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

Rungjindamai, N.[1], Pinruan, U.[1], Choeyklin, R.[1], Hattori, T.[2] and Jones, E.B.G.[1]*

[1]Bioresources Techonology Unit, National Center for Genetic Engineering and Biotechnology, NSTDA, 113 Thailand Science Park, Paholyothin Road, Khlong 1, Khlong Luang, Pathum Thani, 12120, Thailand
[2]Kansai Research Center, Forestry and Forest Products Research Institute, Nagai-Kyutaro 68, Momoyama, Fushimi, Kyoto 612-0855 Japan

Rungjindamai, N., Pinruan, U., Choeyklin, R., Hattori, T. and 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

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*

Article Information
Received 30 October 2008
Accepted 21 November 2008
Published online 30 November 2008
*Corresponding author: E.B.G. Jones; e-mail: remispora@gmail.com

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.

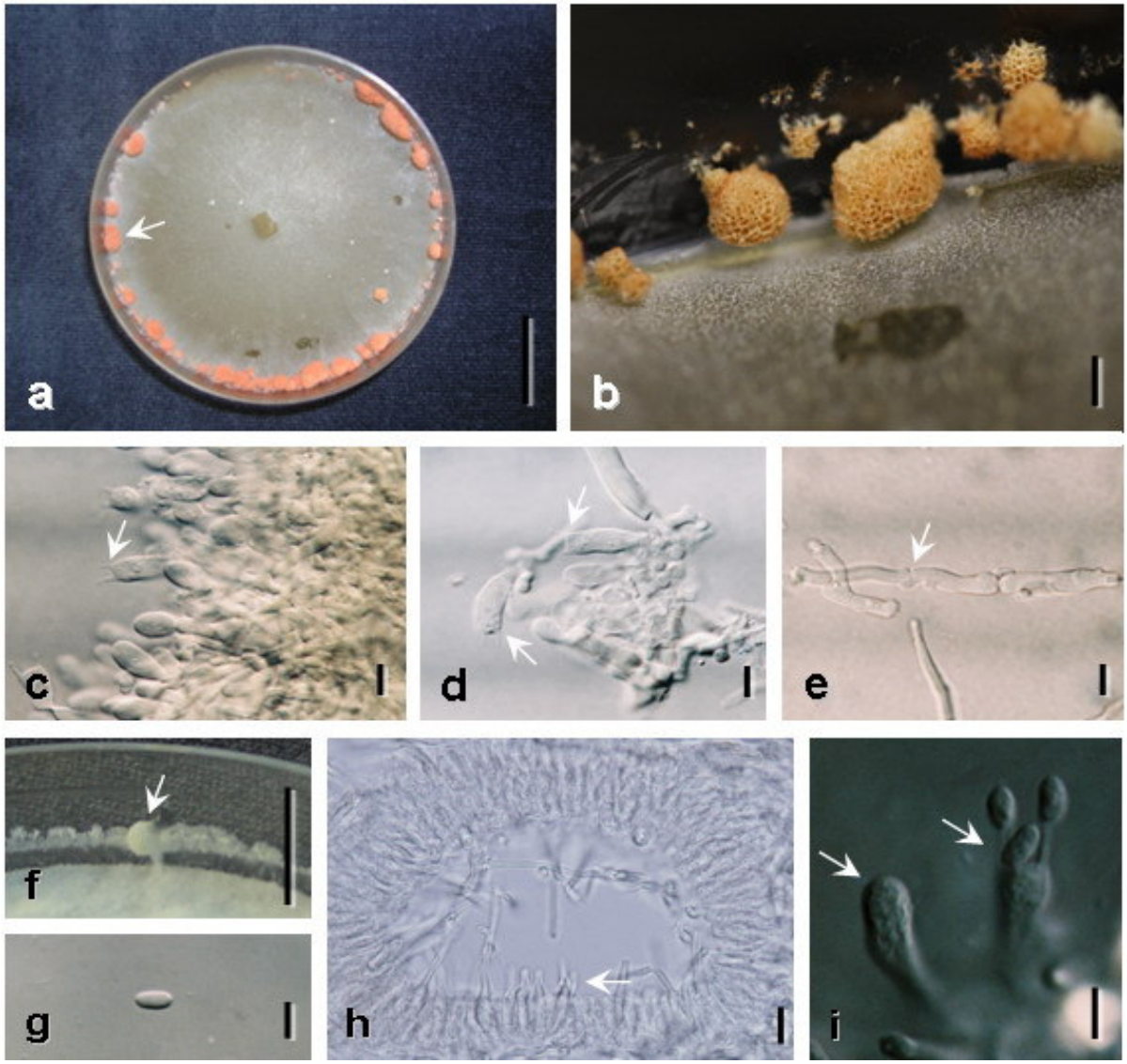

Fig. 1. Isolate 8R 1/1 a-b. Basidiomes on PDA culture formed on side of Petri dish (arrowed). **c-d.** Basidia (arrowed). **e.** Generative hyphae with clamp connection (arrowed). **Isolate 5V 3/3. f.** Basidiomes on PDA culture on Petri dish side (arrowed). **g.** Basidiospore. **h.** Crossection of 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).

