COMPARATIVE FUNGAL DIVERSITY STUDIES ON PALMS IN THAILAND

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(Final Report)

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1. SUMMARY OF REPORT

The biodiversity of fungi on selected palms: *Arenga pinnata, Borassodendron machadonis, Metroxylon sagu, Nypa fructicans*, and *Elaeis guineensis* were studied. Saprophytic fungi were made from 2 field collections on 21-25 July 2008 and 10-16 October 2008, resulting in 64 fungal records, in 26 fungal taxa from fifty-eight samples, but not including 70 basidiomycetes also collected. Two hundred and five axenic saprophytic str ains were isolated and deposited in the BIOTEC Culture Collection (BCC). One hundred and ninety three axenic morpho strains of endophytic fungi from oil palm (*Elaeis guineensis*) were also deposited in 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 deposit in the BIOTEC Culture Collection (BCC).

3. MATERIAL AND METHODS

3.1. Location

This study collected materials in Thai Forest including: Nan Sato Waterfall (Khao Ban That Wildlife Sanctuary Wildlife Sanctuary), Khao Chong Wildlife Development and Conservation Promotion Station, Sai Bor Village, and Sago forest at Na Yong District, Trang Province.

3.2. Sample collection

One collection of *Arenga pinnata* and *Borassodendron machadonis* were made in 21-25 July 2008. Another collection of basidimycetes on *Metroxylon sagu, Nypa fructicans*, and *Elaeis guineensis* in 10-16 October 2008. to undertake an experimental of study of saprophytic fungi on palm material. Material was divided into 3 parts: palm leaf, rachides and petioles under 2 micro-habitats: aerial (dry) and ground contact (damp). Collections of palm material were made and 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.

3.3. Isolation of fungi

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. Axenic cultures are maintained in the BIOTEC Culture Collection (BCC).

3.4. Identification and nomenclature of organisms

Most of the fungi were identified with on the morphology and their 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 et al. (1980), Ellis (1971; 1976) and Matsushima (1975; 1980; 1989; 1993; 1995).

3.5. Data analyses

Percentage abundance of taxa were calculated according to the following formula:

$$\textbf{Percentage abundance of taxon A} = \frac{\text{Occurrence of taxon A} \times 100}{\text{Occurrence of all taxon}}$$

Frequency of occurrence (%)

$$= \frac{\text{Total number of collections of particular taxon encountered}}{\text{Total number samples examined}} \times 100$$

3.6. Molecular study

3.6.1. Selected fungi for study

From the endophye study, the examination of the non sporulating strains 13 strains were identified as basidiomycetes by their clamp connections. Thirteen of these strains were selected for a molecular study.

3.6.2. Growth of fungi for the phylogenetic study

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

3.6.3. 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).

3.6.4. 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.

4. RESULTS

4.1 Biodiversity of fungi on selected palms

The biodiversity of fungi on the selected palms: *Arenga pinnata* and *Borassodendron machadonis* were studied. Saprophytic fungi were made from one field collection on 21-25 July 2008, resulted in 64 fungal records, from fifty-eight samples including *A. pinnata* (10 samples) and *Borassodendron machadonis* (48 samples) yielding 26 fungi. The fungi included: anamorphic fungi (19: 39, 61%) and ascomycetes (7: 25, 39%) (Figure 1). The most frequent taxa were *Stilbohypoxylon elaeicola*, *Spadicoides* sp., *Phaeoisaria clematidis*, *Brachysporiella gayana* and *Linocarpon* sp. Table 1 list the taxa found on the palms.

The percentage of fungi occurring on different parts of palm material was as follows: dry material supported 39% of the fungi recorded, and damp material had 61% (Figure 2). The percentage occurrence of fungi on different parts of palm was 87.5% was on petioles, 1.5% rachides and leaves 11% (Figure 3). Petioles supported the greatest number of collections and diversity. Table 2 records the occurrence of the fungi on different parts of the palm and various niches. Some fungi in this study are illustrated in Figures 4-5.

Fifty seven axenic strains were isolated and deposited in the BIOTEC Culture Collection (BCC) (Table 3).

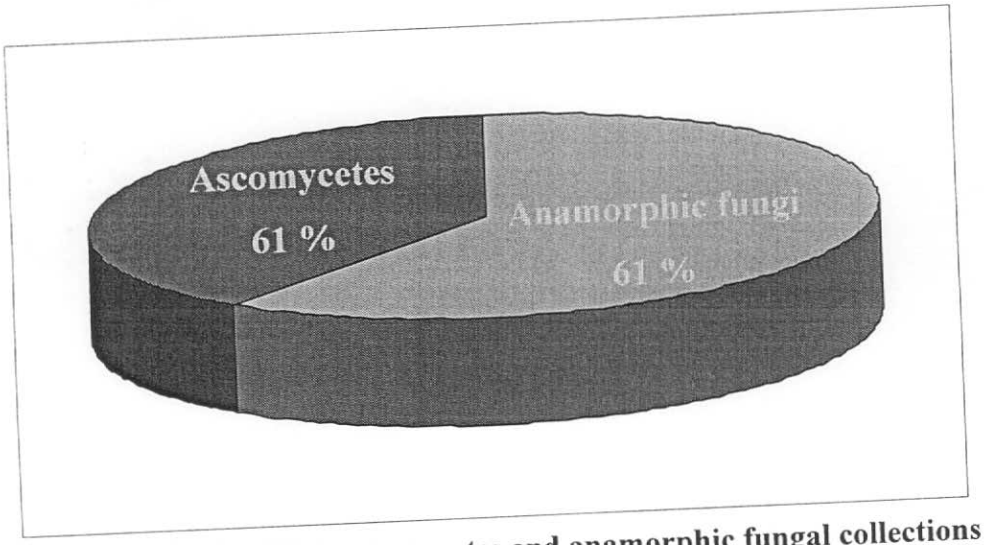

Figure 1. Percentage ascomycetes and anamorphic fungal collections occurring on the two palms

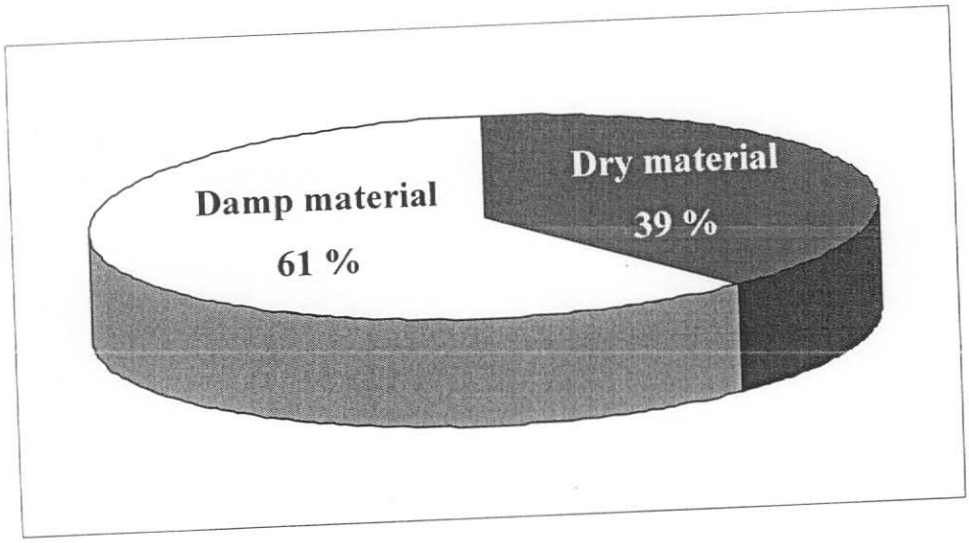

Figure 2. Percentage of fungi occurring on the two palms under different micro-habitats

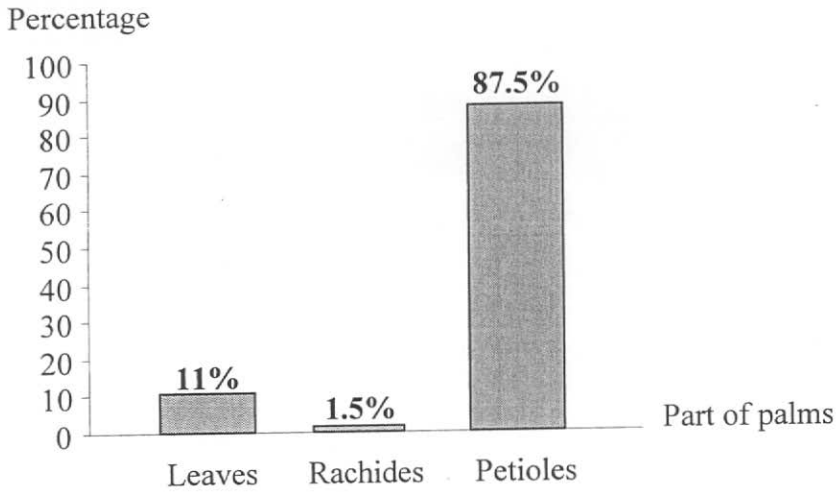

Figure 3. Percentage of fungi occurring on different parts of palm material

4.2. Selected fungi collected during this study

Fungi collected during the study include

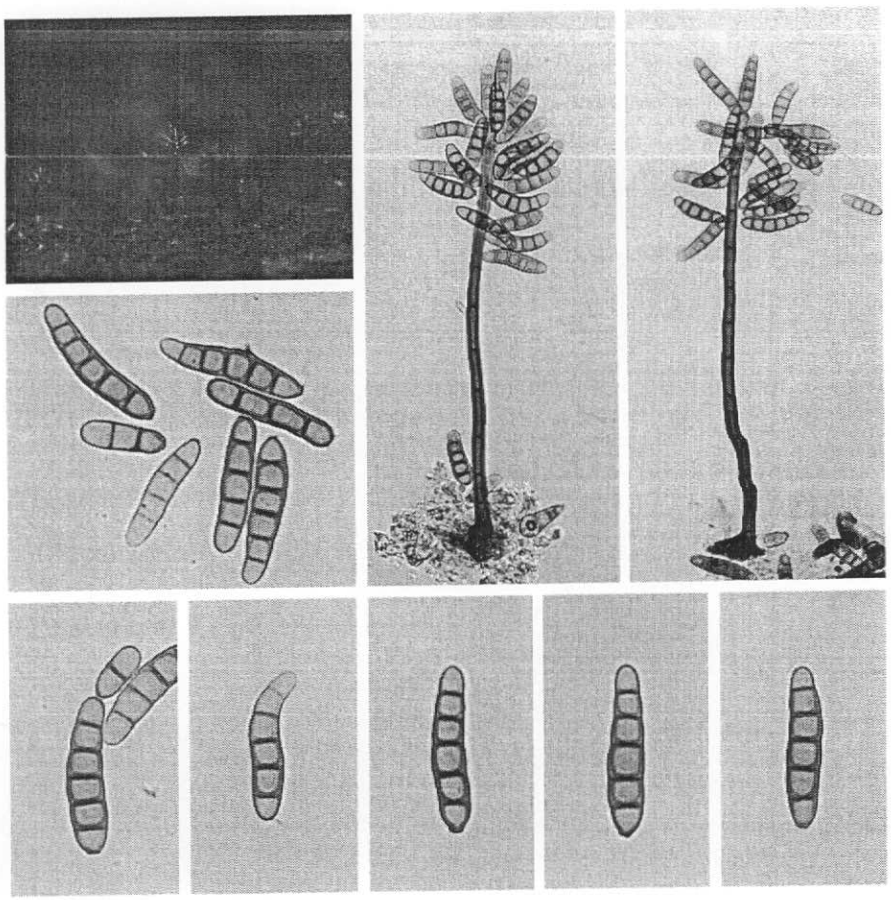

Figure 4. *Spadicoides* sp.

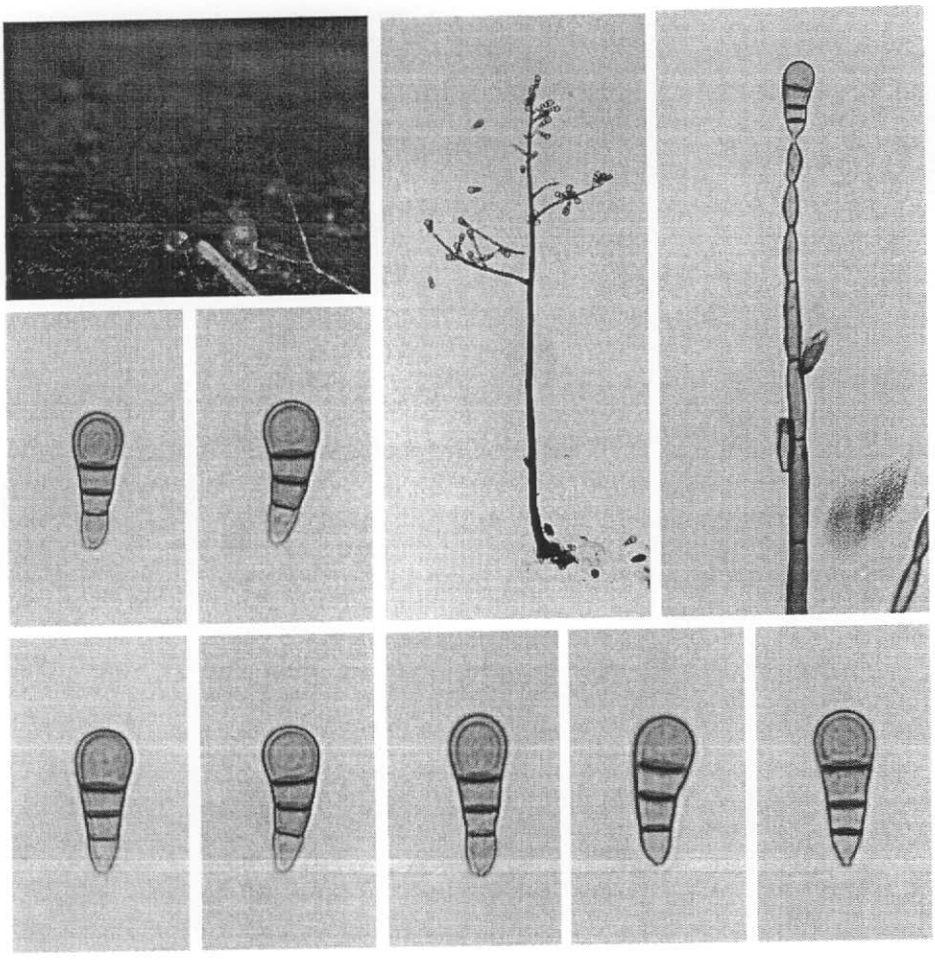

Figure 5. ***Brachysporiella gayana***

Table 1. Fungi occurring on the palms *Arenga pinnata and Borassodendron machadonis*

Fungus name	State	1*	2*	3*
Acrogenospora sp. (AOM00416)	Anamorph	1	1.6	1.7
Astrosphaeriella sp. (AOM00401)	Teleomorph	1	1.6	1.7
Astrosphaeriella sp. (AOM00424)	Teleomorph	1	1.6	1.7
Brachysporiella gayana (AOM00413)	Anamorph	5	7.8	8.6
Brachysporiella sp. (AOM00412)	Anamorph	1	1.6	1.7
Canalisporium caribense (AOM00418)	Anamorph	1	1.6	1.7
Drechslera sp. (AOM00404)	Anamorph	1	1.6	1.7
Gaeumannomyces sp. (AOM00422)	Teleomorph	1	1.6	1.7
Linocarpon sp. (AOM00402)	Teleomorph	4	6.3	6.9
Linocarpon sp. (AOM00425)	Teleomorph	1	1.6	1.7
Phaeoisaria clematidis (AOM00409)	Anamorph	6	9.4	10.3
Pseudobotrytis terrestris (AOM00420)	Anamorph	1	1.6	1.7
Spadicoides sp. (AOM00414)	Anamorph	11	17.2	19.0
Sporidesmiella hyalosperma (AOM00408)	Anamorph	1	1.6	1.7
Sporidesmium sp. (AOM00426)	Anamorph	1	1.6	1.7
Sporoschisma sp. (AOM00421)	Anamorph	1	1.6	1.7
Stilbohypoxylon elaeicola (AOM00411)	Teleomorph	17	26.6	29.3
Trichoderma sp. (AOM00415)	Anamorph	1	1.6	1.7
Unidentified (AOM00403)	Anamorph	1	1.6	1.7
Unidentified (AOM00405)	Teleomorph	1	1.6	1.7
Unidentified (AOM00406)	Anamorph	1	1.6	1.7
Unidentified (AOM00407)	Anamorph	1	1.6	1.7
Unidentified (AOM00410)	Anamorph	1	1.6	1.7
Unidentified (AOM00417)	Anamorph	1	1.6	1.7
Unidentified (AOM00423)	Anamorph	1	1.6	1.7
Xylomyces sp. (AOM00419)	Anamorph	1	1.6	1.7
Total records		64		
Anamorphic fungi		19: 39 (61%)		
Ascomycetes		7: 25 (39%)		
Total species		26		

1* = Number of records

2* = Percentage abundance

3* = Frequency of occurrence

Table 2. Distribution of fungi on different parts of the palms

Name of Fungus	Leaves		Petioles		Rachis		Total
	Dry	Damp	Dry	Damp	Dry	Damp	
Acrogenospora sp. (AOM00416)	–	–	–	1	–	–	1
Astrosphaeriella sp. (AOM00401)	–	–	–	1	–	–	1
Astrosphaeriella sp. (AOM00424)	–	–	–	1	–	–	1
Brachysporiella gayana (AOM00413)	–	–	1	4	–	–	5
Brachysporiella sp. (AOM00412)	–	1	–	–	–	–	1
Canalisporium caribense (AOM00418)	–	–	1	–	–	–	1
Drechslera sp. (AOM00404)	–	1	–	–	–	–	1
Gaeumannomyces sp. (AOM00422)	–	–	1	–	–	–	1
Linocarpon sp. (AOM00402)	–	–	1	3	–	–	4
Linocarpon sp. (AOM00425)	–	–	–	1	–	–	1
Phaeoisaria clematidis (AOM00409)	–	–	4	2	–	–	6
Pseudobotrytis terrestris (AOM00420)	–	–	1	–	–	–	1
Spadicoides sp. (AOM00414)	–	–	4	7	–	–	11
Sporidesmiella hyalosperma (AOM00408)	1	–	–	–	–	–	1
Sporidesmium sp. (AOM00426)	–	–	–	1	–	–	1
Sporoschisma sp. (AOM00421)	–	–	–	1	–	–	1
Stilbohypoxylon elaeicola (AOM00411)	–	–	5	12	–	–	17
Trichoderma sp. (AOM00415)	–	–	–	1	–	–	1
Unidentified (AOM00403)	–	1	–	–	–	–	1
Unidentified (AOM00405)	–	1	–	–	–	–	1
Unidentified (AOM00406)	1	–	–	–	–	–	1
Unidentified (AOM00407)	–	–	–	–	1	–	1
Unidentified (AOM00410)	1	–	–	–	–	–	1
Unidentified (AOM00417)	–	–	1	–	–	–	1
Unidentified (AOM00423)	–	–	1	–	–	–	1
Xylomyces sp. (AOM00419)	–	–	1	–	–	–	1
Total	3	4	21	35	1	0	64

The biodiversity of basidiomycetes from selected palms: *Nypa fructicans, Metroxylon sagu, Calamus* sp., and *Borassodendron machadonis* were studied from two field collections (21-25 July, and 10-16 October 2008), resulting in 70 taxa. The most frequent taxa were in the order Agaricales and Polyporales (Figure 6, Table 3).

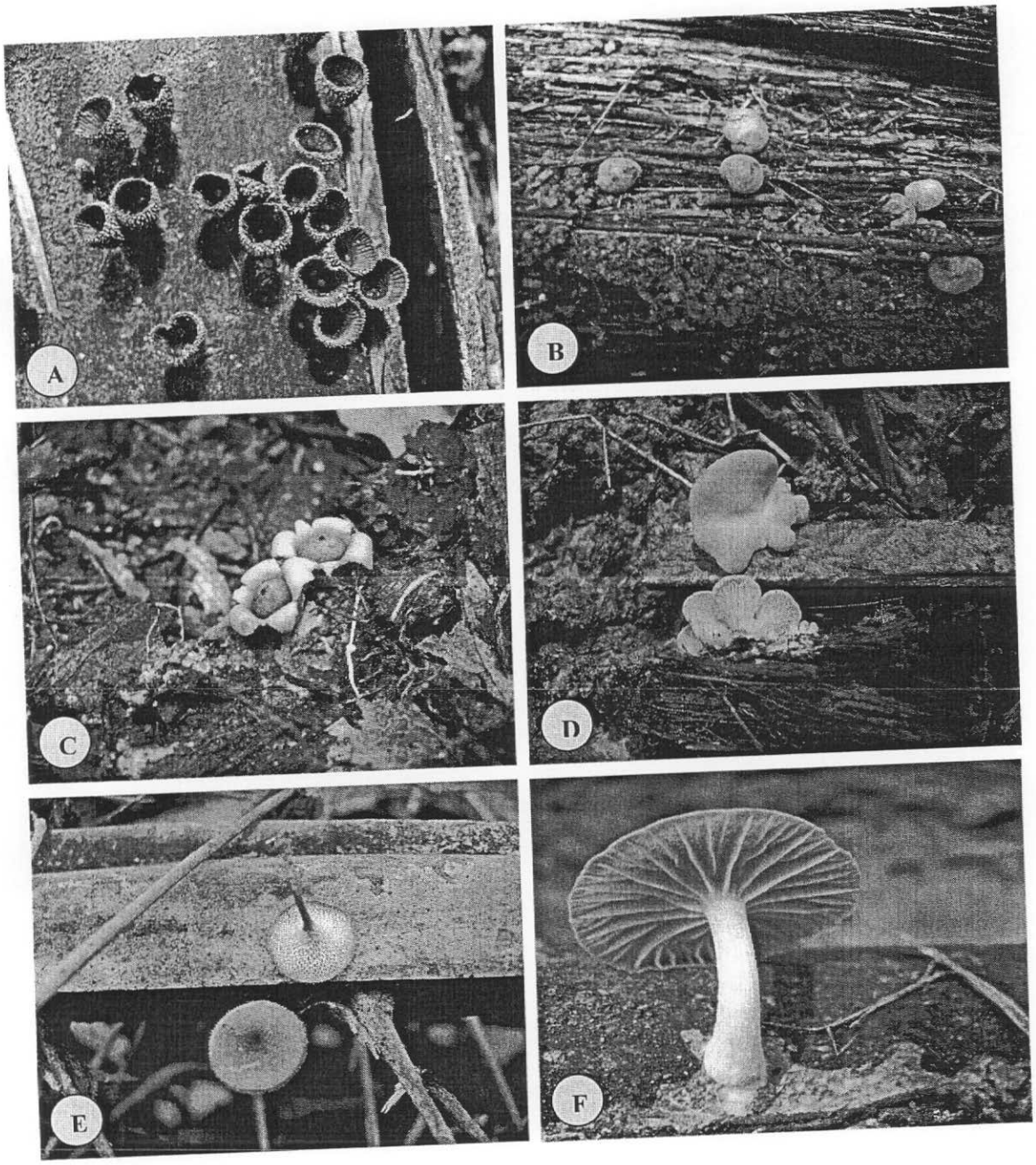

Figure 6. Selected basidiomycetes collected on different palms. A. *Cyathus montagnei*. B. *Mycocalia denudata*. C. *Geastrum* sp. D. *Pleurotus squarrosulus*. E. *Polyporus arcularius*. F. Unidentified Agaricales

4.3 Isolation of endophytic fungi

One hundred and ninety three strains of endophytic fungi from the oil palm, *Elaeis guineensis* were deposited in the BIOTEC Culture Collection (Figure 7, Table 3).

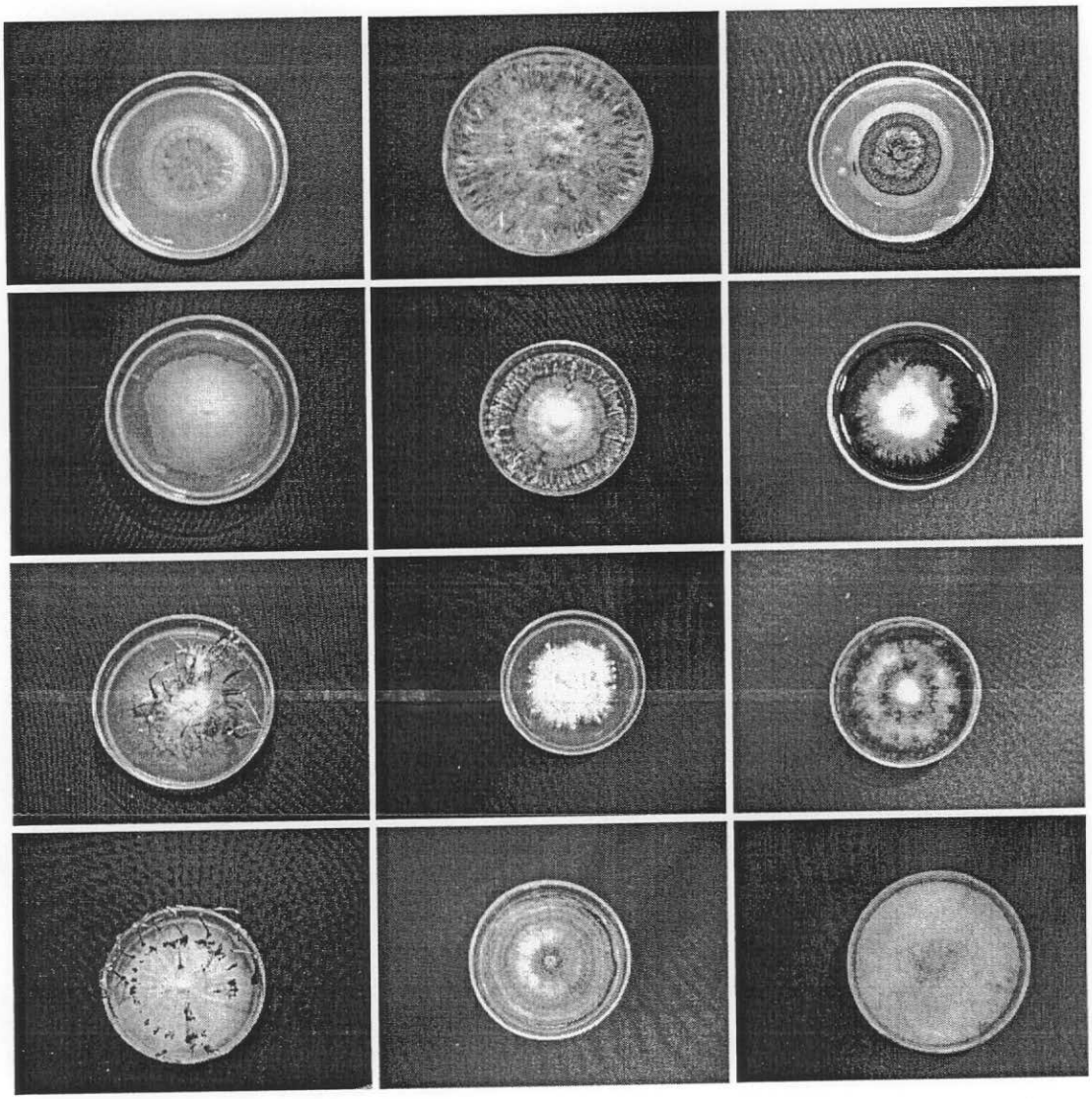

Figure 7. Selected example of the endophytes isolated from *Elaeis guineensis* (growth on PDA)

Table 3. List of axenic strains of saprophytes and endophyte deposited in the BIOTEC Culture Collection (BCC)

No.	Original code	BCC Code	Name	Location	Substrate
1	THP00496	33582	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
2	THP00497	33583	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
3	THP00498	33584	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
4	THP00499	33585	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
5	THP00500	33586	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
6	THP00501	33587	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
7	THP00502	33588	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
8	THP00503	33589	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
9	THP00504	33590	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
10	THP00505	33591	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
11	THP00506	33592	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
12	THP00507	33593	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
13	THP00508	33594	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
14	THP00509	33595	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
15	THP00510	33596	order Agaricales	Thale Ban National Park, Satun	Petiole (*Calamus* sp.)
16	THP00511	33597	order Agaricales	Thale Ban National Park, Satun	Petiole (*Calamus* sp.)
17	THP00512	33598	order Agaricales	Thale Ban National Park, Satun	Petiole (*Calamus* sp.)
18	THP00513	33599	order Agaricales	Thale Ban National Park, Satun	Petiole (*Calamus* sp.)
19	THP00514	33600	order Agaricales	Thale Ban National Park, Satun	Petiole (*Calamus* sp.)
20	THP00515	33601	order Agaricales	Thale Ban National Park, Satun	Petiole (*Calamus* sp.)
21	THP00516	33602	order Agaricales	Thale Ban National Park, Satun	Petiole (*Calamus* sp.)
22	THP00517	33603	order Agaricales	Thale Ban National Park, Satun	Petiole (*Calamus* sp.)
23	THP00518	34465	order Agaricales	Thale Ban National Park, Satun	Petiole (*Calamus* sp.)
24	THP00519	34466	order Agaricales	Thale Ban National Park, Satun	Petiole (*Calamus* sp.)
25	THP00520	34467	order Agaricales	Thale Ban National Park, Satun	Petiole (*Calamus* sp.)
26	THP00521	33604	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
27	THP00522	33605	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)

