	15 Nov. 2006	15 Nov. 2006	Saphan Morana	Phu Hin Rong Kla National Park	Phitsanulok
43	Xy 00489	*Biscogniauxia* sp.	Wood	15 Nov. 2006	15 Nov. 2006	Saphan Morana	Phu Hin Rong Kla National Park	Phitsanulok
44	Xy 00490	*Hypoxylon macrocarpum*	Wood	15 Nov. 2006	15 Nov. 2006	Saphan Morana	Phu Hin Rong Kla National Park	Phitsanulok
45	Xy 00493	*Xylaria* cf. *nigripes*	Soil (termite nest)	26 Sep. 2006	26 Sep. 2006	Chatchanat Farm	Ampure Phu Ruea	Loei
46	Xy 00494	*Hypoxylon* cf. *perforatum*	Wood	26 Sep. 2006	26 Sep. 2006	Chatchanat Farm	Ampure Phu Ruea	Loei

List of Xylariaceae cultures (continued)

47	Xy 00495	*Xylaria* sp.	Wood	26 Sep. 2006	26 Sep. 2006	Chatchanat Farm	Ampure Phu Ruea	Loei
48	Xy 00496	*Xylaria bambusicola*	Bamboo	26 Sep. 2006	26 Sep. 2006	Chatchanat Farm	Ampure Phu Ruea	Loei
49	Xy 00497	*Xylaria* cf. *feejeensis*	Wood	26 Sep. 2006	26 Sep. 2006	Chatchanat Farm	Ampure Phu Ruea	Loei
50	Xy 00498	*Xylaria* sp.	Soil (termite nest)	26 Sep. 2006	26 Sep. 2006	Chatchanat Farm	Ampure Phu Ruea	Loei
51	Xy 00499	*Xylaria* sp.	Wood	26 Sep. 2006	26 Sep. 2006	Chatchanat Farm	Ampure Phu Ruea	Loei
52	Xy 00500	*Xylaria* cf. *multiplex*	Wood	26 Sep. 2006	26 Sep. 2006	Chatchanat Farm	Ampure Phu Ruea	Loei
53	Xy 00501	*Xylaria* sp.	Wood	27 Sep. 2006	27 Sep. 2006	Trail to Kokkaba	Phu Luang Wildlife Sanctuary	Loei
54	Xy 00502	*Xylaria* sp.	Wood	27 Sep. 2006	27 Sep. 2006	Trail to Kokkaba	Phu Luang Wildlife Sanctuary	Loei
55	Xy 00503	*Nemania diffusa*	Wood	15 Nov. 2006	15 Nov. 2006	Phu Hin Rong Kla Km.35	Phu Hin Rong Kla National Park	Phitsanulok
56	Xy 00505	*Xylaria* sp.	Wood	15 Nov. 2006	15 Nov. 2006	Phu Hin Rong Kla Km.35	Phu Hin Rong Kla National Park	Phitsanulok
57	Xy 00506	*Xylaria* sp.	Wood	16 Nov. 2006	16 Nov. 2006	School of Military Politic	Phu Hin Rong Kla National Park	Phitsanulok
58	Xy 00507	*Nemania* sp.	Wood	16 Nov. 2006	16 Nov. 2006	School of Military Politic	Phu Hin Rong Kla National Park	Phitsanulok

List of Xylariaceae cultures (continued)

