12-Months Report

September 2005 – August 2006

Title: Comparative Fungal Diversity Studies on Palms in Thailand (BRT R_148008)

Principal investigator: Prof. E.B.G. Jones

Researcher assistants: Mr.Rattaket Choeyklin

Miss Aom Pinnoi

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COMPARATIVE FUNGAL DIVERSITY STUDIES ON PALMS IN THAILAND (BRT R_148008)

FINAL REPORT

Progress Report No 2: September 2005 – August 2006 (1 Year Report)

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Duration: One year (September 2005 – August 2006)

Date: 31st August, 2006.

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1. SUMMARY OF REPORT:

The biodiversity of fungi on selected palms: *Calamus* sp., *Licuala spinosa*, and *Elaeis guineensis* were studied. Six field collections were made for saprophytic fungi: September, October, and November 2005 and January, March and May 2006; and two for endophytic isolation experiments (November 2005 and May 2006). Saprophytic fungi on *Elaeis guineensis* a total 126 taxa: 28 Ascomycota, 28 anamorphic fungi and 62 Basidiomycota. *Annulatascus velatispora*, *Stilbohypoxylon* sp., *Falciformispora* sp. and *Vanakripa* sp., *Grammothele fuligo* and *Schizophjyllum commune* were common on this palm. On *Calamus* sp. a total of 90 taxa (216 records): 40 Ascomycota, 4 Basidiomycota and 46 anamorphic fungi were recorded. Samples were collected from 4 parts of the palm: with 61% of the fungi recorded from petioles; 38% from rachis and 1% from the trunk. Palm material collected from different habitats were also sampled: dry aerial material yielded 68.5% of the fungi and damp/moist material 31.5%. On this palm, a number of species: AOM 318, *Morenoina palmicola* and *Circinoconis paradoxa*., were common.

Endophytic fungi within petioles and leaves of the fan palm, *Licuala spinosa* from Khuan Khang Hotspring, Trang Province were sampled twice. Two thousand three hundred and forty one isolates were made. Cultures on PDA and CMA were examined periodically for reproductive structures and identified as they sporulated. Many cultures did not sporulate but their distinctive colony morphology and production of sterile stromata, suggested they were xylariaceous species, with 143 morpho types. Six

hundred and forty three axenic morpho strains were characterised and deposited in the BIOTEC Culture Collection (BCC).

2. OBJECTIVES OF THIS STUDY:

1). To undertake a comparative floristic study of saprobic and endophytic fungi on selected terrestrial palms, including the Basidiomycota.

2). To isolate the fungi identified into axenic culture and for deposition in the BIOTEC Culture Collection (BCC).

3). Molecular phylogeny of selected new fungi from peat swamp palms and Xylariaceous endophytic fungi from palms.

Duration: One year (September 2005 – August 2006)

3. MATERIAL AND METHODS

3.1. Location:

Material was collected from Thai Forests including: Khao Yai National Park, Nachon Ratcha Sima Province, Nan Province, Koh Chang National Park, Trat Province, Trang and Krabi Province.

3.2. Sample collection:

Six visits were made in September, October, and November 2005 and January, March and May 2006 to undertake an experimental of study of saprophytic and endophytic fungi on palm material.

3.2.1. Collection of Basidiomycota samples:

A sharp knife or machete was used to remove basidiocarps from substrata. All species were photographed and their color and habitat documented.

3.2.1.1. Specimens preservation

All specimens are oven dried at a temperature of 40-60 °C to kill insects and prevent further fungal growth. When specimens are dry, they are kept in sealed polythene bags, which are labeled with the number or original code given to the fungus,

locality, habitat and collection date. Then they are deposited in the BIOTEC Bangkok Herbarium (BBH).

3.3 Sampling Palm Material

Description of *Calamus* sp.

A robust, climbing palm. Climbing is aided by thorns on the rachides and petioles and spines on the young stems. Leaves are dark green, with numerous long segments. Plants also produce long, specialized, hook-bearing extensions of the rachis or flagella which are modified inflorescences and arise in the leaf axils (Figure 1). The fruit usually contain a single seed.

Figure 1. *Calamus* sp.

Description of *Licuala spinosa* Thunberg.

A widespread fan palm, grows up to more than 15 feet in height. Trunk multiple clumps, slim clustering ending as a dense bush. Leaf circular shaped, with squared-off ends (Figure 2). Flower stalk: from among the leaf bases. Fruit: bright red, the size of a marble (about 0,5 inch in diameter). Seed: small and round.

Figure 2. *Licuala spinosa.*

Description of *Elaeis guineensis* Jacq.

Elaeis guineensis is use in commercial agriculture for the production of palm oil. Mature trees are single-stemmed, and grow to 20 m tall. The leaves are pinnate, and reach between 3-5 m long. A young tree produces about 30 leaves a year. Established trees over 10 years produce about 20 leaves a year. The flowers are produced in dense clusters; each individual flower is small, with three sepals and three petals. The fruit

takes six months to mature from pollination to maturity; it comprises an oily, fleshy outer layer (the pericarp), with a single seed (kernel), also rich in oil. Unlike its relative, the Coconut Palm, the oil palm does not produce offshoots; propagation is by sowing the seeds (Figure 3).

Figure 3. ***Elaeis guineensis.***

Collection of palm material was made, placed in plastic bags and the date of collection recorded. Samples were returned to BIOTEC. Moist tissue paper was placed in the base of plastic boxes to create humid conditions. All the samples were examined under the microscope. The fungi appearing on the samples were isolated into axenic culture using a single spore technique.

3.4. Isolation of fungi:

Media:

Corn meal agar (CMA) supplemented with added antibiotics (streptomycin sulfate 0.5 g/l, penicillin G 0.5 g/l) was used as a standard medium for isolation and sixteen squares are marked on the bottom of the agar plate. Spore suspension were transferred using a sterile Pasteur pipette onto the surface of the CMA plate, with a drop placed above each of the drawn aquares and checked for spore germination on a daily basis.

Basidiomycetes isolation:

Potato Dextrose Agar (PDA) supplemented with added antibiotics [(streptromycin sulfate 0.5 g/L or 100 ppm chloramphenicol (to suppress bacterial growth) + 2 ppm benomyl (to suppress the growth of anamorphic fungi)] was used as a standard medium for basidiomycetes isolation.

Fungal isolation:

Single spore isolations were made from sporulating structures on material incubated in the laboratory or fresh material when isolated in the field laboratory. Isolates were transferred to PDA plates when the fungi had germinated and incubation was at room temperature until growth was observed (Choi et al., 1999). Axenic cultures are maintained in the BIOTEC Culture Collection (BCC).

For the Basidiomycota spores-prints are made immediately on return to thelaboratory. Collected samples are wrapped in paper to keep them moist. If samples dry out, then moist tissue paper is used to cover the samples over night. The spores are then allowed to drop on to transparent paper and these can be used for isolation of the fungus. An alternative method for the isolation of basidiomycetes is to use tissue from the pileus or tube margin. When growth is good the strain is transferred to PDA without antibiotics. Axenic cultures are maintained in the BIOTEC Culture Collection (BCC).

3.5. Endophyte Study

In this work we document the occurrence of endophytic fungi within petioles and leaves of the fan palm, *Licuala spinosa.*

Material and methods

Healthy fan-leaves of *Licuala spinosa* were collected from Khaun Khang hotspring, Trang Province, southern Thailand. Palms of about the same size and age were chosen, leaves with parts of the petiole collected, placed in plastic bags and processed within 5 days.

Discs approximately 5 mm were cut from leaf tissues with a razor blade. Four discs were cut so as to include a major vein and four were cut from the tissue between the veins, therefore, taken from (A) near the leaflet base, and approximately 15 cm from A towards the apex, (B) from A towards to the apex.

To investigate the endophytes living within the petioles, sections were re-assembled in the correct order and were then cut into 5 cm long pieces. A 5 mm segment of tissue was then cut from the apical end of each piece of petiole.

Surface-sterilization techniques which have been widely used for isolation of endophytic fungi involve a sequence of alcohol and sodium hypochlorite: each leaf disc taken from the frond blade was surface sterilized by dipping in 95% ethanol for 1 min, then soaked in sodium hopochlorite (5% available chlorine) for 10 min with a second immersion in 95% ethanol for 30 s then washed with sterile distilled water. The leaf discs were then transferred into Petri dishes (9 cm diam) containing potato dextrose agar (PDA) and Corn Meal Agar (CMA) with added streptomycin sulphate. Four discs were placed in each dish.

The same proceduce was applied to the 5 mm wide petiole segments except that they were dipped in 95% ethanol for 90 s, Chlorax for 15 min, then 95% ethanol for 30 sec and washed with sterile distilled water.

Petri dishes were incubated at 25°C. Fungi that grew from the tissue fragments were subcultured on to PDA and corn meal agar (CMA) in 6 cm diam Petri dishes and incubated as above. Living cultures are deposited at BIOTEC Culture Collection (BCC).

3.6. Identification and nomenclature of organisms:

Most of the fungi were identified with based on the morphology and sporulation on media and fresh material.

The following texts were consulted for basic identification:

Ascomycetes: Hyde et al. (2000), Fröhlich & Hyde (2000).

Coelomycetes: Ainsworth et al. (1973), Nag Raj (1993) and Sutton (1980).

Hyphomycetes: Ainsworth et al. (1973), Carmichael, Kendrick, Conners & Sigler (1980), Ellis (1971; 1976) and Matsushima (1975; 1980; 1989; 1993; 1995).

Basidiomyces: Corner (1983; 1984; 1987; 1989; 1991; 1994), Jülich (1981), Moser (1978), Pegler (1977), Ryvarden and Johansen (1980), Singer (1986) and Teng (1996).

3.7. Molecular study

Growth of fungi for the phylogenetic study

Fungal cultures were grown on Potato Dextrose Broth (PDB) or on Potato Dextrose Agar at 25°C. The fungal biomass in broth was harvested through cheesecloth and washed with sterile distilled water several times and mycelium on PDA scraped off. The biomass was frozen in liquid nitrogen and ground into a fine powder with a mortar and pestle.

DNA extraction, amplification and sequencing:

DNA extraction was performed by following a modified protocol as defined by O'Donnell et al., 1997. Partial sequences from two different regions of the rDNA molecule (characterised by different rates of evolution) were amplified. Primer pairs LROR and LR5 defined by Vilgalys & Hester (1990) were used to amplify a segment of the large 28S subunit. ITS 4 and ITS 5 (as defined by White et al., 1990 were used for ITS-5.8S. DNA sequencing was performed using primers as mentioned above in an Applied Biosystem 3730 DNA Analyzer at the Genome Research Centre (University of Hong Kong).

Phylogenetic analysis:

Sequences generated from different primers were analyzed with other sequences obtained from the GenBank. Multiple alignment was done in BioEdit (Hall, 2005) and Clustal X (Thompson et al., 1997) and analyses were performed in PAUP* 4.0b10 (Swofford, 2002). Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps manually adjusted to optimize alignment. Analyses were done under different optimal conditions.

3.8. Weight loss experiments

Experimental procedure

Experimental bottles were filled with garden soil to a depth of 6-7 cm. Prepared feeder strips of filter paper (dimensions 5 X 2 cm) were laid flat on the soil surface in the decay bottles. Caps of the bottles were drilled, about 1 cm in diameter and plugged with cotton wool, and covered with aluminium foil. Bottles were loosely capped and steam sterilized at 121 °C for 30 minutes. After cooling in an incubator with controlled temperature and humidity (30 °C and 70% relative humidity) for 2 days, the paper feeder strips in each bottle was inoculated with the selected fungus. The fungus was allowed to grow and cover the feeder strips, before the test blocks were introduced into the bottle. Five replicates and a control were prepared for each fungus. Prepared test blocks of *Licuala spinosa* stems, petioles and leaves, oil palm petioles, and bamboo culms dimensions 5x2 cm, were cut and oven-dried for 2 days at 80 °C, and kept in a desiccator until constant weight was reached. They were then placed in the conditioning room at 30°C and 70% relative humidity, each test block was then steam sterilized and placed on the fungus covered paper feeder strips in the decay bottles. After five weeks (two replicates) and ten weeks (three replicates) incubation at 30 °C and 70% relative humidity, they were removed, brushed free of surface mycelium and reweighed. The percentage weight loss due to decay was computed from the difference between the initial and final weight of each test block.

3.9. Inducing Xylariaceous stroma in bottles

Experimental bottles were filled with PDA to a depth of 3 cm and inoculated with a plug of a xylariaceous strain. When the colony covered the surface, the test blocks of *Licuala spinosa* stems were transferred to the bottles. After four weeks, stromata of the xylariaceous strains developed (Figure 5).

4. RESULTS

Three palms were studied and 237 saprophytic fungi recorded and the common species on each palm are listed in Tables 1 and 2.

4.1. FREQUENCY OF OCCURRENCE OF FUNGI ON PALM MATERIAL

Table 1 lists the number of different taxa on the three palms, while Tables 2. lists the common species recorded.

Table 1. The number of fungi occurring on selected palms in Thailand.

	Saprophytic Fungi		Endophytic Fungi	
			Licuala spinosa	
	Calamus sp.	*Elaeis guineensis*	*Experimet 1*	*Experiment 2*
Ascomycetes	40	28	1,229	1,112
Basidiomycetes	4	62		
Anamorphic fungi	46	36		

Number of basidiomycetes on other palms:

1. *Cocos nucifera* = 4
2. *Nypa fructicans* = 12
3. *Arenga pinata* = 2
4. *Metroxylon sagus* = 1
5. Unknown palm = 2

Slides of all the fungi collected have been made and photographs taken of key species for future publications. Dried material has also been prepared for deposition in the BIOTEC Herbarium.

Table 2. The five most common fungi collected on selected palms in Thailand.