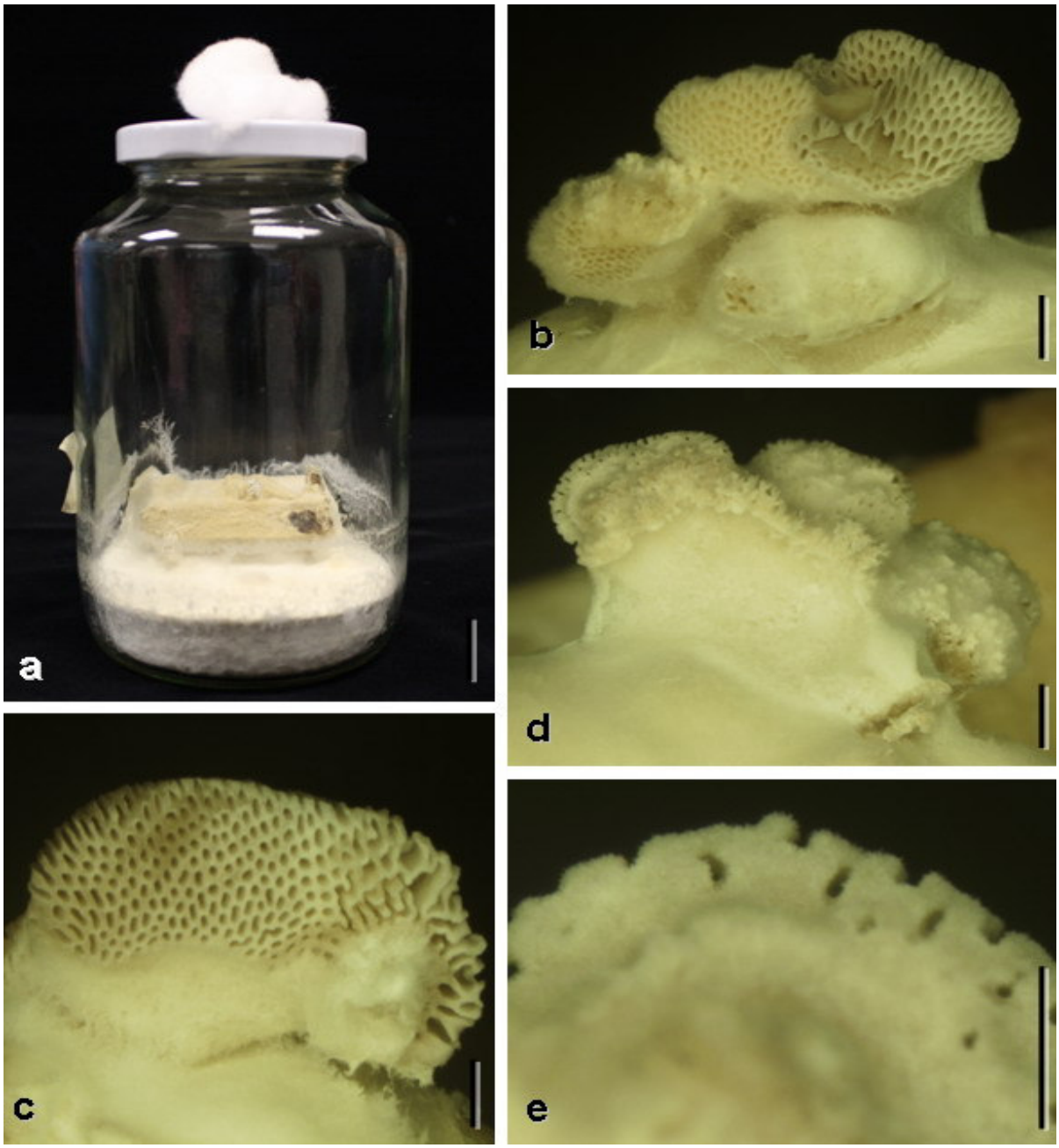

Fig. 2. Isolate 5V 3/3. a. = Basidiomes produced on test blocks of palm petiole in glass bottles containing PDA medium. **b-c.** Lower surface of basidiomes with pores. **d.** Upper surface of basidiomes. **e.** Higher magnification of pores viewed from upper surface of a basidiome. Bars: a = 1.7 cm.,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