59	Xy 00508	*Nemania diffusa*	Wood	16 Nov. 2006	16 Nov. 2006	School of Military Politic	Phu Hin Rong Kla National Park	Phitsanulok
60	Xy 00509	*Hypoxylon* cf. *monticulosum*	Wood	16 Nov. 2006	16 Nov. 2006	School of Military Politic	Phu Hin Rong Kla National Park	Phitsanulok
61	Xy 00510	*Hypoxylon* sp.	Wood	8 Nov. 2006	8 Nov. 2006	Khao Sam Lan Waterfall	Khao Sam Lan National Park	Saraburi
62	Xy 00511	*Hypoxylon monticulosum*	Wood	15 Nov. 2006	15 Nov. 2006	Saphan Morana	Phu Hin Rong Kla National Park	Phitsanulok
63	Xy 00512	*Hypoxylon* sp.	Wood	13 Nov. 2006	13 Nov. 2006	Thungsalang Luang Nature Trail	Thungsalang Luang National Park	Phitsanulok
64	Xy 00513	*Hypoxylon comedens*	Wood	20 Feb. 2007	20 Feb. 2007	Sunanta Waterfall	Khao Nan National Park	Nakhon Si Thammarat
65	Xy 00514	*Hypoxylon* sp.	Wood	20 Feb. 2007	20 Feb. 2007	Sunanta Waterfall	Khao Nan National Park	Nakhon Si Thammarat
66	Xy 00515	*Hypoxylon* sp.	Wood	20 Feb. 2007	20 Feb. 2007	Sunanta Waterfall	Khao Nan National Park	Nakhon Si Thammarat
67	Xy 00516	*Hypoxylon* sp.	Wood	20 Feb. 2007	20 Feb. 2007	Sunanta Waterfall	Khao Nan National Park	Nakhon Si Thammarat
68	Xy 00517	*Kretzschmaria* sp.	Wood	20 Feb. 2007	20 Feb. 2007	Sunanta Waterfall	Khao Nan National Park	Nakhon Si Thammarat
69	Xy 00518	*Hypoxylon* sp.	Wood	20 Feb. 2007	20 Feb. 2007	Sunanta Waterfall	Khao Nan National Park	Nakhon Si Thammarat
70	Xy 00519	*Xylaria* sp.	Wood	20 Feb. 2007	20 Feb. 2007	Si Khit Waterfall	Namtok Si Khit National Park	Nakhon Si Thammarat

List of Xylariaceae cultures (continued)

71	Xy 00520	*Daldinia eschscholzii*	Wood	20 Feb. 2007	20 Feb. 2007	Si Khit Waterfall	Namtok Si Khit National Park	Nakhon Si Thammarat
72	Xy 00521	*Xylaria allantoidea*	Wood	20 Feb. 2007	20 Feb. 2007	Si Khit Waterfall	Namtok Si Khit National Park	Nakhon Si Thammarat
73	Xy 00522	*Xylaria* cf. *obovata*	Wood	20 Feb. 2007	20 Feb. 2007	Si Khit Waterfall	Namtok Si Khit National Park	Nakhon Si Thammarat
74	Xy 00523	*Xylaria grammica*	Wood	20 Feb. 2007	20 Feb. 2007	Si Khit Waterfall	Namtok Si Khit National Park	Nakhon Si Thammarat
75	Xy 00524	*Xylaria allantoidea*	Wood	20 Feb. 2007	20 Feb. 2007	Si Khit Waterfall	Namtok Si Khit National Park	Nakhon Si Thammarat
76	Xy 00525	*Xylaria allantoidea*	Wood	20 Feb. 2007	20 Feb. 2007	Si Khit Waterfall	Namtok Si Khit National Park	Nakhon Si Thammarat
77	Xy 00526	*Xylaria* sp.	Soil (termite nest)	21 Feb. 2007	21 Feb. 2007	Karome Waterfall	Khao Luang National Park	Nakhon Si Thammarat
78	Xy 00527	*Daldinia eschscholzii*	Wood	21 Feb. 2007	21 Feb. 2007	Karome Waterfall	Khao Luang National Park	Nakhon Si Thammarat
79	Xy 00528	*Hypoxylon* sp.	Wood	21 Feb. 2007	21 Feb. 2007	Ai Khieo Waterfall	Khao Luang National Park	Nakhon Si Thammarat
80	Xy 00529	*Hypoxylon* sp.	Wood	21 Feb. 2007	21 Feb. 2007	Ai Khieo Waterfall	Khao Luang National Park	Nakhon Si Thammarat
81	Xy 00530	*Xylaria* sp.	Wood	21 Feb. 2007	21 Feb. 2007	Ai Khieo Waterfall	Khao Luang National Park	Nakhon Si Thammarat
82	Xy 00531	*Hypoxylon comedens*	Wood	20 Feb. 2007	20 Feb. 2007	Sunanta Waterfall	Khao Nan National Park	Nakhon Si Thammarat

List of Xylariaceae cultures (continued)