Calamus sp.	*Elaeis guineensis*
Unidentified anamorphic fungi Aom318	*Annulatascus velatispora*
Morenoina palmicola	*Stilbohypoxylon* sp.
Circinoconis paradoxa	*Falciformispora* sp.
Diaporthe sp.	*Vanakripa* sp.
Helminthosporium sp.	*Lasiodiplidia* sp.
Linocarpon sp.	*Grammothele fuligo*
Phaeosphaeria sp.	*Schizophyllum commune*

4.2. ENDOPHYTE STUDY

Total number of isolates in experiment 1 was 1,229, which comprises 197 morpho types, with 1,112 isolates from experiment 2 with 182 morpho types. The identification of endophytic fungi has proved to be difficult, largely because of the lack of information on the cultural characters of species already described and the none sporulation of many of the isolates. 143 xylariaceous morpho types were made from the 2 experiments and distinguished based on colony morphology. Cultures on PDA and CMA were examined periodically for reproductive structures and identified as they sporulated. The distribution of endophyte isolations on different parst of the palm is shown in Figures 4, 5.

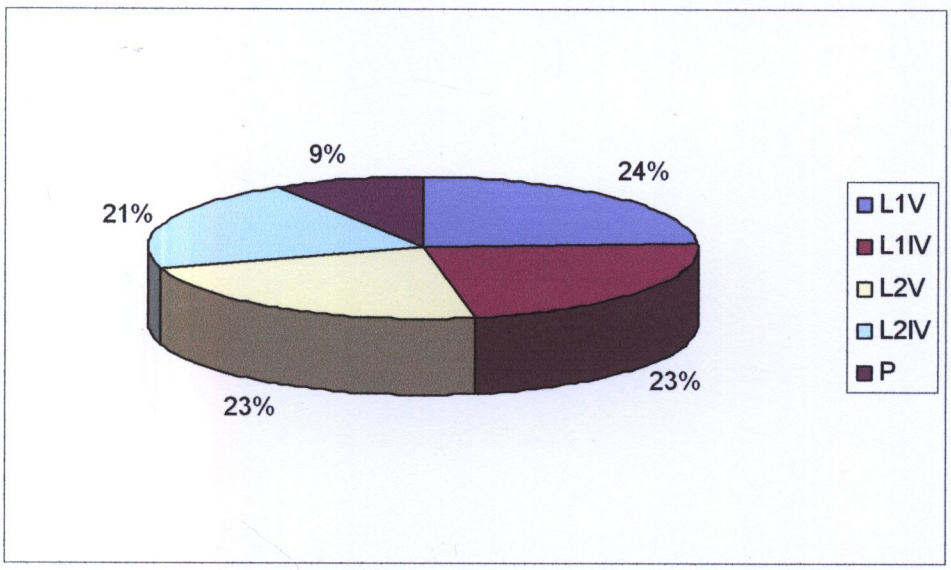

Figure 4. Percentage of isolates recovered from different tissue type of the *Licuala spinosa* palm.

L_1 = base of the leaf to 15 cm., L_2 = from L_1 to the tip of the leaf, IV = Intervein, V = Vein, P = Petiole

Figure 5. Xylariaceous species grown on palm stems.

4.3 WEIGHT LOSS STUDY

Six fungi were inoculated on to different substrata under laboratory conditions: *Ganoderma colossus, Schizophyllum commune, Pycnoporus sanguineus, Grammothele fuligo, Marasmiellus* sp. and an unidentified agaric collected from Malaysia (Figure 6, 7). Percentage weight loss from the selected Basidiomycetes (5 and 10 weeks) are shown in Table 3 and Figure 8.

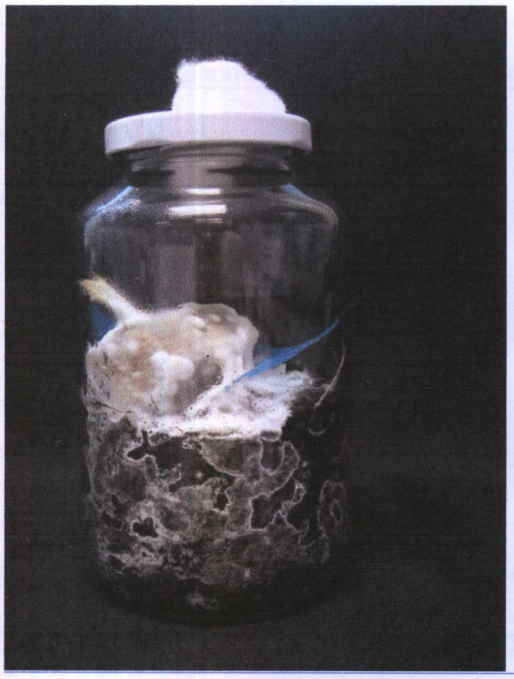

Figure 6. Basidiomycete growing on palm material for 5 weeks.

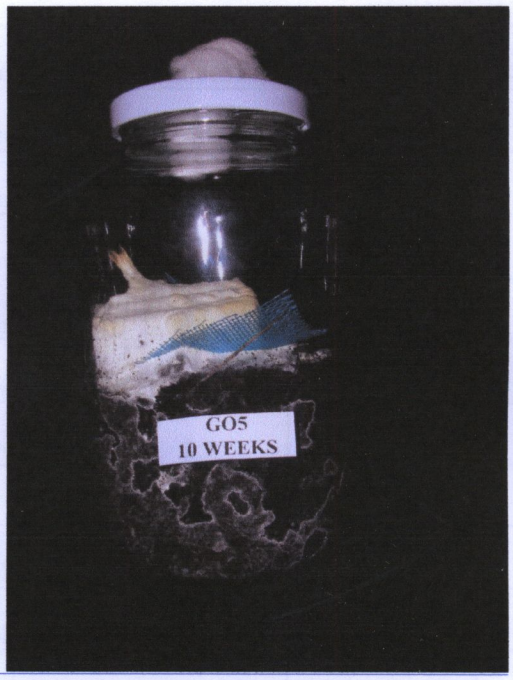

Figure 7. Basidiomycete growing on palm material for 10 weeks.

Table 3. Percentage weight loss of palm and bamboo samples after 5 and 10 weeks exposure.

Fungal name	Type of substrate	Mean (5W)	Mean (10W)	SD(5W)	SD(10W)
unidentified Malaysian agaric No.4	bamboo	8.7	9.8	4.0	0.4
Marasmiellus sp.	bamboo	8.5	8.4	2.3	3.0
Grammothele fuligo	bamboo	9.5	13.8	2.1	1.6
Pycnoporus sanguineus	bamboo	9.2	6.5	1.4	4.3
Ganoderma colossus	bamboo	16.7	23.2	4.7	2.6
Schizophyllum commune	bamboo	7.5	9.0	1.0	0.7
unidentified Malaysian agaric No.4	leaves	38.8	32.3	1.2	8.1
Marasmiellus sp.	leaves	28.3	30.0	6.1	12.0
Grammothele fuligo	leaves	40.0	48.3	0.2	3.0
Pycnoporus sanguineus	leaves	34.6	44.8	0.6	28.8
Ganoderma colossus	leaves	69.7	69.4	0.1	11.2
Schizophyllum commune	leaves	16.1	26.3	4.0	4.6

Table 3 ***cont.***

Fungal name	Type of substrate	Mean (5W)	Mean (10W)	SD(5W)	SD(10W)
unidentified Malaysian agaric No.4	stem	5.9	6.8	3.2	0.6
Marasmiellus sp.	stem	4.7	10.3	2.0	1.7
Grammothele fuligo	stem	28.3	38.4	12.5	6.2
Pycnoporus sanguineus	stem	2.3	5.2	0.1	0.5
Ganoderma colossus	stem	16.7	27.4	3.3	20.1
Schizophyllum commune	stem	3.9	6.1	1.0	1.7
unidentified Malaysian agaric No.4	petioles	17.8	27.7	7.2	3.7
Marasmiellus sp.	petioles	17.1	21.0	1.5	3.4
Grammothele fuligo	petioles	23.3	61.7	6.6	20.2
Pycnoporus sanguineus	petioles	15.8	17.8	1.5	4.5
Ganoderma colossus	petioles	47.2	72.0	1.9	9.7
Schizophyllum commune	petioles	12.6	14.6	1.1	2.0
unidentified Malaysian agaric No.4	Petioles+ rachides	15.1	29.4	6.9	3.2
Marasmiellus sp.	Petioles+ rachides	11.7	15.7	0.1	1.2
Grammothele fuligo	Petioles+ rachides	34.1	51.3	2.8	3.6
Pycnoporus sanguineus	Petioles+ rachides	10.1	14.2	2.4	2.4
Ganoderma colossus	Petioles+ rachides	38.3	56.5	1.9	6.6
Schizophyllum commune	Petioles+ rachides	5.3	10.7	0.1	3.2

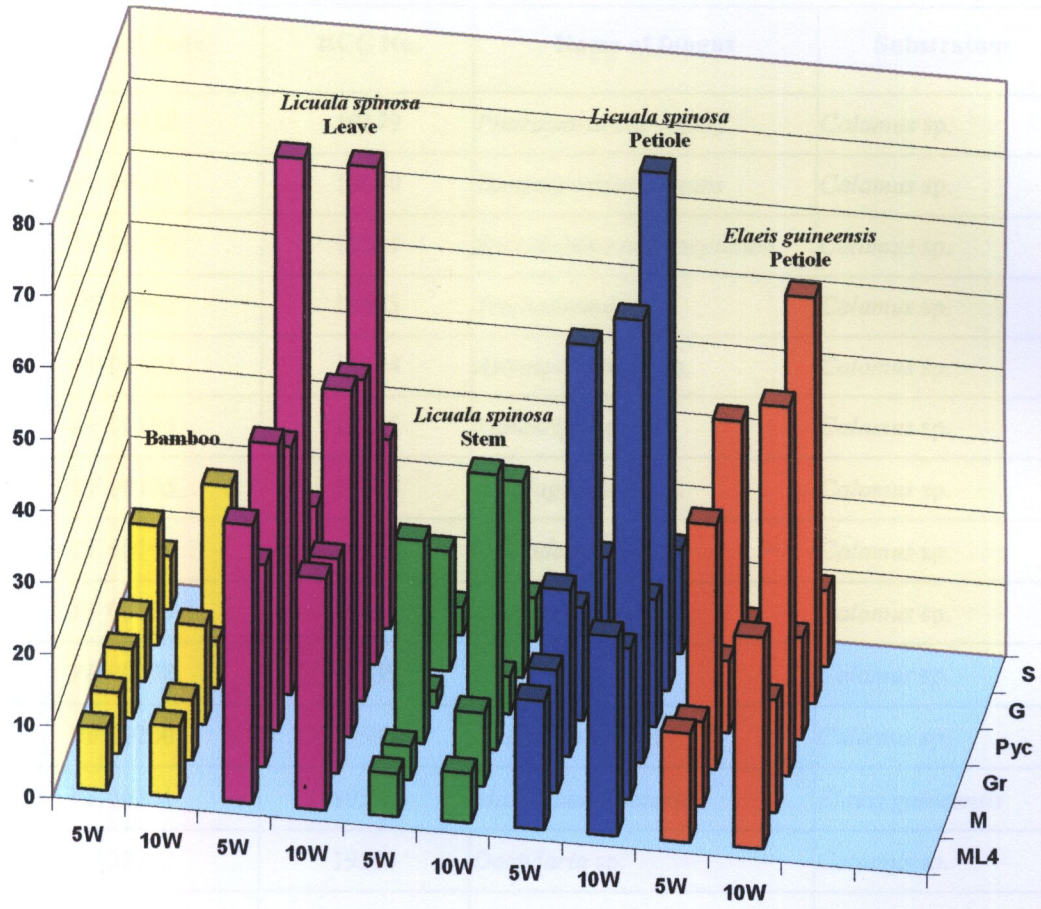

Figure 8. Comparative percentage weight loss from different isolated fungi (5 and 10 weeks).

S = *Schizophyllum commune*, G = *Ganoderma colossus*, Pyc = *Pycnoporus sanguineus*, Gr = *Grammothele fuligo*, M = *Marasmiellus* sp., ML4 = unidentified Malaysian agaric No.4

4.4. ISOLATIONS

Two hundred and thirty seven saprophytic and 2,341 endophytic strains have been isolated and six hundred and forty three strains deposited in the BIOTEC Culture Collection (Table 4). Others will be deposited at a later stage.

Table 4. List of axenic strains deposited in BIOTEC Culture Collection (BCC).