Table 3. Continued

No.	Original code	BCC Code	Name	Location	Substrate
28	THP00523	33606	order Agaricales	Thale Ban National Park, Satun	Petiole (Arecaceae)
29	THP00524	33607	Unidentified	Na Yong, Trang	Petiole (*Metroxylon sagu*)
30	THP00525	33608	Unidentified	Na Yong, Trang	Petiole (*Metroxylon sagu*)
31	THP00526	33609	Unidentified	Na Yong, Trang	Petiole (*Metroxylon sagu*)
32	THP00527	33610	order Polyporales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
33	THP00528	33611	order Polyporales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
34	THP00529	33612	order Polyporales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
35	THP00530	33613	order Polyporales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
36	THP00531	34468	order Polyporales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
37	THP00532	34469	order Polyporales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
38	THP00533	34470	order Agaricales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
39	THP00534	34471	order Agaricales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
40	THP00535	34472	order Agaricales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
41	THP00536	34473	order Agaricales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
42	THP00537	34474	order Agaricales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
43	THP00538	34475	order Agaricales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
44	THP00539	34476	order Agaricales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
45	THP00540	34477	*Grammothele fuligo*	Na Yong, Trang	Petiole (*Metroxylon sagu*)
46	THP00541	34478	*Grammothele fuligo*	Na Yong, Trang	Petiole (*Metroxylon sagu*)
47	THP00542	34479	*Grammothele fuligo*	Na Yong, Trang	Petiole (*Metroxylon sagu*)
48	THP00543	34480	order Agaricales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
49	THP00544	34481	order Agaricales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
50	THP00545	34482	order Agaricales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
51	THP00546	34483	order Polyporales	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
52	THP00547	34484	order Polyporales	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
53	THP00548	34485	order Polyporales	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
54	THP00549	34486	Unidentified	Huai Yot, Trang	Petioles (*Elaeis guineensis*)

Table 3. Continued

No.	Original code	BCC Code	Name	Location	Substrate
55	THP00550	34487	Unidentified	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
56	THP00551	34488	Unidentified	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
57	THP00552	34489	Unidentified	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
58	THP00553	34490	Unidentified	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
59	THP00554	34491	Unidentified	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
60	THP00555	34492	*Pycnoporus sanguineus*	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
61	THP00556	34493	*Pycnoporus sanguineus*	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
62	THP00557	34494	*Pycnoporus sanguineus*	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
63	THP00558	34495	order Agaricales	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
64	THP00559	34496	order Agaricales	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
65	THP00560	34497	order Agaricales	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
66	THP00561	34498	Unidentified	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
67	THP00562	34499	Unidentified	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
68	THP00563	34500	Unidentified	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
69	THP00564		Unidentified	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
70	THP00565		*Ganoderma boninense*	Thale Ban National Park, Satun	dead wood
71	THP00566		order Agaricales	Thale Ban National Park, Satun	branch
72	THP00567		order Polyporales	Thale Ban National Park, Satun	woody bridge
73	THP00568		*Ceriporiopsis resinascens?*	Thale Ban National Park, Satun	dead wood
74	THP00569		*Marasmiellus* sp.	Thale Ban National Park, Satun	dead wood
75	THP00570		order Agaricales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
76	THP00571		*Schizophyllum commune*	Na Yong, Trang	Petiole (*Metroxylon sagu*)
77	THP00572		*Mycocalia denudata*	Na Yong, Trang	Petiole (*Metroxylon sagu*)
78	THP00573		order Agaricales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
79	THP00574		*Schizophyllum commune*	Na Yong, Trang	Petiole (*Metroxylon sagu*)
80	THP00575		order Agaricales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
81	THP00576		order Agaricales	Na Yong, Trang	Petiole (*Metroxylon sagu*)

Table 3. Continued

No.	Original code	BCC Code	Name	Location	Substrate
82	THP00577		*Lentinus* sp.	Na Yong, Trang	woody bridge
83	THP00578		order Agaricales	Na Yong, Trang	Petiole (*Metroxylon sagu*)
84	THP00579		*Marasmiellus* sp.	Na Yong, Trang	Petiole (*Metroxylon sagu*)
86	THP00581		Unidentified	Kantang, Trang	Petioles (*Nypa fructicans*)
87	THP00582		order Agaricales	Kantang, Trang	Petioles (*Nypa fructicans*)
88	THP00583		*Grammothele fuligo*	Kantang, Trang	Petioles (*Nypa fructicans*)
89	THP00584		*Grammothele* sp.	Kantang, Trang	Petioles (*Nypa fructicans*)
90	THP00585		Unidentified	Thale Ban National Park, Satun	soil
91	THP00586		Unidentified	Thale Ban National Park, Satun	wood
92	THP00587		*Marasmius* sp.	Thale Ban National Park, Satun	leaf litter
93	THP00588		*Phellinus pachyphloeus*	Thale Ban National Park, Satun	dead wood
94	THP00589		*Phellinus pachyphloeus*	Thale Ban National Park, Satun	dead wood
95	THP00590		*Phellinus* sp.	Thale Ban National Park, Satun	dead wood
96	THP00591		*Phellinus* sp.	Thale Ban National Park, Satun	dead wood
97	THP00592		*Phellinus* sp.	Thale Ban National Park, Satun	dead wood
98	THP00593		Unidentified	Thale Ban National Park, Satun	dead wood
99	THP00594		Unidentified	Thale Ban National Park, Satun	dead wood
100	THP00595		Unidentified	Thammalung, Satun	branch
101	THP00596		Unidentified	Thammalung, Satun	branch
102	THP00597		Unidentified	Thammalung, Satun	branch
103	THP00598		Unidentified	Thammalung, Satun	woody bridge
104	THP00599		Unidentified	Thammalung, Satun	mangrove wood
105	THP00600		Unidentified	Na Yong, Trang	*Metroxylon sagu*
106	THP00601		Lentinus sp.	Na Yong, Trang	woody bridge
107	THP00602		Unidentified	Na Yong, Trang	*Metroxylon sagu*
108	THP00603		*Marasmiellus* sp.	Na Yong, Trang	*Metroxylon sagu*
109	THP00604		Unidentified	Na Yong, Trang	*Metroxylon sagu*
110	THP00605		*Dichomitus carvernulosus*	Kantang, Trang	Petioles (*Nypa fructicans*)
111	THP00606		*Dichomitus carvernulosus*	Kantang, Trang	Petioles (*Nypa fructicans*)
112	THP00607		*Dichomitus carvernulosus*	Kantang, Trang	Petioles (*Nypa fructicans*)
113	THP00608		*Dichomitus carvernulosus*	Kantang, Trang	Petioles (*Nypa fructicans*)
114	THP00609		order Agaricales	Kantang, Trang	Petioles (*Nypa fructicans*)
115	THP00610		order Polyporales	Kantang, Trang	Petioles (*Nypa fructicans*)

Table 3. Continued

No.	Original code	BCC Code	Name	Location	Substrate
116	THP00611		Unidentified	Kantang, Trang	Petioles (*Nypa fructicans*)
117	THP00612		Unidentified	Kantang, Trang	Petioles (*Nypa fructicans*)
118	THP00613		Unidentified	Kantang, Trang	Petioles (*Nypa fructicans*)
119	THP00614		Unidentified	Kantang, Trang	Petioles (*Nypa fructicans*)
120	THP00615		Unidentified	Kantang, Trang	Petioles (*Nypa fructicans*)
121	THP00616		order Agaricales	Kantang, Trang	Petioles (*Nypa fructicans*)
122	THP00617		*Grammothele* sp.	Kantang, Trang	Petioles (*Nypa fructicans*)
123	THP00618		*Grammothele* sp.	Kantang, Trang	Petioles (*Nypa fructicans*)
124	THP00619		*Grammothele* sp.	Kantang, Trang	Petioles (*Nypa fructicans*)
125	THP00620		*Dichomitus carvernulosus*	Kantang, Trang	Petioles (*Nypa fructicans*)
126	THP00621		*Dichomitus carvernulosus*	Kantang, Trang	Petioles (*Nypa fructicans*)
127	THP00622		*Dichomitus carvernulosus*	Kantang, Trang	Petioles (*Nypa fructicans*)
128	THP00623		*Dichomitus carvernulosus*	Kantang, Trang	Petioles (*Nypa fructicans*)
129	THP00624		*Dichomitus carvernulosus*	Kantang, Trang	Petioles (*Nypa fructicans*)
130	THP00625		*Dichomitus carvernulosus*	Kantang, Trang	Petioles (*Nypa fructicans*)
131	THP00626		*Dichomitus carvernulosus*	Kantang, Trang	Petioles (*Nypa fructicans*)
132	THP00627		Unidentified	Na Yong, Trang	*Metroxylon sagu*
133	THP00628		Unidentified	Na Yong, Trang	*Metroxylon sagu*
134	THP00629		Unidentified	Na Yong, Trang	*Metroxylon sagu*
135	THP00630		Mycocalia denudata	Na Yong, Trang	*Metroxylon sagu*
136	THP00631		Mycocalia denudata	Na Yong, Trang	*Metroxylon sagu*
137	THP00632		*Schizophyllum commune*	Na Yong, Trang	*Metroxylon sagu*
138	THP00633		*Schizophyllum commune*	Na Yong, Trang	*Metroxylon sagu*
139	THP00634		*Schizophyllum commune*	Na Yong, Trang	*Metroxylon sagu*
140	THP00635		*Schizophyllum commune*	Na Yong, Trang	*Metroxylon sagu*
141	THP00636		*Schizophyllum commune*	Na Yong, Trang	*Metroxylon sagu*
142	THP00637		*Schizophyllum commune*	Na Yong, Trang	*Metroxylon sagu*
143	THP00638		Agaricales	Na Yong, Trang	*Metroxylon sagu*
144	THP00639		Agaricales	Na Yong, Trang	*Metroxylon sagu*
145	THP00640		Unidentified	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
146	THP00641		Unidentified	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
147	THP00642		*Hymenochaete* sp.	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
148	THP00643		*Hymenochaete* sp.	Huai Yot, Trang	Petioles (*Elaeis guineensis*)
149	AOM00403.01		Unidentified	Pa-Lian, Trang	*Borassodendron machadonis*

Table 3. Continued

No.	Original code	BCC Code	Name	Location	Substrate
150	AOM00403.02		Unidentified	Pa-Lian, Trang	*Borassodendron machadonis*
151	AOM00403.03		Unidentified	Pa-Lian, Trang	*Borassodendron machadonis*
152	AOM00403.04		Unidentified	Pa-Lian, Trang	*Borassodendron machadonis*
153	AOM00404.01		*Drechslera*	Pa-Lian, Trang	*Borassodendron machadonis*
154	AOM00404.02		*Drechslera*	Pa-Lian, Trang	*Borassodendron machadonis*
155	AOM00404.03		*Drechslera*	Pa-Lian, Trang	*Borassodendron machadonis*
156	AOM00404.04		*Drechslera*	Pa-Lian, Trang	*Borassodendron machadonis*
157	AOM00406.01		*Unidentified*	Pa-Lian, Trang	*Borassodendron machadonis*
158	AOM00406.03		*Unidentified*	Pa-Lian, Trang	*Borassodendron machadonis*
159	AOM00408.01		*Sporidesmiella hyalosperma*	Pa-Lian, Trang	*Borassodendron machadonis*
160	AOM00408.02		*Sporidesmiella hyalosperma*	Pa-Lian, Trang	*Borassodendron machadonis*
161	AOM00408.03		*Sporidesmiella hyalosperma*	Pa-Lian, Trang	*Borassodendron machadonis*
162	AOM00408.04		*Sporidesmiella hyalosperma*	Pa-Lian, Trang	*Borassodendron machadonis*
163	AOM00409.01		*Phaeoisaria clematidis*	Pa-Lian, Trang	*Borassodendron machadonis*
164	AOM00409.02		*Phaeoisaria clematidis*	Pa-Lian, Trang	*Borassodendron machadonis*
165	AOM00409.03		*Phaeoisaria clematidis*	Pa-Lian, Trang	*Borassodendron machadonis*
166	AOM00409.04		*Phaeoisaria clematidis*	Pa-Lian, Trang	*Borassodendron machadonis*
167	AOM00412.01		Unidentified	Pa-Lian, Trang	*Borassodendron machadonis*
168	AOM00412.02		Unidentified	Pa-Lian, Trang	*Borassodendron machadonis*
169	AOM00412.03		Unidentified	Pa-Lian, Trang	*Borassodendron machadonis*
170	AOM00412.04		Unidentified	Pa-Lian, Trang	*Borassodendron machadonis*
171	AOM00414.01		*Spadicoides* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
172	AOM00414.02		*Spadicoides* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
173	AOM00414.03		*Spadicoides* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
174	AOM00414.04		*Spadicoides* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
175	AOM00415.01		*Trichoderma* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
176	AOM00415.02		*Trichoderma* sp.	Pa-Lian, Trang	*Borassodendron machadonis*

Table 3. Continued

No.	Original code	BCC Code	Name	Location	Substrate
177	AOM00415.03		*Trichoderma* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
178	AOM00415.04		*Trichoderma* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
179	AOM00416.01		*Acrogenospora* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
180	AOM00418.01		*Canalisporium* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
181	AOM00418.02		*Canalisporium* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
182	AOM00418.03		*Canalisporium* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
183	AOM00418.04		*Canalisporium* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
184	AOM00419.01		*Xylomyces* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
185	AOM00419.02		*Xylomyces* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
186	AOM00419.03		*Xylomyces* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
187	AOM00419.04		*Xylomyces* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
188	AOM00421.01		*Sporoschisma* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
189	AOM00421.02		*Sporoschisma* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
190	AOM00421.03		*Sporoschisma* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
191	AOM00421.04		*Sporoschisma* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
192	AOM00422.01		*Gaeumannomyces* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
193	AOM00422.02		*Gaeumannomyces* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
194	AOM00422.03		*Gaeumannomyces* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
195	AOM00422.04		*Gaeumannomyces* sp.	Pa-Lian, Trang	*Borassodendron machadonis*
196	AOM00424.01		*Astrosphaeriella* sp.	Na Yong, Trang	*Arenga pinnata*
197	AOM00424.02		*Astrosphaeriella* sp.	Na Yong, Trang	*Arenga pinnata*
198	AOM00424.03		*Astrosphaeriella* sp.	Na Yong, Trang	*Arenga pinnata*
199	AOM00425.01		*Linocarpon* sp.	Na Yong, Trang	*Arenga pinnata*
200	AOM00425.02		*Linocarpon* sp.	Na Yong, Trang	*Arenga pinnata*
201	AOM00425.03		*Linocarpon* sp.	Na Yong, Trang	*Arenga pinnata*
202	AOM00425.04		*Linocarpon* sp.	Na Yong, Trang	*Arenga pinnata*
203	AOM00426.01		*Sporidesmium* sp.	Na Yong, Trang	*Arenga pinnata*
204	AOM00426.02		*Sporidesmium* sp.	Na Yong, Trang	*Arenga pinnata*
205	AOM00426.03		*Sporidesmium* sp.	Na Yong, Trang	*Arenga pinnata*
206	EP 734	26661	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
207	EP 735	26662	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
208	EP 736	28484	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
209	EP 737	27950	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
210	EP 738	26588	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
211	EP 739	26663	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
212	EP 740	26664	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*

Table 3. Continued

No.	Original code	BCC Code	Name	Location	Substrate
213	EP 741	27951	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
214	EP 742	26665	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
215	EP 743	27952	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
216	EP 744	26666	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
217	EP 745	26667	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
218	EP 746	26668	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
219	EP 747	27953	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
220	EP 748	27954	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
221	EP 749		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
222	EP 750	30863	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
223	EP 751		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
224	EP 752	27955	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
225	EP 753	26669	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
226	EP 754	30864	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
227	EP 755	27956	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
228	EP 756		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
229	EP 757	28485	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
230	EP 758	27957	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
231	EP 759	28486	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
232	EP 760	27958	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
233	EP 761	27959	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
234	EP762	28143	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
235	EP763	28144	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
236	EP764	28145	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
237	EP765	28146	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
238	EP766	28147	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
239	EP767	28148	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
240	EP768	28149	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
241	EP769	28150	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
242	EP770	28151	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
243	EP771	28152	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
244	EP772	28153	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
245	EP773	28154	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
246	EP774	28155	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
247	EP775	28156	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
248	EP776	28157	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
249	EP777	28158	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
250	EP778	28159	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
251	EP779	28160	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
252	EP780	28161	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
253	EP781	28162	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
254	EP782	28163	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
255	EP783	28906	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
256	EP784	28487	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
257	EP785	28488	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
258	EP786	28489	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
259	EP787	28490	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
260	EP788	28491	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
261	EP789	28492	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
262	EP790	28493	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
263	EP791	28494	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
264	EP792	28495	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
265	EP793	28496	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
266	EP794	28497	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*

Table 3. Continued

No.	Original code	BCC Code	Name	Location	Substrate
267	EP795	28498	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
268	EP796	28499	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
269	EP797	28500	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
270	EP798	28501	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
271	EP799	28502	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
272	EP800	28907	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
273	EP801	28908	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
274	EP802	28909	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
275	EP803	28910	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
276	EP804	28911	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
277	EP805	28912	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
278	EP806	28913	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
279	EP807	28914	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
280	EP808	28915	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
281	EP809	28916	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
282	EP810	28917	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
283	EP811	28918	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
284	EP812	28919	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
285	EP813	28920	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
286	EP814	28921	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
287	EP815	28922	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
288	EP816	28923	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
289	EP817	28924	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
290	EP818	28925	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
291	EP819	29313	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
292	EP820	29314	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
293	EP821	29315	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
294	EP822	29316	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
295	EP823	29317	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
296	EP824	29318	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
297	EP825	29319	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
298	EP826	29320	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
299	EP827	29321	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
300	EP828	29322	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
301	EP829	29323	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
302	EP830	29324	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
303	EP831	29325	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
304	EP832	29326	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
305	EP833	29327	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
306	EP834	29328	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
307	EP835	29329	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
308	EP836	29330	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
309	EP837	29331	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
310	EP838	29332	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
311	EP839	29877	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
312	EP840	29878	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
313	EP841	29879	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
314	EP842	29880	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
315	EP843	29881	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
316	EP844	29882	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
317	EP845	29883	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
318	EP846	29884	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
319	EP847	29885	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
320	EP848	29886	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*

Table 3. Continued

No.	Original code	BCC Code	Name	Location	Substrate
321	EP849	29887	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
322	EP850	29888	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
323	EP851	29889	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
324	EP852	29890	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
325	EP853	29891	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
326	EP854	29892	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
327	EP855	29893	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
328	EP856	29894	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
329	EP857	29895	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
330	EP858	29896	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
331	EP859	29897	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
332	EP860	30865	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
333	EP861	30866	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
334	EP862	30867	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
335	EP863	30868	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
336	EP864	30869	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
337	EP865	30870	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
338	EP866	30871	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
339	EP867	30872	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
340	EP868	30873	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
341	EP869	30874	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
342	EP870	30875	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
343	EP871	30876	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
344	EP872	30877	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
345	EP873	30878	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
346	EP874	30879	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
347	EP875	30880	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
348	EP876	30881	Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
349	EP900		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
350	EP901		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
351	EP902		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
352	EP903		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
353	EP904		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
354	EP905		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
355	EP906		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
356	EP907		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
357	EP908		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
358	EP909		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
359	EP910		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
360	EP911		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
361	EP912		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
362	EP913		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
363	EP914		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
364	EP915		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
365	EP916		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
366	EP917		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
367	EP918		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
368	EP919		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
369	EP920		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
370	EP921		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
371	EP922		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
372	EP923		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
373	EP924		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
374	EP925		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*

Table 3. Continued

No.	Original code	BCC Code	Name	Location	Substrate
375	EP926		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
376	EP927		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
377	EP928		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
378	EP929		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
379	EP930		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
380	EP931		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
381	EP932		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
382	EP933		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
383	EP934		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
384	EP935		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
385	EP936		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
386	EP937		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
387	EP938		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
388	EP939		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
389	EP940		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
390	EP941		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
391	EP942		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
392	EP943		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
393	EP944		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
394	EP945		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
395	EP946		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
396	EP947		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
397	EP948		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*
398	EP949		Unidentified fungus	Huai Yot, Trang	*Elaeis guineensis*

Total: 205 saprophytes; 193 endophytes

4.4 Phylogenetic study

4.4.1. LSU phylogeny of basidiomycete endophytes

Thirteen LSU rDNA sequences of the basidiomycete endophytes were aligned along with representative taxa from eight major orders of the Basidiomycota comprising the *Agaricales*, *Atheliales*, *Auriculariales*, *Boletales*, *Hymenochaetales*, *Polyporales*, *Russulales* and *Sebacinales* (Figure 8). The endophytic basidiomycetes separated into two major lineages at the ordinal level, eleven isolates within the *Polyporales*, while two are well placed in the *Agaricales*. Two endophytes (8R 1/1 and 8R 1/2) nestled within the Polyporaceae with 90 % BS and 1.00 PP. Isolate 8R 1/1 clustered with *Trametes elegans* with high support (97 %BS and 1.00 PP) (Figure 8 subclade B), while 8R 1/2 formed a clade with *Pycnoporus* sequences, although the statistical support is low (Figure 8 subclade A). Nine endophyte isolates grouped with the *Fomitopsidaceae* with good statistical support (84% BS and 1.00 PP) (Figure 3 subclade C). Four isolates (8V 6/1, 10R 8/1, 7P 3/1 and 7R 9/1) clustered with three *Fomitopsis* species. Two strains (7R 8/1 and 9V 3/1) grouped together with 99 % BS

and 1.00 PP but showed no relationship to any known taxa. These two sequences formed a sister group with various *Piptoporus* species. Three strains including the endophytes 5V 3/3, 2IV 7/1 and 1P 1/1 grouped together with low support and showed no affinity with any subclade. Two endophytic fungi (2IV 2/1 and 2IV 2/2) grouped with members of the *Schizophyllaceae*, in the *Agaricales*, with high statistical support (100 % BS and 1.00 PP) (Figure 8 subclade D).

4.4.2. ITS phylogeny of endophytes within the Polyporaceae

The two endophyte isolates separated into two groups, 8R 1/1 with *Trametes* and 8R 1/2 within the *Pycnoporus* clade, with high support (100% BS and 1.00 PP) (Figure 9). Isolate of 8R 1/1 formed a clade with an unknown fungal endophyte sequence (DQ979682) with 85% BS and 0.98 PP support. This isolate also showed a relationship with *Trametes elegans* (AY68417) with good statistical support (100% BS and 1.00 PP). However, another *Trametes elegans* (isolated as saprobe from Thailand) and *Trametes* species were distantly placed in a lower subclade. Isolate 8R 1/2 grouped with seven sequences of *Pycnoporus sanguineus* which are monophyletic with good support. *Pycnoporus cinnabarinus* and *P. puniceus* clustered in a basal clade, each subclade monophyletic, and with high statistical support for both species (100% BS and 1.00 PP).

4.4.3. ITS phylogeny of endophyte within the Fomitopsidaceae

Four isolates (7R 9/1, 7P 3/1, 8V 6/1 and 10R 8/1) clustered with various *Fomitopsis* species, *i.e. Fomitopsis* sp., *F. palustris* and *F. ostreiformis* with good statistical support (100 % BS and 1.00 PP) (Figure 9 subclade A). Two strains (7R 8/1 and 9V 3/1) are monophyletic with 100 % BS and 1.00 PP and formed a clade with four *Fomitopsis pinicola* strains (Figure 9 suclade B). Finally three isolates (2IV 7/1, 5V 3/3 and 1P 1/1) are monophyletic with high statistical support (99 % BS and 1.00 PP) and grouped with *Fomitopsis meliae* (DQ491421) with good support (100 % BS and 1.00 PP) (Figure 10 subclade C). *Fomes* and *Antrodia* species formed a basal clade.

4.4.4. ITS phylogeny of endophyte within the Schizophyllaceae

Based on ITS sequence analysis, isolates 2 IV 2/1 and 2 IV 2/2 grouped with *Schizophyllum* species with 99% BS and 1.00 PP support (Figure 11).

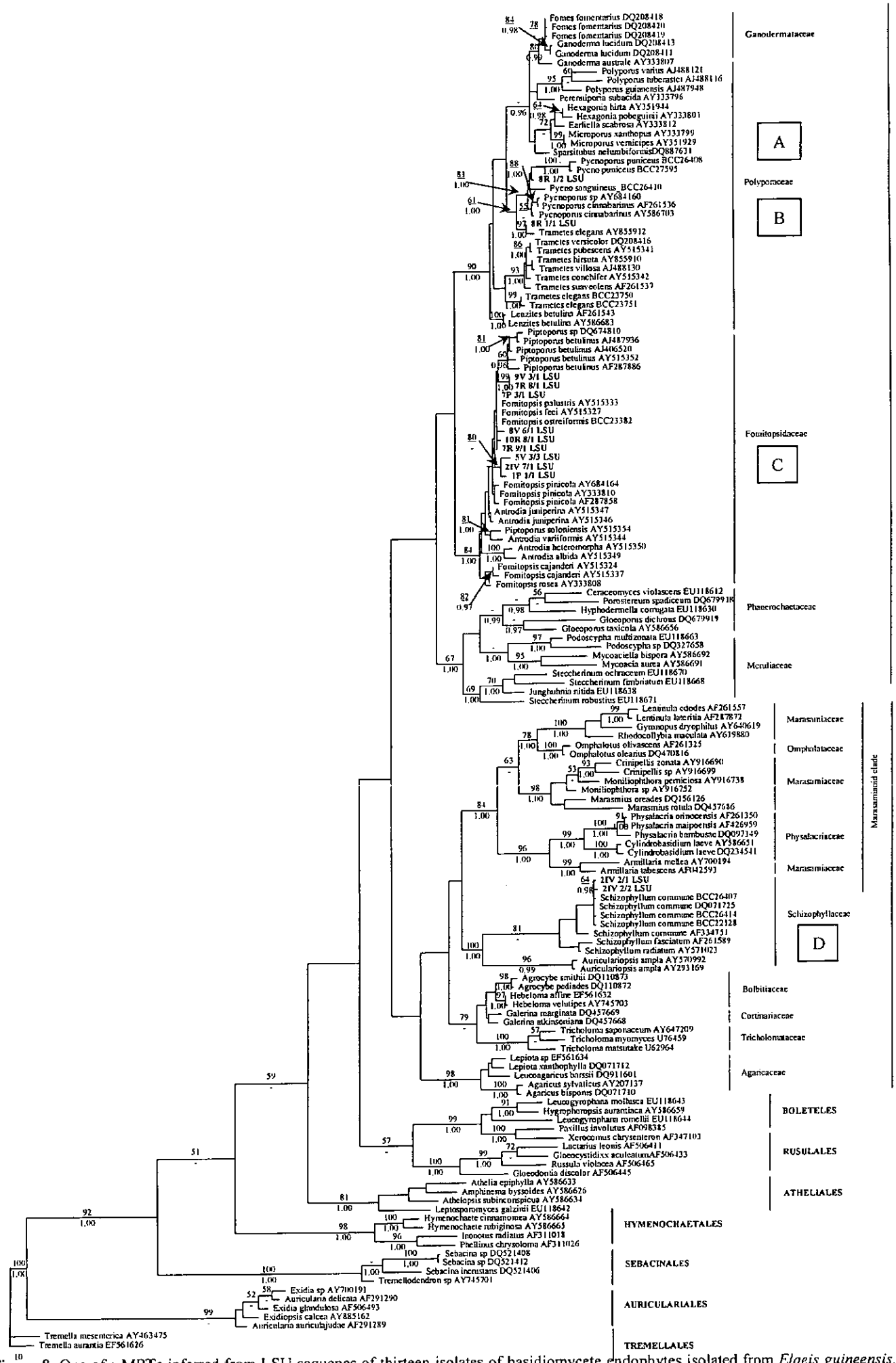

Figure 8. One of 2 MPTs inferred from LSU sequenes of thirteen isolates of basidiomycete endophytes isolated from *Elaeis guineensis*. The MP value (≥50%) and Bayesian PP (≥0.95) are shown above and below the branches, respectively (tree length= 2,595 steps, CI = 0.315, RI=0.758). Basidiomycete endophytes sequenced in this study are printed in bold. Bar = number of changes per nucleotide position.