83	Xy 00532	*Xylaria* sp.	Soil (termite nest)	21 Feb. 2007	21 Feb. 2007	Karome Waterfall	Khao Luang National Park	Nakhon Si Thammarat
84	Xy 00533	*Xylaria* sp.	Wood	17 Mar. 2007	19 Mar. 2007	Khao Jed Yod	Khao Bun Tad Wildlife Sanctuary	Phatthalung
85	Xy 00534	*Hypoxylon comedens*	Wood	17 Mar. 2007	19 Mar. 2007	Khao Jed Yod	Khao Bun Tad Wildlife Sanctuary	Phatthalung
86	Xy 00536	*Xylaria* sp.	Wood	19 Mar. 2007	19 Mar. 2007	Khao Jed Yod	Khao Bun Tad Wildlife Sanctuary	Phatthalung
87	Xy 00537	*Hypoxylon* sp.	Wood	18 Mar. 2007	19 Mar. 2007	Khao Jed Yod	Khao Bun Tad Wildlife Sanctuary	Phatthalung
88	Xy 00538	*Xylaria telfairii*	Wood	18 Mar. 2007	19 Mar. 2007	Khao Jed Yod	Khao Bun Tad Wildlife Sanctuary	Phatthalung
89	Xy 00539	*Xylaria telfairii*	Wood	18 Mar. 2007	19 Mar. 2007	Khao Jed Yod	Khao Bun Tad Wildlife Sanctuary	Phatthalung
90	Xy 00540	*Xylaria* sp.	Wood	18 Mar. 2007	19 Mar. 2007	Khao Jed Yod	Khao Bun Tad Wildlife Sanctuary	Phatthalung
91	Xy 00541	*Xylaria* sp.	Wood	18 Mar. 2007	19 Mar. 2007	Khao Jed Yod	Khao Bun Tad Wildlife Sanctuary	Phatthalung
92	Xy 00542	*Xylaria* sp.	Wood	18 Mar. 2007	19 Mar. 2007	Khao Jed Yod	Khao Bun Tad Wildlife Sanctuary	Phatthalung
93	Xy 00547	*Sarcoxylon* sp.	Wood	11 Jul. 2007	11 Jul. 2007	Pha Ka Jai	Khao Yai National Park	Nakhon Ratchasima
94	Xy 00548	*Xylaria* sp.	Wood	11 Jul. 2007	11 Jul. 2007	Pha Ka Jai	Khao Yai National Park	Nakhon Ratchasima

List of Xylariaceae cultures (continued)

95	Xy 00549	*Xylaria allantoidea*	Wood	11 Jul. 2007	11 Jul. 2007	Pha Ka Jai	Khao Yai National Park	Nakhon Ratchasima
96	Xy 00550	*Xylaria* cf. *nigripes*	Soil (termite nest)	11 Jul. 2007	11 Jul. 2007	Pha Ka Jai	Khao Yai National Park	Nakhon Ratchasima
97	Xy 00551	*Xylaria* cf. *luteostromata*	Wood	11 Jul. 2007	11 Jul. 2007	Pha Ka Jai	Khao Yai National Park	Nakhon Ratchasima
98	Xy 00552	*Hypoxylon subgilvum*	Wood	11 Jul. 2007	11 Jul. 2007	Pha Ka Jai	Khao Yai National Park	Nakhon Ratchasima
99	Xy 00553	*Xylaria* cf. *nigripes*	Soil (termite nest)	11 Jul. 2007	11 Jul. 2007	Pha Ka Jai	Khao Yai National Park	Nakhon Ratchasima
100	Xy 00554	*Xylaria meliacearum*	Wood	26 Jun. 2007	26 Jun. 2007	Ban Krang	Kaeng Krachan National Park	Phetchaburi
101	Xy 00555	*Kretzschmaria* sp.	Wood	26 Jun. 2007	26 Jun. 2007	Ban Krang	Kaeng Krachan National Park	Phetchaburi
102	Xy 00556	*Hypoxylon* sp.	Wood	26 Jun. 2007	26 Jun. 2007	Ban Krang	Kaeng Krachan National Park	Phetchaburi
103	Xy 00557	*Hypoxylon* sp.	Wood	26 Jun. 2007	26 Jun. 2007	Ban Krang	Kaeng Krachan National Park	Phetchaburi
104	Xy 00558	*Xylaria obovata*	Wood	26 Jun. 2007	26 Jun. 2007	Ban Krang	Kaeng Krachan National Park	Phetchaburi
105	Xy 00559	*Xylaria* sp.	Wood	26 Jun. 2007	26 Jun. 2007	Ban Krang	Kaeng Krachan National Park	Phetchaburi
106	Xy 00560	*Xylaria badia*	Wood	26 Jun. 2007	26 Jun. 2007	Ban Krang	Kaeng Krachan National Park	Phetchaburi

List of Xylariaceae cultures (continued)