No.	Original code	BCC No.	Name of fungus	Substratum
1	PF 00188	19579	*Phaeoisaria clamatidis*	*Calamus* sp.
2	PF 00189	19580	*Dictyosporium elegans*	*Calamus* sp.
3	PF 00190	19581	*Sporokrisma nigroseptatum*	*Calamus* sp.
5	PF 00192	19583	*Trichoderma* sp.	*Calamus* sp.
6	PF 00193	19584	*Astrospaheriella* sp.	*Calamus* sp.
7	PF 00194	19585	*Vanakripa* sp.	*Calamus* sp.
8	PF 00195	19586	*Boerlagiomyces* sp.	*Calamus* sp.
9	PF 00196	19587	*Sporodesmiella* sp.	*Calamus* sp.
10	PF 00197	19588	*Cylindrocladium* sp.	*Calamus* sp.
11	PF 00198	19589	*Chalara* sp.	*Calamus* sp.
12	PF 00200	19591	*Diaporthe setulae*	*Calamus* sp.
13	PF 00201	19592	*Massarina bipolaris*	*Elaeis guineensis*
14	PF 00202	19593	*Dactylaria* sp.	*Calamus* sp.
15	PF 00203	19534	*Dictyochaeta* sp.	*Calamus* sp.
16	PF 00206	19597	*Berkleasmium* sp.	*Calamus* sp.
17	PF 00207	19598	*Canalisporium exiguum*	*Calamus* sp.
18	PF 00208	19599	*Pseudorobillarda sojae*	*Calamus* sp.
19	PF 00209	19600	*Thozetella* sp.	*Calamus* sp.
20	PF 00212	20412	Unidentified Hyphomycete 1	*Elaeis guineensis*
21	PF 00213	20413	Unidentified Hyphomycete 1	*Elaeis guineensis*
22	PF 00214	20414	Unidentified Hyphomycete 2	*Elaeis guineensis*
23	PF 00215	20415	Unidentified Hyphomycete 2	*Elaeis guineensis*
24	PF 00216	20416	*Trichoderma* sp.	*Elaeis guineensis*
25	PF 00217	20417	*Trichoderma* sp.	*Elaeis guineensis*
26	PF 00218	20418	*Phaeoisaria clamatidia*	*Elaeis guineensis*
27	PF 00219	20419	*Phaeoisaria clamatidia*	*Elaeis guineensis*
28	PF 00220	20420	Unidentified Hyphomycete 3	*Elaeis guineensis*

Table 4. ***cont.***

No.	Original code	BCC No.	Name of fungus	Substratum
29	PF 00221	20421	Unidentified Hyphomycete 3	*Elaeis guineensis*
30	PF 00222	20422	*Astrosphaeriella* sp.	*Calamus* sp
31	PF 00223	20423	*Astrosphaeriella* sp.	*Elaeis guineensis*
32	PF 00240	20441	*Astrosphaeriella* sp.	*Elaeis guineensis*
33	PF 00241	20442	*Astrosphaeriella* sp.	*Elaeis guineensis*
34	PF 00243	20443	*Dictyosporium* sp.	*Elaeis guineensis*
35	PF 00245	20444	*Berkleasmium* sp.	*Elaeis guineensis*
36	PF 00246	20445	*Eutypa* sp.	*Elaeis guineensis*
37	PF 00247	20446	*Eutypa* sp.	*Elaeis guineensis*
38	PF 00248	20447	*Eutypa* sp.	*Elaeis guineensis*
39	PF 00249	20448	*Chalara* sp.	*Calamus sp.*
40	PF 00250	20449	Unidentified Ascomycete	*Elaeis guineensis*
41	PF 00251	20450	Unidentified Hyphomycete	*Elaeis guineensis*
42	PF 00252	20451	Unidentified Hyphomycete	*Elaeis guineensis*
43	PF 00253	20452	Unidentified Hyphomycete	*Elaeis guineensis*
44	PF 00254	20453	Unidentified Discomycete	*Elaeis guineensis*
45	PF 00255	20454	Unidentified Discomycete	*Elaeis guineensis*
46	PF 00256	20455	Unidentified Discomycete	*Elaeis guineensis*
47	PF 00257	20456	Unidentified Hyphomycete	*Elaeis guineensis*
48	PF 00258	20457	Unidentified Hyphomycete	*Elaeis guineensis*
49	PF 00259	20458	Unidentified Hyphomycete	*Elaeis guineensis*
50	PF 00260		*Massarina bipolaris*	*Elaeis guineensis*
51	PF 00261	20459	*Massarina bipolaris*	*Elaeis guineensis*
52	PF 00262		*Massarina bipolaris*	*Elaeis guineensis*
53	PF 00263	20460	*Astrosphaeriella* sp.	*Elaeis guineensis*
54	PF 00264		*Astrosphaeriella* sp.	*Elaeis guineensis*
55	PF 00265		*Astrosphaeriella* sp.	*Elaeis guineensis*

Table 4. ***cont.***

No.	Original code	BCC No.	Name of fungus	Substratum
56	PF 00266	20463	Unidentified Hyphomycete	*Elaeis guineensis*
57	PF 00267		Unidentified Hyphomycete	*Elaeis guineensis*
58	PF 00268	20464	Unidentified Hyphomycete	*Elaeis guineensis*
59	PF 00269		Unidentified Ascomycete	*Elaeis guineensis*
60	PF 00270	20465	Unidentified Ascomycete	*Elaeis guineensis*
61	PF 00271		*Massarina*-like	*Elaeis guineensis*
62	PF 00272	20466	*Massarina*-like	*Elaeis guineensis*
63	PF 00273	20467	*Massarina*-like	*Elaeis guineensis*
64	PF 00274	20468	Unidentified Hyphomycete	*Elaeis guineensis*
65	PF 00275	20469	Unidentified Hyphomycete	*Elaeis guineensis*
66	PF 00276	20470	Unidentified Hyphomycete	*Elaeis guineensis*
67	PF 00277	20471	*Astrosphaeriella* sp.	*Elaeis guineensis*
68	PF 00278	20472	Unidentified Hyphomycere	*Elaeis guineensis*
69	PF 00279	20473	Unidentified Hyphomycete	*Elaeis guineensis*
70	PF 00280	20474	Unidentified Hyphomycete	*Elaeis guineensis*
71	PF 00281	20475	*Dictyochaeta* sp.	*Elaeis guineensis*
72	PF 00282	20476	*Dictyochaeta* sp.	*Elaeis guineensis*
73	PF 00283	20477	*Sporokrisma nigroseptatum*	*Elaeis guineensis*
74	PF 00285	20478	*Sporokrisma nigroseptatum*	*Elaeis guineensis*
75	PF 00286	20479	*Sporokrisma nigroseptatum*	*Elaeis guineensis*
76	PF 00287	20769	*Massarina bipolaris*	*Elaeis guineensis*
77	PF 00288	20770	*Tetraploa* sp.	*Elaeis guineensis*
78	PF 00289	20771	*Didymosphaeria* sp.	*Elaeis guineensis*
79	PF 00290	20772	Unidentified Hyphomycete	*Elaeis guineensis*
80	PF 00291	20773	*Acrodyctis* sp.	*Elaeis guineensis*
81	PF 00292	20774	*Stilbohypoxylon* sp	*Elaeis guineensis*
82	PF 00293	20775	*Leptosphaeria* sp.	*Elaeis guineensis*

Table 4 *cont.*

No.	Original code	BCC No.	Name of fungus	Substratum
83	PF 00294	20776	*Falciformispora* sp.	*Elaeis guineensis*
84	PF 00295	20777	Unidentified Coelomycete	*Elaeis guineensis*
85	PF 00296	20778	Unidentified Hyphomycete	*Elaeis guineensis*
86	PF 00297	20779	Unidentified Hyphomycete	*Elaeis guineensis*
87	PF 00298	20780	*Massarima* sp.	*Elaeis guineensis*
88	PF 00299	20781	*Vanakripa* sp.	*Elaeis guineensis*
89	PF 00300	20782	*Helocoma* sp.	*Elaeis guineensis*
90	PF 00301	20783	*Lasiodiplodia* sp.	*Elaeis guineensis*
91	PF 00302	20784	*Trichoderma* sp.	*Elaeis guineensis*
92	PF 00303	20785	*Annulatascus* sp.	*Elaeis guineensis*
93	PF 00304	20786	*Berkleasmium* sp.	*Elaeis guineensis*
94	PF 00305	20787	*Dictyosporium* sp.	*Elaeis guineensis*
95	PF 00306	20788	Unidentified Ascomycete	*Elaeis guineensis*
96	AOM 00201.01	15543	*Stictis* sp.	*Caryota urens*
97	AOM 00201.02	15544	*Stictis* sp.	*Caryota urens*
98	AOM 00202.01	15565	*Ellisembia* sp.	*Caryota urens*
99	AOM 00215.01	15946	*Coleodictyospora micronisica*	*Caryota urens*
100	AOM 00217.01	15944	*Astrosphaeriella* sp.	*Caryota urens*
101	AOM 00217.02	15945	*Astrosphaeriella* sp.	*Caryota urens*
102	AOM 00219.01	15947	Unidentified	*Caryota urens*
103	AOM 00222.01	15943	*Monotosporella* sp.	*Caryota urens*
104	AOM 00230.01	15969	*Dictyosporium* sp.	*Caryota urens*
105	AOM00230.02	15970	*Dictyosporium* sp.	*Caryota urens*
106	AOM 00230.03	15971	*Dictyosporium* sp.	*Caryota urens*
107	AOM 00231.01	15972	*Diplococcium* sp.	*Caryota urens*
108	AOM 00231.02	15973	*Diplococcium* sp.	*Caryota urens*
109	AOM 00234.01	16052	Unidentified	*Calamus* sp.

Table 4. ***cont.***

No.	Original code	BCC No.	Name of fungus	Substratum
110	AOM 00234.02	16051	Unidentified	*Calamus* sp.
111	AOM 00234.03	16050	Unidentified	*Calamus* sp.
112	AOM 00235.01	16481	Unidentified	*Calamus* sp.
113	AOM 00235.02	16482	Unidentified	*Calamus* sp.
114	AOM 00235.03	16483	Unidentified	*Calamus* sp.
115	AOM 00237.03	16045	Unidentified	*Calamus* sp.
116	AOM 00238.01	16088	Unidentified	*Calamus* sp.
117	AOM 00238.02	16149	Unidentified	*Calamus* sp.
118	AOM 00238.03	16150	Unidentified	*Calamus* sp.
119	AOM 00238.04	16151	Unidentified	*Calamus* sp.
120	AOM 00242.01	16047	Unidentified	*Calamus* sp.
121	AOM 00242.03	16046	Unidentified	*Calamus* sp.
122	AOM 00244.01	16152	*Astrosphaeriella* sp.	*Eleiodoxa conferta*
123	AOM 00244.02	16153	*Astrosphaeriella* sp.	*Eleiodoxa conferta*
124	AOM 00244.03	16154	*Astrosphaeriella* sp.	*Eleiodoxa conferta*
125	AOM 00244.04	16155	*Astrosphaeriella* sp.	*Eleiodoxa conferta*
126	AOM 00252.01	17000	*Lophiostroma* sp.	*Caryota* sp.
127	AOM 00254.01	17001	*Marssarina*, *Lophiostroma*	*Caryota* sp.
128	AOM 00255.01	17002	*Berkleasmium* sp.	*Caryota* sp.
129	AOM 00255.02	17003	*Berkleasmium* sp.	*Caryota* sp.
130	AOM 00256.04	17004	*Tubeufia* sp.	*Caryota* sp.
131	AOM 00257.01	17005	Unidentified	*Caryota* sp.
132	AOM 00257.02	17006	Unidentified	*Caryota* sp.
133	AOM 00257.03	17007	Unidentified	*Caryota* sp.
134	AOM 00259.01	17008	Unidentified	*Caryota* sp.
135	AOM 00259.02	17009	Unidentified	*Caryota* sp.
136	AOM 00259.03	17010	Unidentified	*Caryota* sp.

Table 4. ***cont.***

No.	Original code	BCC No.	Name of fungus	Substratum
137	AOM 00262.01	17011	*Astrosphaeriella* sp.	*Calamus* sp.
138	AOM 00262.02	17012	*Astrosphaeriella* sp.	*Calamus* sp.
139	AOM 00262.03	17013	*Astrosphaeriella* sp.	*Calamus* sp.
140	AOM 00262.04	17014	*Astrosphaeriella* sp.	*Calamus* sp.
141	AOM 00263.01	17015	Unidentified	*Calamus* sp.
142	AOM 00263.02	17016	Unidentified	*Calamus* sp.
143	AOM 00264.01	17017	Unidentified	*Calamus* sp.
144	AOM 00264.02	17018	Unidentified	*Calamus* sp.
145	AOM 00264.03	17019	Unidentified	*Calamus* sp.
146	AOM 00266.01	17020	Unidentified Ascomycete	*Caryota mitis*
147	AOM 00266.02	17021	Unidentified Ascomycete	*Caryota mitis*
148	AOM 00266.03	17022	Unidentified Ascomycete	*Caryota mitis*
149	AOM00275.01	17027	Unidentified	*Caryota mitis*
150	AOM00275.02	17028	Unidentified	*Caryota mitis*
151	AOM00276.01	17029	Unidentified	*Caryota mitis*
152	AOM00276.02	17030	Unidentified	*Caryota mitis*
153	AOM00276.03	17031	Unidentified	*Caryota mitis*
154	AOM00276.04	17032	Unidentified	*Caryota mitis*
155	AOM 00270.01	17023	*Berkleasmium* sp.	*Calamus* sp.
156	AOM 00270.02	17024	*Berkleasmium* sp.	*Calamus* sp.
157	AOM 00272.01	17025	*Stictis* sp.	*Caryota mitis*
158	AOM 00301.01	20614	Unidentified	*Calamus* sp.
159	AOM 00301.02	20380	Unidentified	*Calamus* sp.
160	AOM 00301.03	20381	Unidentified	*Calamus* sp.
161	AOM 00301.04	20382	Unidentified	*Calamus* sp.
162	AOM 00302.01	20615	Unidentified	*Calamus* sp.
163	AOM 00302.02	20383	Unidentified	*Calamus* sp.

Table 4. ***cont.***

No.	Original code	BCC No.	Name of fungus	Substratum
164	AOM 00302.03	20384	Unidentified	*Calamus* sp.
165	AOM 00302.04	20385	Unidentified	*Calamus* sp.
166	AOM 00306.01		*Berkleasmium* sp.	*Calamus* sp.
167	AOM 00306.02	20316	*Berkleasmium* sp.	*Calamus* sp.
168	AOM 00306.03		*Berkleasmium* sp.	*Calamus* sp.
169	AOM 00306.04		*Berkleasmium* sp.	*Calamus* sp.
170	AOM 00309.01	20617	*Diaporthe* sp.	*Calamus* sp.
171	AOM 00309.02		*Diaporthe* sp.	*Calamus* sp.
172	AOM 00309.03		*Diaporthe* sp.	*Calamus* sp.
173	AOM 00309.04		*Diaporthe* sp.	*Calamus* sp.
174	AOM 00311.01	20618	*Coleodictyospora micronesica*	*Calamus* sp.
175	AOM 00311.02	20386	*Coleodictyospora micronesica*	*Calamus* sp.
176	AOM 00311.03	20387	*Coleodictyospora micronesica*	*Calamus* sp.
177	AOM 00311.04	20388	*Coleodictyospora micronesica*	*Calamus* sp.
178	AOM 00312.01	20619	*Ellisembia* sp.	*Calamus* sp.
179	AOM 00312.02	20389	*Ellisembia* sp.	*Calamus* sp.
180	AOM 00312.03	20390	*Ellisembia* sp.	*Calamus* sp.
181	AOM 00312.04	20391	*Ellisembia* sp.	*Calamus* sp.
182	AOM 00314.01	20620	*Sporidesmium* sp.	*Calamus* sp.
183	AOM 00314.02	20392	*Sporidesmium* sp.	*Calamus* sp.
184	AOM 00314.03	20393	*Sporidesmium* sp.	*Calamus* sp.
185	AOM 00314.04	20394	*Sporidesmium* sp.	*Calamus* sp.
186	AOM 00315.01		*Helminthosporium* sp.	*Calamus* sp.
187	AOM 00315.02		*Helminthosporium* sp.	*Calamus* sp.
188	AOM 00315.03		*Helminthosporium* sp.	*Calamus* sp.
189	AOM 00315.04		*Helminthosporium* sp.	*Calamus* sp.
190	AOM 00316.01	20621	Unidentified Hyphomycete	*Calamus* sp.