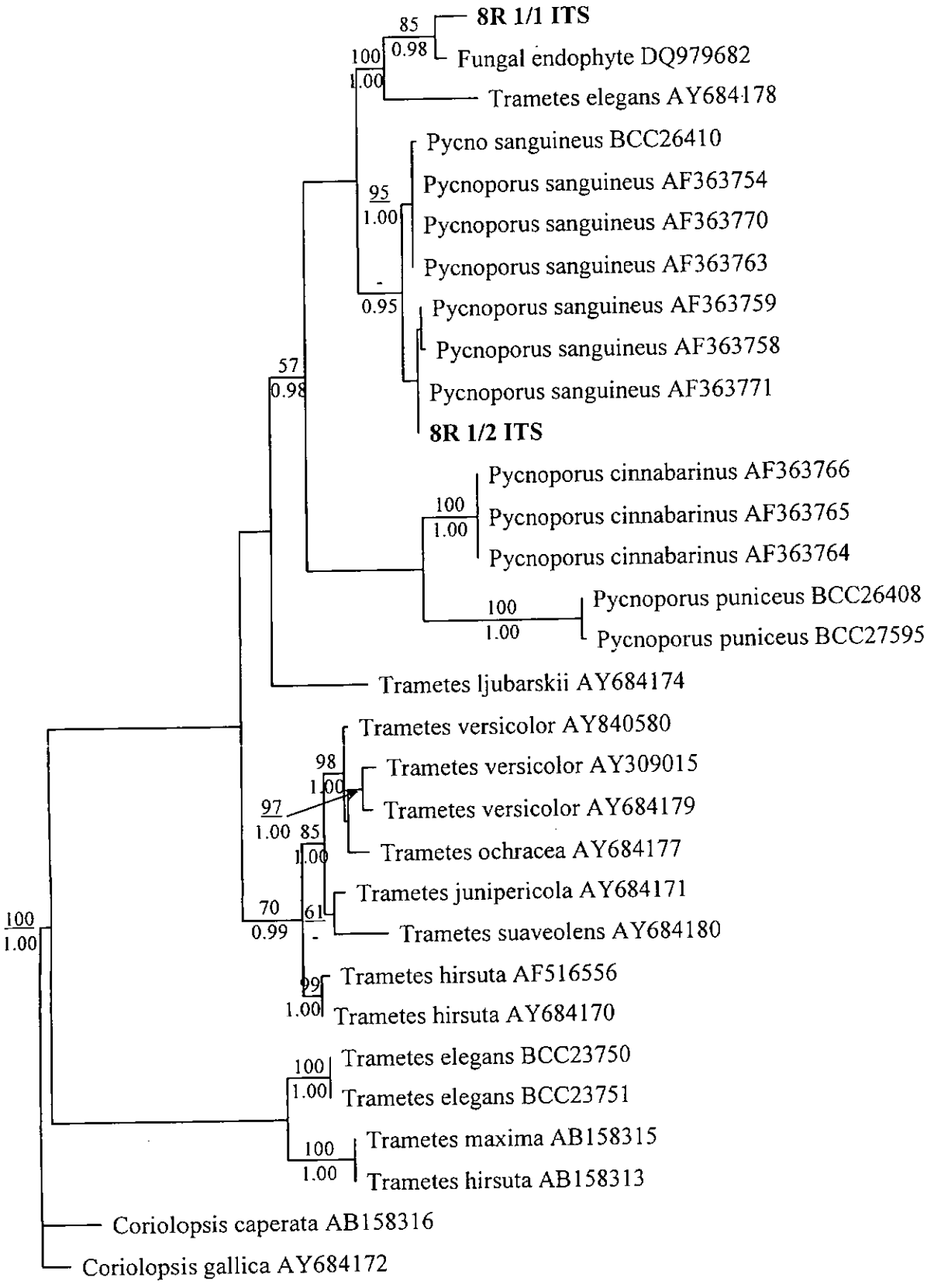

Figure 9. One of 62 MPTs inferred from ITS sequenes of two isolates of the Polyporaceae isolated from *Elaeis guineensis*. The MP value (≥50%) and Bayesian PP (≥0.95) are shown above and below the branches, respectively (tree length= 335 steps, CI = 716, RI=0.884). Basidiomycete endophytes sequenced in this study are printed in bold. Bar = number of changes per nucleotide position.

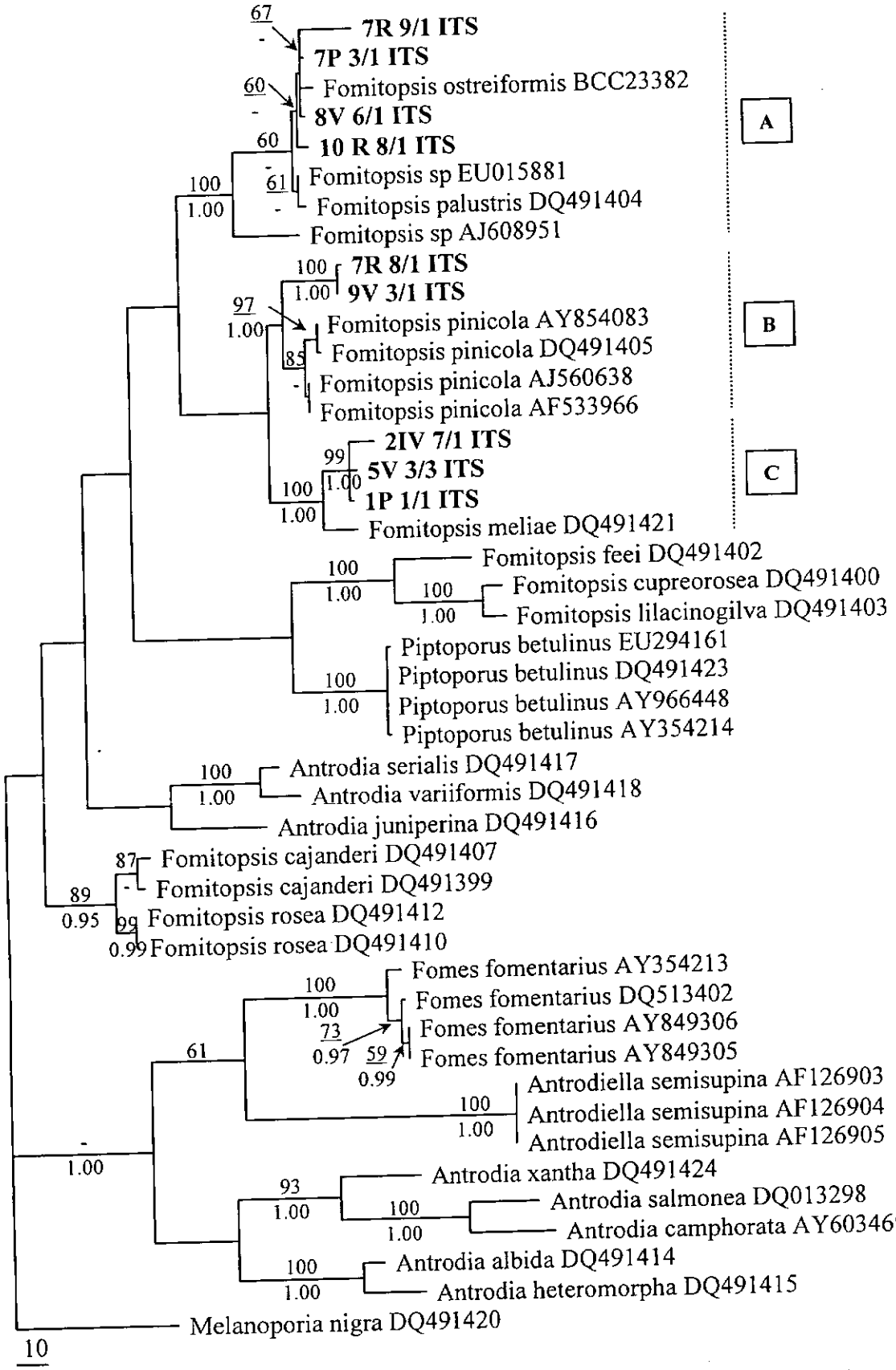

Figure 10. One of 84 MPTs inferred from ITS sequenes of nine isolates of the Fomitopsisdaceae isolated fr *Elaeis guineensis*. The MP value (≥50%) and Bayesian PP (≥0.95) are shown above and below the branches, respectively (tree length= 1,004 steps, CI = 0.574, RI=0.790). Basidiomycete endophytes sequenced in this study are printed in bold. Bar = number of changes per nucleotide position.

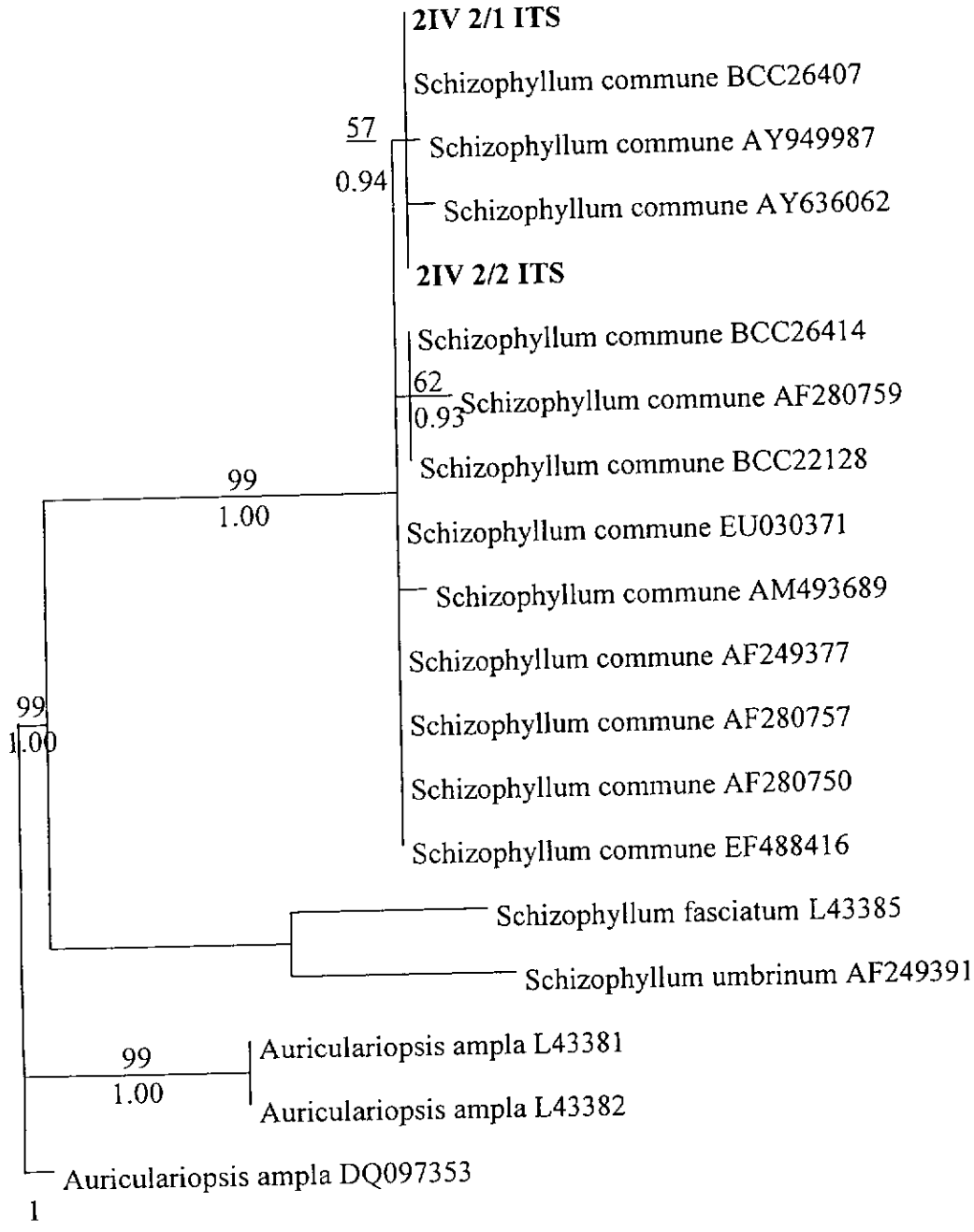

Figure 11. One of 72 MPTs inferred from ITS sequenes of two isolates of the Schizophyllaceae isolated from *Elaeis guineensis*. The MP value (≥50%) and Bayesian PP (≥0.95) are shown above and below the branches, respectively (tree length= 84 steps, CI = 0.942, RI=0.924). Basidiomycete endophytes sequenced in this study are printed in bold. Bar = number of changes per nucleotide position.

5. DELIVERABLES

5.1. Paper published

1. Rungjindamai, N., Pinruan, U., Choeyklin, R. Hattori, T. & Jones, E.B.G. 2008. Molecular characterization of basidiomycetous endophytes isolated from leaves, rachis and petioles of the oil palm, *Elaeis guineensis,* in Thailand. Fungal Diversity 33: 139-161.

5.2. Papers in press

1. Pinnoi, A., Phongpaichit, S., Hyde, K.D. & Jones, E.B.G. 2008. Biodiversity of fungi on *Calamus* (Palmae) in Thailand. Submitted in Mycotaxon.
2. Choeyklin, R., Hattori, T., Jones, E.B.G. & Pang, K.-L. 2009. Phylogenetic relationship of *Ganoderma colossus* and *G. tsunodae* within the family Ganodermataceae. Submitted in Mycoscience.
3. Choeyklin, R., Hattori, T., Jarikhuan, S. & Jones, E.B.G. 2009. Bambusicolous polypores collected in Central Thailand. Submitted in Fungal Diversity.

5.3. Poster Presentation

1. Suetrong, S., Pinruan, U., Sakayaroj, J., Phongpaichit S. & Jones, E.B.G. 2008. A multigene phylogeny of *Falciformispora lignatilis* a bitunicate ascomycete, isolated from oil palm in Thailand. In: China-Japan Pan Ascia Paciffic Mycology Forum, Changchun, China, 28 July- 5 August 2008.

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Molecular characterization of basidiomycetous endophytes isolated from leaves, rachis and petioles of the oil palm, *Elaeis guineensis*, in Thailand

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Most endophytes isolated from plants and algae are members of the Ascomycota or their anamorphs, with only a few reports of basidiomycetous endophytes, these often being orchid mycorrhizas. Fungal endophyets were isolated from healthy leaves, rachis and petioles of the oil palm *Elaeis guineensis* in a Thai plantation. In two experiments 892 and 917 endophytes were isolated yielding 162 and 178 morphotypes, respectively. Non-sporulating isolates were grouped into 162 morphotypes according to their colony morphology. Many of these morphotypes were shown to be basidiomycetes as clamp connections were present and some produced basidia and basidiospores in culture. Thirteen basidiomycetous morphotypes were therefore further characterized by molecular analysis using ribosomal DNA sequences. The LSU region was used to clarify the ordinal taxonomic level status of these isolates. The phylogenetic position of the basidiomycetous endophytes was separated into two major lineages, two and eleven in the *Agaricales* and *Polyporales*, respectively. Based on ITS sequence analysis the two *Agaricales* strains grouped with *Schizophyllum* species and showed a close relationship with *S. commune*. Within the *Polyporales* two and nine strains had an affinity with the *Polyporaceae* and *Fomitopsidaceae*, respectively. One of the endophytic *Polyporaceae* strains was monophyletic with seven sequences of *Pycnoporus sanguineus*, while another isolate grouped with a fungal endophyte DQ979682 and *Trametes elegans*. The largest fungal assemblage was within the *Fomitopsidaceae*, four endophytic isolates clustered with *Fomitopsis* species (*F. ostreiformis, F. palustris*), two and three isolates grouped with *Fomitopsis pinicola* and *Fomitopsis meliae*, respectively. Numerous genera of the Basidiomycota are reported herein as endophytes and are the first report of basidiomycete endophytes from oil palm. Our analysis demonstrated that LSU and ITS data are powerful tools to resolve the taxonomy of basidiomycetous endophytes. The biological role of these endophytes is discussed.

Key words: Agaricomycotina, Basidiomycota, *Elaeis guineensis*, endophyte, *Fomitopsis*, *Pycnoporus*, rDNA phylogeny, systematics, *Schizophyllum*, *Trametes*

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Introduction

The oil palm *Elaeis guineensis*, a native of West Africa, was introduced to Java by the Dutch and by the British into Malaysia in 1910. Oil palms are widely planted in Thailand and were introduced in 1920 and have been cultivated on a commercial basis since 1968 (Likhitekaraj and Tummakate, 2000). The oil palm is a source of edible vegetable oil yielding some 28 million tones' in 2004 (Stevenson, 2006). Nowadays, demand of oil palm consumption is increasing as a precursor in biodiesel production. Therefore oil palm has become an important economic plant for industrial exploitation as an alternative energy source. However, in recent years, oil palms have been prone to fungal attack by *Ganoderma boninense* and a number of studies have been undertaken to find biofungicides that can control infestations (Abdullah, 2000; Ariffin *et al.*, 2000; Flood *et al.*, 2000, 2005; Likhittekaraj and Tummakate, 2000; Paterson, 2007). Sieber *et al.* (1991) and Petrini *et al.*

(1992) have also explored the concept and use of endophytic fungi in biocontrol.

Oil palm plantations are now extensive in Asia with old fronds cut off to rot between the trees. The leaves quickly rot within 8 weeks but the rachis and petioles take longer and are colonized by a wider range of saprobic fungi (Choeyklin, unpublished data). In our search for a biofungicide to control *Ganoderma* attack, we have been isolating and screening both saprobic and endophytic fungi colonizing the various parts of the oil palm. Understanding the fungal community of oil palm could facilitate the basic knowledge of disease management of this crucial commercial plant (Evans *et al.*, 2003; Evans, in press). In two experiments, 892 and 917 endophytes were isolated yielding 13 and 6 basidiomycetous isolates, respectively.

Most endophytes are ascomycetes and their anamorphs (Carroll, 1988; Rodrigues, 1994; Sridhar and Raviraja, 1995; Gonthier *et al.*, 2006; Arnold, 2007), with only a limited number of papers published on the basidiomycetes (Petrini, 1986; Chapela and Boddy, 1988a, b; Oses *et al.*, 2006; Sánchez Márquez *et al.*, 2007). The latter are widely reported as endophytes from diverse host plants and geographical areas, worldwide (Table 2). Basidiomycetes have been reported as endophytes of grasses, (Sánchez Marquez, 2007), orchids (Hadley, 1975), various liverworts (Ligrone *et al.*, 1993; Duckett *et al.*, 2006; Russell and Bulman, 2005; Duckett and Ligrone, 2008a, b) and from the cocoa tree, *Theobroma cacao* and *Th. giliteri* (Evans *et al.*, 2003; Crozier *et al.*, 2006; Thomas *et al.*, 2008).

A few palms have been studied for endophytes: *Euterpe olaeracea* (Rodriques, 1994), *Sabal bermudana* and *Livistona chinensis* (Southcott and Johnson, 1997), *Trachycarpus fortunei* (Taylor *et al.*, 1999), *Licuala* species (Fröhlich *et al.*, 2000) and *Phoenix dactylifera* (Gomez-Vidal, 2006). All species isolated were ascomycetes or their anamorphs. However, Guo *et al.* (2001) detected a basidiomycetous endophyte in *Livistona chinensis* by extracting DNA directly from the palm tissue. However it was not isolated using traditional methodology, and the taxon could not identified further to a lower taxonomic level, as there were to few 5.8S sequences available in the GenBank.

In this study molecular techniques were employed to characterize the endophytic basidiomycete assemblage isolated from the oil palm. Partial large subunit (LSU) of nuclear ribosomal DNA was selected for a preliminary experiment so as to characterize their higher taxonomic placement, as this region is well represented in the GenBank. Therefore a dataset "backbone" of major clades of the homobasidiomycetes was established based on published data (Moncalvo *et al.*, 2002; Binder *et al.*, 2005; Hibbett *et al.*, 2007; Thomas *et al.*, 2008). The internal transcribed spacer (ITS) was further generated in order to define and confirm their lower taxonomic position.

The overall objective of this study is to isolate endophytes from *Elaeis guineensis* so as to develop a biocontrol management strategy for the palm pathogen *Ganoderma boninense.* In this paper we focus on (i) report the diversity of basidiomycetous endophytes isolated from *E. guineensis* and (ii) to characterize these using phylogenetic evidence.

Materials and methods

Sample selection

Ten plants of *Elaeis guineensis* from a site at Sai Bor oil palm plantations, Trang Province were selected for sampling in April and Septmeber 2007. Ten fronds from each plant were removed, bagged and returned to the laboratory.

Endophyte isolation and culture maintenance

Palms of about the same size were selected, leaves attached to parts of the petiole collected, placed in plastic bags and processed on return to the laboratory. Ten discs were cut so as to include a major vein and ten cut from tissue between the veins.

For palm petioles and rachis, sections were made of each, and 5 cm long pieces removed from each section. A 5 mm segment of tissue was randomly cut to ten discs from each piece of petiole and rachis.

Surface sterilization of the leaf discs was carried out by dipping in 95% ethanol for 1 minute, then soaking in sodium hypochloride (3% available chlorine) for 5 minutes and with

a second immersion in 95% ethanol for 30 seconds, followed by washing in sterile distilled water. Leaf discs were transferred to Petri dishes (9 cm diam.) containing potato dextrose agar (PDA) and corn meal agar (CMA) with added streptomycin sulphate. Five discs were placed in each dish. The same procedure was applied to the 5 mm segments from the petiole and rachis, but were dipped in 95% ethanol for 90 seconds, Chlorox for 7 minutes, then 30 seconds in ethanol, and then washed in sterile distilled water. Petri dishes were incubated at 25°C for up to one week, and mycelium growing from the tissues sub-cultured on to PDA and CMA in 6 cm diam Petri dischs and incubated at 25°C. Isolates were identified by their sporulation structures on the media, while non sporlulating strains were characterized by their colony morphology into morphotypes.

From examination of the non sporulating strains 19 strains were identified as basidiomycetes by their clamp connections. Thirteen of these strains were selected for this molecular study.

DNA extraction and PCR amplification

Fungi were inoculated on potato dextrose agar (PDA) for three weeks and then transfered into potato dextrose broth (PDB) at room temperature for one week. Mycelium was filtered and washed with sterilized water. Biomass was frozen and ground into fine powder with mortar and pestle. Genomic DNA was extracted using CTAB method (O' Donnell *et al.*, 1997) with some modification. Partial large subunit (LSU) and complete internal transcribed spacer (ITS) were amplified with fungal specific primer: LROR, LR7 and ITS5, ITS4, respectively (White *et al.*, 1990, Bunyard *et al.*, 1994) using Fermentas, *Tag* DNA Polymerase (recombinant) kit (Fermentas, Ontario, Canada). The PCR amplification cycles were performed following White *et al.* (1990) and Bunyard *et al.* (1994) with a DNA Engine DYAD ALD 1244 Thermocycler (MJ Research, Waltham, MA). Amplified PCR fragments were purified with NucleoSpin Extract DNA purification kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instruction and then sequenced by Macrogen (Seoul, Korea) using the same primers as for amplification.

Sequence alignment and phylogenetic analysis

LSU and ITS regions were employed to search the closest sequences from the GenBank database (http://www.ncbi.nlm.nih.gov) using a BLAST search (Altschul *et al.*, 1990). The LSU region was initially blasted in order to determine the familial and ordinal level. The phylogenetic construction of LSU sequence was performed based on the study of Moncalvo *et al.* (2002) and Hibbett *et al.* (2007). Further LSU sequences from different major classes, orders and families of the Agaricomycetes were included in data matrix. The ITS region was used to clarify the generic and species level of the isolates. Our endophytic sequences were compared with relatedness from BLAST search. DNA sequences were multiple aligned using Clustal W 1.6 (Thompson *et al.*, 1994) and adjusted manually to maximize alignment using BioEdit 7.5.0.3 (Hall, 2006).

The aligned dataset was subsequently analysed using MP in PAUP* 4.0b10 (Swofford, 2002), for the most parsimonious trees (MPTs). Heuristic searches algorithm with tree-bisection-reconnection (TBR) branch swapping, 100 replicates of random stepwise sequence addition, were performed. Gaps were treated as missing data and given equal weight. The tree length, consistency indices (CI) and retention indices (RI) were calculated for each tree generated. The Kishino-Hasegawa (K-H) test was used for estimation of the best tree topology (Kishino and Hasegawa, 1989).

Bayesian phylogenetic inference was calculated with MrBayes 3.0b4 with general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Huelsenbeck and Ronquist, 2001). Four Markov chains were run from random starting trees for 5 M generations and sampled every 100 generations. The first 500K generations were discarded as burn-in of the chain. A majority rule consensus tree of all remaining trees was calculated.

Statistical support for the internal branches was estimated by bootstrapping

Table 1. New sequences generated in this study and their collection data.

Fungal code	Source	Host plant	Plant part	Site of collection	Basidiomycete structure	GenBank accession number	
						LSU	ITS
8R 1/1	BCC30874	*Elaeis guineensis*	Rachis	Sai Bor oil palms plantation, Trang	Basidiomes (poroid)	FJ372693	FJ372671
8R 1/2	BCC29328	*Elaeis guineensis*	Rachis	Sai Bor oil palms plantation, Trang	Basidiomes (poroid)	FJ372694	FJ372672
1P 1/1	BCC30875	*Elaeis guineensis*	Petioles	Sai Bor oil palms plantation, Trang	Clamp connection	FJ372695	FJ372673
2IV 7/1	BCC28151	*Elaeis guineensis*	Intervein	Sai Bor oil palms plantation, Trang	Basidiomes (poroid)	FJ372696	FJ372674
5V 3/3	BCC30880	*Elaeis guineensis*	Vein	Sai Bor oil palms plantation, Trang	Basidiomes (poroid)	FJ372697	FJ372675
7R 8/1	BCC30881	*Elaeis guineensis*	Rachis	Sai Bor oil palms plantation, Trang	Clamp connection	FJ372698	FJ372676
9V 3/1	BCC30879	*Elaeis guineensis*	Vein	Sai Bor oil palms plantation, Trang	Clamp connection	FJ372699	FJ372677
7P 3/1	BCC30873	*Elaeis guineensis*	Petioles	Sai Bor oil palms plantation, Trang	Basidiomes (poroid)	FJ372700	FJ372678
7R 9/1	BCC30877	*Elaeis guineensis*	Rachis	Sai Bor oil palms plantation, Trang	Clamp connection	FJ372701	FJ372679
8V 6/1	BCC30866	*Elaeis guineensis*	Vein	Sai Bor oil palms plantation, Trang	Clamp connection	FJ372702	FJ372680
10R 8/1	BCC30876	*Elaeis guineensis*	Rachis	Sai Bor oil palms plantation, Trang	Clamp connection	FJ372703	FJ372681
2IV 2/1	BCC30878	*Elaeis guineensis*	Intervein	Sai Bor oil palms plantation, Trang	Clamp connection	FJ372704	FJ372682
2IV 2/2	BCC28497	*Elaeis guineensis*	Intervein	Sai Bor oil palms plantation, Trang	Clamp connection	FJ372705	FJ372683
Fomitopsis ostreiformis	BCC23382	Saprobic on wood		Khao Yai National Park, Nakhon Ratchasima	*	FJ372706	FJ372684
Pycnoporus puniceus	BCC26408	Saprobic on wood		Tammarang Pier, Satun	*	FJ372707	FJ372685
Pycnoporus puniceus	BCC27595	Saprobic on wood		Tammarang Pier, Satun	*	FJ372708	FJ372686
Pycnoporus sanguineus	BCC26410	Oil palm		Sai Bor oil palm plantation, Trang	*	FJ372709	FJ372687
Schizophyllum commune	BCC22128	Oil palm fruits		Sai Bor oil palm plantation, Trang	*	FJ372710	FJ372688
Schizophyllum commune	BCC26407	Saprobic on mangrove wood		Hat Khanom - Mu Ko Thale Tai Nation Park, Surat Thani	*	FJ372711	FJ372689
Schizophyllum commune	BCC26414	Bamboo		Bamboo Garden, Prachin Buri	*	FJ372712	FJ372690
Trametes elegans	BCC23750	Saprobic on wood		Khao Luang Naional Park, Nakhon Si Thammarat	*	FJ372713	FJ372691
Trametes elegans	BCC23751	Saprobic on wood		Khao Luang Naional Park, Nakhon Si Thammarat	*	FJ372714	FJ372692

* All isolated and identified from fresh basidiomes.

Table 2. Selected list of basidiomycetous endophytes reported in the literature.

Plant host	Order	Fungal identification	Host plant	Reference
Orchid	***Cantharellales***	*Ceratobasidium cornigerum*	*Plantanthera obtusata*	Currah and Sherburne, 1992
		Ceratobasidium obscurum	*Amerorchis rotundifolia*	Currah and Sherburne, 1992
		Epulorhiza anaticula	*Calypso bulbosa*	Currah and Sherburne, 1992
		Epulorhiza repens	*Platanthera obtusata*	Currah and Sherburne, 1992
		Epulorhiza repens	*Acianthus* spp.	Bougoure *et al.*, 2005
		Moniliopsis anomala	*Coeloglossum viride*	Currah and Sherburne, 1992
		Sistotrema sp.	*Pipperia unalascensis*	Currah and Sherburne, 1992
		Thanatephorus pennatus	*Calypso bulbosa*	Currah and Sherburne, 1992
		Tulasnella calospora	*Diuris maculata*	Warcup, 1971
		Tulasnella sp.	*Neuwiedia veratrifolia*	Kristiansen *et al.*, 2004
		Thanatephorus sp.	*Neuwiedia veratrifolia*	Kristiansen *et al.*, 2004
		Thanatephorus sp.	*Pterostyulis* spp.	Bougoure *et al.*, 2005
	Sebacinales	*Sebacina vermifera*	*Nicotiana attenuata*	Barazani *et al.*, 2007
		Sebacina vermifera	*Caladenia* spp.	Warcup, 1971
			Glossodia major	
			Elythranthera brunonis	
			Elythranthera emarginata	
			Eriochilus cucullatus	
		Sebacina sp.	*Bletilla ochracea*	Tao *et al.*, 2008
		Sebacina sp.	*Platanthera obtusata*	Currah and Sherburne, 1992
Liverworts	***Cantharellales***	*Tulasnella* sp.	*Cryptothallus mirabilis*	Bidartondo *et al.*, 2003
			Aneura pinguis	
		Tulasnella sp.	*Aneura pinguis*	Kottke *et al.*, 2003
	Sebacinales	*sebacinoid*	*Lophozia incisa*	Weiss *et al.*, 2004
		sebacinoid	*Lophozia sudetica*	Weiss *et al.*, 2004
		sebacinoid	*Calypogeia muelleriana*	Weiss *et al.*, 2004
		sebacinoid	*Lophozia ibcisao*	Kottke *et al.*, 2003
		sebacinoid	*Lophozoia sudetica*	Kottke *et al.*, 2003
	Incertae sedis	Basidiomycete associations	*Jungermanniales*	Duckett *et al.*, 2006

Table 2 (continued). Selected list of basidiomycetous endophytes reported in the literature.