107	Xy 00561	*Xylaria* cf. *cubensis*	Wood	26 Jun. 2007	26 Jun. 2007	Ban Krang	Kaeng Krachan National Park	Phetchaburi
108	Xy 00562	*Xylaria cubensis*	Wood	26 Jun. 2007	26 Jun. 2007	Ban Krang	Kaeng Krachan National Park	Phetchaburi
109	Xy 00563	*Xylaria* sp.	Wood	26 Jun. 2007	26 Jun. 2007	Ban Krang	Kaeng Krachan National Park	Phetchaburi
110	Xy 00564	*Xylaria* sp.	Wood	26 Jun. 2007	26 Jun. 2007	Ban Krang	Kaeng Krachan National Park	Phetchaburi
111	Xy 00565	*Xylaria* sp.	Wood	26 Jun. 2007	26 Jun. 2007	Ban Krang	Kaeng Krachan National Park	Phetchaburi
112	Xy 00566	*Xylaria badia*	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
113	Xy 00567	*Xylaria obovata*	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
114	Xy 00568	*Xylaria allantoidea*	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
115	Xy 00569	*Xylaria badia*	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
116	Xy 00570	*Xylaria hyperythra*	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
117	Xy 00571	*Xylaria* sp.	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
118	Xy 00572	*Xylaria* cf. *heliscus*	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima

List of Xylariaceae cultures (continued)

119	Xy 00573	*Xylaria* cf. *heliscus*	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
120	Xy 00574	*Hypoxylon lenormandii*	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
121	Xy 00575	*Nemania* sp.	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
122	Xy 00576	*Nemania* sp.	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
123	Xy 00577	*Nemania* sp.	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
124	Xy 00578	*Xylaria* sp.	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
125	Xy 00579	*Hypoxylon fendleri*	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
126	Xy 00580	*Whalleya microplaca*	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
127	Xy 00581	*Nemania diffusa*	Wood	10 Jul. 2007	10 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
128	Xy 00582	*Xylaria* sp.	Wood	18 Mar. 2007	19 Mar. 2007	Khao Jed Yod	Khao Bun Tad Wildlife Sanctuary	Phatthalung
129	Xy 00583	*Kretzschmaria* sp.	Wood	27 Jun. 2007	27 Jun. 2007	Paneontung	Kaeng Krachan National Park	Phetchaburi
130	Xy 00584	*Kretzschmaria* sp.	Wood	27 Jun. 2007	27 Jun. 2007	Paneontung	Kaeng Krachan National Park	Phetchaburi

List of Xylariaceae cultures (continued)

131	Xy 00585	*Xylaria* sp.	Soil (termite nest)	28 Jul. 2007	28 Jul. 2007	Pa La U Water fall	Kaeng Krachan National Park	Phetchaburi
132	Xy 00586	*Kretzschmaria* sp.	Wood	28 Jul. 2007	28 Jul. 2007	Pa La U Water fall	Kaeng Krachan National Park	Phetchaburi
133	Xy 00587	*Xylaria* cf. *allantoidea*	Wood	28 Jul. 2007	28 Jul. 2007	Pa La U Water fall	Kaeng Krachan National Park	Phetchaburi
134	Xy 00588	*Xylaria cubensis*	Wood	28 Jul. 2007	28 Jul. 2007	Pa La U Water fall	Kaeng Krachan National Park	Phetchaburi
135	Xy 00589	*Xylaria* sp.	Wood	28 Jul. 2007	28 Jul. 2007	Pa La U Water fall	Kaeng Krachan National Park	Phetchaburi
136	Xy 00590	*Xylaria globosa*	Wood	28 Jul. 2007	28 Jul. 2007	Pa La U Water fall	Kaeng Krachan National Park	Phetchaburi
137	Xy 00591	*Xylaria* sp.	Wood	11 Jul. 2007	11 Jul. 2007	Pha Ka Jai	Khao Yai National Park	Nakhon Ratchasima
138	Xy 00592	*Xylaria fockei*	Wood	11 Jul. 2007	11 Jul. 2007	Pha Ka Jai	Khao Yai National Park	Nakhon Ratchasima
139	Xy 00593	*Xylaria telfairii*	Wood	11 Jul. 2007	11 Jul. 2007	Pha Ka Jai	Khao Yai National Park	Nakhon Ratchasima
140	Xy 00594	*Xylaria hyperytha*	Wood	11 Jul. 2007	11 Jul. 2007	Pha Ka Jai	Khao Yai National Park	Nakhon Ratchasima
141	Xy 00595	*Xylaria escharoidea*	Soil (termite nest)	20 Jun. 2007	20 Jun. 2007	Garden	Ampure Bueng Kum	Bangkok
142	Xy 00596	*Xylaria* sp.	Wood	7 Jun. 2007	7 Jun. 2007	Nam Nao Study trail	Nam Nao National Park	Phetchabun