Table 4. ***cont.***

No.	Original code	BCC No.	Name of fungus	Substratum
191	AOM 00316.02	20622	Unidentified Hyphomycete	*Calamus* sp.
192	AOM 00316.03	20395	Unidentified Hyphomycete	*Calamus* sp.
193	AOM 00316.04	20396	Unidentified Hyphomycete	*Calamus* sp.
194	AOM 00317.01	20623	*Diplocladiella* sp.	*Calamus* sp.
195	AOM 00317.02	20397	*Diplocladiella* sp.	*Calamus* sp.
196	AOM 00317.03	20398	*Diplocladiella* sp.	*Calamus* sp.
197	AOM 00317.04	20399	*Diplocladiella* sp.	*Calamus* sp.
198	AOM 00318.01	20624	Unidentified Hyphomycete	*Calamus* sp.
199	AOM 00318.02	20400	Unidentified Hyphomycete	*Calamus* sp.
200	AOM 00318.03	20401	Unidentified Hyphomycete	*Calamus* sp.
201	AOM 00318.04	20402	Unidentified Hyphomycete	*Calamus* sp.
202	AOM 00319.01	20625	*Astrosphaeriella visuvius*	*Calamus* sp.
203	AOM 00319.02	20626	*Astrosphaeriella visuvius*	*Calamus* sp.
204	AOM 00319.03		*Astrosphaeriella visuvius*	*Calamus* sp.
205	AOM 00319.04		*Astrosphaeriella visuvius*	*Calamus* sp.
206	AOM 00321.01		Unidentified	*Calamus* sp.
207	AOM 00321.02		Unidentified	*Calamus* sp.
208	AOM 00321.03		Unidentified	*Calamus* sp.
209	AOM 00321.04		Unidentified	*Calamus* sp.
210	AOM 00323.01	20627	*Sporidesmium altum*	*Calamus* sp.
211	AOM 00323.02	20405	*Sporidesmium altum*	*Calamus* sp.
212	AOM 00323.03	20406	*Sporidesmium altum*	*Calamus* sp.
213	AOM 00323.04	20407	*Sporidesmium altum*	*Calamus* sp.
214	AOM 00324.01	20628	*Sporidesmium* sp.	*Calamus* sp.
215	AOM 00325.01	20629	*Sporidesmium* sp.	*Calamus* sp.
216	AOM 00325.02	20408	*Sporidesmium* sp.	*Calamus* sp.
217	AOM 00327.01	20630	*Sporidesmium* sp.	*Calamus* sp.

Table 4. ***cont.***

No.	Original code	BCC No.	Name of fungus	Substratum
218	AOM 00327.02	20409	*Sporidesmium* sp.	*Calamus* sp.
219	AOM 00327.03	20410	*Sporidesmium* sp.	*Calamus* sp.
220	AOM 00328.01		*Melanographium citri*	*Calamus* sp.
221	AOM 00328.02		*Melanographium citri*	*Calamus* sp.
220	AOM 00328.03		*Melanographium citri*	*Calamus* sp.
221	THP00122	18542	*Dictyopanus* sp.	Unidentified palm
222	THP00123	18543	*Grammothele* sp.	Unidentified palm
223	THP00124	18544	*Grammothele* sp.	Unidentified palm
224	THP00125	18545	*Cymatoderma* sp.	*Calamus* sp.
225	THP00126	18546	*Cymatoderma* sp.	*Calamus* sp.
226	THP00128	18548	Unidentified	Unidentified palm
227	THP00129	18549	Unidentified	Unidentified palm
228	THP00130	18550	Unidentified	Unidentified palm
229	THP00131	18551	Unidentified	*Calamus* sp.
230	THP00194	19702	*Grammothele* sp.	*Salacca* sp.
231	THP00206		*Pleurutus* sp.	*Elaeis guineensis*
232	THP00207		*Pleurotus* sp.	*Elaeis guineensis*
233	THP00208		Unidentified	*Elaeis guineensis*
234	THP00209		*Marasmius* -like	*Elaeis guineensis*
235	THP00210		*Marasmius* -like	*Elaeis guineensis*
236	THP00211		*Marasmiellus*.-like	*Nypa fructicans*
237	THP00212		*Marasmiellus* sp.	*Nypa fructicans*
238	THP00213	20831	*Grammothele fuligo*	*Metroxylon sagu*
239	THP00214	20832	*Marasmiellus* sp.	*Nypa fructicans*
240	THP00219	22084	*Ganoderma* sp.	*Elaeis guineensis*
241	THP00220	22085	*Ganoderma* sp.	*Elaeis guineensis*
242	THP00221	22086	*Ganoderma* sp.	*Elaeis guineensis*

Table 4 ***cont.***

No.	Original code	BCC No.	Name of fungus	Substratum
243	THP00222	22087	*Ganoderma* sp.	*Elaeis guineensis*
244	THP00223	22088	Unidentified Basidiomycete	*Elaeis guineensis*
245	THP00224	22089	Unidentified Basidiomycete	*Elaeis guineensis*
246	THP00225	22090	Unidentified Basidiomycete	*Elaeis guineensis*
247	THP00226	22091	Unidentified Basidiomycete	*Elaeis guineensis*
248	THP00247	22112	*Grammothele fuligo*	*Metroxylon sagu*
249	THP00248	22113	*Grammothele fuligo*	*Metroxylon sagu*
250	THP00249	22114	*Grammothele fuligo*	*Metroxylon sagu*
251	THP00250	22115	*Grammothele fuligo*	*Metroxylon sagu*
252	THP00255	22120	Unidentified Basidiomycete	*Elaeis guineensis*
253	THP00256	22121	Unidentified Basidiomycete	*Elaeis guineensis*
254	THP00257	22122	Unidentified Basidiomycete	*Elaeis guineensis*
255	THP00258	22123	Unidentified Basidiomycete	*Elaeis guineensis*
256	THP00259	22124	*Thelephora* sp.	*Elaeis guineensis*
257	THP00260	22125	*Thelephora* sp.	*Elaeis guineensis*
258	THP00263	22128	Unidentified Basidiomycet	*Elaeis guineensis*
259	THP00264	22129	Unidentified Basidiomycete	*Elaeis guineensis*
260	THP00265	22130	Unidentified Basidiomycete	*Elaeis guineensis*
261	THP00266	22131	Unidentified Basidiomycete	*Elaeis guineensis*
262	THP00267	22132	Unidentified Basidiomycete	*Elaeis guineensis*
263	THP00268	22133	Unidentified Basidiomycete	*Elaeis guineensis*
264	THP00269	22314	*Ganoderma australe*	*Elaeis guineensis*
265	THP00270	22315	*Ganoderma australe*	*Elaeis guineensis*
266	THP00271	22316	*Ganoderma australe*	*Elaeis guineensis*
267	THP00272	22317	*Ganoderma australe*	*Elaeis guineensis*
268	THP00273	22318	*Ganoderma australe*	*Elaeis guineensis*
269	THP00274	22319	*Ganoderma australe*	*Elaeis guineensis*

Table 4 *cont.*

No.	Original code	BCC No.	Name of fungus	Substratum
270	THP00275	22320	*Ganoderma australe*	*Elaeis guineensis*
271	THP00276	22321	*Ganoderma australe*	*Elaeis guineensis*
272	THP00277	22322	*Ganoderma australe*	*Elaeis guineensis*
273	THP00278	22323	*Polyporus arcularius*	*Elaeis guineensis*
274	THP00279	22324	*Ganoderma* sp.	*Elaeis guineensis*
275	THP00280	22325	*Ganoderma* sp.	*Elaeis guineensis*
276	THP00282	22327	*Schizophyllum commune*	*Elaeis guineensis*
277	THP00286	22331	*Ganoderma* sp.	*Elaeis guineensis*
278	THP00287	22332	*Grammothele fuligo*	*Elaeis guineensis*
279	THP00288	22333	*Grammothele fuligo*	*Elaeis guineensis*
280	THP00290	22335	*Marasmius* sp.	*Elaeis guineensis*
281	THP00291	22336	*Marasmius* sp.	*Elaeis guineensis*
282	THP00292	22337	*Marasmius* sp.	*bamboo*
283	THP00295	22340	*Ganoderma* sp.	*Elaeis guineensis*
284	THP00296	22341	*Ganoderma* sp.	*Elaeis guineensis*
285	THP00297	22342	*Schizophyllum commune*	*Elaeis guineensis*
286	THP00298	22343	*Schizophyllum commune*	*Elaeis guineensis*
287	THP00299	22344	*Schizophyllum commune*	*Elaeis guineensis*
288	THP00300	22345	*Ganoderma australe*	*Elaeis guineensis*
289	THP00301	22346	*Ganoderma australe*	*Elaeis guineensis*
290	THP00304	22349	*Ganoderma* sp.	*Cocos nucifera*
291	THP00305	22350	*Ganoderma* sp.	*Cocos nucifera*
292-643	EP00148-499		Unidentified	*Elaeis guineensis*

4.5 Phylogenetic study

Group of fungi	Progress
Astrosphaeriella	Writing up paper
Berkleasmium	Paper submitted to Mycologia
Nemania	Writing up paper
Xylariaceous strains	20 strains sequenced and a further 10 will be sequenced over the next 6 months

5. DELIVERABLES:

5.1. Published papers

1. Pinnoi, A., S. Lumyong, K.D. Hyde & E.B.G. Jones. 2006. Biodiversity of fungi on the palm *Eleiodoxa conferta* in Sirindhorn peat swamp forest, Narathiwat, Thailand. Fungal Diversity 22: 205-218.

5.2 Papers in press

1. Pinnoi, A., R. Jeewon, J. Sakayaroj, K.D. Hyde & E.B.G. Jones. *Berkleasmium crunisia* sp. nov. and its teleomorphic affinities to the Pleosporales based on 18S, 28S and ITS-5.8S rDNA sequence analyses. Submitted to Mycologia.
2. Pinruan, U., S. Lumyong, K.D. Hyde & E.B.G. Jones. 2006. Occurrence of fungi on tissues of the peatswamp palm *Licuala longecalycata*. Fungal Diversity 23: xx-xx.

5.3. Papers in preparation

1. *Baipadsphaeria* gen. nov., a new freshwater ascomycete from decaying palm leaves.
2. *Thaliomyces setulis* gen. et. sp. nov. and its anamorph on palms.
3. Phylogenetic relationship of *Nemania eleiodoxae*.
4. Phylogeny of *Astrosphaeriella* species

5.4. Oral Presentations

1. Pinruan, U. 2005. A new peat swamp fungus: *Flammispora bioteca*. In: Special presentation of Mycological Research in Thailand. 7 December 2005, BIOTEC Building, Science Park, Thailand.
2. Jones, E.B.G. 2006. Progress in the documentation of Asian fungal diversity. In: 8th International Mycological Congress, 20-25 August 2006, Cairns Convention Centre, Cairns, Australia (Invited talk).
3. Jones, E.B.G. 2006. Bioactive compounds of Marine Fungi. Invited Talk. Trends in Biotechnology 3, 4-6 September, Putrajaya, Malaysia.
4. Jones, E.B.G. 2006. Marine endophytes: A source of new chemical natural products: A review. With co authors U. Pinruan, A. Pinoi & S. Stanley (Invited Special Plenary Talk). 24-28 July, 13th International Marine Fouling and Corrosion Congress, Rio di Janero, Brazil.

5.5. Poster Presentations

1. Jones, E.B.G., K.D. Hyde, S. Lumyong, U. Pinruan & A. Pinnoi. 2005. Biodiversity of fungi on palms in Sirindhorn Peat Swamp Forest, Narathiwat, Thailand. In: BioThailand 2005. 2-5 November 2005, Queen Sirikit National Convention Center, Bangkok, Thailand.
2. Pinruan, U., J. Sakayaroj, S. Lumyong & E.B.G. Jones. 2005. Two new ascomycetes from peat swamp palm. In: 9th BRT Annual Conference 2005. 10-13 October 2005, Sofitel Raja Ochid Hotel, Khon Kaen, Thailand.
3. Choeyklin, R., K.-L. Pang, T. Hattori, V. Sabaratnam, & E.B.G. Jones. 2006. Basidiomycetes on palms and bamboo in Thailand, with special reference to the phylogeny of *Ganoderma colossus* and *Ganoderma tsunodae*. In: 8th International Mycological Congress, 20-25 August 2006, Cairns Convention Centre, Cairns, Australia.
4. Pinnoi, A., R. Jeewon, K.D. Hyde, S. Phongpaichit, & E.B.G. Jones. 2006. Phylogeny of a putative new *Nemania* speces: *Nemania eleiodoxae*. In: 8th International Mycological Congress, 20-25 August 2006, Cairns Convention Centre, Cairns, Australia.
5. Pinuan, U., A. Pinnoi, K.D. Hyde, R. Jeerwon & E.B.G. Jones. 2006. Endophytes of a peat swamp palm: *Licuala longicalycata* and *L. spinosa*. In: 8th

International Mycological Congress, 20-25 August 2006, Cairns Convention Centre, Cairns, Australia.