Plant host	Order	Fungal identification	Host plant	Reference
Monocotyledon and Dicotyledon	*Agaricales*	*Agaricales* sp. 1	*Theobroma gileri*	Evans *et al.*, 2003
		Coprinellus sp.1-2	*Theobroma cacao*	Crozier *et al.*, 2006
		Coprinellus sp.	*Theobroma gileri*	Thomas *et al.*, 2008
		Crinipellis roreri	*Theobroma gileri*	Evans *et al.*, 2003
		gloeosterioid sp.	*Theobroma cacao*	Crozier *et al.*, 2006
		Melanotus subcuneiformis	*Theobroma cacao*	Thomas *et al.*, 2008
		Psilocybe sp.	*Theobroma gileri*	Evans *et al.*, 2003
		Schizophyllum sp.	*Theobroma gileri*	Thomas *et al.*, 2008
		Schizophyllum commune	*Pinus tabulaeformis*	Wang *et al.*, 2005
	Auriculariales	*Auriculariales* sp.	*Theobroma cacao*	Crozier *et al.*, 2006
	Boletales	*Coniophora puteana*	*Fagus sylvatica*	Chapela and Boddy, 1998
	Hymenochaetales	*hymenochaetoid* sp. 1-2	*Theobroma cacao*	Crozier *et al.*, 2006
		Inonotus sp.	*Theobroma cacao*	Crozier *et al.*, 2006
		Fomitiporia sp.	*Pinus taeda*	Arnold *et al.*, 2007
	Polyporales	*Byssomerulius* sp.	*Theobroma cacao*	Crozier *et al.*, 2006
		Coriolopsis sp.	*Theobroma gileri*	Evans *et al.*, 2003
		corticioid sp. 1-9	*Theobrom cacao*	Crozier *et al.*, 2006
		cf *Daedeleopsis* sp.	*Theobroma gileri*	Evans *et al.*, 2003
		Ganoderma sp.	*Theobroma gileri*	Thomas *et al.*, 2008
		hymenochaetoid sp. 1-2	*Theobroma cacao*	Crozier *et al.*, 2006
		Inonotus sp.	*Theobroma cacao*	Crozier *et al.*, 2006
		Lentinus sp.	*Theobroma cacao*	Crozier *et al.*, 2006
		Lentinus sp. 1-2	*Theobroma gileri*	Thomas *et al.*, 2008
		Meripilus sp.	*Theobroma gileri*	Thomas *et al.*, 2008
		Oxyporus sp.	*Theobroma cacao*	Crozier *et al.*, 2006
		Perenniporia sp.	*Theobroma gileri*	Evans *et al.*, 2003
		Phanerochaete sp.	*Theobroma cacao*	Crozier *et al.*, 2006
		phlebioid sp.	*Theobroma cacao*	Crozier *et al.*, 2006
		Piptoporus sp.	*Theobroma gileri*	Thomas *et al.*, 2008
		Polyporaceae sp. 1-2	*Theobroma gileri*	Thomas *et al.*, 2008
		Polyporaceae sp. 1-3	*Theobroma cacao*	Crozier *et al.*, 2006
		Podoscypha sp.	*Theobroma gileri*	Thomas *et al.*, 2008
		Pycnoporus sp. 1-2	*Theobroma cacao*	Crozier *et al.*, 2006

Table 2 (continue). Selected list of basidiomycetous endophytes reported in the literature.

Plant host	Order	Fungal identification	Host plant	Reference
Monocotyledon and Dicotyledon		*Pycnoporus* sp. 1-2	*Theobroma cacao*	Crozier *et al.*, 2006
		cf. Pycnoporus sp.	*Theobroma gileri*	Thomas *et al.*, 2008
		Trametes sp.	*Theobroma gileri*	Evans *et al.*, 2003
		Trametes hirsuta	*Podophyllum hexandrum*	Puri *et al.*, 2006
	Russulales	*Lachnocladiaceae* sp.	*Theobroma gileri*	Thomas *et al.*, 2008
		Wrightoporia sp.	*Theobroma gileri*	Thomas *et al.*, 2008
	Sebacinales	*Piriformospora indica*	*Hordeum vulgare*	Waller *et al.*, 2005
	Incertae sedis	Basidiomycetes sp. 1-4	*Theobroma gileri*	Evans *et al.*, 2003
		Basidiomycete spp.	*Theobroma gileri*	Evans *et al.*, 2003
		Basidiomycete P1-9	*Livistona chienesis*	Guo *et al.*, 2001
		Bjerkkandera sp.	*Drimys winteri*	Oses *et al.*, 2006
		Mycelia sterilia	*Theobroma gileri*	Evans *et al.*, 2003
		Tulasnella	*Cryptothallus mirabilis*	Bidartondo *et al.*, 2003
		Unidentified basidiomycete	*Prumnopitys andina*	Oses *et al.*, 2006

analysis (Felenstein, 1985) with 1K replications (ten replicates of random stepwise sequence addition, TBR branch swapping) and PP were performed. The MP BS values (≥50%) and Bayesian PPs (≥0.95) are shown above and below the tree branches, respectively. The rDNA sequences, consisting of LSU and ITS were submitted into the GenBank database (Table 1). The accession numbers for all sequences derived from the GenBank database are included in the phylogenetic trees. The new sequences generated for basidiomycetous endophytes are shown in Table 1.

Results

Morphology of selected basidiomycetous endophyte isolates

In this study 13 endophyte isolates from *Elaeis guineensis*, were morphologically identified as basidiomycetes based on the presence of clamp connections or basidia/ basidiospores in the cultures (Table 1, Figs 1 and 2).

Isolate 8R 1/1

Upper surface, mat white at first, becoming cream, orange, reddish to brightly reddish-orange colour, downy, floccose, sometimes thin translucent (Fig. 1a), reverse plate at first uncharged then becomes yellowish-brown, producing small resupinate poroid fruit bodies, pale to bright orange at the margin of the colony and on the Petri-dish side (Fig. 1b), pores round, 3-5 pores/mm with clamp connection (Fig. 1e). Hyphal system, trimitic, generative hyphae with clamp connections, binding hyphae hyaline, highly branched, thick-walled, 2.5-3 µm wide, and skeletal hyphae, hyaline, unbranched, very thick-walled to solid. Basidia clavate, hyaline, thin-walled, 28-28.5 × 8-8.5 µm (Figs 1c-d). Basidiospores ellipsoid, hyaline, thin-walled, 5-5.5 × 3-3.5 µm. Isolated from palm rachis.

Isolate 5V 3/3

Upper surface white cottony mycelium on PDA, reverse plate concolorous with front plate. Hyphal system dimitic generative hyphae with clamp connections, hyaline in Melzer's reagent, thin-walled, 2-4 µm wide, skeletal hyphae hyaline in Melzer's reagent, unbranced, thick-walled to nearly solid, 2-3 µm wide. Basidia clavate, hyaline, thin-walled 20-25 × 5-5.5 µm (Fig. 1f). Basidiospore narrow-ellipsoid, hyaline, thin-walled, 5-5.5 × 2.5 µm (Figs 1g-i).

Cultures were grown on PDA medium in glass bottles with test blocks of palm petioles added once good growth was established (Fig. 2a). After 12 months 5V 3/3 produced small "basidiomes" that were poroid in appearance (Figs 2b-e). Long term exposure of inoculated palm petioles have been exposed under field coniditions, to stimulate fruit body initiation. No results are currently available.

Small fruit bodies produced on oil palm petioles (8 × 5 × 4 mm) in bottle (Figs 2a-d), dimidiate, pileus surface covered with cream coloured mycelium, tubes 3 mm long, pale yellowish-brown, pores round to angular (Figs 1h, 2b-c), white colour, pores cream when young becoming pale grayish-brown to pale yellowish-brown. Mycelium very dense on substratum before forming fruit bodies. Isolated from vein of palm leaf.

LSU phylogeny of basidiomycete endophytes

A phylogenetic tree was constructed from a dataset consisting of 135 sequences aligned with *Tremella mesenterica* and *T. aurantiaca* as the outgroup. A total of 1,337 characters, 422 are parsimony informative, 114 are parsimony uninformative and 801 are constant characters (tree length 2,595, C.I. = 0.315, R.I. = 0.758). Maximum parsimony analysis yielded two maximum parsimonious trees. Thirteen LSU rDNA sequences of the basidiomycete endophytes were aligned along with representative taxa from eight major orders of the Basidiomycota comprising the *Agaricales*, *Atheliales*, *Auriculariales*, *Boletales*, *Hymenochaetales*, *Polyporales*, *Russulales* and *Sebacinales* (Fig. 3). The endophytic basidiomycetes separated into two major lineages at the ordinal level, eleven isolates within the *Polyporales*, while two are well placed in the *Agaricales*.

Within the *Polyporales*, 66 LSU sequences from five families, representing the *Fomitopsidaceae*, *Ganodermataceae*, *Meruliaceae*, *Polyporaceae* and *Phanerochaetaceae*, were incorporated in this analysis. Two endophytes (8R 1/1 and 8R 1/2) nestled within the *Polyporaceae* with 90 % BS and 1.00 PP.

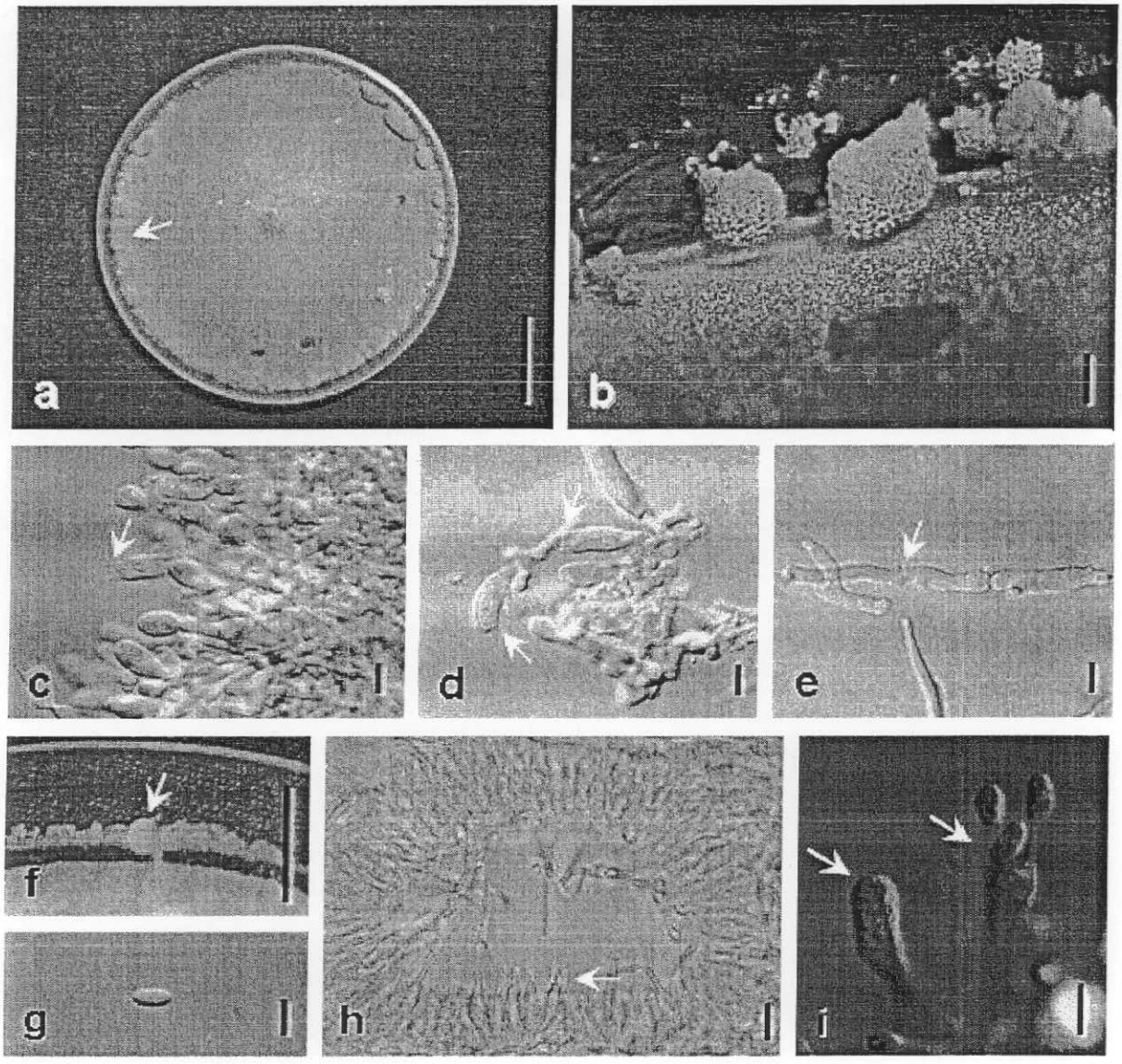

Fig. 1. Isolate 8R 1/1 a-b. Basidiomes on PDA culture formed on side of Petri dish (arrowed). **c-d.** Basidia (arrowed). **e.** Generative hyphae with clamp connection (arrowed). **Isolate 5V 3/3. f.** Basidiomes on PDA culture on Petri dish side (arrowed). **g.** Basidiospore. **h.** Crossection of a pore in culture material with cystidia (arrowed). **i.** Basidia with basidiospores (arrowed), Bars: a = 1 cm, b = 1 mm, c-e = 5 μm, f = 1 cm, g, i = 5 μm, h = 10 μm

Isolate 8R 1/1 clustered with *Trametes elegans* with high support (97 %BS and 1.00 PP) (Fig. 3 subclade B), while 8R 1/2 formed a clade with *Pycnoporus* sequences, although the statistical support is low (Fig. 3 subclade A). Nine endophyte isolates grouped with the *Fomitopsidaceae* with good statistical support (84% BS and 1.00 PP) (Fig. 3 subclade C). However, the statistical support within this group is low. Four isolates (8V 6/1, 10R 8/1, 7P 3/1 and 7R 9/1) clustered with three *Fomitopsis* species. Two strains (7R 8/1 and 9V 3/1) grouped together with 99 % BS and 1.00 PP but showed no relationship to any known taxa. These two sequences formed a sister group with various *Piptoporus* species. Three strains including the endophytes 5V 3/3, 2IV 7/1 and 1P 1/1 grouped together with low support and showed no affinity with any subclade. Two endophytic fungi (2IV 2/1 and 2IV 2/2) grouped with members of the *Schizophyllaceae*, in the *Agaricales*, with high statistical support (100 % BS and 1.00 PP) (Fig. 3 subclade D).

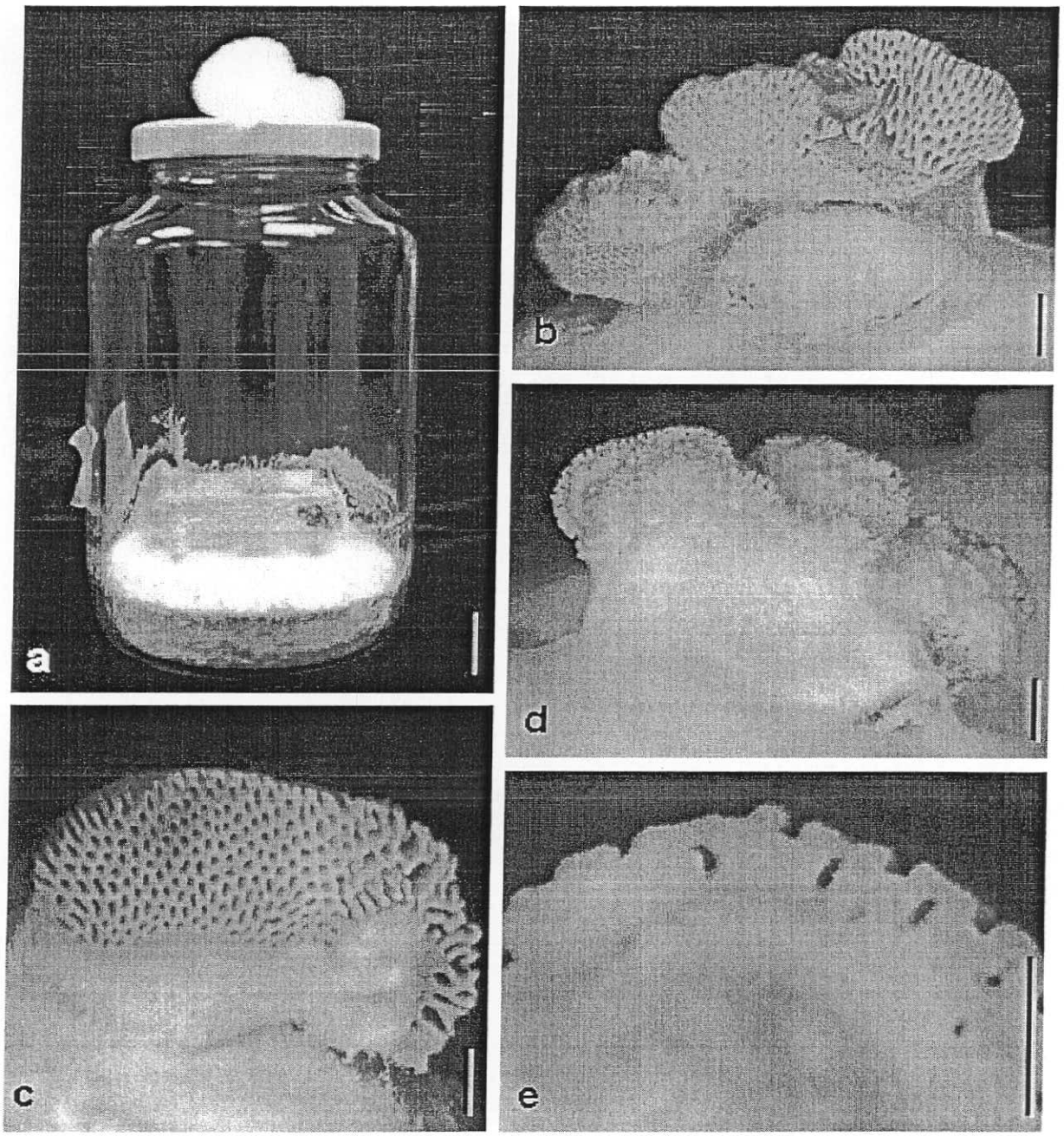

Fig. 2. Isolate 5V 3/3. a. = Basidiomes produced on test blocks of palm petiole in glass bottles containing PDA medium. **b-c.** Lower surface of basidiomes with pores. **d.** Upper surface of basidiomes. **e.** Higher magnification of pores viewed from upper surface of a basidiome. Bars: a = 1.7 cm., b, d = 1 mm, c, e = 5 mm.

ITS phylogeny of endophytes within the Polyporaceae

A phylogenetic tree was constructed from a dataset consisting of 28 sequences aligned with *Coriolopsis caperata* and *C. gallica* as the outgroup. A total of 656 characters, 151 are parsimony informative, 35 are parsimony uninformative and 470 are constant characters (tree length 335, C.I. = 0.716, R.I. = 0.884) (Fig. 4). The two endophyte isolates separated into two groups, 8R 1/1 with *Trametes* and 8R 1/2 within the *Pycnoporus* clade, with high support (100% BS and 1.00 PP). Isolate 8R 1/1 formed a clade with an unknown fungal endophyte sequence (DQ979682) with 85% BS and 0.98 PP support. This isolate also showed a

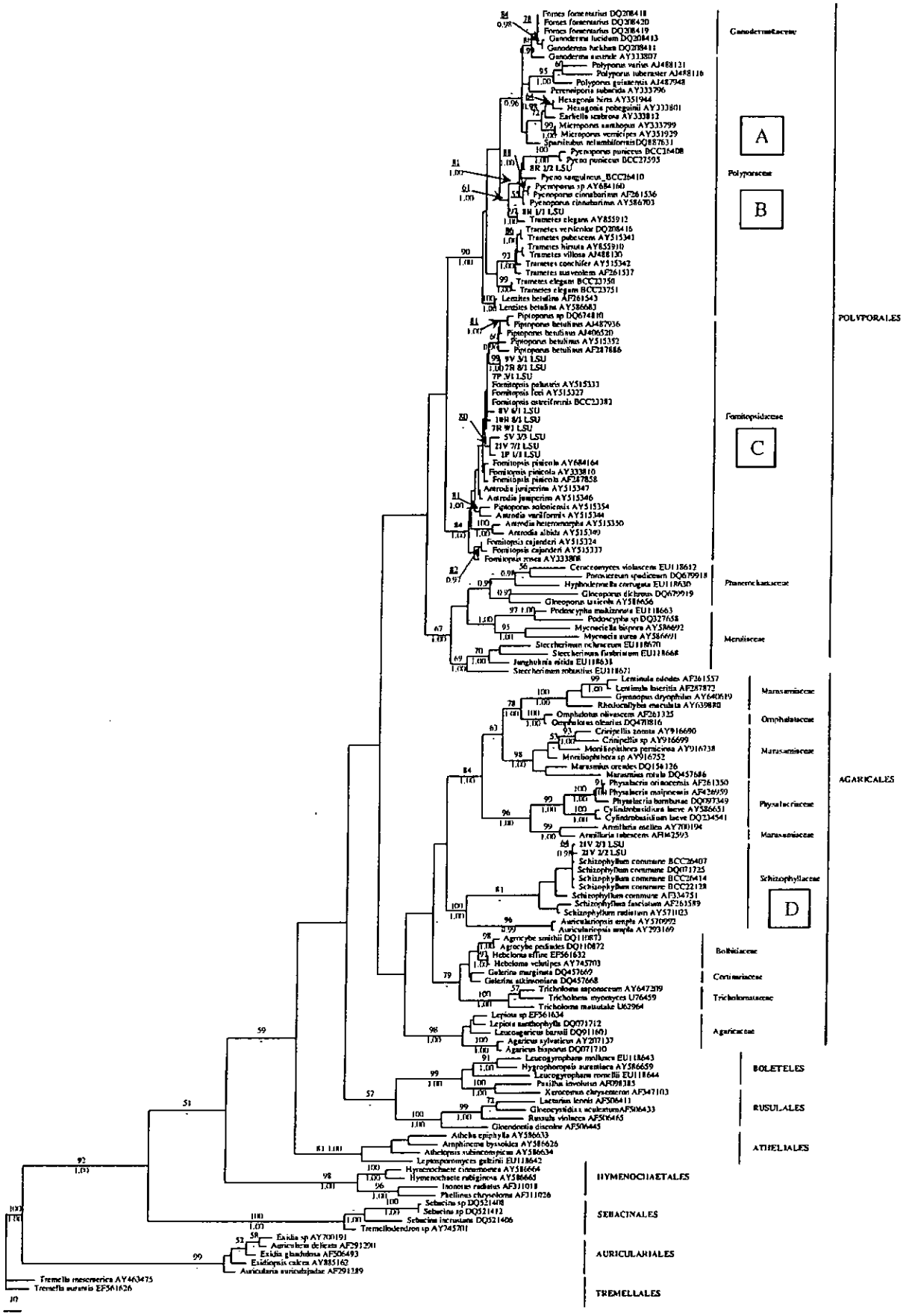
Fomes fomentarius DQ208418
Fomes fomentarius DQ208420
Fomes fomentarius DQ208419
Ganoderma lucidum DQ208413
Ganoderma hickban DQ208411
Ganoderma australe AY333807
Polyporus varius AJ488121
Polyporus tuberaster AJ488116
Polyporus guianensis AJ487948
Perenniporia subacida AY333796
Hexagonia hirta AY351944
Hexagonia pobeguinii AY333801
Earliella scabrosa AY333812
Microporus xanthopus AY333799
Microporus vernicipes AY351939
Spongiporus leucomallellus DQ887631
Pycnoporus puniceus BCC35408
Pycno puniceus BCC27595
8R 1/2 LSU
Pycno sanguineus_BCC26410
Pycnoporus sp AY684160
Pycnoporus cinnabarinus AF261536
Pycnoporus cinnabarinus AY586703
8R 1/1 LSU
Trametes elegans AY855912
Trametes versicolor DQ208416
Trametes pubescens AY515341
Trametes hirsuta AY855910
Trametes villosa AJ488130
Trametes conchifer AY515342
Trametes suaveolens AF261537
Trametes elegans BCC23750
Trametes elegans BCC23751
Lenzites betulina AF261543
Lenzites betulina AY586683
Piptoporus sp DQ674810
Piptoporus betulinus AJ487936
Piptoporus betulinus AJ406520
Piptoporus betulinus AY515392
Piptoporus betulinus AF287886
9V 3/1 LSU
7R 8/1 LSU
7P 3/1 LSU
Fomitopsis pahasris AY515333
Fomitopsis feei AY515327
Fomitopsis estreifrensis BCC23382
8V 6/1 LSU
10H 8/1 LSU
7R 9/1 LSU
8V 3/3 LSU
21V 7/2 LSU
1P 1/1 LSU
Fomitopsis pinicola AY684164
Fomitopsis pinicola AY333810
Fomitopsis pinicola AF287858
Antrodia juniperina AY515347
Antrodia juniperina AY515346
Piptoporus soloniensis AY515354
Antrodia variiformis AY515344
Antrodia heteromorpha AY515350
Antrodia albida AY515349
Fomitopsis cajanderi AY515324
Fomitopsis cajanderi AY515337
Fomitopsis rosea AY333808
Ceraceomyces violascens EU118612
Punctularia spediceum DQ679918
Hyphodermella corrugata EU118630
Gleoporus dichrous DQ679919
Gleoporus taiwanensis AY586656
Podoscypha multizonata EU118663
Podoscypha sp DQ327658
Mycoacia bispora AY586692
Mycoacia aurea AY586691
Steccherinum ochraceum EU118670
Steccherinum fimbriatum EU118668
Junghuhnia nitida EU118638
Steccherinum robustius EU118671
Lentinula edodes AF261537
Lentinula lateritia AF287872
Gymnopus dryophilus AY640519
Rhodocollybia maculata AY639880
Omphalotus olivascens AF261325
Omphalotus olearius DQ471816
Crinipellis zonata AY916690
Crinipellis sp AY916699
Moniliophthora perniciosa AY916738
Moniliophthora sp AY916752
Marasmius crinales DQ156126
Marasmius rotula DQ457686
Physalacria orinocensis AF261350
Physalacria malpiensis AF286959
Physalacria bambusae DQ937349
Cylindrobasidium laeve AY586651
Cylindrobasidium laeve DQ234541
Armillaria ostoyae AY700194
Armillaria tabescens AF162593
21V 3/3 LSU
21V 3/2 LSU
Schizophyllum commune BCC26407
Schizophyllum commune DQ071725
Schizophyllum commune BCC26414
Schizophyllum commune BCC22128
Schizophyllum commune AF334751
Schizophyllum fasciatum AF263589
Schizophyllum radiatum AY571103
Auriculariopsis ampla AY570992
Auriculariopsis ampla AY293169
Agrocybe smithii DQ110873
Agrocybe pediades DQ110872
Hebeloma affine EF561632
Hebeloma velutipes AY745703
Galerina marginata DQ457669
Galerina sikkimensis DQ457668
Tricholoma saponaceum AY647259
Tricholoma myomyces U76459
Tricholoma matsutake U62964
Lepiota sp EF561634
Lepiota xanthophylla DQ071712
Leucoagaricus barnardi DQ911691
Agaricus sylvaticus AY207137
Agaricus bisporus DQ071710
Leucogyrophana mollusca EU118643
Hygrophoropsis aurantiaca AY586659
Leucogyrophana romellii EU118644
Paxillus involutus AF098385
Xerocomus chrysenteron AF347103
Lactarius lennis AF506411
Gloeocystidiellum aculeatum AF506433
Russula violacea AF506465
Gloeodontia discolor AF506445
Athelia epiphylla AY586633
Amphinema byssoides AY586626
Athelopsis subinconspicua AY586634
Leptosporomyces galzinii EU118642
Hymenochaete cinnamomea AY586664
Hymenochaete rubiginosa AY586665
Inonotus radiatus AF311011
Phellinus chrysoloma AF311026
Sebacina sp DQ521408
Sebacina sp DQ521412
Sebacina incrustans DQ521406
Tremellodendron sp AY745701
Exidia sp AY700191
Auricularia delicata AF291200
Exidia glandulosa AF506493
Exidiopsis calcea AY885162
Auricularia auriculajudae AF291289
Tremella mesenterica AY463475
Tremella aurantia EF561626
A
B
C
D
POLYPORALES
Ganodermataceae
Polyporaceae
Fomitopsidaceae
Phanerochaetaceae
Meruliaceae
AGARICALES
Marasmiaceae
Omphalotaceae
Marasmiaceae
Physalacriaceae
Marasmiaceae
Schizophyllaceae
Bolbitiaceae
Cortinariaceae
Tricholomataceae
Agaricaceae
BOLETELES
RUSULALES
ATHELIALES
HYMENOCHAETALES
SEBACINALES
AURICULARIALES
TREMELLALES

Fig. 3. One of 2 MPTs inferred from LSU sequenes of thirteen isolates of basidiomycete endophytes isolated from *Elaeis guineensis*. The MP value (≥50%) and Bayesian PP (≥0.95) are shown above and below the branches, respectively (tree length= 2,595 steps, CI = 0.315, RI=0.758). Basidiomycete endophytes sequenced in this study are printed in bold. Bar = number of changes per nucleotide position.

relationship with *Trametes elegans* (AY68417) with good statistical support (100% BS and 1.00 PP). However, another *Trametes elegans* (isolated as a saprobe from Thailand) and *Trametes* species were distantly placed in a lower subclade. Isolate 8R 1/2 grouped with seven sequences of *Pycnoporus sanguineus* which are monophyletic with good support. *Pycnoporus cinnabarinus* and *P. puniceus* clustered in a basal clade, each subclade monophyletic, and with high statistical support for both species (100% BS and 1.00 PP).