List of Xylariaceae cultures (continued)

143	Xy 00597	*Xylaria* sp.	Wood	7 Jun. 2007	7 Jun. 2007	Nam Nao Study trail	Nam Nao National Park	Phetchabun
144	Xy 00598	*Xylaria* sp.	Wood	7 Jun. 2007	7 Jun. 2007	Nam Nao Study trail	Nam Nao National Park	Phetchabun
145	Xy 00599	*Xylaria* sp.	Wood	8 Jun. 2007	8 Jun. 2007	Nam Nao Study trail	Nam Nao National Park	Phetchabun
146	Xy 00600	*Xylaria* sp.	Soil (termite nest)	8 Jun. 2007	8 Jun. 2007	Nam Nao Study trail	Nam Nao National Park	Phetchabun
147	Xy 00612	*Xylaria grammica*	Wood	23 Aug. 2007	23 Aug. 2007	Study Trail	Khlong Lan National Park	Kamphaeng Phet
148	Xy 00613	*Xylaria* sp.	Wood	24 Aug. 2007	24 Aug. 2007	Study Trail	Khlong Lan National Park	Kamphaeng Phet
149	Xy 00614	*Xylaria* sp.	Wood	24 Aug. 2007	24 Aug. 2007	Study Trail	Khlong Lan National Park	Kamphaeng Phet
150	Xy 00615	*Xylaria cubensis*	Wood	24 Aug. 2007	24 Aug. 2007	Study Trail	Khlong Lan National Park	Kamphaeng Phet
151	Xy 00616	*Daldinia eschscholzii*	Wood	24 Aug. 2007	24 Aug. 2007	Study Trail	Khlong Lan National Park	Kamphaeng Phet
152	Xy 00617	*Xylaria* sp.	Soil (termite nest)	23 May 2007	23 May 2007	Study Trail	Mae Ka Sa National Park	Tak
153	Xy 00618	*Xylaria* sp.	Wood	24 Aug. 2007	24 Aug. 2007	Study Trail	Mae Wong National Park	Kamphaeng Phet
154	Xy 00619	*Hypoxylon comedens*	Wood	28 May 2007	28 May 2007	Pun Churee Study Trail	Doi Inthanon National Park	Chiang Mai

List of Xylariaceae cultures (continued)

155	Xy 00620	*Hypoxylon comedens*	Wood	26 Jun. 2007	26 Jun. 2007	Ban Krang	Kaeng Krachan National Park	Petchaburee
156	Xy 00628	*Xylaria* sp.	Wood	23 Aug. 2007	23 Aug. 2007	Study Trail	Khlong Lan National Park	Kamphaeng Phet
157	Xy 00629	*Xylaria* sp.	Wood	23 Aug. 2007	23 Aug. 2007	Study Trail	Khlong Lan National Park	Kamphaeng Phet
158	Xy 00630	*Xylaria grammica*	Wood	13 Jul. 2007	13 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
159	Xy 00631	*Xylaria* cf. *allantoidea*	Wood	13 Jul. 2007	13 Jul. 2007	KM 33	Khao Yai National Park	Nakhon Ratchasima
160	Xy 00632	*Xylaria globosa*	Wood	24 Aug. 2007	24 Aug. 2007	KM 35	Khao Yai National Park	Nakhon Ratchasima
161	Xy 00636	*Hypoxylon comedens*	Wood	28 May 2007	22 Jun. 2007	Pun Churee Study Trail	Doi Inthanon National Park	Chiang Mai
162	Xy 00637	*Xylaria* sp.	Soil (termite nest)	28 Jun. 2007	28 Jun. 2007	Pa La U Waterfall	Kaeng Krachan National Park	Phetchaburi
163	Xy 00638	*Hypoxylon comedens*	Wood	23 Jun. 2007	23 Jun. 2007	Paneontung	Kaeng Krachan National Park	Phetchaburi
164	Xy 00641	*Xylaria* sp.	Wood	24 Aug. 2007	24 Aug. 2007	KM 35	Khao Yai National Park	Nakhon Ratchasima

Appendix II

Presentation

Project "A study on the termite-associated *Xylaria* in Thailand" was presented by abstract and poster.