FUTURE WORK:

1. Fungi collected so far will be identified to species level.
2. Fungal isolation work to continue.
3. Phylogenetic trees of selected fungi will be constructed.
4. Writing up manuscripts for publication.

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Biodiversity of fungi on the palm *Eleiodoxa conferta* in Sirindhorn peat swamp forest, Narathiwat, Thailand

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This study focuses on the saprobic fungi occurring on decaying palm material of *Eleiodoxa conferta* at Sirindhorn peat swamp forest, Narathiwat Province, Thailand. In this survey, 462 fungal records were made from seven field collections in May, June, September and November (2001) and February, May and November (2002). Two hundred and fifty-one records were identified to species level, 176 to generic level while 35 records were unidentified. Of the 112 taxa identified 43 (38%) were ascomycetes, 67 (60%) anamorphic fungi and 2 (2%) basidiomycetes. Different parts of *E. conferta* support differing fungi: dry (aerial) material supported 17% of the fungal records, damp (moist and on the surface of the soil) material 34.5%, while submerged wet material had the most fungal records (48.5%). The percentage abundances of fungi on different parts of *E. conferta* were petioles 53%, rachides 30% and leaves 17%. Many of the taxa collected are new to science. *Eleiodoxa conferta* has been shown to support a rich diversity of fungi that differ significantly from those on terrestrial and brackish water palms. Eight new species and one genus have been described from this palm, while 12 taxa await description.

Key words: biodiversity, habitat preference, palm fungi, peat swamp, tissue specificity

Introduction

A wide range of fungi have been documented from palms primarily from tropical locations (Hyde, 1996a,b,c; Hyde *et al.*, 1997; Taylor and Hyde, 2003). Up to 1994, *ca.* 1,580 fungi had been recorded from palms including 650 ascomycetes, 270 basidiomycetes and 660 anamorphic fungi, with 75% of the fungi on palms being new records to science (Hyde *et al.*, 1997).

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Anthostomella, *Astrosphaeriella*, *Capsulospora*, *Linocarpon*, *Neolinocarpon* and *Oxydothis* are common genera on terrestrial palm material (Fröhlich and Hyde, 2000; Yanna, 2001a,b; Taylor and Hyde, 2003).

Studies on palm fungal diversity have focused on saprobic terrestrial species, with fewer on endophytes and pathogens (Fröhlich and Hyde, 2000). In aquatic habitats, the brackish water palm *Nypa fruticans* has been examined for fungi with *Astrosphaeriella*, *Linocarpon* and *Oxydothis* being the most common genera (Hyde and Alias, 1999). Freshwater peat swamps are often rich in palms species but little information is available on the fungi colonizing such substrata (Shearer and Crane, 1986).

The objectives of this study were determine the fungal diversity on *Eleiodoxa conferta*; determine the diversity and distribution of fungi on different parts of *Eleiodoxa conferta*; determine the effect of dry, damp, and wet microhabitats on fungal diversity on *Eleiodoxa conferta*; compare the fungal diversity on freshwater palm, *Eleiodoxa conferta*, with those on brackish water and terrestrial palms and compare the fungal diversity on palm and other substrates from freshwater habitats.

Materials and methods

Sample collection

Eleiodoxa conferta was collected at Sirindhorn Research and Nature Study Center (Sirindhorn Peat Swamp Forest), Narathiwat Province, Thailand (See Fig. 1). Collections of *Eleiodoxa conferta* were divided into 3 parts: palm leaves, rachides and petioles, and from 3 microhabitats: wet (constantly submerged), damp (moist and on the surface of the soil), and dry (aerial part) microhabitats and then placed in plastic bags. Collections were made in May, June, September and November (2001) and February, May and November (2002). Samples were returned to the laboratory where the material was incubated in plastic boxes on sterile moist tissue. The material was kept moist and examined periodically for fungal fruiting structures, and species identified. One thousand and seventy one samples were collected over the study period: May (2001) 109 samples; June (2001) 271; September (2001) 105; November (2001) 121; February (2002) 215; May (2002) 160 and November (2002) 90.

Isolation

Single spore isolations were made from sporulating structures on material incubated in the laboratory or from fresh material when isolated in the field

Fig. 1. Sirindhorn peat swamp forest arrow the palm *Eleiodoxa conferta.*

laboratory. The isolation medium was Corn Meal Agar (CMA), with added antibiotics (Streptomycin 0.5 g/l, Penicillin G 0.5 g/l), and germinating spores transferred to Potato Dextrose Agar (PDA), and incubated at room temperature until growth was observed.

Data analyses: Percentage abundance of a taxon was calculated according to the following formula:

Percentage abundance of taxon A = $\dfrac{\text{Occurrence of taxon A} \times 100}{\text{Occurrence of all taxa}}$

Similarity index (Magurran, 1988)

$2N / N_1 + N_2$ (comparing fungi between 2 vertical positions)
$3N / N_1 + N_2 + N_3$ (Overall: comparing fungi between 3 vertical positions)
Where,
N = Number of fungi commonly occurring at multiple levels
N_1 = total number of fungal species on level 1
N_2 = total number of fungal species on level 2
N_3 = total number of fungal species on level 3

Results

Abundance of fungi on the palm Eleiodoxa conferta

Four-hundred and sixty-two fungal records were made from six field collections (Table 1). One hundred and twelve taxa were collected and identified with 43 (38% all of records) ascomycetes, 67 (60%) anamorphic fungi and 2 (2%) basidiomycetes (Table 1). The most common taxa were *Cancellidium applanatum* (6.9% of all records), *Xylomyces aquaticus* (5.8%), *Astrosphaeriella* sp. (5.6%), *Stilbohypoxylon moelleri* (5.2%), *Lophiostoma frondisubmersa* (5%), *Microthyrium* sp. (5%), *Morenoina palmicola* (4.5%), *Phaeoisaria clematidis* (4.1%), *Nemania eleiodoxae* (3%), and *Jahnula appendiculata* (2.8%) (Table 1). Forty species (36% of total species) were represented by only one record and can be regarded as infrequent or rare. Twenty-three taxa remained unidentified: 6 ascomycetes, 12 hyphomycetes, 3 coelomycetes, and 2 basidiomycetes.

Abundance of fungi on palm material under different microhabitats

Percentage abundance of fungi on different parts of the *E. conferta* were as follows: dry material supported 17%, damp material 34.5%, while the wet material supported the most fungi 48.5%, with *Xylomyces aquaticus* (19 records), *Microthyrium* sp. (18), *Astrosphaeriella* sp. (15), and *Jahnula appendiculata* (12) being the most common taxa (data not shown). On dry material a few records of the following species were made: *Morenoina palmicola* (12 records), *Nemania eleiodoxae* (8), and *Capnodiastrum/Kamatia* sp. (5); while on damp material: *Cancellidium applanatum* (12 records), *Gaeumannomyces* sp. (6), and *Berkleasmium typhae* (5) were the most common fungi (data not shown).

Percentage coverage of fungi on different parts of the palm

The percentage coverage of fungi on different parts of *E. conferta* were petioles 53%, leaves 17%, and rachides 30%. The following species appeared exclusively or primarily on the petioles of the palm: *Stilbohypoxylon moelleri* (12 records), *Morenoina palmicola* (10), *Nemania eleiodoxae* (9), *Astrosphaeriella* sp. 4 (6), *Capnodiastrum/Kamatia* sp. (6), *Gaeumannomyces* sp. (6) *Coleodictyospora micronesica* (5), *Delortia palmicola* (4) and *Nawawia filiformis* (4). Fewer fungi were recorded on the palm rachides, the most common being *Phaeoisaria clematidis* (9), *Microthyrium* sp. (8 records), *Berkleasmium typhae* (6), and *Sporidesmium* sp. 1 (4). The following species were only collected once on leaf material and diversity was low *Acrocalymma*

Table 1. Abundance of fungi on the palm *Eleiodoxa conferta* at the peat swamp forest, Narathiwat (species listed in order of percentage abundance).

Fungus	Number of records	Percentage abundance
Cancellidium applanatum	32	6.9
Xylomyces aquaticus	27	5.8
Astrosphaeriella sp.*	26	5.6
Stilbohypoxylon moelleri	24	5.2
Lophiostoma frondisubmersa	23	5.0
Microthyrium sp.	23	5.0
Morenoina palmicola	21	4.5
Phaeoisaria clematidis	19	4.1
Nemania eleiodoxae *	14	3.0
Jahnula appendiculata	13	2.8
Gaeumannomyces sp.*	12	2.6
Berkleasmium typhae	10	2.2
Nawawia filiformis	9	1.9
Capnodiastrum/ Kamatia sp.	9	1.9
Coleodictyospora micronesica	8	1.7
Annulatascus sp. 1	8	1.7
Astrosphaeriella sp. 4	7	1.5
Submersisphaeria palmae	7	1.5
Oxydothis rattanicola	6	1.3
*Didymobotryum biseptata**	6	1.3
Delortia palmicola	5	1.1
Septomyrothecium sp. 1	5	1.1
Sporidesmium sp.*	5	1.1
Stictis sp.	5	1.1
Brachysporiella gayana	4	0.9
Bionectria sp.	3	0.6
Custingophora undulatistipes	3	0.6
Helicomyces roseus	3	0.6
Stachybotrys albipes	3	0.6
Thozetella sp.*	3	0.6
Annulatascus velatispora	3	0.6
Dactylella sp. 1	3	0.6
Fluviatispora reticulata	3	0.6
Trichoderma sp.	3	0.6
Dactylaria flammulicornuta	3	0.6
Arthrobotrys sp.	2	0.4
Astrosphaeriella sp. 3	2	0.4
Capsulospora frondicola	2	0.4
Chlamydospore type 2	2	0.4
Chloridium sp.	2	0.4
Diplococcium asperum	2	0.4
Dischloridium sp.	2	0.4
Linocarpon sp.*	2	0.4

Table 1 continued. Abundance of fungi on the palm *Eleiodoxa conferta* at the peat swamp forest, Narathiwat (species listed in order of percentage abundance).

Fungus	Number of records	Percentage abundance
Massarina-like	2	0.4
Penicillium sp.	2	0.4
Pestalosphaeria austroamericana	2	0.4
Phialogeniculata sp.	2	0.4
Pleurophragmium sp. 1	2	0.4
Septomyrothecium sp. 2	2	0.4
Sporidesmium-like	2	0.4
Acrocalymma medicaginis	1	0.2
Anthostomella sp.*	1	0.2
Annulatuscus sp. 2 *	1	0.2
Apioclypea apiosporioides	1	0.2
Astrosphaeriella angustispora	1	0.2
Astrosphaeriella sp. 1	1	0.2
Astrosphaeriella sp. 2	1	0.2
Cancellidium-like 1	1	0.2
Cancellidium-like 2	1	0.2
*Chaetoporthe eleiodoxae**	1	0.2
Chaetopsina sp.	1	0.2
Chalara siamense	1	0.2
Chlamydospore type 1	1	0.2
Dactylaria uliginicola	1	0.2
Dactylella sp. 2	1	0.2
Diaporthe sp.	1	0.2
Gnomonia sp.	1	0.2
Goidanichiella fusiforma	1	0.2
Gonytrichum macrocladum	1	0.2
Haplographium state of *Hyaloscypha dematiicola*	1	0.2
Helicoma sp.	1	0.2
Helicosporium sp.	1	0.2
Helicoubisia coronata	1	0.2
Heteroconium sp.	1	0.2
Lophodermium sp.	1	0.2
Melanographium citri	1	0.2
Monotosporella rhizoidea	1	0.2
Munkovalsaria sp.*	1	0.2
Ophiostoma sp.	1	0.2
Orbilia sp.	1	0.2
Ornatispora sp.	1	0.2
Pleurophragmium sp. 2	1	0.2
Septomyrothecium sp. 2	1	0.2
Sporidesmium sp. 2*	1	0.2
Septomyrothecium sp. 2	1	0.2

Table 1 continued. Abundance of fungi on the palm *Eleiodoxa conferta* at the peat swamp forest, Narathiwat (species listed in order of percentage abundance).

Fungus	Number of records	Percentage abundance
Sporidesmium sp. 2*	1	0.2
Tubeufia claspisphaeria	1	0.2
Unisetosphaeria penguinoides	1	0.2
Vanakripa minutiellipsoidea	1	0.2
Verticillium sp.	1	0.2
Wiesneriomyces-like	1	0.2
Unidentified ascomycetes (6 taxa)	14	#
Unidentified basidiomycete (2 taxa)	5	#
Unidentified coelomycetes (3 taxa)	3	#
Unidentified hyphomycetes (12 taxa)	13	#
Total records	462	100
Ascomycetes	43	38
Basidiomycetes	2	2
Anamorphic fungi	67	60
Total species	**112**	**100**

* New species awaiting description
# Data not presented

sp., *Annulatascus* sp. 2, *Astrosphaeriella angustispora*, *Helicoubisia coronata*, *Lophodermium* sp., *Melanographium* sp., *Septomyrothecium* sp. 2, *Stachybotrys albipes*, and *Verticillium* sp.

Some fungi were found on all parts of the palm: e.g. *Astrosphaeriella* sp., *Cancellidium applanatum*, *Lophiostoma frondisubmersa*, *Microthyrium* sp., *Nawawia filiformis*, and *Xylomyces aquaticus*. Saprobes found under every microhabitat included: *Nemania eleiodoxae*, *Astrosphaeriella* sp. 4, *Berkleasmium typhae*, *Capnodiastrum/Kamatia* sp., *Coleodictyospora micronesica*, *Delortia palmicola*, *Gaeumannomyces* sp., *Morenoina palmicola*, *Phaeoisaria clematidis*, and *Stilbohypoxylon moelleri*.