ITS phylogeny of endophyte within the Fomitopsidaceae

A phylogenetic tree was constructed from a dataset consisting of 35 sequences aligned with *Melanoporia nigra* as the outgroup. A total of 720 characters, 295 are parsimony informative, 76 are parsimony uninformative and 349 are constant characters (tree length = 1,004, C.I. = 0.574, R.I. = 0.790,). In order to resolve the phylogentic position of the endophyte isolates within the *Fomitopsidaceae*, *Fomitopsis* and the related genera: *Antrodia, Antrodiella, Fomes* and *Piptoporus*, were integrated into this ITS sequence alignment (Fig. 5). Four isolates (7R 9/1, 7P 3/1, 8V 6/1 and 10R 8/1) clustered with various *Fomitopsis* species, *i.e. Fomitopsis* sp., *F. palustris* and *F. ostreiformis* with good statistical support (100 % BS and 1.00 PP) (Fig. 5 subclade A). Two strains (7R 8/1 and 9V 3/1) are monophyletic with 100 % BS and 1.00 PP and formed a clade with four *Fomitopsis pinicola* strains (Fig. 5 suclade B). Finally three isolates (2IV 7/1, 5V 3/3 and 1P 1/1) are monophyletic with high statistical support (99 % BS and 1.00 PP) and grouped with *Fomitopsis meliae* (DQ491421) with good support (100 % BS and 1.00 PP) (Fig. 5 subclade C). *Fomes* and *Antrodia* species formed a basal clade.

ITS phylogeny of endophyte within the Schizophyllaceae

A phylogenetic tree was constructed from a dataset comprising 16 sequences aligned with *Auriculariopsis ampla* as the outgroup. A total of 613 characters, 34 are parsimony informative, 43 are parsimony uninformative and 536 are constant characters (tree length = 86, C.I. = 0.942, R.I. = 0.924). Based on ITS sequence analysis, isolates 2 IV 2/1 and 2 IV 2/2 grouped with *Schizophyllum* species with 99% BS and 1.00 PP support (Fig. 6).

Discussion

Occurrence of basidiomycetes as endophytes

Arnold (2007) in her review of the diversity of foliar endophytic fungi, highlights the expansion of our knowledge of published papers on non-grass endophytes: 1.2 per year (1971-1990) to 15 per year (2001 to early 2007). These publications largely document ascomycetes and their anamorphs with hardly a mention of basidiomycete endophytes. Further more, the Dothideomycetes and Sordariomycetes are the major foliar endophyte species (Arnold *et al.*, 2007; Sánchez Marquez, 2007).

In recent years however, basidiomycetes have increasingly been reported in the literature. These fall into three categories. Firstly, endomycorrhizal basidiomycetes of orchids (Bernard, 1909; Hadley, 1975; Warcup and Talbot, 1980; Warcup, 1988, 1991). Most were non-sporulating basidiomycete taxa akin to *Rhizoctonia sensu lato* (or "orchidaceous rhizoctonia") (Currah and Sherburne, 1992). These orchidaceous endophytes were further characterized by their septal pore ultrastructure. For example, dolipore septa with dome-shaped septal pore caps: *Ceratobasidium cornigerum, C. obscurum, Moniliopsis anomala, Thanatephorus pennatus* and *Sistotrema* sp. (Currah and Sherbouren, 1992) (Table 2). More recently isolated basidiomycete orchid endophytes have been characterized at the molecular level (Kristiansen *et al.*, 2004; Tao *et al.*, 2008).

The second group of endophytic basidiomycetes is reported from liverworts (Ligrone *et al.*, 1993; Kottke *et al.*, 2003; Duckett *et al.*, 2006; Duckett and Ligrone, 2005, 2008a, b). Most observations examined

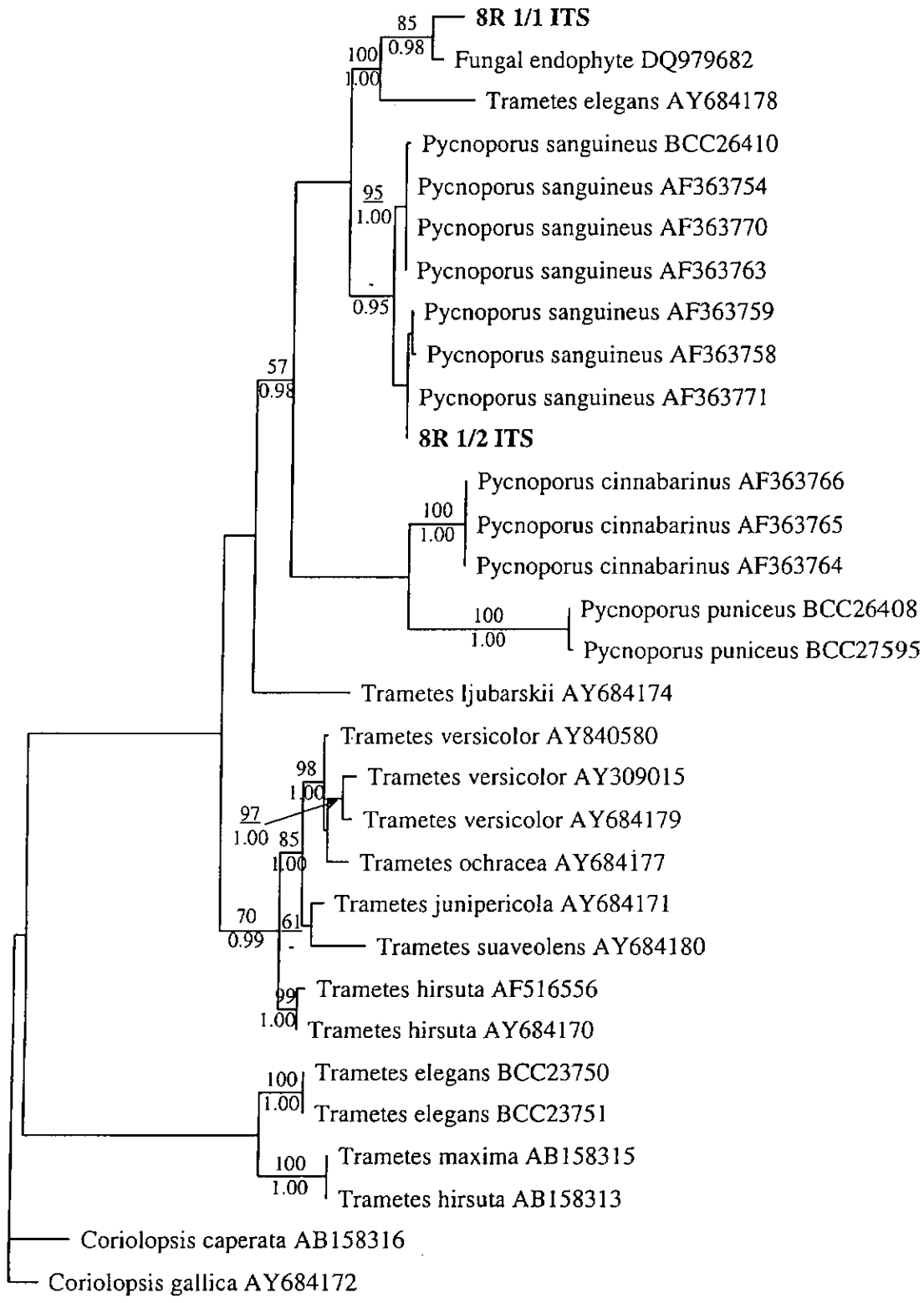

Fig. 4. One of 62 MPTs inferred from ITS sequenes of two isolates of the *Polyporaceae* isolated from *Elaeis guineensis*. The MP value (≥50%) and Bayesian PP (≥0.95) are shown above and below the branches, respectively (tree length= 335 steps, CI = 716, RI=0.884). Basidiomycete endophytes sequenced in this study are printed in bold. Bar = number of changes per nucleotide position.

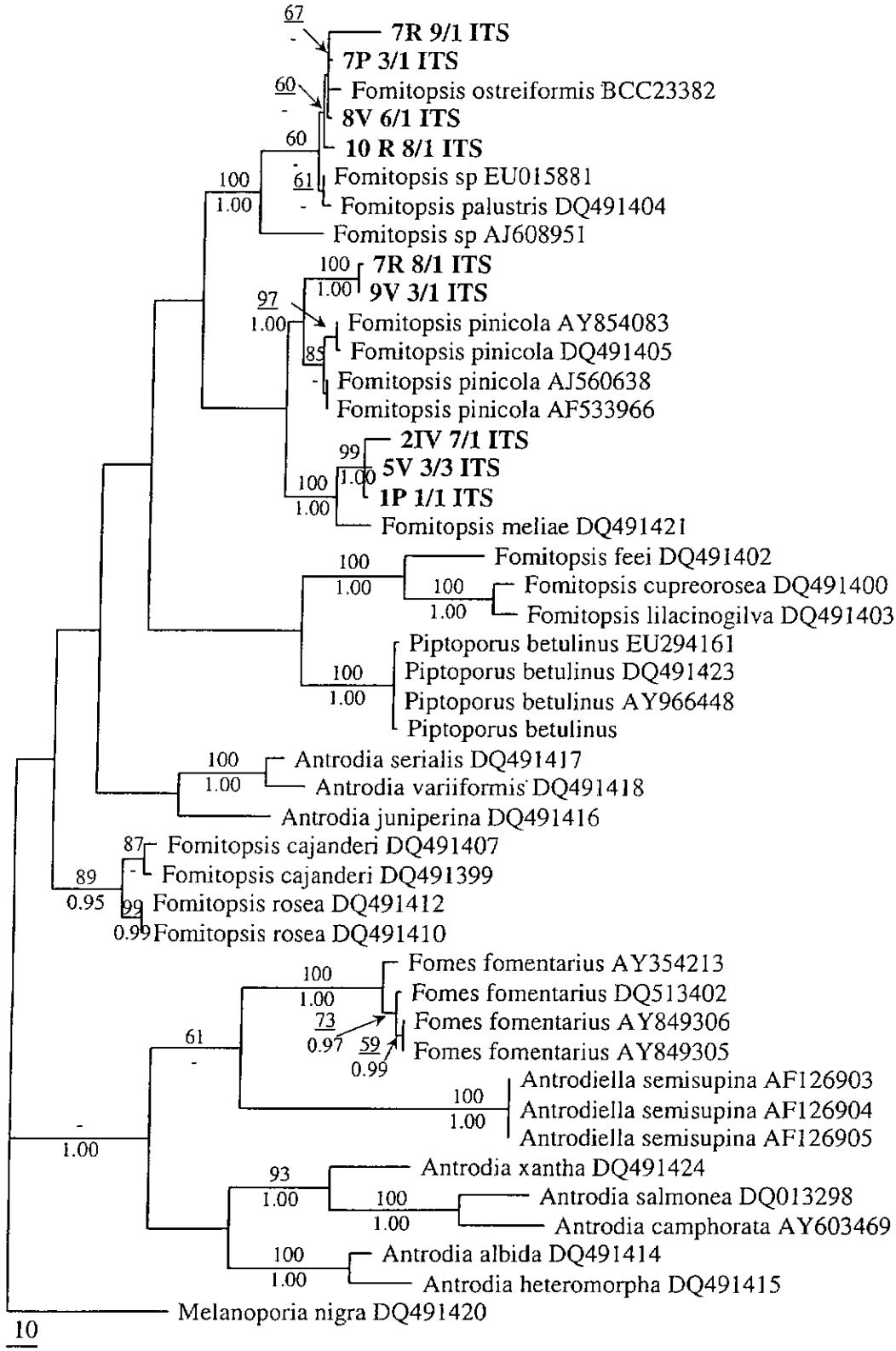

Fig. 5. One of 84 MPTs inferred from ITS sequenes of nine isolates of the *Fomitopsisdaceae* isolated from *Elaeis guineensis*. The MP value (≥50%) and Bayesian PP (≥0.95) are shown above and below the branches, respectively (tree length= 1,004 steps, CI = 0.574, RI=0.790). Basidiomycete endophytes sequenced in this study are printed in bold. Bar = number of changes per nucleotide position.

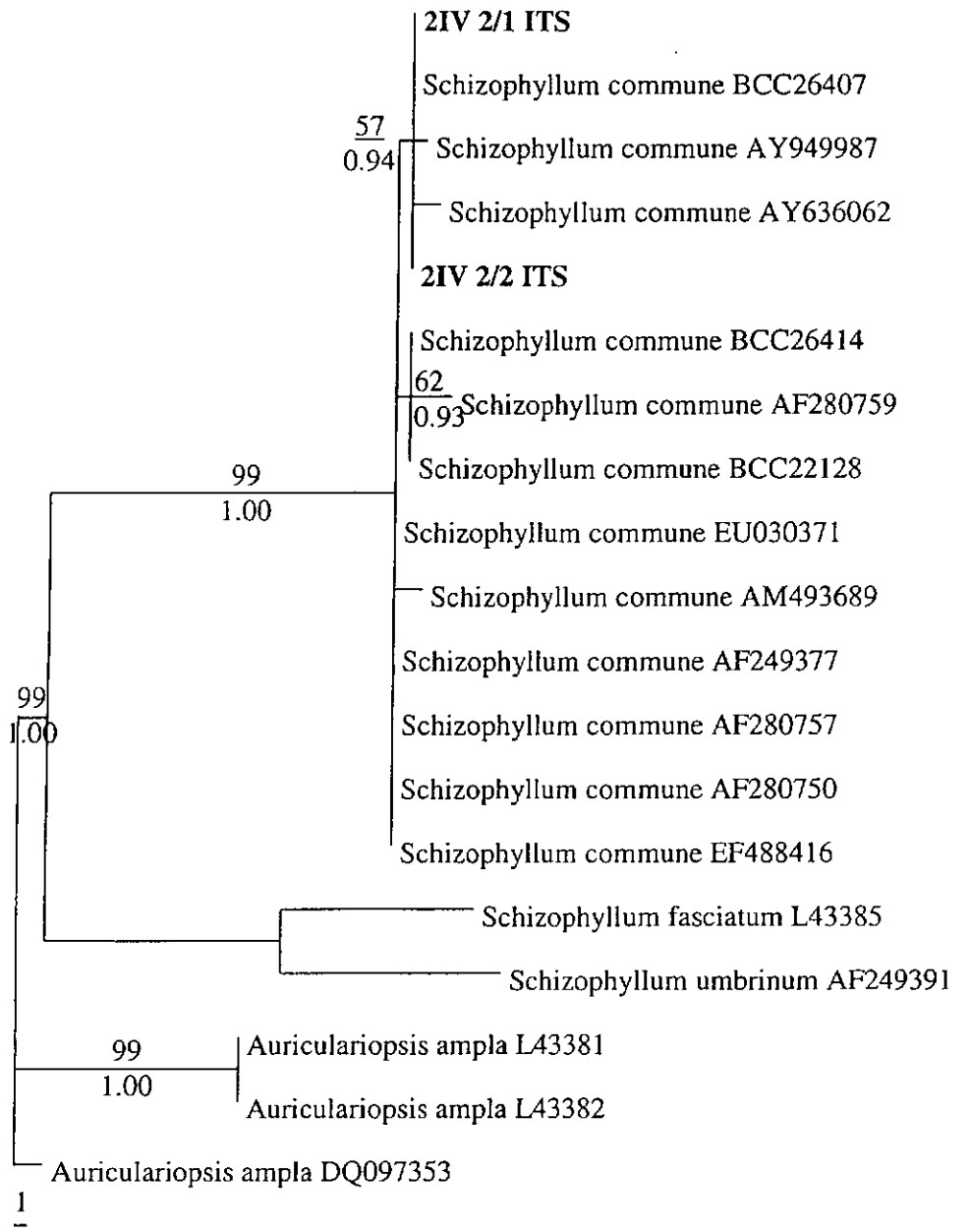

Fig. 6. One of 72 MPTs inferred from ITS sequenes of two isolates of the *Schizophyllaceae* isolated from *Elaeis guineensis*. The MP value (≥50%) and Bayesian PP (≥0.95) are shown above and below the branches, respectively (tree length= 84 steps, CI = 0.942, RI=0.924). Basidiomycete endophytes sequenced in this study are printed in bold. Bar = number of changes per nucleotide position

the ultrastructure of the fungal endophytes within the cells of the hosts, while Kottke *et al.* (2003) used both septal pore ultrastructure and molecular studies to resolve the identity of these basidiomcyete endophytes. For example, the mycobiont from the liverwort *Aneura pinguis* clustered in the *Tulasnella* clade, while microbionts of *Calypogeia muelleriana, Lophozia incisa* and *L. sudetica*, grouped with the *Sebacinaceae* (Kottke *et al.*, 2003).

There are various definitions of what constitutes an endophyte (e.g. Arnold, 2007). Generally mycorrhizal fungi are excluded (Rogers, 2000), as they are restricted to plant roots and derive nutrients from the soil by

specialized interfaces (Schulz and Boyle, 2005). Endophytes on the otherhand do not require nutrients from the soil, and live asymptomatically within roots, stems, leaves, and in this study rachis and petioles, of healthy plants (Brundrett, 2002).

Group 1 and 2 may best regarded as symbiotic associations (Nebel *et al.*, 2004), but published papers often refer to them as endophytes (Duckett *et al.*, 2006; Tao *et al.*, 2008) Nebel *et al.* (2004) hypothesise that the symbiotic fungal plant associations were established long before the evolution of roots and true mycorrhizal associations.

The third group of basidiomycete endophytes are those associated with monocotyledons and dicotyledonous plants (Table 2). These have been detected either by direct isolation from the host tissue or by Denaturing Gradient Gel Electrophoresis (DGGE) analysis of non-culturable fungi (Tao *et al.*, 2008). However, few basidiomycetes have been identified by the use of the latter technique (e.g. Duong *et al.*, 2006).

The greatest endophyte basidiomycete diversity has been that from the cocoa plant (*Theobroma cacao*) (Crozier *et al.*, 2006) and *Theobroma gileri* (Evans *et al.*, 2003; Thomas *et al.*, 2008) (Table 2). They detail eight and two genera in the *Agaricales* and *Russulales*, respectively, while the greater number belong in the *Polyporales* (29). However, there is little overlap with those isolated in the current study: *Schizophyllum* sp. and *Pycnoporus* sp. 1, 2 (Crozier *et al.*, 2006; Thomas *et al.*, 2008), while Evans *et al.* (2003) and Puri *et al.* (2002) isolated a *Trametes* sp. The palm endophytes could be assigned with confidence to *Schizophyllum commune* and *Pycnoporus sanguineus*, both also collected as saprobes of senescent palm fronds in Thailand. This suggests that members of the *Polyporales* could be dominant endophytic basidiomycetes within woody plants. In our investigation, *Fomitopsis* species are the most diverse and largest fungal assemblage in oil plam, *Elaeis guineensis*.

Identification of endophytes from oil palm

Three saprobic *Pycnoporus* species were also sequenced to see if they were related to the isolated endophytes. Isolate BCC 26410 was isolated from decaying oil palm fronds (from the same location as the endophyte study), and groups with *P. sanguineus*. The two other strains (BCC 26408, BCC 27595 isolated from decaying wood in Thailand, Table 1) were identified as *P. puniceus* and formed a sister group to *P. cinnabarinus*. *Pycnoporus puniceus* is a rarely collected species and this is the first record for Thailand. However, it has been reported from Malaysia (Ryvarden and Johansen, 1980).

Schizophyllum commune was also isolated as a saprobe (BCC22128 from oil palm, BCC26407 from a mangrove tree, and BCC 26414 from bamboo) and used in our analysis. All group with other *S. commune* sequences from the GenBank. This is an extremely common basidiomycete in Thailand, occurring on a wide range of substrata although not particularly active in wood degradation (Ujang *et al.*, 2007). It is worldwide in distribution and James *et al.* (2001) have identified three genetically discrete populations: eastern hemisphere; North America and Central America, South America and Caribbean, but they did not sequence any Asian strains. Strain 2 IV 2/1 sporulated on the isolation plug plated out on PDA.

Isolate 8R 1/1 forms a well supported group with *Trametes elegans*, and an unidentified endophyte sequence from the GenBank. The taxonomic status of the *T. elegans* in our analysis may be questioned, but it is the same sequence as that used by Tomšovsky *et al.* (2006) in their study into the molecular phylogeny of European *Trametes* species. They concluded that *T. elegans* belongs in the genus *Trametes*, and confirmed the monophyly of the genus *Pycnoporus* within the paraphyletic *Trametes* clade. However, the colony morphology of isolate 8R 1/1 was identical to 8R 1/2 (*P. sanguineus*) which raises the question of the identity of this strain. Two strains of *Trametes* (BCC23750, BCC23751 isolated as saprobic on wood collected from Khao Luang National Park) were also included in our study and form a clade with good support, but do not group with the endophytic isolate 8R 1/1.

The greatest number of palm endophytes grouped in the *Fomitopsidaceae*, *Polyporales* and the genus *Fomitopsis*. These are reported for the first time as endophytes (Table 2). *Fomitopsis* species are active brown rot fungi and cosmopolitan in their distribution in boreal and temperate zones (Ryvarden and Gilbertson, 1993; Kim *et al.*, 2005, 2007). *Fomitopsis* is phylogenetically heterogeneous, which Kim *et al.* (2005) divided into three subgroups, but none well-supported by bootstrap support. Kim *et al.* (2007) described a new *Fomitopsis* (*F. incarnatus*) which groups with *F. rosea* (*Rhodofomes*) and *F. cajanderi*, in a well-supported clade. However, the phylogenetic position of the *Fomitopsis* species is not fully resolved.

In our phylogenetic analysis, *Fomitopsis* species separated in to three clades: (1). Four isolates (7R 9/1, 7P 3/1, 8V 6/1, 10R 8/1) forming a subclade with *F. ostreiformis*, with *F. palustris* as a sister group. However, Kim *et al.* (2005) report *F. feei* and *F. palustris* grouping together with *Piptoporus portentosus*, and *Daedalea quercina*, but the relationship was not resolved. (2). Two isolates (7R 8/1, 9V 3/1) formed a well supported sister group to *F. pinicola*. However Kim *et al.* (2005) show that *F. pinicola* formed a monophyletic group with *Piptoporus betulinus* as a sister group. (3). Three isolates (1P 1/1, 2IV 7/1, 5V 3/3) group with *Fomitopsis meliae* with high support, which has an affinity with *F. pinicola*, *P. betulinus* and *F. palustris* (Kim *et al.*, 2007), and this is also reflected in our study. *Fomitopsis meliae* is sometimes regarded as a synonym of *Fomes meliae* (Index Fungorum) but does not belong in that genus because it is a brown rot species (Hattori, pers. comm.) *Fomitopsis meliae* is often regarded as an allied species of *F. palustris* (Kim *et al.*, 2007) and refered by Kotlaba and Pouzar (1990) to the genus *Pilatoporus*. However, in our data *F. meliae* and *F. palustris* are not monophyletic. *Fomitopsis meliae* is an American species and occurs in tropical Asia as well. Of some 43 recognized *Fomitopsis* species (Index Fungorum), *F. pinicola* and *F. pseudopetchii* are known from Thailand, both collected in the north of the country (Hjortstrom and Ryvarden, 1982; Phanichapol, 1968), while Corner (1989) reported *F. euosma* and *F. pseudopetchii* from Malaysia. Therefore the data recorded here adds to our knowledge of *Fomitopsis* in tropical areas.

Induction of basidiomycete fruiting bodies

Initially our basidiomycete isolates did not sporulate under laboratory conditions, but eventually five strains produced minute poroid basidiomes (Figs 1a-b, 2b-e). The endophyte strains were inoculated with test blocks of palm petioles and small basidiomes formed after 12 months of incubation.

Fruiting body induction in basidiomycetes is variable with *Schizophyllum commune* producing prolific basidiomes on sawdust media in plastic bags (Thaithatgoon *et al.*, 2004; Vikineswary *et al.*, 2007) after 4 weeks. Lomascolo *et al.* (2002) induced basidium-producing areas of *Pycnoporus* strains as "reddish-orange granules" on malt extract broth after 4-5 weeks incubation at 20-24°C. Similar observations are repeated here (Figs 1a-b). Basidiomycete endophytes may well have been overlooked in previous studies as the mycelium was not examined for the presence of clamp connections, or the induction of fruiting bodies under laboratory conditions. For the latter, a prolonged incubation period may be necessary.

Role of endophytic basidiomycetes

The documentation of a wider range of basidiomycetes as endophytes raises the questions as to their role in nature. Chapela and Boddy (1988a,b) pointed out that endophytes (particularly basidiomycetes), may be precursors to a saprobic phase. They drew attention to the rapid growth of these fungi on senescence of the woody tissue, and ultimately a saprobic regime. This hypothesis has been revisited by Oses *et al.* (2006), who isolated two basidiomycete endophytes from Chilean tree species (*Drimys winter* and *Prumnopitys andina*) and evaluated their ability to produce lignocellulotytic enzymes. The *Bjerkandera* sp. produced phenoloxidase and cellulase, with a weight loss of wood

chips of 13.3%. The unidentified basidiomycete (probably a *Rhizocontia* sp.) was unable to cause weight loss of the wood chips. Oses *et al.* (2006) concluded that "basidiomycetes are able to develop a non-selective white rot decay pattern", a strategy that may confer an advantage in the colonization of senescent woody tissue. This hypothesis may be correct, however, in two studies on saprobes on palms in Thailand only two and three basidiomycete taxa were identified (Pinnoi *et al.*, 2006; Pinruan *et al.*, 2007), while Fröhlich *et al.* (2000) reported none in their study of endophytic palm fungi from Australia and Brunei. This result was probably due to a bias towards ascomycetes and their anamorphs.

Hyde (2001) suggests there is compelling evidence that endophytic fungi become saprobes, while others support the hypothesis that they may be latent pathogens (Photita *et al.*, 2001; Duong *et al.*, 2006). Arnold (2007) however, cautions such conclusions until substrates are sampled to the point of statistical completion.

Most endophytic basidiomycetes are white rot species (Oses *et al.*, 2006; Thomas *et al.*, 2008), while *Fomitopsis* species are brown rot fungi. Wood decay fungi are able to produce a wide range of lignocellulosic enzymes (Pointing *et al.*, 2000; Lomascolo *et al.*, 2002; Oses *et al.*, 2006; Munusamy *et al.*, 2008), and their presence as endophytes is a useful strategy later when the host dies (Tao *et al.*, 2008).

Potential of bioactive metabolites from basidiomycetes

The potential use of these endophytes as biocontrol organisms against the oil palm pathogen, *Ganoderma boninense*, is dependent on the isolated endophytes producing bioactive secondary metabolites (Evans *et al.*, 2003). Endophytes have been shown to be a rich source of bioactive metabolites (Strobel, 2002; Strobel *et al.*, 2001; Ezra *et al.*, 2004; Kim *et al.*, 2004; Maria *et al.*, 2005; Schulz *et al.*, 2002, 2007; Wiyakrutta *et al.*, 2004; Tejesvi *et al.*, 2007; Phongpaichit *et al.*, 2007; Pongcharoen *et al.*, 2008). However, endophytes have yet to be screened for bioactive compounds, but their saprobic counterparts are known to have such activity (Kupka *et al.*, 1981; Rosa *et al.*, 2003; Zjawiony, 2004; Valdicia *et al.*, 2005).

Conclusion

The objective of this study was to explore the diversity of basidiomycete endophytes, and characterize the isolates from oil palm. Of the 13 isolates studied, three strains can be identified with confidence as *Schizophyllum commune* (2) and *Pycnoporus sanguineus* (1) while a fourth strain falls within the *Pycnoporus* clade. Of the nine remaining isolates, four showed an affinity with *Fomitopsis ostreiformis*, three with *Fomitopsis meliae* and two with *F. pinnicola*. Further resolution of the *Fomitopsis* strains requires wider taxon sampling and a range of genes.

Recent studies indicate that basidiomycetes are part of the endophytic community and careful examination of sterile cultures for clamp connections and minute fruit bodies may yield further taxa. Further studies may well confirm that basidiomycete endophytes are host specific as outlined in this paper. Orchids, liverworts and woody plant hosts appear to support taxonomic diverse endophytic taxa.

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MYCOTAXON

BIODIVERSITY OF FUNGI ON CALAMUS (PALMAE) IN THAILAND

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ABSTRACT

A study of saprotrophic microfungi associated with the palm *Calamus* sp. in Thai forests yielded 88 species, with 40 ascomycetes (45.5%), and 48 anamorphic taxa (54.5%) from 212 fungal collections. The most common fungi were *Tetraploa* sp. (14.1% of all records), *Morenoina palmicola* (11.8%), *Circinoconis paradoxa* (5.2%), *Diaporthe* sp. (4.7%), and *Helminthosporium* sp. (4.7%). The percentage of fungi occurring on dry versus damp materials were 68.5% and 31.5%, respectively, with 61% of fungi occurring on petioles and 39% on rachides. The fungi occurring on *Calamus* sp. are compared with those recorded on other palms in Australia, Brunei, Hong Kong and Thailand.

Key words: biodiversity, palm fungi, tissue preference.

INRODUCTION

Several studies have been undertaken on saprotrophic fungi from Thai palms: Pilantanapak (2005) reported 81 taxa from *Nypa fruticans* of which 22 were new records for the principality; Aramsiriujiwet (1996) collected 29 hyphomycetes from terrestrial palms in Southern Thailand (*Borassus flabellifer, Caryota* sp., *Cocos nucifera, Cyrostachys lakka, Corypha lecomtei, Elaeis guineensis*, and *Roystonea*

regina), while Sarapat (2003) recorded 111 species from twelve palm species sampled in Sirindhorn peat swamp forest. Hidiyat *et al.* (2006) reported 4 species of *Oxydothis* from palms in Chiang Mai Province, and three of these were new to science. Subsequently Pinnoi *et al.* (2006) and Pinruan *et al.* (2007) documented fungi on the peat swamp palms *Eleiodoxa conferta* and *Licuala longicalycata,* respectively, from the peat swamp at Sirindhorn, yielding 114 and 147 species.

MATERIALS AND METHODS

Sample collection

Four collections of *Calamus* spp. were made in January, April, July and November (2006). Material was divided into 2 parts: palm rachides and petioles under 2 conditions: aerial (dry) and ground contact (damp). Collections were made at Khoa Yai National Park, Thaleban National Park and Klong Tom hot waterfall in Thailand. Samples were placed in plastic bags and the dates and locations recorded. Samples were returned to the laboratory and incubated in moist plastic boxes at 25°C for 1 week and observed.