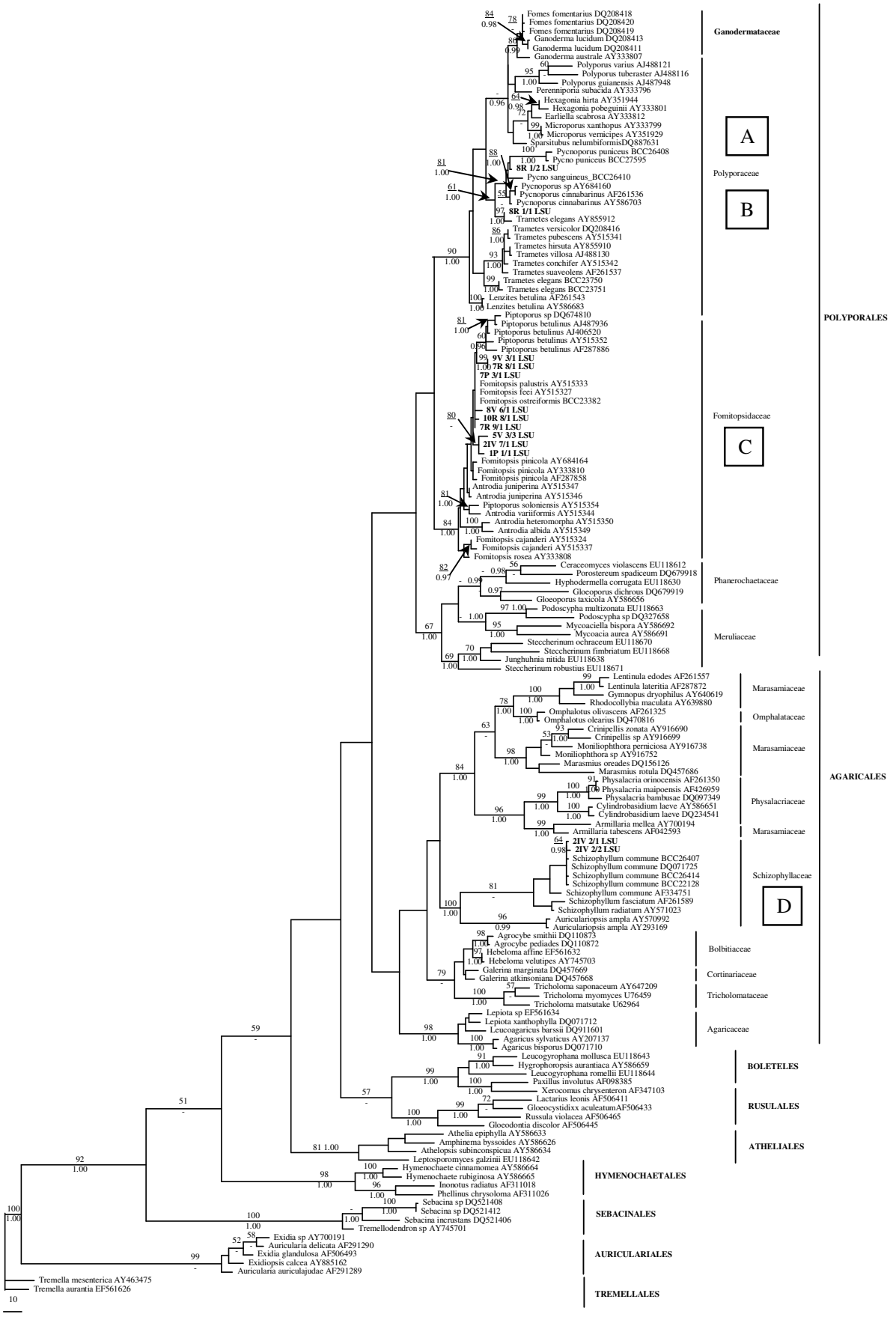
Fomes fomentarius DQ208418
Fomes fomentarius DQ208420
Fomes fomentarius DQ208419
Ganoderma lucidum DQ208413
Ganoderma lucidum DQ208411
Ganoderma australe AY333807
Polyporus varius AJ488121
Polyporus tuberaster AJ488116
Polyporus guianensis AJ487948
Perenniporia subacida AY333796
Hexagonia hirta AY351944
Hexagonia pobeguinii AY333801
Earliella scabrosa AY333812
Microporus xanthopus AY333799
Microporus vernicipes AY351929
Sparsitubus nelumbiformisDQ887631
Pycnoporus puniceus BCC26408
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 AY515352
Piptoporus betulinus AF287886
9V 3/1 LSU
7R 8/1 LSU
7P 3/1 LSU
Fomitopsis palustris AY515333
Fomitopsis feei AY515327
Fomitopsis ostreiformis BCC23382
8V 6/1 LSU
10R 8/1 LSU
7R 9/1 LSU
5V 3/3 LSU
2IV 7/1 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
Porostereum spadiceum DQ679918
Hyphodermella corrugata EU118630
Gloeoporus dichrous DQ679919
Gloeoporus taxicola AY586656
Podoscypha multizonata EU118663
Podoscypha sp DQ327658
Mycoaciella bispora AY586692
Mycoacia aurea AY586691
Steccherinum ochraceum EU118670
Steccherinum fimbriatum EU118668
Junghuhnia nitida EU118638
Steccherinum robustius EU118671
Lentinula edodes AF261557
Lentinula lateritia AF287872
Gymnopus dryophilus AY640619
Rhodocollybia maculata AY639880
Omphalotus olivascens AF261325
Omphalotus olearius DQ470816
Crinipellis zonata AY916690
Crinipellis sp AY916699
Moniliophthora perniciosa AY916738
Moniliophthora sp AY916752
Marasmius oreades DQ156126
Marasmius rotula DQ457686
Physalacria orinoensis AF261350
Physalacria maipoensis AF426959
Physalacria bambusae DQ097349
Cylindrobasidium laeve AY586651