1. ประเสริฐ ศรีกิติกุลชัย อาทิตย์ คนสนิท โทมัส เลสโซล และ เจนนิเฟอร์ เหลืองสะอาด, 2550, การศึกษาเบื้องต้นของราไซลาเรียที่มีความสัมพันธ์กับดินปลวกในประเทศไทย, การประชุมวิชาการประจำปีโครงการ BRT ครั้งที่ 11, 15-18 ตุลาคม 2550, อุดรธานี, ประเทศไทย.

ผลงานเผยแพร่สู่สาธารณะ

เรื่อง "เห็ดมูลช้าง หรือ *Poronia gigantea*" ส่งโครงการ BRT พร้อม รายงาน 6 เดือน

Poster presentation in the BRT annual meeting

การศึกษาเบื้องต้นของราไซลาเรีย ที่มีความสัมพันธ์กับดินปลวกในประเทศไทย

ประเสริฐ ศรีกิติกุลชัย[1] อาทิตย์ คนสนิท[1] โทมัส เลสโซล[2] และ เจนนิเฟอร์ เหลืองสอาด[1]

[1] ศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ (ไบโอเทค) 113 อุทยานวิทยาศาสตร์ประเทศไทย ถ.พหลโยธิน ต.คลองหนึ่ง อ.คลองหลวง จ.ปทุมธานี 12120 โทร. 02-5646700 ต่อ3525 e-mail : prasert@biotec.or.th

[2] ภาควิชาราวิทยา มหาวิทยาลัยโคเปนเฮเกน ประเทศเดนมาร์ก

กิตติกรรมประกาศ

"ผลงานวิจัยนี้ได้รับทุนสนับสนุนจากโครงการพัฒนาองค์ความรู้และศึกษานโยบายการจัดการทรัพยากรชีวภาพในประเทศไทย ซึ่งร่วมจัดตั้งโดยสำนักงานกองทุนสนับสนุนการวิจัยและศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ รหัสโครงการ BRT R_25002"

บทนำ

ป่าไม้ในประเทศไทยเป็นแหล่งที่มีความอุดมสมบูรณ์ซึ่งสามารถพบความหลากหลายของสิ่งมีชีวิตอยู่เป็นจำนวนมาก โดยเฉพาะอย่างยิ่งจุลินทรีย์

ราไซลาเรียเป็นราที่พบได้ทั่วไปในพื้นที่ป่าไม้ของประเทศไทย รากลุ่มนี้โดยทั่วไปมักพบเจริญเติบโตอยู่บนซากไม้ เมล็ด ใบไม้ มูลสัตว์ และบนพื้นดิน โดยเฉพาะอย่างยิ่งราไซลาเรียที่เจริญอยู่บนพื้นดินซึ่งคาดว่าน่าจะมีความสัมพันธ์กับรังปลวก และเป็นกลุ่มที่มีการศึกษาอยู่น้อยมากดังนั้นงานวิจัยชิ้นนี้จึงจัดทำขึ้นเพื่อศึกษาความสัมพันธ์ของราในกลุ่มนี้

วัตถุประสงค์

1. เพื่อสำรวจและเก็บรวบรวมราวงศ์ Xylariaceae เพื่อการศึกษาวิจัยและการใช้ประโยชน์
2. เพื่อศึกษาความสัมพันธ์ของราไซลาเรียที่เจริญบนดินปลวก

วิธีการทดลอง

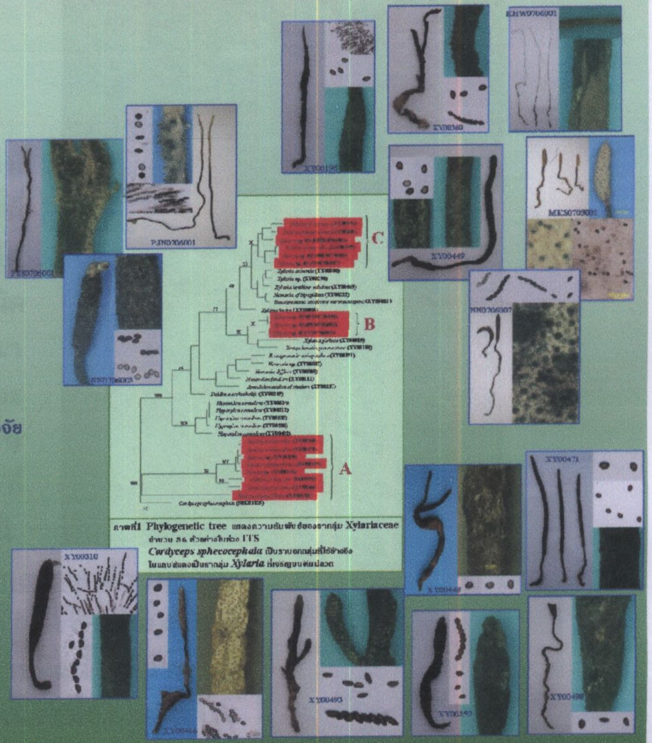
1. สำรวจและเก็บรวบรวมตัวอย่างราวงศ์ Xylariaceae ที่เจริญบน Substrates ต่าง ๆตัดแยกจนได้เชื้อบริสุทธิ์
2. ศึกษาความสัมพันธ์ของราโดยการสกัด DNA จากเชื้อบริสุทธิ์หรือจากตัวอย่างราไซลาเรียที่เก็บได้