Fungi that were recorded equally on petioles and rachides include *Annulatascus velatispora*, *Astrosphaeriella* sp. 4, Basidiomycete 1, *Berkleasmium typhae*, *Coleodictyospora micronesica*, *Custingophora undulatistipes*, *Didymobotryum biseptata*, *Gaeumannomyces* sp., *Helicomyces roseus*, *Jahnula appendiculata*, *Morenoina palmicola*, *Oxydothis rattanicola*, *Phaeoisaria clematidis*, *Stilbohypoxylon moelleri*, *Submersisphaeria palmae*, and *Thozetella* sp.

Discussion

The results presented raise a number of questions with respect to the diversity of fungi on the palm *E. conferta*. Are the species recorded unique to

the peat swamp forest and how similar is the fungal community to that on terrestrial palms?

Are the fungi on Eleiodoxa conferta *different to those on terrestrial palms?*

Ascomycetes are common on *Eleiodoxa conferta* as in the terrestrial palms *Oraniopsis appendiculata* and *Livistona australis* (Taylor and Hyde, 2003) with *Astrosphaeriella* species common to all three. However, genera such as *Arecomyces*, *Linocarpon* and *Oxydothis*, generally common on terrestrial palms, were not a dominant group on *E. conferta*. Similar differences were observed with the fungi *Brachysporiella*, *Linocarpon*, *Oxydothis* and *Trichoderma* common fungi on the palm *Oncosperma horridum* (Yanna *et al.*, 2001a), but rarely found on *E. conferta*.

Comparisons of ten most dominant fungi on terrestrial palms and *E. conferta* showed little overlap in species (data not show). A variety of factors may account for the differences observed, habitats, host-specificity, location, temperature, and rainfall (Fröhlich and Hyde, 2000; Taylor and Hyde, 2003).

Fungi common to palms are often non-specific in their host species associations. However, not only are cases of host species specificity notable (e.g. *Oxydothis alexandrarum* is commonly collected on, and thus far exclusive to *Archontophoenix alexandrae*), but also differences in the composition of assemblages of different palms has been noted (Yanna *et al.*, 2001a,b; Taylor and Hyde, 2003). At which level specificity occurs, e.g. host genus, subtribe, tribe, subfamily, is not yet obvious, but should become apparent as the mycota of more palm hosts are systemically investigated.

Comparison of fungi colonizing the brackish water palm Nypa fruticans *with* Eleiodoxa conferta

Nypa fruticans is a palm that grows in brackish water and extends into freshwater zones and the fungi colonizing it have been well documented by Hyde (1992), Hyde and Alias (1999) and Hyde *et al.* (1999). Sixty-four fungi have been recorded on *Nypa* from Brunei, Malaysia and Thailand, and the ten most common species collected in Brunei are listed in Table 2. Pilantanapak (2003) and Pilantanapak *et al.* (2005) have undertaken a quantitative study of the fungi growing on *Nypa* in Kamnanyiam, Samut Songkhram, Thailand and reported a wide variety of species. Some were present at a high frequency of occurrence: *Aniptodera nypae* (14%), *Astrosphaeriella striataspora* (26.4%), *Trichocladium nypicola* (34.8%), *Helicorhoidion nypicola* (34%), *Linocarpon nipae* (30.8%), *Oxydothis nypae* (26.8%), while others occurred at a lower

Table 2. Ten most common fungi on *Nypa fruticans* in Brunei (Hyde, 1992) and *Eleiodoxa conferta*.

Nypa fruticans	*Eleiodoxa conferta*
Linocarpon appendiculatum	*Cancellidium applanatum*
Astrosphaeriella striataspora	*Xylomyces aquaticus*
Oxydothis nypae	*Astrosphaeriella* sp.*
Lignincola laevis	*Stilbohypoxylon moelleri*
Linocarpon nipae	*Lophiostoma frondisubmersa*
Lulworthia grandispora	*Microthyrium* sp.
Halocyphina villosa	*Morenoina palmicola*
Helicascus nypae	*Phaeoisaria clematidis*
Fasciatispora nypae	*Nemania eleiodoxae**
Carinispora nypae	*Jahnula appendiculata*
Ascomycetes = 9 species	Ascomycetes = 7 species
Basidiomycetes = 1 species	Anamorphic fungi = 3 species
Total = 10 species	Total = 10 species

* New species awaiting description

frequency: *Lulworthia grandispora* (3.6%), *Neolinocarpon globosicarpum* (4%), *Aniptodera limnetica* (6%), *Dictyosporium elegans* (6.3%), and *Lignincola laevis* (8.8%). Some species were present at a very low frequency and included: *Linocarpon appendiculatum* and *Cirrenalia pygmea.*

A comparison of the fungi colonizing *Eleiodoxa conferta* with *Nypa fruticans* shows that there are few species/genera in common: *Astrosphaeriella*, *Linocarpon* and *Oxydothis*. However, the genera *Carinispora*, *Fasciatispora*, *Halocyphina*, *Helicascus*, *Lignincola* and *Lulworthia*, which are common on *Nypa,* have not been recorded on *E. conferta* (Table 2). These genera are more commonly found on substrata in marine habitats (Poonyth *et al.*, 1999) and may require sodium chloride for growth, while those on *E. conferta* may not be salt tolerant. The latter may be more tolerant to acidic waters, while marine fungi tend to occur in more alkaline waters.

Comparison of fungi on Eleiodoxa conferta *with freshwater fungi*

Fungi occurring on *Eleiodoxa conferta* in a peat swamp can be compared with those on different substrata in freshwater streams and rivers (Table 3). Common fungi in freshwater habitats include: *Aquaticola*, *Aniptodera*, *Dictyochaeta*, *Dictyosporium*, *Helicomyces*, *Savoryella* and *Sporoschisma* (Ho *et al.*, 1999a, b; Sivichai *et al.*, 2000; Luo *et al.*, 2004; Tsui *et al.*, 2004) but these genera are not common or even reported on *E. conferta*, and these differences can be attributed to habitat and the substrata sampled. Nevertheless,

Table 3. Ten most common fungi on wood in freshwater habitats in Hong Kong and Thailand. (Ho *et al.*, 2001, 2002; Sivichai, 1999)

Machilus velutina **Natural wood Hong Kong**	***Dipterocarpus alatus*** **Natural wood Thailand**	***Pinus velutina*** **Natural wood Hong Kong**
Savoryella lignicola	*Helicomyces roseus*	*Massarina ingoldiana*
Aniptodera chesapeakensis	*Trematosphaeria* sp.	*Sporoschisma nigroseptatum*
Sporoschisma floriformis	Sporodochial	*Spirosphaera floriformis*
Aquaticola rhomboida	*Dictyochaeta* sp.	*Aniptodera chesapeakensis*
Dictyosporium elegans	*Ophioceras dolichostomum*	*Lophiostoma bipolare*
Lophiostoma ingoldianum	Discomycete	*Aquaticola rhomboida*
Xylomyces chlamydosporus	*Bombardia* sp.	*Dictyosporium elegans*
Dictyosporium digitatum	Unidentified ascomycete	*Dictyosporium digitatum*
Cercophora appalachianensis	Pycnidial fungus 1	*Sporoschisma uniseptatum*
Kameshwaromyces globosus	*Tubeufia cylindrothecia*	*Savoryella lignicola*

Submerged test block ***Dipterocarpus alatus*** **Thailand**	**Submerged test block** ***Xylia dolabriformis*** **Thailand**
Trematosphaeria sp.	*Savoryella aquatica*
Unidentified ascomycete	*Trematosphaeria* sp.
Helicomyces roseus	*Biflagellospora gracilis*
Anthostomella aquatica	*Helicomyces roseus*
Sporodochial	*Dactylaria* sp.
Unidentified hyphomycete	*Scutisporus brunneus*
Dactylaria sp.	*Volutella* sp.
Ellisembia brachypus	*Dictyochaeta* sp.
Pycnidial fungus 1	*Ellisembia opaca*
Discomycete species	*Biflagellospora papillata*

fungi on *E. conferta* such as *Cancellidium applanatum*, *Xylomyces aquaticus*, *Phaeoisaria clematidis* and *Nawawia filiformis* were also found on other substrata in freshwater habitats but at a lower frequency than the fungi mentioned above.

Why are fungi more abundant on wet palm material?

Wet palm material was found to support more fungal records than damp or dry material. A number of factors may account for this: a water logged substratum, and the acidity of the water. Most fungi require a high relative

humidity for spore infection, spore germination, growth, and reproduction (Magan and Lacey, 1984). A further factor that may account for this is the acidic nature of the water in the peat swamp forest (pH 3-6 depending on season). pH has been shown to affect the growth of fungi for example some prefer alkaline conditions for growth: urea or ammonia fungi; fungi in tree holes (Hilton and Mill, 1986; Kladwang *et al.*, 2003). Preference for acidic conditions for the growth is more widely documented (Sabine and Eleanora, 2000).

Webster (1956) examined the moisture content of erect stems of the terrestrial grass *Dactylis* and showed that the atmosphere around the basal parts was often saturated with water up to about 10 cm above soil level. There was a steep decline in moisture content with increasing height above the soil level. As the stems of most grasses remain erect, the decline in the humidity gradient is marked (Dix and Webster, 1995; Van Ryckegem and Verbeken, 2005a,b). However, they found that some fungi were better able to colonize the upper internodes, with a lower humidity, than those confined to the lower regions of the grass. This could account for the variation in fungal communities on different parts of the grass with low or high moisture contents and may account for the vertical distribution of fungi on such a substratum. Similarly distinct zonation of fungi with height above water has been reported for the brackish water marsh grasses *Spartina* and *Juncus roemarianus* (Gessner and Kohlmeyer, 1976; Kohlmeyer and Volkmann-Kohlmeyer, 2002) and the marine angiosperms e.g. *Posidonia oceanica* and *Cymodocea nodosa* (Cuomo *et al.*, 1982). This has been attributed to the salinity of the water and degree of drying out of the aerial portions of the grasses.

Why are fungi more abundant on petioles of the palm?

Fungi were more prevalent on palm petioles (53%) than on the rachides (30%) and leaves (17%) and this may be accounted for by their anatomical structure. Leaves contain mainly parenchymatous cells that are thin-walled, with chloroplasts and rich in starch, while rachides and petioles have more sclerenchyma associated with the vascular bundles. Thus, the thicker cell walls may yield more nutrients for the sustained growth of fungi, in particular cellulose.

As with the grasses (Dix and Webster, 1995; Van Ryckegem and Verbeken, 2005a,b), there is a gradation in the water content of various parts of the palm. The base of the palm (petiole) is generally submerged or in contact with the peat swamp water and is therefore either waterlogged or high in moisture content and thus more suitable for fungal colonization. Petioles also contain vascular bundles that may take up water and retain moisture for a

longer time (Fisher *et al.*, 2002). The aerial dried palm leaves contain less moisture and are subject to a more rapid drying out than the rachides and petioles.

Tissue-specificity has been widely observed (e.g. in infructescences of *Protea* sp.: Lee *et al.*, 2005) and possible reasons for tissue-specificity, or recurrence, has been suggested for saprobic microfungi from palms (Fröhlich and Hyde, 2000; Yanna *et al.*, 2001a,b). Palm petioles are more robust in terms of structure than leaves and do not decompose as rapidly, thus allowing time for a more complex fungal population to form and for a succession of different fungi to develop (Fröhlich and Hyde, 1999). Furthermore, endophytes have been shown to be tissue-recurrent (Kumar and Hyde, 2004) and therefore may account for tissue recurrent saprobes if they change lifestyles at plant senescence.

In conclusion, the peat swamp palm *Eleiodoxa conferta* has been shown to support a rich fungal diversity comprising few dominant species and many rare species. This type of distribution is typical of other biodiversity studies that significantly differ from those on terrestrial and brackish water palms. This diversity is reflected in the number of new taxa described (*Chalara siamense*, *Custingophora undulatistipes*, *Dactylaria flammulicornuta*, *Dactylaria palmae*, *Dactylaria uliginicola*, *Goidanichiella fusiforma*, *Submersisphaeria palmae*, *Unisetosphaeria penguinoides* and *Vanakripa minutiellipsoidea*) while others await study and description (Hyde *et al.*, 2002; McKenzie *et al.*, 2002; Pinnoi *et al.*, 2003a,b, 2004).

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Phylogeny of a putative new species: *Nemania eleiodoxae*

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Abstract

Nemania eleiodoxae is described from decaying petioles of the palm *Eleiodoxa conferta* from Sirindhorn peat swamp forest, Narathiwat, Thailand. *Nemania eleiodoxae* is illustrated compared with similar species and illustrated. Sequence analyses from 18S, 28S rDNA and ITS region were analysed phylogenetically using Maximum Parsimony (MP) and Markov Chain Monte Carlo (MCMC) analysis. The new species belongs in the Xylariaceae based on molecular and morphological evidence.

Description

Ascomata superficial, solitary or gregarious, black, carbonaceous (Fig. 1), in vertical section 825-1375 um diam., 250-375 um high, subglobose, periphysate, ostiolar canal (Fig. 3). Peridium 22.5-62.5 um (x=32 um, n=15) wide, comprising several layers of compressed cells, black (Fig. 3). Paraphyses 2 um wide, hyphae-like, septate, numerous and embedded in a gelatinous matrix (Figs. 2, 4). Asci 107.5-155 x 6.2-10 um (x=131 x 8.5 um, n=25), 8-spores, cylindrical, pedicellate, unitunicate, apically truncate 5-2.5 um with a J+ (Figs. 5, 8). Ascospores 17.5 23 x 4.5-6.2 um, uniseriate, inequilaterally ellipsoidal, slightly curved, brown, unicellular, smooth walled, germ slit extending the full-length of ascospore. Thin mucilaginous sheath present (Figs. 9, 14).