Isolation

Sporulating fungi were observed under a stereomicroscope and isolated into axenic culture using a single spore technique (Choi *et al.*, 1999). 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 good growth was established. Cultures and dry material are deposited in BIOTEC Culture Collection (BCC) and BIOTEC Bangkok Herbarium (BBH), respectively.

Data analyses

Percentage abundance of taxa was calculated according to the following formula:

$$\textbf{Percentage abundance of taxon A} = \frac{\text{Occurrence of taxon A} \times 100}{\text{Occurrence of all taxon}}$$

RESULTS

Abundance of occurrence of fungi on *Calamus*.

Two-hundred and twelve fungal records made from the four field collections yielded 88 species (Ascomycota 40 species, 45.5% and anamorphic fungi 48species, 54.5%) (Figure 1). The most common fungi were *Tetraploa* sp. (14.1% of all records), *Morenoina palmicola* (11.8%), *Circinoconis paradoxa* (5.2%), *Diaporthe* sp. (4.7%), and *Helminthosporium* sp. (4.7%) (Table 1a).

The percentage occurrence of fungi on different parts of *Calamus* spp. were as follows: dry material supported 68.5% of the fungi recorded, and damp material had 31.5% (Figure 1b), with 61% on the petioles, and 39% on the rachides (Figure 1c).

Fungi found only on petioles were: *Melanographium citri* (4 records), *Astrosphaeriella* sp. 1 (4). *Astrosphaeriella vesuvius* (4), AOM 324 (3), and *Coleodictyospora micronesica* (2) while only *Lachnellula* sp. (2) occurred on the rachides. Fungi found on both petioles and rachides included: *Anthostomella* sp., *Circinoconis paradoxa*, *Diaporthe* sp., *Diplococcium* sp., *Exserticlava vasiformis*, *Goidanichiella fusiformis*, *Helminthosporium* sp., *Linocarpon* sp., *Morenoina palmicola*, *Pheosphaeria* sp., and *Sporidesmium* sp.

Forty–two taxa were found only on dry material, but only 15 taxa on damp material. Fungi occurring in both micro–habitats included: *Anthostomella* sp., *Diaporthe* sp., *Exserticlava vasiformis*, *Goidanichiella fusiformis*, *Helmithosporium* sp., *Linocarpon* sp., *Morenoina palmicola*, *Phaeosphaeria* sp., and *Sporidesmium* sp.

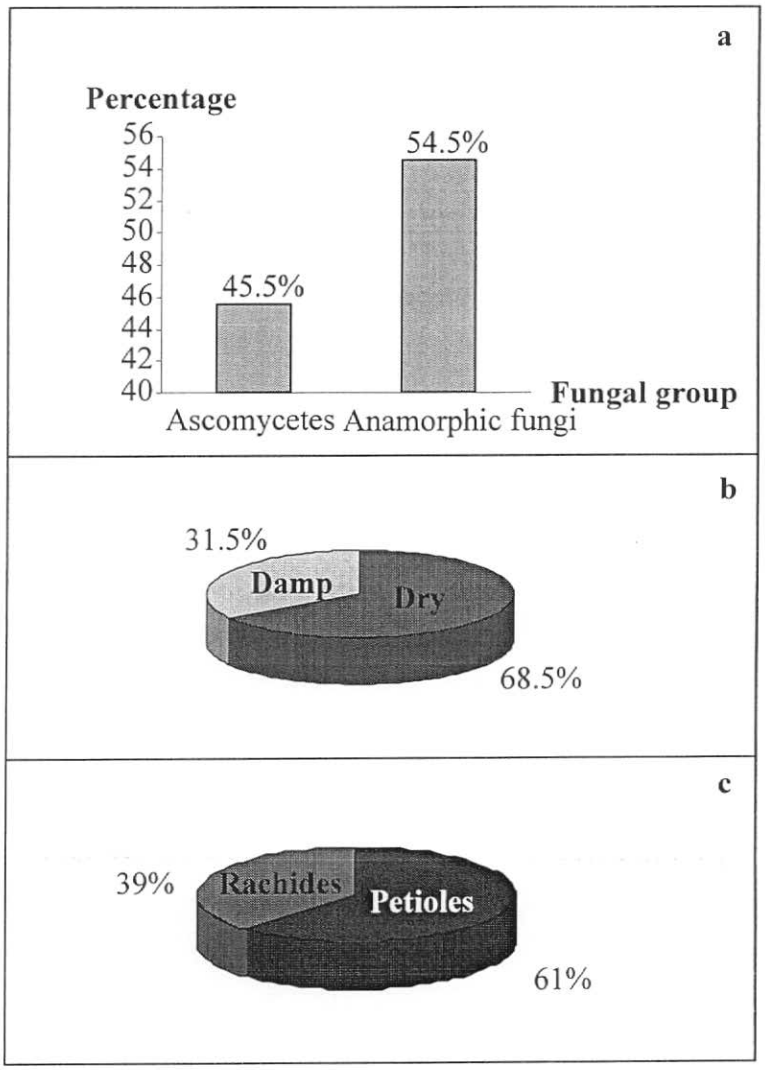

Figure 1 a. Percentage occurrence of ascomycetes, and anamorphic fungi recorded on samples of *Calamus* sp. **b.** Percentage of fungi occurring under different conditions. **c.** Percentage of fungi occurring on different parts of palm material.

Table 1. Percentage abundance of saprotrophic fungi on the terrestrial palm *Calamus*

Fungus	1*	Fungus	1*
Tetraploa sp.	14.2	*Dictyosporium* sp. 1	0.5
Morenoina palmicola	11.8	*Dictyosporium* sp. 2	0.5
Circinoconis paradoxa	5.2	*Diplocladiella* sp.	0.5
Diaporthe sp.	4.7	*Ellisembia* sp.	0.5
Helminthosporium sp.	4.7	*Ellisembia* sp.	0.5
Linocarpon sp.	3.8	*Helicoma* sp.	0.5
(AOM 301)	3.8	*Helminthosporium senseletii*	0.5
Phaeosphaeria sp.	2.8	*Helminthosporium* sp.	0.5
Anthostomella sp.	1.9	*Hyphodiscova jaipurensis*	0.5
Astrosphaeriella sp. 1	1.9	*Linocarpon* sp.	0.5
Goidanichiella fusiformis	1.9	*Orbilia* sp.	0.5
Melanographium citri	1.9	*Oxydothis* sp.	0.5
Diplococcium sp.	1.4	*Oxydothis* sp.	0.5
(AOM 238)	1.4	*Oxydothis* sp. 1	0.5
(AOM 329)	1.4	*Oxydothis* sp. 2	0.5
(AOM 324)	1.4	*Oxydothis* sp. 3	0.5
Coleodictyospora micronesica	0.9	*Pithomyces* sp.	0.5
Cordana triseptata	0.9	*Thozetella* sp.	0.5
Exserticlava vasiformis	0.9	*Sporidesmium altum*	0.5
Lachnellula sp.	0.9	*Sporidesmium* sp. 1	0.5
Sporidesmium sp.	0.9	*Sporidesmium* sp. 2	0.5
Acrodictys erecta	0.5	*Sporidesmium* sp. 3	0.5
Astrosphaeriella sp. 2	0.5	*Sporidesmium* sp. 4	0.5
Astrosphaeriella vesuvius	0.5	*Sporoschisma* sp.	0.5
Berkleasmium micronesicum	0.5	*Stictis* sp. 1	0.5
Berkleasmium crunisia	0.5	*Stictis* sp. 2	0.5
Berkleasmium sp.	0.5	Unidentified (27 taxa)	13.5
Brachysporiella gayana	0.5	*Verticillium* sp.	0.5
Capnodiastrum sp.	0.5	*Volutella ramkumarii*	0.5
Chaetosphaeria sp.	0.5		
Cylindrocladium sp.	0.5	**Anamorphic fungi**	**54.5**
Dactylaria sp.	0.5	**Ascomycetes**	**45.5**

1* = percentage abandance

DISCUSSION

Hawksworth (1991) estimated fungal diversity at 1.5 million species worldwide, but to date only approximately 80,000 species have been described (Kirk *et al.*, 2001). This prompted Hyde (2001) to speculate as to where these missing fungi might be found. Many locations, habitats and substrata have not been examined for the occurrence of fungi, many may occur as endophytes (Wei *et al.*, 2007; Sánchez Márquez *et al.*, 2007), while others are non cultureable (Duong *et al.*, 2007). This has led to intensive studies on fungal diversity worldwide and in particular in Asian and

South American regions (e.g. Desjardin and Ovrebo, 2006; Le *et al.*, 2007; Nuytinck *et al.*, 2006).

In an analysis of fungal communities on *Calamus* in northern Queensland, Australia (Fröhlich and Hyde, 2000) and the present study, 17 and 88 genera, respectively were reported, but only six genera were common to both localities: *Anthostomella*, *Diaporthe*, *Lachnellula*, *Linocarpon*, *Morenoina* and *Oxydothis*. Eighty-five genera occurred only on the peat swamp palms *Eleiodoxa conferta* and *Licuala longicalycata* when compared to those on *Calamus* species (Table 2). This indicates the great variation that occurs between the different palms and their habitats as seen in previous studies (Fröhlich and Hyde, 2000, Taylor and Hyde, 2003).

The fungal community on *Calamus* spp. in this study also differs from that on the terrestrial palms from Brunei and Hong Kong SAR, in having more ascomycetes than anamorphic fungi (Table 3). Only the genera *Astrosphaeriella* and *Helminthosporium* were common to this study and terrestrial palms in Hong Kong and Brunei (Table 3). One reason that more ascomycetes may occur on palm material is their ability to withstand desiccation, larger size of the resource allowing for a wide variety of taxa to colonize it. Often the fronds remain attached to the tree in an aerial position where they decay. The ascomycete fruiting bodies are usually covered in hardened clypei (e.g. *Oxydothis* spp; *Astrosphaeriella* spp.) which reduces drying out of substratum. Subsequently when the fronds become wet ascomata absorb water and start to release spores (Hyde, pers. obers.). {is the ratio od asco to hypho lower in wet palms? – this would support my argument).

Several studies, of different habitats and hosts show dissimilar fungal communities (Goh and Hyde, 1996; Wong *et al.*, 1998; Ho *et al.*, 2000; Kane *et al.*, 2002; Tsui and Hyde, 2003; Tsui *et al.*, 2003; Shearer *et al.*, 2007; Kodsueb *et al.*, 2008a,b). Of key importance is the low overlap between different habitats (Cai *et al.*, 2006; Pinnoi *et al.*, 2006, Pinruan *et al.*, 2007; Kodsueb *et al.*, 2008a,b). Fungal colonization may depend on environmental conditions such as climate, temperature, humidity, and these usually differ between different habitats (Baker and Meeker, 1972).

Fungal diversity in tropical regions is greater than temperate regions (Goh and Hyde, 1996; Wong *et al.*, 1998; Ho *et al.*, 2000; Kane *et al.*, 2002; Tsui and Hyde,

2003; Tsui *et al.*, 2003; Hyde *et al.*, 2007; Shearer *et al.*, 2007). Pinruan (2004) suggested that a number of factors affect fungal diversity including: number of samples collected, portion of plant material sampled (such as rachis, petiole or inflorescence), collecting times, different hosts, different habitats, climate, nutrient status of host, presence of inhibitory compounds, fungal competition for resource, and the status of the host in the country.

In the present study, approximately an equal number of ascomycetes and anamorphic fungi were recorded. Ascomycetes are prevalent on peat swamp palms; where the relative humidity of the habitat may be a key factor in determining the fungal community. The anatomy and structure of ascomycetes is more complicated than for anamorphic fungi, so they may need a longer time and suitable environment to produce ascomata. Consequently the nature of the substratum in terms of dimension, composition and size of resource is also relevant. Therefore the combination of a large resource combined with suitable environmental conditions are important in the development of a varied fungal community.

Calamus petioles supported a greater species diversity than rachides and this may be accounted for by the larger surface area, and tissues composed of lignocellulose. There is a marked difference in the anatomical structure of palm tissues: thin walled parenchymatous cells in leaves and thick-walled cells in petioles and rachides that are cellulose rich and contain lignin (Pinruan *et al.*, 2007; Hyde *et al.*, 2007).

Petioles contain vascular bundles and a larger surface area that may take up water and retain moisture for a longer time. Tran *et al.* (2006) suggest that a large leaf retains more moisture than a similar layer of small leaves. This may affect tissue-specificity, a topic rarely discussed (Yanna *et al.*, 2001a; Paulus *et al.*, 2003). Host substrata contain a wide variety of compounds, some of which may attract fungal colonization (Boddy and Watkinson, 1995; Pinruan *et al.*, 2007), and some may inhibit or are toxic for fungal growth e.g. phenolic compounds (Yanna *et al.*, 2001a; Pinruan *et al.*, 2007).

The overlap in fungal diversity on different hosts is quite low (Cai *et al.*, 2006; Pinnoi *et al.*, 2006, Pinruan *et al.*, 2007; Kodsueb *et al.*, 2008a,b) and possible reasons for is may be tissue-specificity, or recurrence (Fröhlich and Hyde 2000; Yanna *et al.*

2001a,b; Zhou and Hyde, 2001; Taylor and Hyde, 2003). Hyde and Alias (2000) report 41 fungi that are unique to *Nypa fruticans,* with different parts of the palm supporting different fungi. A similar observation was made by Pinruan *et al.* (2007) for the palm *Licuala longicalycata* and equally different palm tissues and microhabitats supported distinct fungal communities. Hyde *et al.* (2007) suggested that "currently, lack of knowledge of the full extent of fungal specificity or recurrence because of incomplete sampling and because no systematically collected data is available for microfungal assemblages on other closely related plant taxa", may account for the observation made.

Competition between fungi is another factor that may account for the observed specificity. Dix and Webster (1995) observed stronger competition between species occurring on the lower than the upper portions of grasses after stem collapse. Pinnoi (2004) reported some ascomycetes, such as *Stilbohypoxylon eleiodoxae* produced inhibition zones with other fungi.

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Table 2 A comparison of fungi reported from this study with terrestrial and peat swamp palm at the genera level.

Genus name	1*	2*	3*	4*	Genus name	1*	2*	3*	4*
Acrocalymma	–	–	+	–	*Cylindrocladium*	–	+	–	–
Acrodictys	–	+	–	–	*Dactylaria*	–	+	+	+
Annulatascus	–	–	+	+	*Delortia*	–	–	+	+
Anthostomella	+	+	+	+	*Diaporthe*	+	+	+	–
Apioclypea	–	–	+	–	*Dictyochaeta*	–	–	–	+
Arecomyces	+	–	–	+	*Dictyosporium*	–	–	–	+
Arecophila	+	–	–	+	*Dictyosporium*	–	+	–	–
Arthrinium	–	–	–	+	*Didymobotryum*	–	–	+	–
Arthrobotrys	–	–	+	+	*Didymosphaeria*	–	–	–	+
Ascominuta	–	–	–	+	*Diplocladiella*	–	+	–	–
Aspergillus	–	–	–	+	*Diplococcium*	–	+	+	–
Astrocystis	+	–	–	+	*Durispora*	+	–	–	–
Astrosphaeriella	–	+	+	+	*Ellisembia*	–	+	–	–
Bactrodesmium	–	–	–	+	*Endocalyx*	–	–	–	+
Baipadsphaeria	–	–	–	+	*Eutypa*	+	–	–	–
Berkleasmium	–	+	+	+	*Exserticlava*	–	+	–	–
Bionectria	–	–	+	–	*Fasciatispora*	+	–	–	–
Brachysporiella	–	+	+	–	*Flammispora*	–	–	–	+
Canalisporium	–	–	–	+	*Fluviatispora*	–	–	+	–
Cancellidium	–	–	+	+	*Frondisphaeria*	+	–	–	–
Candelabrum	–	–	–	+	*Gaeumannomyces*	–	–	+	–
Capnodiastrum	–	+	+	–	*Gliocladium*	–	–	–	+
Capsulospora	+	–	+	–	*Glomerella*	–	–	–	+
Carinispora	–	–	–	+	*Glonium*	–	–	–	+
Caryospora	–	–	–	+	*Gnomonia*	–	–	+	–
Cenangiumella	+	–	–	–	*Goidanichiella*	–	+	+	–
Chaetoporthe	–	–	+	–	*Gonytrichum*	–	–	+	+
Chaetopsina	–	–	+	–	*Guignadia*	+	–	+	+
Chaetospermum	–	–	–	+	*Haematonectria*	+	–	+	–
Chaetosphaeria	–	+	–	+	*Haplographium*	–	–	+	–
Chalara	–	–	+	+	*Helicoma*	–	+	+	+
Chloridium	–	–	+	–	*Helicomyces*	–	–	+	–
Circinoconis	–	+	–	–	*Helicosporium*	–	–	+	+
Coleodictyospora	–	+	+	–	*Helicoubisia*	–	–	+	–
Cordana	–	+	–	–	*Helminthosporium*	–	+	–	–
Cosmospora	+	–	–	–	*Herpotrichia*	+	–	–	–
Craspedodidymum	–	–	–	+	*Heteroconium*	–	–	+	–
Cryptophailoidea	–	–	–	+	*Hydropisphaera*	+	–	–	–
Custingophora	–	–	+	–	*Hyphodiscova*	–	+	–	–
Cyanopulvis	+	–	–	–	*Hypoxylon*	+	–	–	–

Table 2 *cont.* A comparison of fungi reported from this study with terrestrial and peat swamp palm at the genera level.

Genus name	1*	2*	3*	4*	Genus name	1*	2*	3*	4*
Ijuhya	+	–	–	–	*Phaeodothis*	+	–	–	+
Jahnula	–	–	+	+	*Phaeoisaria*	–	+	+	+
Koorchaloma	–	–	–	+	*Phialogeniculata*	–	–	+	–
Lachnellula	+	+	–	–	*Phomatospora*	–	–	–	+
Lachnum	+	–	–	–	*Phruensis*	–	–	–	+
Lanceispora	–	–	–	+	*Pithomyces*	–	+	–	–
Lasiodiplodia	–	–	–	+	*Pleurophragmium*	–	–	+	–
Lasionectria	+	–	–	–	*Pseudorobillarda*	–	–	–	+
Linocarpon	+	+	+	+	*Rosellinia*	–	–	–	+
Lophiostoma	–	–	+	+	*Septomyrothecium*	–	–	+	–
Lophodermium	–	–	+	–	*Solheimia*	–	–	–	+
Manokwaria	+	–	–	–	*Sorokinella*	+	–	–	–
Massarina	–	–	–	+	*Spadicoides*	–	–	–	+
Melanographium	–	+	+	+	*Sporidesmiella*	–	–	–	+
Microthyrium	–	–	+	+	*Sporidesmium*	–	+	+	–
Mollisia	+	–	–	–	*Sporoschisma*	–	+	–	–
Monotosporella	–	–	+	+	*Stachybotrys*	–	–	+	+
Morenoina	+	+	+	–	*Stictis*	–	+	+	–
Munkovalsaria	–	–	+	–	*Stilbohypoxylon*	+	–	+	+
Mycomicrothelia	+	–	–	–	*Strossmayeria*	+	–	–	–
Myelosperma	+	–	–	+	*Submersisphaeria*	+	–	+	+
Nawawia	–	–	+	+	*Terriera pandani*	+	–	–	–
Nectria	–	–	–	+	*Tetraploa*	–	+	–	–
Nemania	–	–	+	–	*Thailandiomyces*	–	–	–	+
Niesslia	–	–	–	+	*Thozetella*	–	+	+	+
Ochronectria	+	–	–	–	*Trichoderma*	–	–	+	+
Ophioceras	+	–	–	+	*Tubeufia*	+	–	+	+
Ophiostoma	–	–	+	–	*Unisetosphaeria*	–	–	+	–
Orbilia	–	+	+	+	*Vanakripa*	–	–	+	–
Ornatispora	–	–	+	–	*Verticillium*	–	+	+	+
Oxydothis	+	+	+	+	*Volutella*	–	+	–	–
Pemphidium	+	–	–	–	*Wiesneriomyces*	–	–	–	+
Penicillium	–	–	+	+	*Xylaria*	+	–	–	–
Pestalosphaeria	–	–	+	–	*Xylomyces*	–	–	+	+
Petrakiopsis	–	–	–	+	**Total species**	**40**	**37**	**68**	**75**

* 1 = *Calamus* (Fröhlich and Hyde, 2000)

2 = *Calamus* (this study)

3 = *Eleiodoxa conferta* (Pinnoi *et al.*, 2006)

4 = *Licuala longicalycata* (Pinruan *et al.*, 2007)

Table 3 Comparison of fungi on terrestrial palms with those on *Calamus* spp.

***Calamus* spp.**	***Arenga engleri* (Hong Kong SAR)**	***Livistona chinensis* (Hong Kong SAR)**	***Phoenix hanceana* (Hong Kong SAR)**
Tetraploa sp.	*Piricauda cochinensis*	*Astrosphaeriella bakeriana*	*Diplococcium stoveri*
Morenoina palmicola	*Diplococcium stoveri*	*Lachnum palmae*	*Endocalyx cinctus*
Circinoconis paradoxa	*Helminthosporium solani*	*Appendicospora hongkongensis*	*Cryptophiale udagawae*
Diaporthe sp.	*Melanographium palmicola*	*Monodictys putredinis*	*Penzigomyces nodipes*
Helminthosporium sp.	*Melanographium selenioides*	*Oxydothis elaeicola*	*Thozetella effusa*
Linocarpon sp.	*Monodictys putredinis*	*Trichoderma harzianum*	*Pseudospiropes simplex*
(AOM 301)	*Oxydothis ragai*	*Neolinocarpon australiense*	*Dictyochaeta simplex*
Phaeosphaeria sp.	*Pestalotiopsis palmarum*	*Fasciatispora petrakii*	*Serenomyces shearii*
Anthostomella sp.	*Guignardia manokwaria*	*Corynesporopsis isabelicae*	*Capsulospora brunneispora*
Astrosphaeriella sp.	*Dischoridium roseum*	*Dictyosporium elegans*	*Harknessia globosa*
Ascomycetes = 6 species	Ascomycetes = 2 species	Ascomycetes = 6 species	Ascomycetes = 2 species
Anamorphic fungi = 4	Anamorphic fungi = 8	Anamorphic fungi = 4	Anamorphic fungi = 8
Total = 10	Total = 10	Total = 10	Total = 10
***Calamus* spp.**	***Arenga undulatifolia* (Brunei)**	***Oncosperma horridum* (Brunei)**	***Salacca affinis* (Brunei)**
(AOM 318)	*Piricauda cochinensis*	*Linocarpon livistinae*	*Zygosporium minus*
Morenoina palmicola	*Melanographium selemiodes*	*Craspedodydimum nigroseptatum*	*Linocarpon livistinae*
Circinoconis paradoxa	*Trichoderma harzianum*	*Zygosporium minus*	*Peltistromella anomala*
Diaporthe sp.	*Zygosporium minus*	*Monotosporella setosa* var. *macrospora*	*Helicosporium griseum*
Helminthosporium sp.	*Pleurophragmium* sp.	*Neolinocarpon australiense*	*Volutella ciliata*
Linocarpon sp.	*Helmithosporium velutimum*	*Trichoderma harzianum*	*Oxydothis luteaspora*
(AOM 301)	*Volutella ciliata*	*Oxydothis luteaspora*	*Periconiella* sp.
Phaeosphaeria sp.	*Peltistromella anomala*	*Oxydothis licualae*	*Arecomyces bruneiensis*
Anthostomella sp.	*Stachylidium* sp.	*Oxydothis elaeicola*	*Sporidesmium parvum*
Astrosphaeriella sp.	*Anthostomella minutoides*	*Brachysporiella gayana*	*Codinaea intermedia*
Ascomycetes = 6 species	Ascomycetes = 1 species	Ascomycetes = 5 species	Ascomycetes = 3 species
Anamorphic fungi = 4	Anamorphic fungi = 9	Anamorphic fungi = 5	Anamorphic fungi = 7
Total = 10	Total = 10	Total = 10	Total = 10

Bambusicolous polypores collected in Central Thailand

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The following seven polypores were recorded on bamboo culms in Central Thailand: *Flavodon flavus*, *Grammothele fuligo*, *Irpex lacteus*, *Perenniporia bambusicola* sp. nov., *Piptoporus roseovinaceus* sp. nov., *Rigidoporus* cf. *lineatus*, and *Serpula similis*. *Perenniporia bambusicola* is characterized by orange pores turning dark violet to black in KOH, orange mycelial strands and sheet and oblong, apically truncate basidiospores. A key to the world species of *Perenniporia* with resupinate basidiocarps and bright colored pore surface is provided. *Piptoporus roseovinaceus* is compared to *P. soloniensis*, but its hyphal system is monomitic in the trama while the latter has dimitic tubes.

Key words: Bambusoideae, Basidiomycetes, host specificity, polyporaceae, wood-inhabiting fungi

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Introduction

Bamboos are widely distributed in Thailand in several forest types, especially mixed deciduous forest and as understory shrubs and in bamboo forests. Eighty two species of bamboo belonging to 15 genera are documented in Thailand with *Dendrocalamus brandisii*, *Dendrocalamus* sp., *Bambusa blumeana*, *Thyrsostachys oliveri*, and *Cephalostachyum pergracile* being the most common species (Rungnapha *et al.*, 2001). Hyde *et al.* (2002) based on literature search and scanning the "Index of Fungi" (http://nt.ars-grin.gov.fungaldatabase) listed more than 1,100 species reported on bamboo worldwide including 630 ascomycetes, 150 basidiomycetes and 330 anamorphic fungi. Many basidiomycetes were rusts and smuts of the genera *Puccinia*, *Stereostratum* and *Uredo* causing spots on leaves.

Boidin *et al.* (1986) and Sotome *et al.* (2007) have reported polypores on bamboo, while Coelho *et al.* (2006) list 56 worldwide. Only limited information is available on Thai polypores, while in this study 124 basidiomycete collections were made on bamboo.

The purpose of this study is to describe two new polypore species collected on bamboo, in the genera *Perenniporia* and *Piptoporus* and to present a key to *Perenniporia* species with resupinate basidiocarps with a yellow to orange pore surface.

Materials and methods

Intensive collections of polypores and other basidiomycetes on bamboo culms were made mainly in Central Thailand. Main collecting

sites were as follows: Prachin Buri Prov.: The Bamboo Park; Wang Bon. Nakhon Ratchasima Prov.: Khao Yai National Park. Nan Prov.: Phu Fa Phattana Centre (Huai Pha Phueng); Huai Pla Pung. Nakhon Si Thammarat Prov.: Khao Luang National Park.

Macroscopic characters were described based on fresh and dried specimens. Microscopic characters were made from dried specimens, examining free-hand cut sections mounted in Melzer's reagent or in 5% (w/v) KOH solution after staining in 1% (w/v) phloxine solution. A non-amyloid and non-dextrinoid reaction was described as IKI-. Basidiospores were measured mounted in Melzer's reagent. The following abbreviations are used in the text: L, mean spore length; W, mean spore width (side view); r, the ratio of length/width of a basidiospore; R, mean of r. The term (n = x/y) means x measurements of basidiospores from y specimens. In presenting the spore size, 5% of the measurements at each end are given in parentheses (Dai and Niemelä 1997). Examined specimens were deposited in BBH or TFM.

Taxonomy

Flavodon flavus (Klotzsch) Ryvarden, Norw. J. Bot. 20: 3 (1973).

Specimens examined: THAILAND, Prachin Buri Prov., The Bamboo Park, 27 September 2005, coll. R. Choeyklin (BBH 19092); the same place, 20 September 2006, coll. R. Choeyklin (BBH 19090); the same place, 6 December 2006, coll. R. Choeyklin (BBH 19091; 19283).

Remarks: This is a common species in SE Asia, frequently found on hardwood trees, but also occasionally reported on bamboo in Papua New Guinea (Quanten, 1997). For a detailed description, see Ryvarden and Johansen (1980).

Grammothele fuligo (Berk. & Broome) Ryvarden, Trans. Br. mycol. Soc. 73: 15 (1979).

Specimens examined: THAILAND, Prachin Buri Prov., The Bamboo Park, 6 December 2006, coll. R. Choeyklin (BBH 19763).

Remarks: This species is restricted to monocotyledons (Ryarden and Johansen, 1980), and has been recorded on bamboo in India (Virdi, 1990) and Costa Rica (Carranza-Morse, 1991). For a detailed description, see Ryvarden and Johansen (1980).

Irpex lacteus (Fr.) Fr., Elench. Fung. 1: 142 (1828).

Specimens examined: THAILAND, Prachin Buri Prov. Wang Bon, June 2006, coll. R. Choeyklin (BBH 19101).

Remarks: This species occurs most frequently on hardwood trees (Gilbertson and Ryvarden, 1986), but has also been recorded on bamboo by Coelho *et al.* (2006). For a detailed description, see Gilbertson and Ryvarden (1986).

Perenniporia bambusicola Choeyklin, T. Hatt. & E.B.G. Jones, **sp. nov.** (Fig. 1, 3-7)
MycoBank: 511874

Etymology: bambusicola (Latin), growing on bamboo.

Basidiocarpia resupinata. *Pori angularia*, aurantiaca, 6–8/mm. *Systema hypharum* dimitica. *Hyphae generativae* hyalinae, fibulatae. *Hyphae skeletales* arboriformes, hyalinae, haud dextrinoideae. *Basidiosporae* oblongae, truncatae, infirme dextrinoideae, 3.8–5.8 × 1.8–2.5 µm.