ตารางที่ 1 แสดงรายชื่อราทั้ง 36 ตัวอย่างที่นำมาสกัด DNA เพื่อทำ Sequencing

Scientific Name	Code	substrate
Xylaria sp.	KHW0706001	[illegible]
Xylaria sp.	MKS0708001	ดินปลวก
Xylaria sp.	NND706007	[illegible]
Xylaria sp.	RND706002	[illegible]
Xylaria sp.	NND706007	[illegible]
Xylaria sp.	PJN0706001	[illegible]
Xylaria sp.	PSS0706001	ดินปลวก
Xylaria [illegible]	XY00080	ท่อนไม้
Xylaria globosa	XY00089	ท่อนไม้
Xylaria [illegible]	XY00098	ท่อนไม้
Hypoxylon [illegible]	XY00111	ท่อนไม้
Kretzschmaria pavimentosa	XY00180	ท่อนไม้
Biscogniauxia [illegible]	XY00191	ท่อนไม้
Xylaria cf. nigripes	XY00196	ดินปลวก
Hypoxylon [illegible]	XY00213	ท่อนไม้
Daldinia [illegible]	XY00210	ท่อนไม้
Annulohypoxylon cf. stygium	XY00217	ท่อนไม้
Nemania cf. bipapillata	XY00230	ท่อนไม้
Hypoxylon [illegible]	XY00240	ท่อนไม้
Xylaria sp.	XY00242	ท่อนไม้
Xylaria cf. piperiformis	XY00310	ดินปลวก
Xylaria cf. piperiformis	XY00311	[illegible]
Xylaria sp.	XY00330	ดินปลวก
Xylaria cf. piperiformis	XY00399	[illegible]
Xylaria cf. piperiformis	XY00360	ดินปลวก
Hypoxylon [illegible]	XY00402	ท่อนไม้
Xylaria escharoidea	XY00416	ดินปลวก
Xylaria [illegible]	XY00449	ดินปลวก
Xylaria [illegible]	XY00466	[illegible]
Xylaria [illegible]	XY00469	ฝัก เมล็ดพืช
Xylaria [illegible]	XY00471	[illegible]
Biscogniauxia [illegible] var microspora	XY00488	ท่อนไม้
Xylaria cf. nigripes	XY00493	[illegible]
Xylaria sp.	XY00498	ดินปลวก
Nemania sp.	XY00507	ท่อนไม้
Nemania [illegible]	XY00509	ท่อนไม้
Hypoxylon [illegible]	XY00534	ท่อนไม้
Hypoxylon [illegible]	XY00536	ท่อนไม้
Cordyceps sphecocephala	NBU12138	แมลง

Procedures to determine ITS rDNA sequence

- DNA extraction
- PCR amplification of ITS rDNA
- Agarose gel eletrophoresis
- Purification of PCR products
- Sequencing
- Data Analyzing



ภาพที่1 Phylogenetic tree แสดงความสัมพันธ์ของรากลุ่ม Xylariaceae จำนวน 36 ตัวอย่างในท่อน ITS *Cordyceps sphecocephala* เป็นรานอกกลุ่มที่ใช้อ้างอิง ในแถบสีแดงเป็นรากลุ่ม *Xylaria* ที่เจริญบนดินปลวก