The 18S rDNA dataset contained 30 taxa including 889 characters with 140 parsimony informative sites, 102 parsimony uninformative sites and 647 constant characters. The dataset was analysed under ST Matrix with gaps treated as new state criteria, which yielded 1 parsimonious tree of 903.7 steps with CI, RI, RC and HI of 0.646, 0.716, 0.462 and 0.354 respectively. Bootstrap values (generated from 1000 replicates) and Bayesian posterior probabilities were generated from 3000000 generarion. *Nemania eleiodoxae* is a member of the family Xylariaceae, clustered with *Rosellinia necatrix*, *Astrocystis cocoes*, *Xylaria polymorpha*, *Xylaria acuta* and *Xylaria curta* (FIG 15).

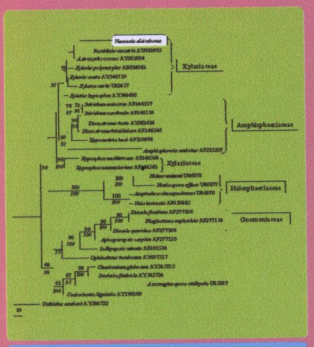
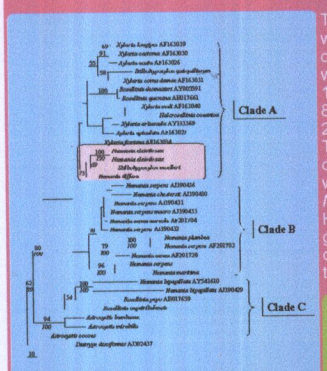


FIG 15 Phylogenetic tree based on 18S rDNA sequences. The tree was rooted with *Dothidea sambuci* and constructed under the Maximum Parsimony criterion with transition: transversion ratio as estimated by symmetric step matrix using (ST Matrix2:2). The number at each branch point represents percentage bootstrap support calculated from 1000 replicates (upper) and Bayesian posterior probabilities (below). Branch lengths are proportional to the numbers of nucleotide substitutions and are measured by scale bar (Bar; 10 % sequence divergence).

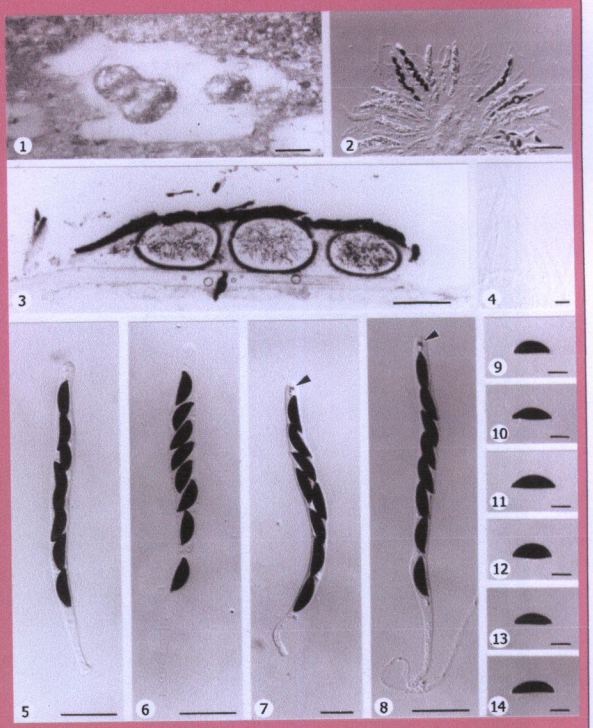


The ITS data consisted of 35 taxa with *Diatrype disciformis* as an outgroup. The aligned dataset was 494 characters, out of which 193 were parsimony informative, 87 parsimony uninformative and 214 constant characters. The ITS tree confirms monophyly of the two *N. eleodoxae* strains, with *Stilbohypoxylon moelleri* and *N. diffusa* as a sister group. However, *N. eleiodoxae* does not group within the main *Nemania* clade, but is basal to the *Xylaria* clade (FIG 17).

FIG 17. Phylogenetic tree based on ITS1-5.8S-ITS2 sequences. The tree was rooted with *Diatype disciformis* and constructed under unweighted Maximum Parsimony criterion. The number at each branch point represents percentage bootstrap support calculated from 1000 replicates (upper) and Bayesian posterior probabilities (below). Branch lengths are proportional to the numbers of nucleotide substitutions and are measured by scale bar (Bar; 10 % sequence divergence).

Introduction

Nemania species have been described (Xylariaceae, Xylariales) from a wide range of substrata and world wide in distribution (Kirk et al., 2001). A survey of fungi on *E. conferta* yielded 114 species including an undescribed *Nemania* sp.



The 28S DNA matrix consisted of 40 taxa with *Dothidea sambuci* as an outgroup. The aligned dataset was 836 characters, out of which 220 were parsimony informative, 80 parsimony uninformative and 536 constant characters. The tree show 915 steps with CI, RI, RC and HI of 0.477, 0.697, 0.332 and 0.523 respectively. Bootstrap values (generated from 1000 replicates) and Bayesian posterior probabilities were generated from 3000000 generarion. *Nemania eleiodoxae* is closely related to *Rosellinia necatrix* and *Astrocystis cocoes* but this clade has weak maximum parsimony bootstrap support, however they have Bayesian posterior probabilities support of 66%. *Astrocystis cocoes* and *Rosellinia necatrix* clustered together with Bayesian posterior probabilities support 80% (FIG 16).

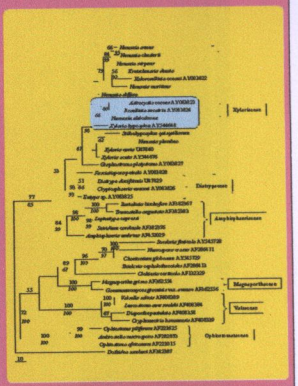


FIG 16. Phylogenetic tree based on partial 28S rDNA sequences. The tree was rooted with *Dothidea sambuci* and constructed under the Maximum Parsimony criterion. The number at each branch point represents percentage bootstrap support calculated from 1000 replicates (upper) and Bayesian posterior probabilities (below). Branch lengths are proportional to the numbers of nucleotide substitutions and are measured by scale bar (Bar; 10 % sequence divergence).

Discussion

Morphlogically (stromata, asci and ascospores) *Nemania eleiodoxae* is similar to *N. maritima* and *N. confluens*. However, *N. eleiodoxae* differs from *N. maritima* in having longer asci 107.5-155 x 6.2 10 um vs. 80-100 x 6 9 um, and ascospores 17.5-23 x 4.5-6.2 um vs. 9-12 x 5 6(-6.5) um. It is distinguished from *N. confluens* in having narrower asci 107.5-155 x 6.2-10 um vs. 105-125 x 10-12 um, longer and narrower ascospores 17.5-23 x 4.5 6.2 um vs. 15-18 x 8 9 um. Further more *N.eleiodoxae* is distinguished from other *Nemania* species in having a thin mucilaginous sheath. *N. confluens* also differs from *N. maritima* in having soft to woody inter-perithecial tissue, bigger, brown to dark brown ascospores, with a longer germ slit longer. *N. maritima* has carbonized inter-perithecial tissue, smaller, light brown to brown, and a germ slit shorter than 2/3 the spore-length or it may be seemingly lacking.

Granmo et al. (1999) and Ju and Rogers (2000) respectively have suggested that *N. confluens* and *N. maritima* should be segregated from *Nemania*. *N. maritima* because of its lack of an anamorph, production of the teleomorph in culture and its maritime habitat on mangrove wood. However, no strains of *N. confluens* were available for sequencing and further studies are required to resolve its taxonomic position within the genus. The position of *N. eleiodoxae* in the genus may also be questioned as the ascospores have pointed apices and are surrounded by a thin mucilaginous sheath.

Acknowledgments

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Endophytes of peat swamp palms: *Licuala longecalycata* and *L. spinosa*

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INTRODUCTION

Endophytes are defined as fungi colonizing healthy plant tissue without causing overt symptoms in or apparent injury to the host (Bills, 1996). Endophytes are generally not considered organ-specific, and it is likely that many of the species isolated from stems also occur in leaves. It is recognized that many fungi referred to as endophytes represent latent infections by plant pathogenic fungi. Many endophytic fungi remain quiescent within their hosts until the host dies (Dix and Webster, 1995).

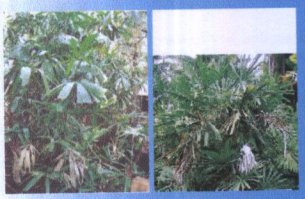

Figure 1. *Licuala longecalycata* palm (left) and *L. spinosa* (right).

Xylaria species are common endophytes in many tropical plants, including palms, orchids, bromeliads, aroids, and ferns (Dreyfuss & Petrini, 1984; Rodrigues & Samuels, 1990; Rodrigues, 1994; Richardson & Currah, 1995). Many groups of fungi exist as endophytes, though most are ascomycetes. Well-known examples are clavicipitaceous species that inhabit grasses. However, the ecology and distribution of most groups of endophytic fungi remain poorly known.

One hypothesis for the role of xylariaceous endophytes is that they are quiescent colonizers and will later become saprobes and decompose cellulose and lignin when the plant begins to senesce (Petrini *et al*., 1995; Whalley, 1996). However, growing evidence suggests that some xylariaceous fungi may exist solely as endophytes (Rogers, 2000).

MATERIAL AND METHODS

The diversity of endophytic fungi was estimated in fronds of the fan palms (Licuala longecalycata and L. spinosa). All fronds were washed in running tap water.

The diversity of endophytic fungi was estimated in fronds of the fan palms (*Licuala longecalycata* and *L. spinosa*). All fronds were washed in running tap water. Plant tissues were rinsed with 95% ethanol for 1 minutes, then surface disinfected with sodium hypochloride solution (4% available Cl-) for 15 minutes (petioles) and 10 minutes (leaves), rinsed once in 95% ethanol for 30 seconds, and finally, in sterile distilled water. Palm discs (5 per plate) were then transferred to Petri dishes containing potato dextrose agar (PDA) with streptomycin sulphate to inhibit bacterial growth. Endophytes grew out of the after a week at 25-28 C. Following incubation, fungal isolates recovered from each fragment were purified and grouped on the basis of phenotypic characteristics, e.g. sporulation, colony morphology, colony colour and growth rate. Isolates representing each fungal group of interest were selected for further identification by morphological traits (classic taxonomy) and/or rDNA sequencing.

Figure 2. Colonies of selected endophytes on potato dextrose agar

MOLECULAR STUDY

DNA extraction, amplification and sequencing : DNA extraction was performed by following a modified protocol as defined and outlined by Jeewon et al 2004. Partial sequences from two different regions of the rDNA molecule (characterised by different rates of evolution) were amplified. Primer pairs LROR and LR5 primer pairs were used to amplify a segment of the large 28S subunit and ITS 4 and ITS 5 were used to generate nucleotides from the complete ITS including 5.8S regions.

Phylogenetic analysis . Sequences generated from different primers were analyzed with other sequences obtained from the GenBank. Multiple alignment was done in BioEdit and Clustal X and analyses were performed in PAUP* 4.0b10. Maximum Parsimony and Markov chain Monte Carlo analysis were conducted using heuristic searches as implemented in PAUP. Analyses were done under different parameters. Clade stability was assessed in a bootstrap analysis with 1000 replicates, random sequence additions with maxtrees set to 1000 and other default parameters as implemented in PAUP*. Kishino-Hasegawa (KH) tests and Templeton tests were performed in order to determine whether trees inferred from the different tree building methods were significantly different. Trees were viewed in Treeview.

RESULTS

A. ISOLATION OF STRAINS

	L. longecalycata	L. spinosa
Total isolates	147	1289
Number of morpho strains	-*	195
Xylariaceous morphs	22	75

* Morpho types not documented

In our study of fungi colonizing peat swamp palms, no saprobic Xylaria species have been recovered (Pinnoi *et al*., 2006; Pinruan *et al*., 2007), although over 350 species have been identified

B. INDUCTION OF SPORULATION OF XYLARIACEOUS STRAINS.

Growth of 10 selected xylariaceous strains on palm trunks resulted in the formation of sterile stromata, that were morphologically distinct. Thus confirming they were different species

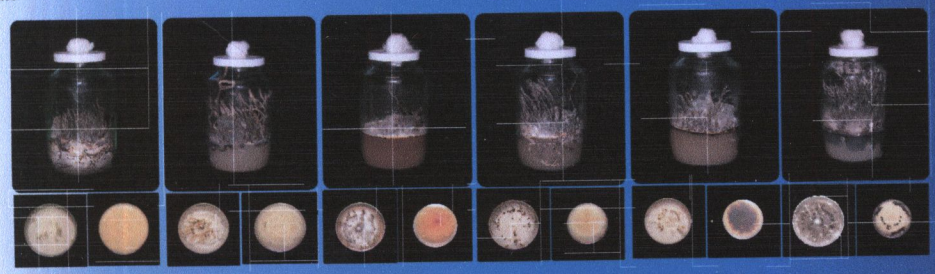

Figure 3. Morph 130 Figure 4. Morph 144. Figure 5. Morph 158. Figure 6 . Morph 161. Figure 7. Morph 165. Figure 8. Morph 190.

C. MOLECULAR SEQUENCE IDENTIFICATION OF THE SELECTED XYLARIACEOUS STRAINS.

Ten sterile thought to be xylariaceous species were sequenced and aligned (Figs 9-10). ITS sequence data confirms that the selected strains are referable to the Xylariaceae (8 strains) and Clypeosphaeriaceae (2 strains) (Xylariales) (Fig. 9). Within the Xylariaceae clade three subclades are recognized:

1. Strain 3LV3.2 groups with *Halorosellinia oceanica* and Kretschmaria deusta while strain 3LV3.1 groups with *Nemania diffusa* (Clade A, Fig. 10).
2. Strains 5L3V2.1 and 1L3V1.1 group with *Xylaria curta* with high bootstrap support (Clade C).
3. Strains 4L3V2.1 and *Nemania eleiodoxae* are basal to the main Xylariaceae clade (Clade C).
4. *Nemania maritima* groups with *Astrocystis cocoes*, while 2L4V4.1 is basal to this group (Clade D).