Basidiocarps annual, resupinate, effused. *Marginal sterile zone* fimbriate, orange to pale orange, up to 1 mm wide, often lacking. *Pore surface* even, orange when fresh, drying dark orange to orange brown, grayish orange or not discolored; pores angular, 6–8/mm; dissepiments thin and entire. *Tubes* concolorous with the pore surface, tough-fibrous to leathery, up to 1 mm deep, often shallow. *Context* almost lacking, cream to light orange. *Mycelial strands* flat and sheet-like, often conspicuous, orange to cream.

Hyphal system dimitic. *Tramal generative hyphae* with clamp-connections, occasionally branched, hyaline, 1.2–2.2 µm wide. *Tramal vegetative hyphae* arboriform with stalk and side branches, thick-walled, hyaline, IKI- to slightly dextrinoid in mass, with granules discoloring into violet in KOH solution, up to 2.0 µm wide at the base. *Hyphae composing mycelial strands* similar to *P. aurantiaca*, see Decock and Ryvarden (1999). *Basidia* only one seen, clavate, 13 µm long, 7.8 µm wide. Cystidia absent. *Basidiospores* flat, oblong ellipsoid in the side view, ellipsoid and truncate in the front view, thick-walled, hyaline, slightly dextrinoid, (3.5–)3.8–5.8 ×

(1.5–)1.8–2.5(–2.8) µm (side view) × (2.2–)2.5–3.6(–3.9) µm (front view), L = 4.7 µm, W_1 = 2.0 µm (side view), W_2 = 3.1 µm (front view), R = 2.3 µm (n = 60/2).

Specimens examined: THAILAND, Prachin Buri Prov., The Bamboo Park, on *Gigantochloa albociliata* (Munro) Kurz (*Bambusoideae*), 28 June 2006, coll. R. Choeyklin (BBH 19093; holotype); the same place and the same date (BBH 19096; 19097; 19384); the same place, 20 September 2006 (BBH 19094; 19098); the same place, 6 December 2006 (BBH 19095; BBH 19284); the same place, 11 June 2007 (BBH, 19099); Chanthaburi Prov., Khao Kitchakoot Nat. Park, on bamboo, 28 May 1997, coll. M. Núñez (TFM F-23198).

Remarks: This species is peculiar with a restricted occurrence on bamboo. After intensive collections in Central Thailand, it is hitherto known only on bamboo culms and thus possibly specific to bamboo. Additionally, the vivid orange pore surface turning dark violet to black with KOH and orange coloured mycelial strands are good field characters. Sometimes, mycelial strands are widespread on the substrates and more conspicuous than the basidiocarps.

This species is morphologically closely related *P. aurantiaca* (A. David & Rajchenb.) Decock & Ryvarden and *P. xantha* Decock & Ryvarden sharing similar yellow to orange pores discoloring into violet with KOH solution, tiny and truncate basidiospores and small arboriform hyphae in the trama (Decock and Ryvarden, 1999). However, *P. aurantiaca* has wider basidiospores (3–4 µm wide; David and Rajchenberg, 1985; Decock and Ryvarden, 1999) while *P. bambusicola* has flat and oblong basidiospores measuring 1.8–2.5 µm wide in side view. In *P. xantha*, basidiospores are also wider, and additionally the pores are more yellowish and no mycelial strands are produced (Decock and Ryvarden, 1999).

There are several species of *Perenniporia* with resupinate basidiocarps and vividly yellow to orange pore surface, and a key to the world species are provided below.

A key to the worldwide of *Perenniporia* with resupinate basidiocarps and yellow-orange pores

1. Basidiospores shorter than 5.5 µm on average..... 2
1. Basidiospores longer than 5.5 µm on average..... 6

2. Pore surface unchanged or slightly darker with KOH solution.....3
2. Pore surface violet to almost black with KOH solution; basidiospores IKI- to weakly dextrinoid..... 4

3. Pore surface bright yellow to light brown, 7–9/mm. Vegetative hyphae arboriform, IKI- to slightly dextrinoid in mass. Basidiospores ovoid to truncate, slightly thick walled, weakly to moderately dextrinoid, 4–5 × 3–4 µm. Known from SE Asia, on Dipterocarpaceae trees (Hattori and Lee, 1999 as '*P. dipterocarpicola*', Decock, 2001)..... .....***P. corticola*** (Corner) Decock
3. Pore surface creamy to yellow or light brown, 8–10/mm. Vegetative hyphae arboriform, IKI-. Basidiospores subglobose to ellipsoid, apically sub- to distinctly truncate, thick-walled, IKI- to distinctly dextrinoid, 3–3.8 × 2.5–3.2 µm. Known from Philippines and Japan (Decock, 2001)..... .....***P. straminea*** (Bres.) Ryvarden

4. Basidiospores flat, short cylindrical in side view, ellipsoid and truncate in front view. Pore surface orange, 6–8/mm. Usually with distinct mycelial strands. Vegetative hyphae arboriform, IKI- to weakly dextrinoid in mass. Basidiospores thick-walled, slightly dextrinoid, 3.8–5.8 × 1.8–2.5 (side view) x 2.5–3.6 µm (front view). Known from Thailand. On bamboo ..... ***P. bambusicola***
4. Basidiospores ellipsoid and truncate 5

5. Pores bright yellow when fresh, 6–8/mm, without mycelial strands. Vegetative hyphae arboriform, IKI- to slightly dextrinoid. Basidiospores ellipsoid, truncate, thick-walled, IKI- to slightly dextrinoid, 4.2–5.8 × 3.2–4.2 µm. Known from S America and SE Asia. On hardwoods. (Decock and Ryvarden, 1999) ***P. xantha***
5. Pores orange when fresh, 7–8/mm, with or without orange mycelial strands. Vegetative hyphae arboriform, IKI- to slightly dextrinoid. Basidiospores ellipsoid, truncate, thick-walled, IKI- to slightly dextrinoid, 4.2–5.5 × 3.0–4.0 µm. Known from S America. On hardwoods. (Decock and Rycarden, 1999)..... .....***P. aurantiaca***

6. Basidiospores thin-walled, ellipsoid and IKI-, 6–7.5 x 4–5 µm. Pore surface yellow, 4–5/mm. Vegetative hyphae unbranched to branched, IKI-. Widespread in N Hemisphere. On hardwoods. (Gilbertson and Ryvarden, 1987)..... ***P. tenuis*** (Schwein.) Ryvarden
6. Basidiospores thick-walled, more or less dextrinoid... 7

7. Pore surface yellow, 5–8/mm, often effused-reflexed, marginal sterile zone reddish. Vegetative hyphae

frequently branched, dextrinoid. Basidiospores thick-walled, ellipsoid, slightly truncate, dextrinoid, 5–7 x 3–5 μm. Known from temperate areas of E Asia. On *Maackia* and other hardwoods. (Núñez and Ryvarden, 2001).... ***P. maackiae*** (Bondartsev & Ljub.) Parmasto

7. Pores 4-6/mm, without reddish marginal zone 8

8. Vegetative hyphae distinctly arboriform, IKI-. Pore surface yellow, 4–5/mm. Basidiospores thick-walled, broadly ellipsoid, dextrinoid, 5.6–7.7 × 4.1–5.9 μm. Known from S America. On dead wood. (Decock and Ryvarden, 1999)..... .***P. chromatica*** (Berk. & Cooke) Decock & Ryvarden

8. Vegetative hyphae unbranched to branched, but not arboriform, weakly to strongly dextrinoid. Pore surface bright yellow or cream, 4–6/mm. Basidiospores thick-walled, broadly ellipsoid, weakly to strongly dextrinoid, 5–6.5 × 3.5–4.5 μm. Widespread in N Hemisphere. On hardwoods. (Gilbertson and Ryvarden, 1987)..... .....***P. meddula-panis*** (Jacq. : Fr.) Donk

Piptoporus roseovinaceus Choeyklin, T. Hatt. & E.B.G. Jones, sp. nov. (Fig. 2, 8–10)
MycoBank: 511875

Etymology: roseus + vinaceus (Latin), after the rose to wine colored pileus.

Basidiocarpia sessilia. *Pilei* dimidiati vel flabelliformes, velutini vel hirsuti, rosei vel vinacei. *Pori* rosei, angulares, 3–4/mm. *Systema hypharum* dimiticum in contextu, monomiticum in tramate. *Hyphae generativae* fibulatae. *Hyphae skeletales* hyalinae, IKI-. *Basidiosporae* prelate ellipsoideae vel ellipsoideae, IKI-, 4.8–6.0 × 3.8–4.5 μm.

Basidiocarps annual, sessile, single. *Pilei* dimidiate to flabelliform, applanate, to triquetrous, pileus surface velutinous to hirsute drying scrupose, with irregular or radial ridges, azonate, pink to reddish violet in fresh condition, drying light orange to grayish orange; pileus margin undulating, rounded. *Pore surface* even to partly nodulose, pinkish to pink, darker on bruising in fresh condition, drying sordid white to grayish orange; pores angular, 3–4/mm; dissepiments moderately thick and entire. *Context* fleshy in fresh condition, drying fibrous-corky, light in weight, without a crust, white to pale orange, up to 10 mm thick. *Tubes* soft in fresh condition drying brittle, sordid white to grayish orange, up to 2 mm deep.

Hyphal system dimitic in context, monomitic in trama. *Contextual generative hyphae* with clamp-connections, unbranched to occasionally branched, thin-walled, hyaline, 1.5–7 μm wide (in KOH solution). *Contextual skeletal hyphae* straight to sinuous, often irregularly swelled, thick-walled to almost solid, abundantly seen in Melzer's reagent, but swelled and dissolved in KOH solution, hyaline, IKI-, 3–10 μm wide. *Tramal generative hyphae* with clamp-connections, occasionally branched, hyaline, 1.5–3 μm wide. *Basidia* collapsed. *Cystidia* absent. *Basidiospores* short ellipsoid to ellipsoid, thin-walled, hyaline, IKI-, (4.2–)4.8–6.0(–6.8) × (3.7–)3.8–4.5(–4.8) μm, 1.1 = r = 1.6, L = 5.5 μm, W = 4.1 μm, R = 1.4 (n = 50/1).

Specimens examined: THAILAND, Prachin Buri Prov. The Bamboo Park, on dead bamboo culms, 28 September 2002, coll. R. Choeyklin (BBH 19084).

Other specimens examined: *Piptoporus soloniensis* (Duby : Fr.) Pilát, JAPAN, Tottori Pref., Mt. Daisen, 26 September 1986, coll. Y. Abe, (TFM F-14485); Kouchi Pref., Monobe, Nishikuma, 13 Nov. 1991, coll. T. Hattori (TFM F-16426); Nagano Pref., Kiso, Kaida, 9 September 1994, coll. T. Hattori (TFM F-17210).

Remarks: This species is close to *Piptoporus soloniensis* (Fr.) Pilát, a species with a distribution mainly in the temperate area. It has also vivid coloured pileus surface, hyphal characters in context and short ellipsoid basidiospores, but the latter has orange, cream to whitish pileus surface, buff to pinkish context and fibrous-corky tubes with a dimitic hyphal system as in context (Gilbertson and Ryvarden, 1987).

Piptoporus soloniensis is now widely accepted in *Piptoporus* P. Karst. because of the sessile basidiocarps light in weight when dry, the light colored and corky context, the dimitic hyphal system in the context, the negative reaction with Melzer's reagent, and the decay type (Gilbertson and Ryvarden, 1997; Ryvarden and Gilbertson, 1994). Kim *et al.* (2005) suggested that *P. soloniensis* is phylogenetically not related to *P. betulinus* (Bull.: Fr.) P. Karst., the type species of *Piptoporus*, but no nomenclatural conclusion was made for the placement of *P. soloniensis*. Before emendation of *Piptoporus* and other related genera based on phylogenetic analyses, we prefer to keep *P. soloniensis* and *P. roseovinaceus*, most possibly allied to the former, in this genus.

Tyromyces armeniacus (Corner) T. Hatt. and *T. incarnatus* Imazeki (= *T. roseipileus*

Corner) also have pink to reddish basidiocarps and fleshy context and were reported from Southeast Asia, but have a monomitic hyphal system in the context (Corner, 1989; Hattori, 2003a, 2003b).

Rigidoporus cf. ***lineatus*** (Pers.) Ryvarden, Norw. J. Bot. 19: 236 (1972).

Pileus dimidiate, applanate, pileus surface glabrous, concentrically sulcate, light brown up to 2 cm wide. *Pore surface* grayish orange, pores angular, 8–10/mm. *Context* fleshy-leathery in fresh condition, drying woody, without a crust, up to 1.5 mm thick. *Tubes* rigid, concolourous with pore surface, up to 1 mm deep. *Hyphal system* mono-dimitic. *Cystidial hyphae* abundant in trama, encrusted with crystals. *Basidiospores* globose to subglobose, thin-walled, hyaline, IKI-, 4–5 × 3.5–4.5 µm.

Specimens examined: THAILAND, Nakhon Si Thammarat Prov., Khao Luang National Park, 11 October 2006, coll. R. Choeyklin (BBH 19103).

Remarks: This is similar to *R. lineatus*, but basidiocarps and basidiospores are smaller than in the typical form. This form was also collected on bamboo in Malaysia and is possibly distinct from *R. lineatus*. For the time being, we leave this as *R.* cf. *lineatus* because there are several names that have been considered synonyms of *R. lineatus* but some of them have different morphology from the typical form (Hattori, 2001).

Serpula similis (Berk. & Broome) Ginns, Mycologia 63: 231 (1971).

= *Serpula eurocephala* (Berk. & Broome) W.B. Cooke, auct. non Berk. & Broome, W.B. Cooke: Mycologia 49:212, 1957.

Specimens examined: Thailand, Prachin Buri Prov., The Bamboo Park, 27 September 2005, coll. R. Choeyklin (BBH 19087); the same place, 28 June 2006, coll. R. Choeyklin (BBH 19088; 19089).

Remarks: This is widely distributed in SE Asia, and commonly seen on bamboo (Cooke, 1957) but also on hardwoods. For detailed descriptions, see Cooke (1957) as '*S. eurocephala*' and Ginns (1971).

Discussion

Among the species listed here, *F. flavus* and *I. lacteus* are more frequently reported on hardwood trees (Gilbertson and Ryvarden, 1986; Ryvarden and Johansen, 1980), and these species are suggested to have wide host range. *Grammothele fuligo* has a peculiar host range, specific to monocotyledons, and more frequently collected on palms in Thailand.

Serpula similis is most probably a paleotropical species, frequently collected on bamboo, both in the wild and in buildings, but also on other wood such as *Leucaena glauca* (Cooke 1957) suggesting that it has a preference for *Bambusoideae* but is not a specialist.

Perenniporia bambusicola is an outstanding species with a vivid orange pore surface and conspicuous mycelial strands. It is hitherto known only on bamboo, in two localities in Thailand and Yunnan in southern China (Decock, personal communication). Therefore, it might be restricted to and a specialist on bamboo. *Perenniporia aurantiaca*, a closely related species, is hitherto known only from South America (David and Rajchenberg, 1985; Decock and Ryvarden, 1999) and so far known only on hardwood trees.

Rigidoporus cf. *lineatus* can be another species that has specificity or preference for bamboo. This is similar to *R. lineatus*, but its basidiocarps are usually less than 1 cm long, and basidiospores are mostly less than 5 µm long contrasting that they are 4.5–6 µm long in *R. lineatus* (Gilbertson and Ryvarden, 1987; Ryvarden and Johansen, 1980). Detailed studies are needed to resolve its identity from *R. lineatus* and its nomenclature. *Piptoporus roseovinaceus* is hitherto known only from the holotype, and its host range is unclear.

A number of other polypores on bamboo culms in our collections are not discussed here. Some of them may be new to science, but we refrain from describing them as new because of the limited number of specimens and their quality.

After a world comprehensive survey of polypores on bamboo, Coelho *et al.* (2006) suggested that only 14 out of 57 species are specific to bamboo as a substrate. Several polypores growing on bamboo culms are

expected from tropical Asia including Thailand, but hitherto limited information is available from this area. More intensive collections and further studies may reveal more polypores specific to bamboo in tropical Asia.

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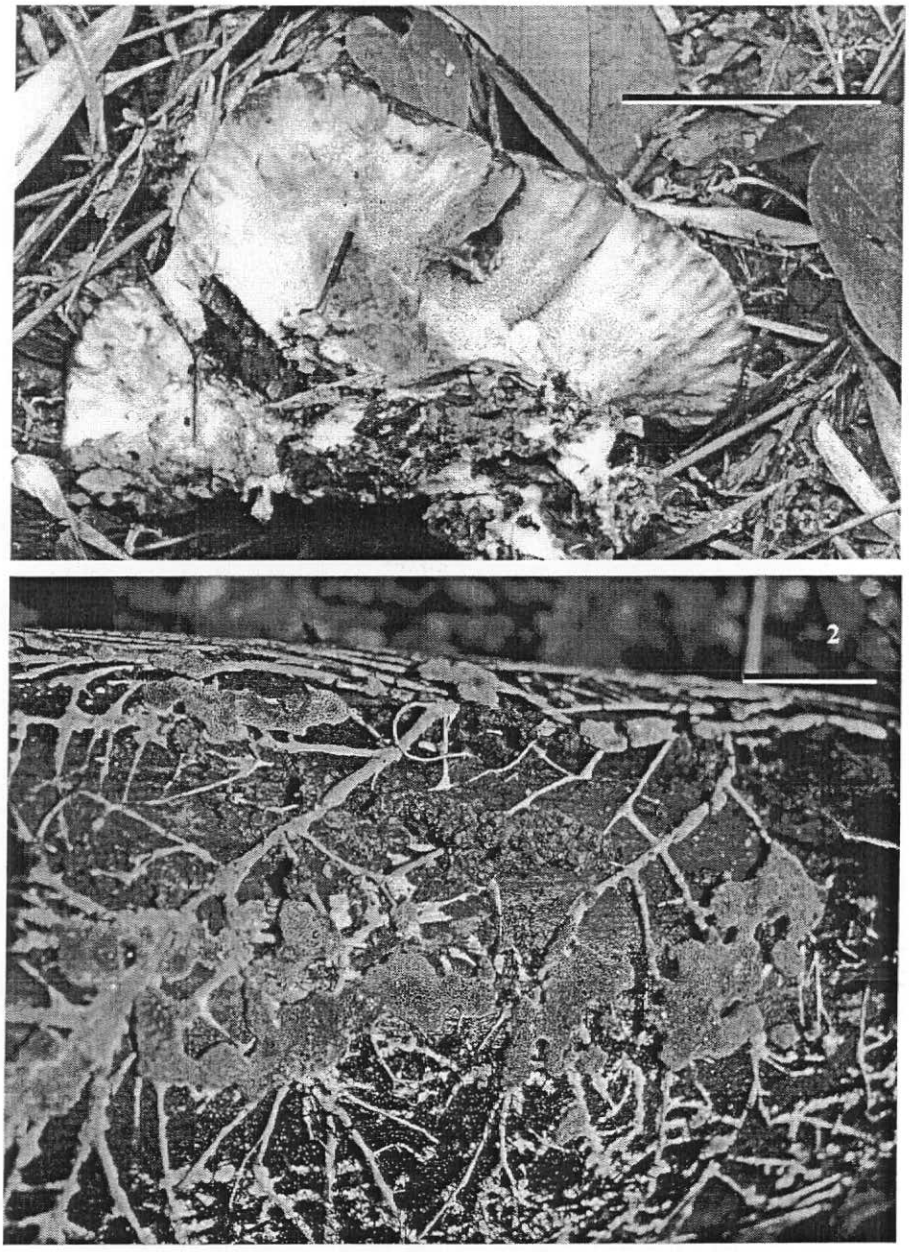
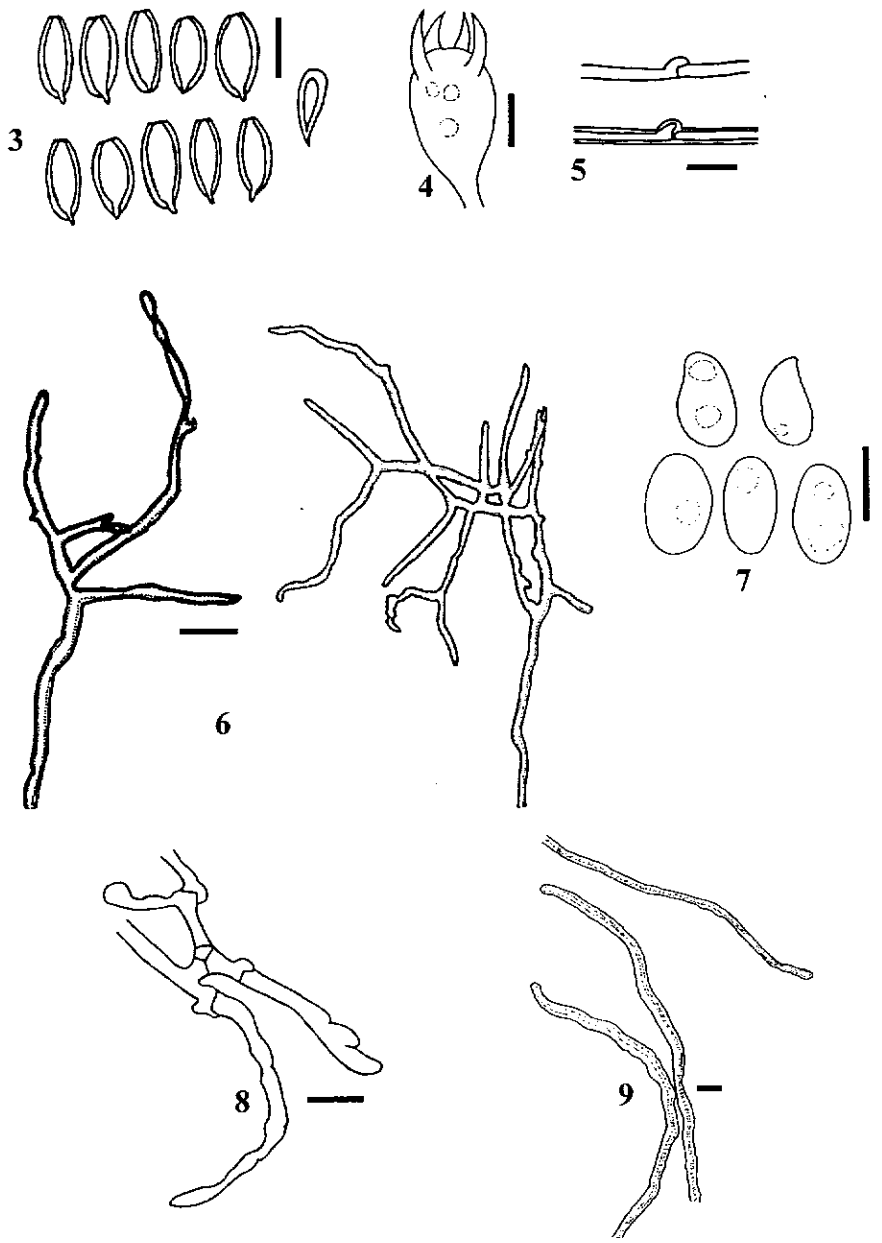


Figs 1–2. Basidiocarps (holotypes). **1.** *Piptoporus roseovinaceus* sp. nov.
2. *Perenniporia bambusicola* sp. nov. Bars: 1. = 5 cm; 2. = 2 cm



Figs 3–7. Line drawings of *Perenniporia bambusicola* sp.nov. **3.** Basidospores. **4.** Basidia. **5.** Generative hyphae with clamp-connections from trama. **6.** Arboriform vegetative hyphae with stalk and side branches from trama. **Figs 7–9.** Line drawings of *Piptoporus roseovinaceus* sp. nov. **7.** Basidiospores. **8.** Generative hyphae with clamp-connections from trama. **9.** Skeletal hyphae from context. **Bars:** 3–9 = 5 µm.

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(6) Number of text page

(7) Abstract

Phylogenetic analyses of the ITS regions of the nuclear rDNA and mitochondrial SSU rDNA revealed that both *G. colossus* and *G. tsunodae* are distinct from *Ganoderma* s.s. We accept *Tomophagus* to accommodate *T. colossus*. *Trachyderma* Imazeki (1952) is a genus typified by *T. tsunodae* (= *G. tsunodae*), with *Trachyderma* Norm. (1853), as an earlier homonym, therefore we propose a new name *Leucoganoderma* to replace *Trachyderma* Imazeki. *Tomophagus* is distinct from *Ganoderma* by the white and soft fibrous context consisting of unbranched to scarcely branched vegetative hyphae and finely reticulate basidiospores. *Leucoganoderma* is distinct from *Ganoderma* by the white and fleshy context consisting of conspicuously branched vegetative

hyphae and finely reticulate basidiospores. White and fleshy context and reticulate basidiospores are considered important characters at the generic level within the Ganodermataceae.

(8) Key words: basidiospore morphology, *Humphreya*, hyphal character, *Tomophagus colossus*, *Trachyderma tsunodae*

Introduction

Ganoderma colossus is an infrequently reported basidiomycete widely distributed in the tropics including Africa, the neotropics, Australia, South and S.E. Asia, but is also known from Florida, Japan and more recently from Taiwan (Furtado 1965; Gilbertson and Ryvarden 1986; Imazeki 1953; Ryvarden and Johansen 1980; Steyaert 1972; Wu and Zhang 2003). Morphologically, this species differs from most of other *Ganoderma* species in the soft and white context and reticulate endospore surface (Wu and Zhang 2003). Murrill (1905), based on the morphology of *G. colossus*, transferred it to a new genus *Tomophagus* Murrill, although it was not universally accepted (Steyaert 1972, Ryvarden 1991).

Ganoderma tsunodae (Lloyd) Trott. is hitherto known only from Japan (Imazeki 1939) and China (Zhao 1989). This species differs in many aspects from *Ganoderma* by its watery-fleshy and white context, drying to woody hard, and yellowish basidiospores that are paler than those of other *Ganoderma* spp. Imazeki (1952) described *Trachyderma* to typify this species and also transferred *Ganoderma subresinosum* (Murrill) Humph. to this genus because they share similar context characters. However, the former species is usually placed in *Ganoderma* on the basis of basidiospore morphology (Moncalvo and Ryvarden 1997; Núñez and Ryvarden 2000; Ryvarden 1991) and in addition, *Trachyderma* Imazeki (1952) is a later homonym of *Trachyderma* Norm. (1853; Ryvarden 1991). Hattori and Ryvarden (1994) suggested that species with loose and soft consistency should be separated from *Ganoderma, Tomophagus* is the proper genus to accommodate them.

Steyaert (1972) described a new genus *Magoderma* to accommodate *G. subresinosum* and some allied species with basidiospores similar to *Amauroderma* but with a cuticular structure distinct from *Amauroderma*. Corner (1983) combined *G. subresinosum* with *Amauroderma* and this combination is now widely accepted (Zhao 1989; Moncalvo and Ryvarden 1997).

Sequence analysis of the internal transcribed spacer (ITS) regions of *G. colossus* and *G. tsunodae* showed that they formed a basal clade to those of other *Ganoderma* species, but with weak support (Moncalvo 2000). Moncalvo (2000) did not reclassify them but suggested they might be better separated into genera as advocated by Imazeki (1952) and Murrill (1905).

A new collection of *G. colossus* on coconut palm washed up on the shore at Morib mangrove, Malaysia, and the isolation of a number of strains, enabled us to reexamine its morphology and phylogenetic relationship within the Ganodermataceae. We also examined the morphology of *G. tsunodae*, another member with white and fleshy context.

The objective of this study is to describe detailed morphologies of *G. colossus* and *G. tsunodae*, and to examine their phylogenetic relationship with other *Ganoderma* species and selected *Amauroderma* species.

Materials and methods

Molecular studies

Fungal isolates (Table 1) were grown in GYP medium (4 g/L glucose, 4 g/L yeast extract, 2 g/L peptone), made up with distilled water. Mycelium (~100 mg) was harvested by filtration, washed twice with sterile distilled water, blotted dry by filter paper and immediately frozen in liquid nitrogen. Mycelium pellets were ground into fine powder using a mortar and pestle.

Genomic DNA was extracted using the DNeasy Plant DNA Extraction Kit (Qiagen) according to the manufacturer's instructions. The mitochondrial SSU rRNA gene was amplified from genomic DNA using primers BMS05, BMS65 and BMS113 (Hong et al. 2002). While ITS regions of the nuclear rRNA gene clusters were amplified using ITS5 and ITS4R (White et al. 1990). PCR reactions were performed in 50 µL using FINNZYMES, DyNAzymeTM II DNA Polymerase Kit (MACHEREY-NAGEL, Product code F-551S) in a Perkin Elmer thermal cycler.

The amplification cycle consisted of an initial denaturation step of 94 °C for 2 min followed by 35 cycles of (i) denaturation (94 °C for 1 min), (ii) annealing (55 °C for 1.5 min) and (iii) elongation (72 °C for 2.5 min) and a final 10 min elongation step at 72 °C. The PCR products were analysed by agarose gel electrophoresis and purified using a NucleoSpinR Plant DNA Purification Kit (MACHEREY-NAGEL, Catalogue No. 740 570. 50) according to the manufacturer's instructions. PCR products were sent to Macrogen Inc., Korea, for direct sequencing.

Returned sequences were checked for ambiguity, assembled and deposited in GenBank (Table 1). Several sequences of *Ganoderma* species and other related fungi were downloaded from GenBank for phylogenetic positionings of *G. colossus* and *G. tsunodae*. Sequences were programme-aligned in Clustal W 1.6 (Thompson *et al.* 1994) and manually adjusted in Se-Al v1.0a1 (Rambaut 1999) then deposited in TreeBASE (accession SXXXX). Both ITS and mitochondrial SSU rDNA datasets were entered into PAUP* 4.0b10 for maximum parsimony and parsimony bootstrap analyses (Swofford 2002). Heuristic searches were run for both datasets with the following settings: gaps treated as missing data, starting tree(s) obtained via stepwise addition, random sequence addition of 1,000 replicas, a tree-bisection-reconnection (TBR) branch-swapping algorithm, MULTREES off. A thousand parsimony analyses were used to reflect the support of the clades with the same settings with the exception that 10 replicas of random sequence addition were used. In parallel, Bayesian analyses were performed on both datasets in MrBayes v3.1.2: GTR+I+γ model, 5 million generations in 4 chains with sampling every 100 generations, discarding the first 25% of the trees (Huelsenbeck and Ronquist 2005; Ronquist and Huelsenbeck 2003).