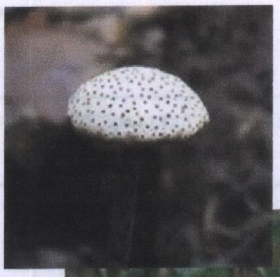
ผลการทดลอง

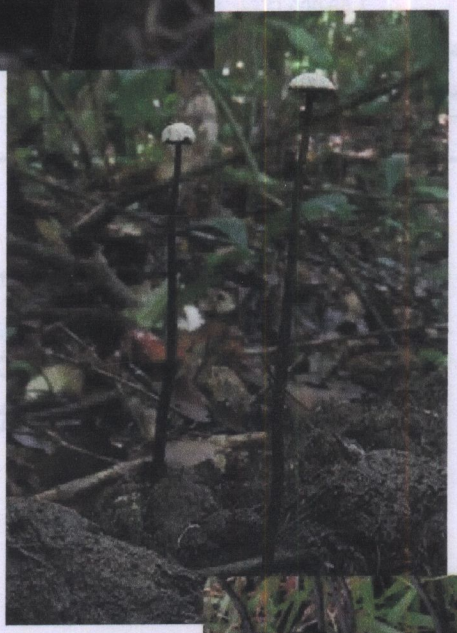
1. สำรวจพบราในวงศ์ Xylariaceae 8 สกุลคือ Annulohypoxylon, Biscogniauxia, Daldinia, Hypoxylon, Kretzschmaria, Nemania, Rhopalostroma และ Xylaria
2. ความสัมพันธ์ของราไซลาเรียที่เจริญบนดินปลวก สามารถจำแนกได้เป็น 3 กลุ่มคือ กลุ่ม A, B และ C ซึ่งจะเห็นว่าวิวัฒนาการของราในกลุ่มนี้อาจจะเริ่มจาก กลุ่ม A ซึ่งเจริญบนดินก่อนและมีการปรับตัวเพื่อให้สามารถเจริญบน substrate อื่น ๆได้ดังจะเห็นได้จากกลุ่ม B ซึ่งสามารถพบวิวัฒนาการร่วมกัน และหลังจากนั้นจะมีการปรับตัวเพื่อกลับมาเจริญเติบโตบนดินอีกครั้งหนึ่งดังจะเห็นได้จากกลุ่ม C ซึ่งมีวิวัฒนาการแยกออกมาจาก substrate อื่นอีกครั้งหนึ่ง

สรุปผลการทดลอง

การสำรวจและเก็บรวบรวมตัวอย่างราวงศ์ Xylariaceae นั้นจะทำให้พบความหลากหลายทางชีวภาพของราในสกุลนี้เพิ่มมากขึ้น ซึ่งจะเป็นประโยชน์อย่างมากต่อการศึกษาวิจัยความหลากหลายทางชีวภาพและวิวัฒนาการของราตลอดจนถึงและการนำไปใช้ประโยชน์ในด้านต่าง ๆอีกด้วย

เห็ดมูลช้าง หรือ *"Poronia gigantea"*





Order : ***Xylariales***

Family : ***Xylariaceae***

Genus : ***Poronia***

ตลอดช่วงฤดูฝนในป่าฝนเขตร้อนอย่างประเทศไทย มักพบราชนิดหนึ่งที่เจริญเติบโตอยู่ตามมูลช้าง ที่เรียกว่า "เห็ดมูลช้าง" หรือ "***Poronia gigantea***" ซึ่งราชนิดนี้จะมีก้านยาวประมาณ 15 เซนติเมตร หนาประมาณ 0.3-0.5 เซนติเมตร โดยส่วนใหญ่จะพบในขณะที่เป็นดอกอ่อนซึ่งมีลักษณะเป็นกระเปาะสีขาวที่ส่วนบนของดอกเป็นที่กำเนิดสปอร์แบบไม่อาศัยเพศที่เรียกว่าโคนิเดีย (conidia) มีลักษณะเป็นสปอร์ขนาดเล็กสีขาวคล้ายผงแป้ง (pruinose) แต่เมื่อเจริญเต็มที่จะเปลี่ยนเป็นอับสปอร์ (perithecia) ที่มีลักษณะเป็นจุดเล็ก ๆ สีดำ ราชนิดนี้จะมีก้านยาวเหนียวเป็นมันวาว สีดำสนิท และบริเวณโคนจะมีลักษณะเป็นก้อนกลมหนา (sclerotium) ซึ่งเป็นต้นกำเนิดเส้นใยที่สามารถหยุดการเจริญเติบโตได้ในช่วงที่มีสภาวะไม่เหมาะสมได้

ถ่ายภาพโดย : Dr.Thomas Læssøe
สถานที่ : อุทยานแห่งชาติเขาใหญ่
วันที่ : 11 กรกฎาคม 2550