Within the Clypeosphaeriaceae two strains (BL3V3 and 2L41V3.1 group with Clypeopshaeria philly with high bootstrap support.

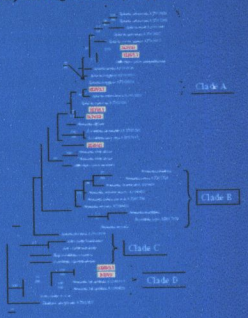

Figure 9. Phylogenetic tree based on ITS1-5.8S-ITS2 rDNA sequences. The tree was rooted with *Diatrype disciformis* and constructed under the Unweighted Maximum Parsimony criterion. The number at each branch point represents percentage bootstrap support calculated from 1000 replicates (upper) and Bayesian posterior probabilities (below). Branch lengths are proportional to the numbers of nucleotide substitutions and are measured by scale bar (Bar; 10 % sequence divergence).

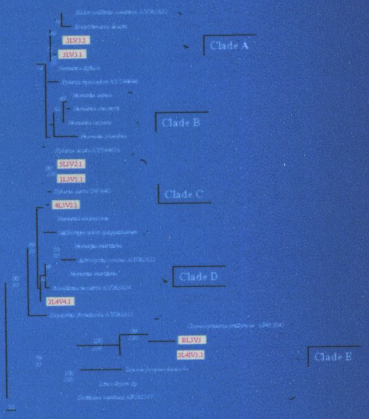

Figure 10. Phylogenetic tree based on 28S rDNA sequences. The tree was rooted with *Dothidia sambuci* and constructed under the Unweighted Maximum Parsimony criterion. The number at each branch point represents percentage bootstrap support calculated from 1000 replicates (upper) and Bayesian posterior probabilities (below). Branch lengths are proportional to the numbers of nucleotide substitutions and are measured by scale bar (Bar; 10 % sequence divergence).

CONCLUSIONS

The palms *L. longecalycata* and *L. spinosa* have been shown to support a wide range of endophytes, a siginificant number being xylariaceous species. Sequence data (LSU and ITS rDNA) confirms that ten strains of these are referable to the either the Xylariaceae or Clypeosphaeriaceae (Xylariales). Growth of these strains on palm stems in culture, demonstrated their ability to produce sterile stromata, all with a different morphology, indicating they were separate species.

Future work will include sequencing of further sterile xylariaceous strains in order to resolve their identified.

ACKNOWLEDGEMENTS

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Basidiomycetes on palms and bamboo in Thailand, with special reference to the phylogeny of *Ganoderma colossus* and *G. tsunodae*

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INTRODUCTION

Rostrup (1902) was the first to describe the larger basidiomycetes of Thailand, with subsequent studies by Heim (1962), Hjortstam and Ryvarden (1982), and Bandoni (1998). More recent studies are reviewed by Desjardin et al. (2004), who estimate that some 300 species are now known for the principality and probably accounting for only 20% if its estimated basidiomycete

OBJECTIVES

1.To document saprobic basidiomycetes on palms and bamboo in Thailand.
2.To determine the phylogenetical relationships of *Ganoderma colossus* and *Ganoderma tsunodae*.

DIVERSITY OF BASIDIOMYCETES ON BAMBOO AND PALMS IN THAILAND

Collection of samples

Basidiocarps on bamboo and palm material were collected from a variety of national park forests throughout Thailand i.e. Khao Yai National Park, Nakhon Ratcha-Sima; Trang and Krabi Province (with particular emphasis on oil palm, *Elaeis guineensis*); Nan Province; Koh Chang National Park, Trat Province and Mushroom Research Center,Chiang Mai Province. All specimens were deposited in the BIOTEC Bangkok Herbarium (BBH).

Isolation of basidiomycetes

Single spore isolates were made of all species on : Malt Extract Agar or Potato Dextrose Agar with the addition of antibiotics. Cultures are maintained in the BIOTEC Culture Collection (BCC).

RESULTS

BASIDOMYCETES RECORDED ON BAMBOO AND PALMS IN THAILAND

Basidiomycetes on bamboo	Basidiomycetes on palms	
Campanella junghunii	*Aurificaria indica*	*Cymatoderma* sp
Dictyophora indusiata	*Coriolopsis? sanguinaria*	*Dictyoparus* sp.
Favolaschia tonkinensis	*Dictyophora indusiata*	*Ganoderma* sp. 1
Flavodon flavus	*Flavodon cervinogilvus*	*Ganoderma* sp. 2
Grammothele fuligo	*Ganoderma australe*	*Hymenochaete* sp. 1
Hexagonia apiaria	*Ganoderma cf. boninense*	*Hymenochaete* sp. 2
Marasmius siccus	*Ganoderma colossus*	*Trametes* sp. (new species?)
Schizophyllum commune	*Grammothele fuligo*	*Trametes* sp.
Serpula eurocephala	*Hexagonia cf. tenuis*	Corticiaceae: 12 unidentified species
Crinipellis sp.	*Lenzites acutus*	
Dictyopanus sp.	*Microporus xanthopus*	
Entoloma sp.	*Phellinus noxius*	
Marasmius sp. 1	*Phlebia strigosozonata*	
Marasmius sp. 2	*Polyporus arcularius*	
Perenniporia sp. (new species?)	*Pycnoporus sanguineus*	
Pterula sp.	*Rigidoporus lineatus*	
Ramaria sp.	*Schizophyllum commune*	
Stereopsis sp.	*Trametes cf. hirsuta*	
Agaricales: 10 unidentified species	*Ceriporiopsis* sp. ?	
Corticiaceae: 3 unidentified species	*Coriolopsis* sp.	
Total 31 species	**Total 36 species**	

ILLUSTRATIONS OF SELECTED THAI BASIDIOMYCETES ON PALMS AND BAMBOO

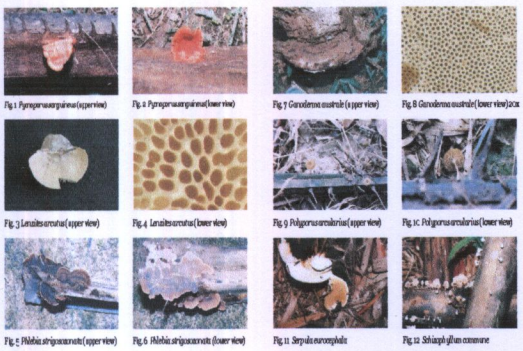

Fig.1 *Pyroporus sanguineus* (upper view) Fig. 2 *Pyroporus sanguineus* (lower view) Fig. 7 *Ganoderma australe* (upper view) Fig. 8 *Ganoderma australe* (lower view) 2cm
Fig. 3 *Lenzites acutus* (upper view) Fig. 4 *Lenzites acutus* (lower view) Fig. 9 *Polyporus arcularius* (upper view) Fig. 10 *Polyporus arcularius* (lower view)
Fig. 5 *Phlebia strigosozonata* (upper view) Fig. 6 *Phlebia strigosozonata* (lower view) Fig. 11 *Serpula eurocephala* Fig. 12 *Schizophyllum commune*

Fig. 23 Consensus tree of the maximum parsimonious tree of the nuclear ITS regions of the rRNA gene of *Ganoderma* spp.

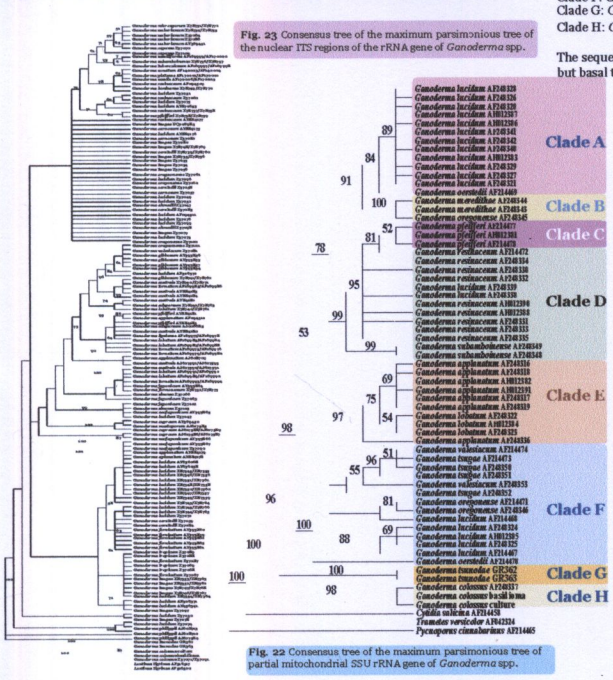

Fig. 22 Consensus tree of the maximum parsimonious tree of partial mitochondrial SSU rRNA gene of *Ganoderma* spp.

PHYLOGENETIC RELATIONSHIPS OF *GANODERMA* SPECIES

Ganoderma colossus

Ganoderma colossus was collected on coconut palm in Morib mangrove, Malaysia (June 2005) (Figs. 13 and 14), and isolated into axenic culture (BCC 17711, BCC 18005, BCC 18006, BCC 18007, BCC 18008). An unusual feature of the strain was the massive production of golden brown spiny-verrucose asexual spores (Figs. 16).

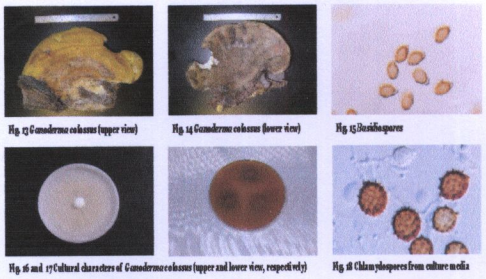

Fig. 13 *Ganoderma colossus* (upper view) Fig. 14 *Ganoderma colossus* (lower view) Fig. 15 Basidiospores
Fig. 16 and 17 Cultural characters of *Ganoderma colossus* (upper and lower view, respectively) Fig. 18 Chlamydospores from culture media

Morphological features

Ganoderma colossus

Basidiocarp annual, dimidiate to semi-circular, 200 wide x 300 long x 73 mm thick, pilear surface yellowish-brown to yellow, smooth, laccate to dull, pileus margin thin, cutis very thin, context chalky, pale brown and light in weight (Figs. 13 and 14); **Pores** 5-13 mm long, round to angular, 1-3 pores/mm, dark brown when dried; **Basidiospores** truncate to sub-globose, thick-walled (double layers), exporeidium smooth and hyaline, endoperidium, 11-14 x 9-11 mm (N=38) (Figs. 15); **Hyphal system** dimitic; generative hyphae hyaline, thin-walled with clamp connections, skeletal hyphae thick-walled, solid, 2.5-5 mm wide. Chlamydospores in basidiocarps not present. **Cultural characters** colony pale brown and covered with white mycelium, reverse darker, producing golden-brown spores in culture on both PDA and MEA, guttation of brown droplets on mycelium (Figs. 16 and 17). Chlamydospores smooth, rough, warted or spiny, globose, subglobose or fusiform, 6-9 mm (N=41), thin-walled when young and thick-walled when old, hyaline to brown, spores produced on the apex of hyphal branches or intercalary on hyphae, hyaline when young and pale brown to brown when mature, clamp connections present, colony pale brown and covered with white mycelium, reverse darker, produce gold-brown spores in culture on both PDA and MEA, guttation of brown droplets on mycelium (Figs. 18).

Ganoderma tsunodae

Basidiocarp annual, dimidiate, flabelliform to spathulate, applanate, pilear surface dark cinnamon to light brown, glabrous, context white; **Pores** 10-15 mm long, round to angular, 4-5 pores/mm, pore surface white, fibrous-corky and rigid when dried; **Basidiospores** ellipsoid to truncate, pale yellow, double wall; **Hypal system** dimitic.
Cultural characters colony effuse, cottony, mycelium white at the center and cream at the margin, and producing a yellow pigment in agar (Figs. 20 and 21). No asexual spores.

Fig. 19 *Ganoderma tsunodae* Fig. 20 and 21 Cultural character of *Ganoderma tsunodae* (upper and lower view, respectively)

Phylogeny of *Ganoderma colossus* and *Ganoderma tsunodae*

The nuclear ITS regions of the rRNA gene and partial mitochondrial SSU rRNA gene of both species using (maximum parsimony analyses) confirmed that they were distinct from other *Ganoderma* spp.

Mitochondrial SSU of gene rRNA

SSU sequence data identified 9 clades within the genus
Clade A: *Ganoderma lucidum* with *Ganoderma oerstedii* as a sister group
Clade B: *Ganoderma meredithiae* and *Ganoderma oregonense*
Clade C: *Ganoderma pfeifferi*
Clade D: *Ganoderma resinaceum* with *Ganoderma subamboinense* as a sister group
Clade E: *Ganoderma applanatum* with *Ganoderma lobatum* as a sister group
Clade F: *Ganoderma tsugae*, *Ganoderma lucidum* and other *Ganoderma* species
Clade G: *Ganoderma tsunodae*
Clade H: *Ganoderma colossus*

The sequence data places both *Ganoderma tsunodae* and *Ganoderma colossus* in the *Ganoderma* clade but basal to the other species (Fig. 22).

ITS region of rRNA gene

The inclusion of more sequences from the GenBank of the ITS rRNA gene, and more species, places *Ganoderma colossus* and *Ganoderma tsunodae* basal to all other *Ganoderma* species. Support for other species is weak and demonstrates the variability in species identification and quality of the downloaded sequences (Fig. 23).

CONCLUSION

1. Sixty-seven basidiomycetes were recorded on palm and bamboo collected in Thailand.
2. Only three basidiomycetes were common to both palms and bamboo.
3. Sequence data places *Ganoderma colossus* and *Ganoderma tsunodae* basal to all other *Ganoderma* species.
4. *Ganoderma* sequences from the GenBank indicate that some species were incorrectly identified; as they appear in more than one clade.
5. Molecular and morphological evidence indicates that *Ganoderma colossus* and *Ganoderma tsunodae* differ from other *Ganoderma* species and their taxonomic status needs to be reviewed.
6. Prolific asexual spore development in *Ganoderma colossus* is note worthy within the genus.

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