Morphological studies

Macroscopic characters were described based on fresh and dried specimens of *G. colossus* and *G. tsunodae*. Microscopic characters were based on dried specimens, examining free-hand sections mounted in Melzer's reagent or in 5% (w/v) KOH solution. A non-dextrinoid and non-amyloid reaction was described as IKI-. Basidiospores mounted in Melzer's reagent were measured. The following abbreviations are used in the text: L, mean spore length; M, mean spore width; r, the ratio of length/width of a basidiospore; R, mean of r. The term (n = x/y) means x measurements of basidiospores from y specimens. Examined specimens were deposited in BBH (BIOTEC Bangkok Herbarium, Thailand) or TFM (Forestry and Forest Products Research Insitute, Japan).

Cultural characters were studied on 2% malt agar plates or potato dextrose agar plates at room temperature and described according to Nobles (1965). Extracellular oxidase reactions were tested with 1-naphthol ethanol solution and tyrosine ethanol suspension according to Käärik (1965).

Herbarium accession numbers, locations and GenBank sequence accession numbers for isolates of *Ganoderma colossus*, *G. tsunodae* and *Amauroderma* species used in this study are given in Table 1.

For comparative studies on basidiospores, contextual hyphae and cultural characters, several specimens and cultures of Ganodermataceae were also examined.

Results

Molecular phylogenetic studies

The ITS analysis of *Ganoderma* sequences in the GenBank highlights the problems of the identification of deposited strains and a detailed discussion of the data is not profitable, as such species as *G. tsugae* appears in many clades (Fig. 1). However, *G. colossus*, *G. tsunodae* and *A. subresinosum* formed basal groups to the tree, all well supported with 100% bootstrap values, and are outside of detected clades consist of other *Ganoderma* spp.

Parsimony analyses of the mitochondrial SSU data for 61 sequences reveal 9 clades with good parsimony bootstrap support and posterior probability (Fig. 2). Well established clades include *G. pfeifferi/G. resinaceum* (A); *G. subamboinense* (B); *G. lucidum* (C); *G. meredithae* (D); *G. tsugae/ G. valesiacum* clade (F); and *G applanatum/lobatum/G.boninense* clade (E). *Ganoderma* species except for *G. tsunodae* and *G. colossus* consist a clade (the core *Ganoderma* clade) with high support (BT = 82, PP = 1.0), but *G. tsunodae* and *Amauroderma* species form a separate clade (G; BT = 73, PP = 0.99) as a sister group of the core *Ganoderma* clade. In addition, *G. colossus* consist a highly supported clade (H) at the base of all Ganodermataceae examined in this study. The *Perennipora* species were placed outside of the clade consist of all the Ganodermataceae species examined here.

Morphological characters and Taxonomy

Tomophagus colossus (Fr.) Murrill, Torreya 5: 197, 1905. Figs. 3-6, 9, 22

=*Polyporus colossus* Fr. Nov. Symb. Mycol. 56, 1851.

=*Ganoderma colossus* (Fr.) Baker, Fungi Malay. 425, 1918.

Basidiocarp sessile, dimidiate to semicircular, 200 wide x 300 long x 73 mm thick, pilear surface yellowish-brown to yellow, glabrous, slightly laccate to dull, margin obtuse, pilear cuticle very thin. Context chalky to soft-fibrous, white to pale brown and light in weight. Tubes brittle, dark brown when dried, 2-13 mm long. Pores round to angular, cream to pale brown, dark brown when dried, 3-4/mm.

Hyphal system dimitic; contextual generative hyphae not seen; contextual vegetative hyphae thick-walled, usually with a distinct lumen, straight, unbranched to rarely branched, hyaline, IKI- to weakly dextrinoid, up to 5 μm wide; tramal generative hyphae not seen; tramal vegetative hyphae thick-walled to solid, sinuous, unbranched to occasionally branched, hyaline, IKI- to weakly dextrinoid, up to 5 μm wide. Contextual hyphae of pilear cuticle swollen at the apical end to nearly clavate and ornamented, hyaline, 30-32.5 x 7.5-12.5 μm. Chlamydospores in basidiocarps not present. Basidiospores ovoid to sub-globose, occasionally truncate, double walled, the inner wall yellowish brown to brown and finely reticulate, the outer wall smooth and hyaline, 12.5-20.0 x 8.8-11.3 μm, $1.38 \leq r \leq 1.78$, L = 16.2 μm, W = 10.1 μm, R = 1.61 (n = 50/1).

Specimens examined: Malaysia. Morib, on dead coconut (*Cocos nucifera*) trunk, coll. E.B. Gareth Jones, 27 Feb. 2006 (BBH 18767); Japan. Miyazaki, on *Diospyros kaki*, coll. T. Hashimoto, 26 Oct. 1947 (TFM F-5334).

Cultural characters: Growth moderate (approximately 20 mm/w), plates covered in 3-4 weeks. Advancing zone even, appressed. Mat at first white then pale brown and covered with white mycelium producing golden-brown spores in culture on both PDA and MEA, cottony to felty, often with guttation of brown droplets on mycelium. Reverse unchanged to darker. Odour none. Hymenophore development not seen within 6 weeks. Generative hyphae from margin and aerial mycelium with clamp-connections, hyaline, 1.6-4 μm wide. Fiber hyphae sparse, slightly thick-walled, mostly unbranched, hyaline, 0.8-1.8 μm wide. Chlamydospores ellipsoid to subglobose, smooth, thin-walled and hyaline when young, subglobose to globose, thick-walled,

warty or spiny and pale brown to brown when old, 15.8-26.5 μm in diam (n = 30/1), produced on the apex of hyphal branches or intercalary. Extracellular peroxidase activities: 1-naphthol, +; tyrosine, -.

Species code: 2, 3, 8, 34, 36, (38), 39, 43, 56 (Nobles 1965).

Leucoganoderma tsunodae (Lloyd) Choeyklin, T. Hatt. & E.B.G. Jones, comb. nov.

Figs. 7-8, 9, 19

MycoBank no.: MB512312

Basionym: *Polyporus tsunodae* Lloyd, Mycol. Writ. 5:792, 1918.

= *Ganoderma tsunodae* (Lloyd) Trott., Syll. Fung. 23:139, 1925.

= *Trachyderma tsunodae* (Lloyd) Imazeki, Bull. Gov. Forest Exp. Sta. 57:97, 1952.

For macroscopic characters, see Zhao (1989) and Hattori and Ryvarden (1994) as '*G. tsunodae*'. Coloured photographs were provided by Imazeki et al (1988) and Wu and Dai (2005). Hyphal system dimitic; generative hyphae not seen; contextual vegetative hyphae conspicuously and repeatedly branched, narrow hyphal tips prominent, thick-walled to solid, hyaline, IKI-, up to 6 μm wide; tramal vegetative hyphae similar to contextual vegetative hyphae, up to 4 μm wide. Basidiospores ellipsoid to ovoid, truncate or not, double walled, inner wall yellow, finely reticulate, outer wall hyaline and smooth, 17.5-22.5 x 12.5-17.5μm, $1.25 \leq r \leq 1.80$, L = 21.4 μm, W = 14.8, R = 1.45 (n = 50/1).

Specimens examined: Japan. Prov. Kozuke, coll. K. Tsunoda, 8 Jul. 1917 (lectotype; BPI US0307263); Tottori Pref., Mt. Daisen, on dead wood of *Fagus crenata* (?), coll. T. Hattori, 4 Aug. 1989 (TFM F-15117); Ibaraki Pref., Mt. Tsukuba, on *Fagus crenata*, coll. T. Hattori, 9 Sep. 1999 (TFM F-19295).

Cultural characters: Growth moderate (31-42 mm/w), plates covered in 2-3 weeks. Advancing zone bayed, appressed. Mat at first white then becoming brown near the center, felty,

brown part becoming crustose. Reverse unchanged. Odour none. Hymenophore development not seen within 6 weeks. Generative hyphae from margin and aerial mycelium with clamp-connections, hyaline, brown in crustose areas, 1.8-5 μm wide. Fiber hyphae slightly thick-walled, repeatedly branched, hyaline, up to 1.8 μm wide. Cuticular cells present in crustose areas, thick-walled, at first hyaline becoming brown. Interlocking hyphae present in crustose areas, scattered, thick-walled, brown.

Species code: 2, 3, 8, 10, 11, 32, (36), 37, 38, (42), 43 (Nobles 1965).

Leucoganoderma Choeyklin, T. Hatt. & E.B.G. Jones, nom. nov.

MycoBank no.: MB512299

Replaced synonym: *Trachyderma* Imazeki, Bull. Gov. Forest Exp. Sta. 57:97, 1952 (non *Trachyderma* Norman, Nyt. Magazin for Naturvid. VII, 229, 1853).

Type species: *Leucoganoderma tsunodae* (Lloyd) Choeyklin, T. Hatt. & E.B.G. Jones.

Etymology: Greek, *leuco* (= white) + *Ganoderma* (= a genus name).

Basidiocarps annual, lignicolous, sessile; pileus surface irregularly rough, non-laccate; context white, fleshy in fresh condition drying woody hard, with a thin crust; hymenophore poroid. Crust composed of interwoven hyphae without palisade cells; hyphal system dimitic with generative hyphae and repeatedly branched vegetative hyphae; basidiospores large (up to 22 μm long in the type species), ovoid, often truncate, double-walled, endospore surface finely reticulate, yellow.

It is distinct from *Ganoderma* by the white and fleshy context becoming woody hard after dried (light to dark brown and fibrous-corky in *Ganoderma*), repeatedly branched vegetative hyphae (arboriform with a long stalk in *Ganoderma*) and yellow and finely reticulate basidiospores (brown and echinulate to verrucose in *Ganoderma*). *Tomophagus* has soft-fibrous context consist of mostly unbranched vegetative hyphae, a crust with hymeniform structure,

brown basidiospores and chlamydospores in the culture. *Amauroderma* (including *Magoderma*) has globose to ellipsoid basidiospores without an apical thickening or a truncate apex, and less branched hyphae. *Humphreya* has a funnel-shaped stipitate basidiocarp with a long stalk, a thick and sharply defined crust and basidiospores with distinctly reticulate ridges.

Leucoganoderma tsunodae is hitherto the only species accepted in the genus.

Morphologial characters of specimens and cultures of allied species examined for comparison

Amauroderma subresinosum (Murrill) Corner, Beih. Nova Hedwig. 75:93, 1983. Fig. 11

Vegetative hyphae hyaline, unbranched to arboriform, with or without side branches; basidiospores double-walled, endospores pale brown, finely echinulate; for other characters, see Corner (1983) and Steyaert (1972) as '*Magoderma subresinosum*'.

Specimen examined: Thailand. Thung Cho Watershed Management Unit, Mae Taeng, Chiang Mai Province, on wood under soil, coll. R. Choeyklin, 28 Jul. 2004 (BHH 17466).

Cultural characters: No chlamydospore observed. Generative hyphae with clamp-connections, vegetative hyphae occasionally branched (examined culture: ML 50, Table 1).

Amauroderma subrugosum (Bres. & Pat.) Torrend, Broteria ser. bot. 18: 128, 1920. Fig. 12

Vegetative hyphae hyaline, unbranched to arboriform with side branches; basidiospores double-walled, endospores pale brown, finely echinulate; for other characters, see Corner (1983) and Ryvarden and Johansen (1980) as '*Amauroderma rugosum*'.

Specimen examined: Thailand. The Mushroom Research Centre, Mae Taeng, Chiang Mai, on wood under soil, coll. R. Choeyklin, 21 Jul. 2004 (BBH 16266); Khao Yai National Park, Nakhon RatchaSima, on wood under soil, coll. R. Choeyklin, 29 Jun. 2006 (BBH 17824); Khao Yai National Park, Nakhon RatchaSima, on wood under soil, coll. R. Choeyklin, 29 Jun. 2006 (BBH 17844); Headquarter Nature Trail (Across the Lodge Bridge), Hala Bala Wildlife Sanctuary, Waeng, Narathiwat, on wood under soil, coll. R. Choeyklin, 29 May 2000 (BBH

19073); Khao Yai National Park, Nakhon RatchaSima, on wood under soil, coll. R. Choeyklin, 11 Jun. 2007 (BBH 19085); Khao Yai National Park, Nakhon RatchaSima, on wood under soil, coll. R. Choeyklin, 11 Jun. 2007 (BBH 19086).

Cultural characters: No chlamydospore observed. For other characters, see Chang et al. (1996) as 'A. rugosum' (examined culture: BCC16655, Thailand; ML56, Malaysia)

Ganoderma australe (Fr.) Pat., Bull. Soc. Myc. Fr. 4:1712, 1887. Figs. 13, 17

Vegetative hyphae olive brown, arboriform, with a long stalk and side branches; basidiospores double-walled, endospores yellowish brown, verrucose; for other characters, see Corner (1983) and Ryvarden and Johansen (1980).

Specimen examined: Thailand. Khao Yai National Park, Nakhon RatchaSima, on dead wood, coll. R. Choeyklin, 10 Apr. 2006 (BBH 17838); Rani Waterfall, Thale Ban National Park, Satun, on dead wood, coll. R. Choeyklin, 19 Aug. 2006 (BBH 17841); Rani Waterfall, Thale Ban National Park, Satun, on dead wood, coll. R. Choeyklin, 19 Aug. 2006 (BBH 17846); Huai Pla Pung Waterfall, Bo Kluea, Nan, on dead wood, coll. R. Choeyklin, 20 Sep. 2005 (BBH 17847); Mu Ko Chang National Park, Trat, on dead coconut (*Cocos nucifera*) trunk, coll. R. Choeyklin, 4 Oct. 2005 (BBH 19072); Tambon Khlong Thom Nuea, Khlong Thom, Krabi, on dead oil palm (*Elaeis guineensis*), coll. R. Choeyklin, 4 May 2006 (BBH 19074); Hat Chao Mai, Hat Chao Mai National Park, Trang, on dead *Casuarina equisetifolia*, coll. R. Choeyklin 15 Nov. 2006 (BBH 19078); Rani Waterfall, Thale Ban National Park, Satun, on dead wood, coll. R. Choeyklin, 11 Nov. 2006 (BBH 19079); Ban Khiriwong, Phrom Khiri, Nakhon Si Thammarat, on dead wood, coll. R. Choeyklin, 11 Oct. 2006 (BBH 19082).

Ganoderma boninense Pat., Bull Soc. Mycol. Fr. 5:72, 1889. Figs. 14, 21

Vegetative hyphae olive brown, arboriform, with a long stalk and simple side branches; basidiospores double-walled, endospores light brown, finely echinulate; for other characters, see Steyaert (1967).

Specimen examined: Thailand. Ban Nuea Khlong, Nuea Khlong, Krabi, on dead oil palm (*Elaeis guineensis*), coll. R. Choeyklin, 4 May 2006 (BBH 19068); Ban Thong Krut, Ko Samui, Surat Thani, on dead coconut (*Cocos nucifera*) stump, coll. R. Choeyklin, 13 Oct. 2006 (BBH 19071).

Malaysia. Morib, on dead coconut (*Cocos nucifera*) trunk, coll. E.B. Gareth Jones, 27 Feb. 2006 (BBH 19069, BBH 19070).

Ganoderma lucidum (W. Curt : Fr.) P. Karst., Rev. Mycol. 3:17, 1881. Figs. 15, 20

Vegetative hyphae hyaline to light brown, arboriform, with an indistinct stalk and branches; basidiospores double-walled, endospores yellowish brown, distinctly echinulate; for other characters, see Gilbertson and Ryvarden (1986).

Specimen examined: Japan. Tokyo, coll. K. Aoshima, 12 Aug. 1965 (TFM F- HATTORI WILL CONFIRM THIS SOON).

Ganoderma philippii Bres. & Henn., Syll. Fung. 9:180, 1881. Figs. 16, 18

Vegetative hyphae olive brown, arboriform, with a long stalk and side branches; basidiospores double-walled, endospores yellowish brown, verrucose; for other characters, see Corner (1983) and Steyaert (1972).

Specimen examined: Malaysia. N. Sembilan, Pasoh, coll. T. Hattori, 18 Mar. 1997 (TFM F-17831).

Discussion

Our mitochondrial SSU analysis revealed that both of *G. colossus* and *G. tsunodae* do not nest within the core *Ganoderma* clade. Morphological characters of the above 2 species are also distinct from the members of the core *Ganoderma* clade (*Ganoderma sensu stricto*), and then we accept *Tomophagus* to accommodate *T. colossus* and described *Leucoganoderma* for *L. tsunodae*. The most outstanding character of *T. colossus* and *L. tsunodae* is the white to pale coloured and soft fleshy context in fresh condition within Ganodermataceae. Most *Ganoderma* spp., including subgen. *Elfvingia,* have light to dark brown and fibrous-corky context. All *Ganoderma* species examined here have olivaceous to brown arboriform vegetative hyphae with a long stalk and scattered to frequent branches. On the other hand, vegetative hyphae are hyaline, unbranched to inconspicuously branched and scarcely interwoven in *T. colossus* that make its context white and soft-fibrous-fleshy to chalky. In *L. tsunodae*, most parts of the vegetative hyphae are repeatedly branched. In dried specimens, many of the branched hyphae are highly interwoven, sometimes agglutinated, and then not easily squashed even in KOH solution. Because of the hyphal characters above, context of the dried specimens of *L. tsunodae* become woody hard.

Another important character of *T. colossus* is conspicuous occurrence of chlamydospores in the culture (Stalpers 1978). Steyaert (1972) and Gilbertson and Ryvarden (1986) described the occurrence of chlamydospores in the context, but it was not observed from the specimens examined here. Occurrence of chlamydospores in the context is perhaps variable according to the specimens. Among the Ganodermataceae species with white and fleshy context examined here, *T. colossus* is the only species that produces chlamydospores in culture. Presence of chlamydospores may be a character with phylogenic importance for those with white and fleshy context as in *Ganoderma s. s.* as suggested by Hong and Jung (2004).

Basidiospore characteristics are usually considered most important in defining genera of the Ganodermataceae (Moncalvo and Ryvarden 1997; Steyaert 1972). Basidiospores of *T.*

colossus are usually taken for those of typical *Ganoderma* (Furtado 1965). However, as suggested by Wu and Zhang (2003), the endospore surface of the basidiospores of *T. colossus* is reticulate and distinct from the echinulate to verrucose surface in the basidiospores of *Ganoderma* spp. They concluded that this basidiospore character supports the separation of *Tomophagus* from *Ganoderma*. *Ganoderma trengganuense* Corner also has a white and soft context and reticulate basidiospores (Corner 1983), and it may be a related species to *T. colossus*. *Humphreya* Steyaert has more distinctly reticulate basidiospores with reticulate to disjointed ridges (Steyaert 1972). *Humphreya* spp. also have white to pale and fleshy context as in *Tomophagus* (Furtado 1967; Steyaert 1972) and are perhaps more related to *Tomophagus* than to *Ganoderma s.s.*

Careful examination revealed that the endospore surface of the basidiospores of *L. tsunodae* is also finely reticulate and distinct from those of *Ganoderma* spp. Additionally, as suggested by Imazeki (1939, 1952), basidiospores of *L. tsunodae* are larger and paler than most *Ganoderma* spp. and spore prints are lemon yellow contrasting to those of most *Ganoderma* spp. that are more or less brown. Similarly large and yellowish basidiospores are also seen in some species with white to pale and fleshy context as in *G. asperulatum* (Murrill) Bres. and *Humphreya* spp. (Furtado 1967; Steyaert 1972) though their phylogenic position within the Ganodermataceae is still unknown. *Leucoganoderma tsunodae* grouped with *Amauroderma* species in the mitochondrial SSU rDNA tree. They share white and fleshy context in fresh condition and Imazeki (1952) put *L. tsunodae* and *A. subresinosum* in the same genus *Trachyderma*. However, basidiospores of *L. tsunodae* are ovoid and have distinctly thickened and often truncate apex as in *Ganoderma* spp. contrasting that those of *Amauroderma* species are globose to ellipsoid and lack apical thickenings. Additionally, the ITS tree did not show close relationship *of L. tsunodae* and *Amauroderma* species. Considering the above results, we conclude to keep *L. tsunodae* distinct from *Amauroderma*.

There are some other species of *Ganoderma* with light coloured context, such as *G. mirabile* (Lloyd) Humphrey and *G. weberianum* (Bres. & Henn.) Stey., but their basidiospores are similar to other *Ganoderma* spp. and their context is corky (Corner 1983; Steyaert 1972). They possibly represent members of *Ganoderma s.s.* though confirmation through sequence analysis is desirable.

Tomophagus colossus is widely distributed in tropical and subtropical areas and has a wide host range. The Malaysian collection examined here was made on a coconut palm, a feature it shares with some *Ganoderma* spp. that are specific or recurrent on palms (Steyaert 1967). On the contrary, *L. tsunodae* is restricted to cool temperate areas of East Asia, and occurs most frequently on *Fagus* spp. It should be noted that this is the only species of Ganodermataceae outside of the core *Ganoderma* clade distributed in temperate areas of the Northern Hemisphere. This species may have phylogeographic importance among this family.

The morphology of basidiospores and structure of the crust are considered important characters in delineating genera and subgenera of the Ganodermataceae (Imazeki 1939; Moncalvo and Ryvarden 1997; Steyaert 1972). Additionally, we suggest that context characters induced from hyphal structures are another important character. Currently cultures are not available for many of the Ganodermataceae species with white and a fleshy context to enable comment on their phylogenetic relationship within the Ganodermataceae, and further studies are warranted.

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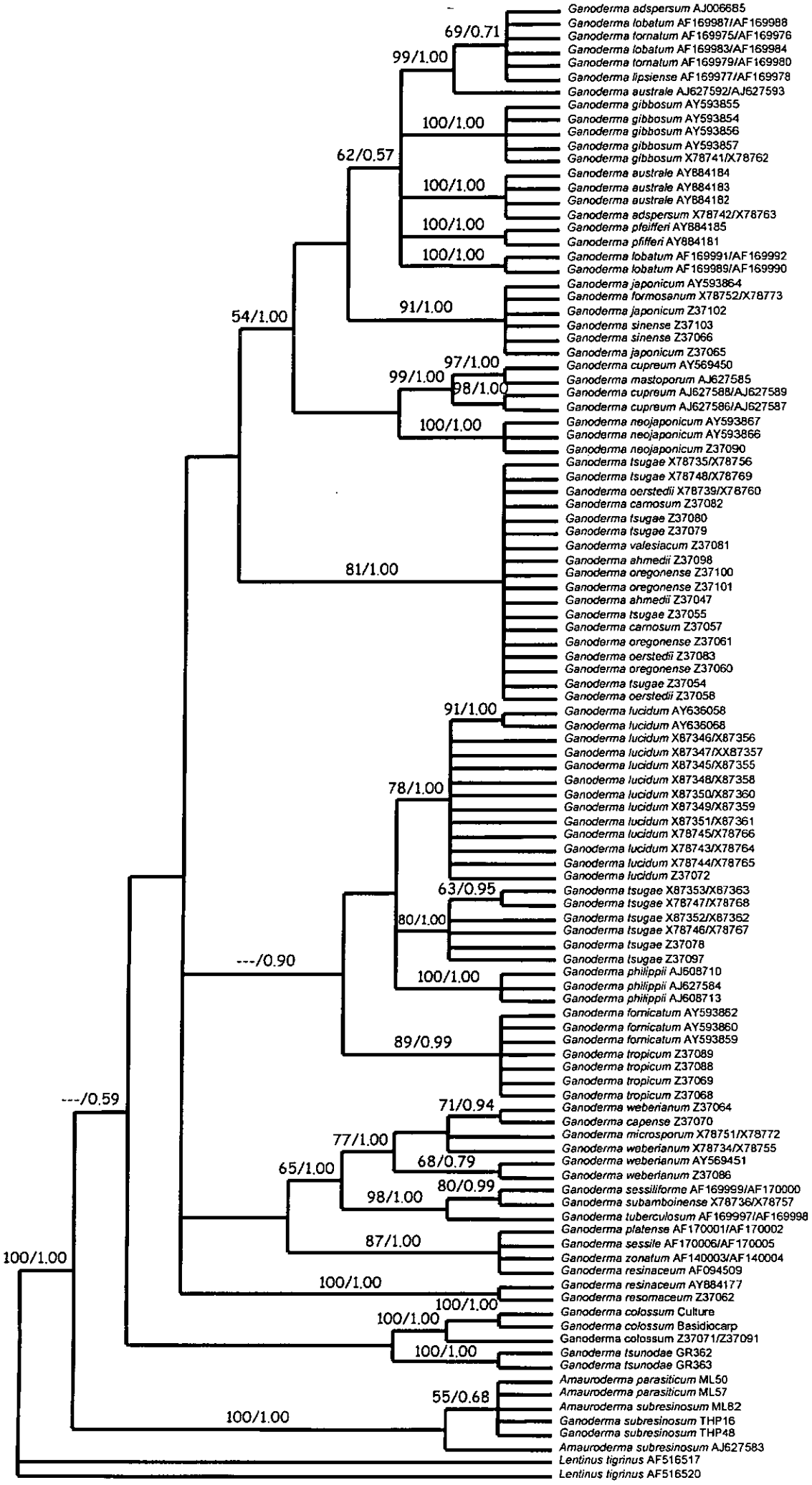

Fig. 1. Consensus tree of 4,906 most parsimonious trees with a tree length of 624 steps, a consistency index of 0.559 and a retention index of 0.864. Parsimony bootstrap values and posterior probabilities are shown above and below the branch, respectively

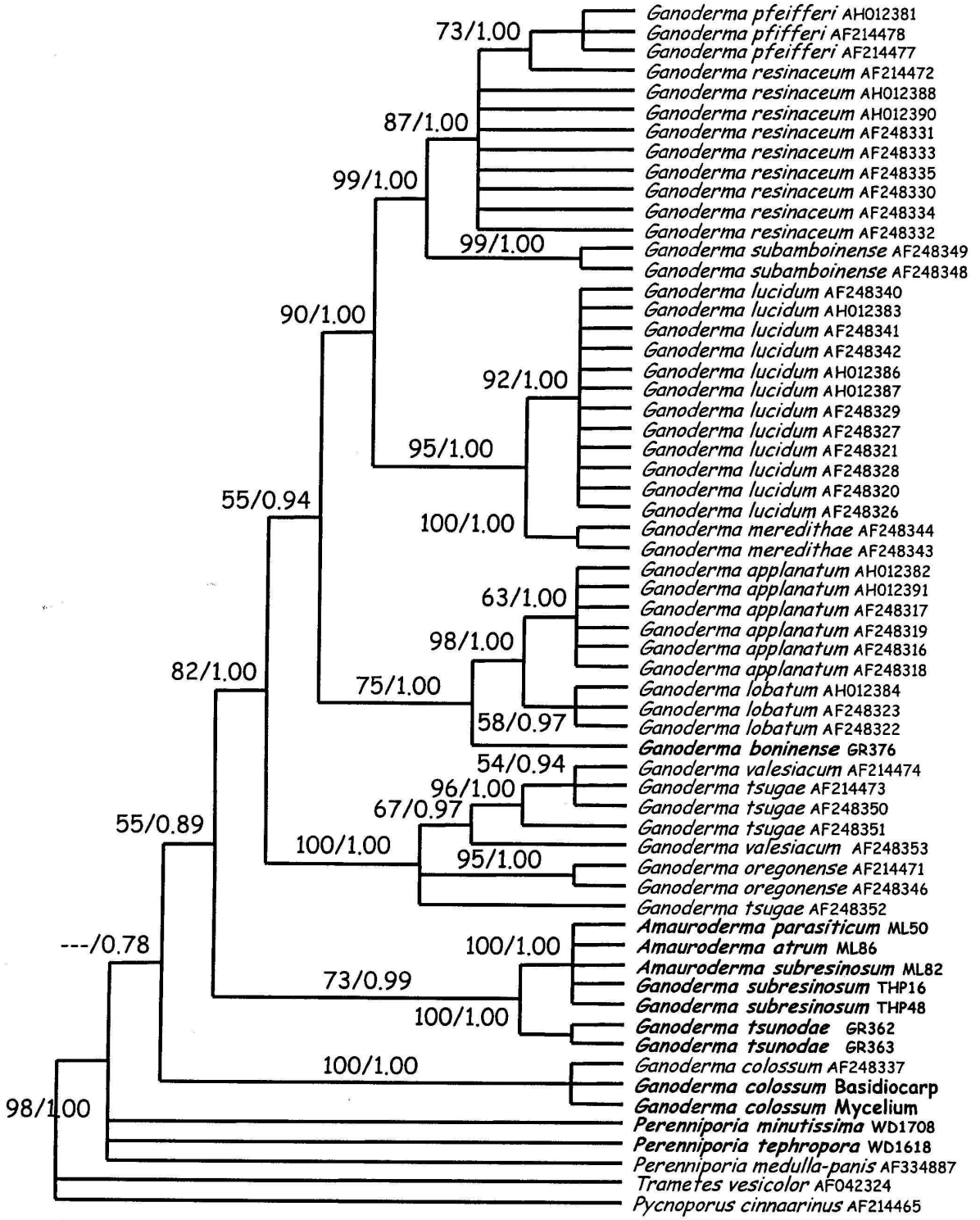

Fig. 2. Consensus tree of 977 most parsimonious trees with a tree length of 530 steps, a consistency index of 0.775 and a retention index of 0.907. Parsimony bootstrap values and posterior probabilities are shown above and below the branch, respectively