Cylindrobasidium laeve DQ234541
Armillaria mellea AY700194
Armillaria tabescens AF042593
2IV 2/1 LSU
2IV 2/2 LSU
Schizophyllum commune BCC26407
Schizophyllum commune DQ071725
Schizophyllum commune BCC26414
Schizophyllum commune BCC22128
Schizophyllum commune AF334751
Schizophyllum fasciatum AF261589
Schizophyllum radiatum AY571023
Auriculariopsis ampla AY570992
Auriculariopsis ampla AY293169
Agrocybe smithii DQ110873
Agrocybe pediades DQ110872
Hebeloma affine EF561632
Hebeloma velutipes AY745703
Galerina marginata DQ457669
Galerina atkinsoniana DQ457668
Tricholoma saponaceum AY647209
Tricholoma myomyces U76459
Tricholoma matsutake U62964
Lepiota sp EF561634
Lepiota xanthophylla DQ071712
Leucoagaricus barssii DQ911601
Agaricus sylvaticus AY207137
Agaricus bisporus DQ071710
Leucogyrophana mollusca EU118643
Hygrophoropsis aurantiaca AY586659
Leucogyrophana romellii EU118644
Paxillus involutus AF098385
Xerocomus chrysenteron AF347103
Lactarius leonis AF506411
Gloeocystidixx aculeatumAF506433
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 AF311018
Phellinus chrysoloma AF311026
Sebacina sp DQ521408
Sebacina sp DQ521412
Sebacina incrustans DQ521406
Tremellodendron sp AY745701
Exidia sp AY700191
Auricularia delicata AF291290
Exidia glandulosa AF506493
Exidiopsis calcea AY885162
Auricularia auriculajudae AF291289
Tremella mesenterica AY463475
Tremella aurantia EF561626
10
Ganodermataceae
A
Polyporaceae
B
Fomitopsidaceae
C
Phanerochaetaceae
Meruliaceae
POLYPORALES
Marasamiaceae
Omphalataceae
Marasamiaceae
Physalacriaceae
Marasamiaceae
Schizophyllaceae
D
AGARICALES
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

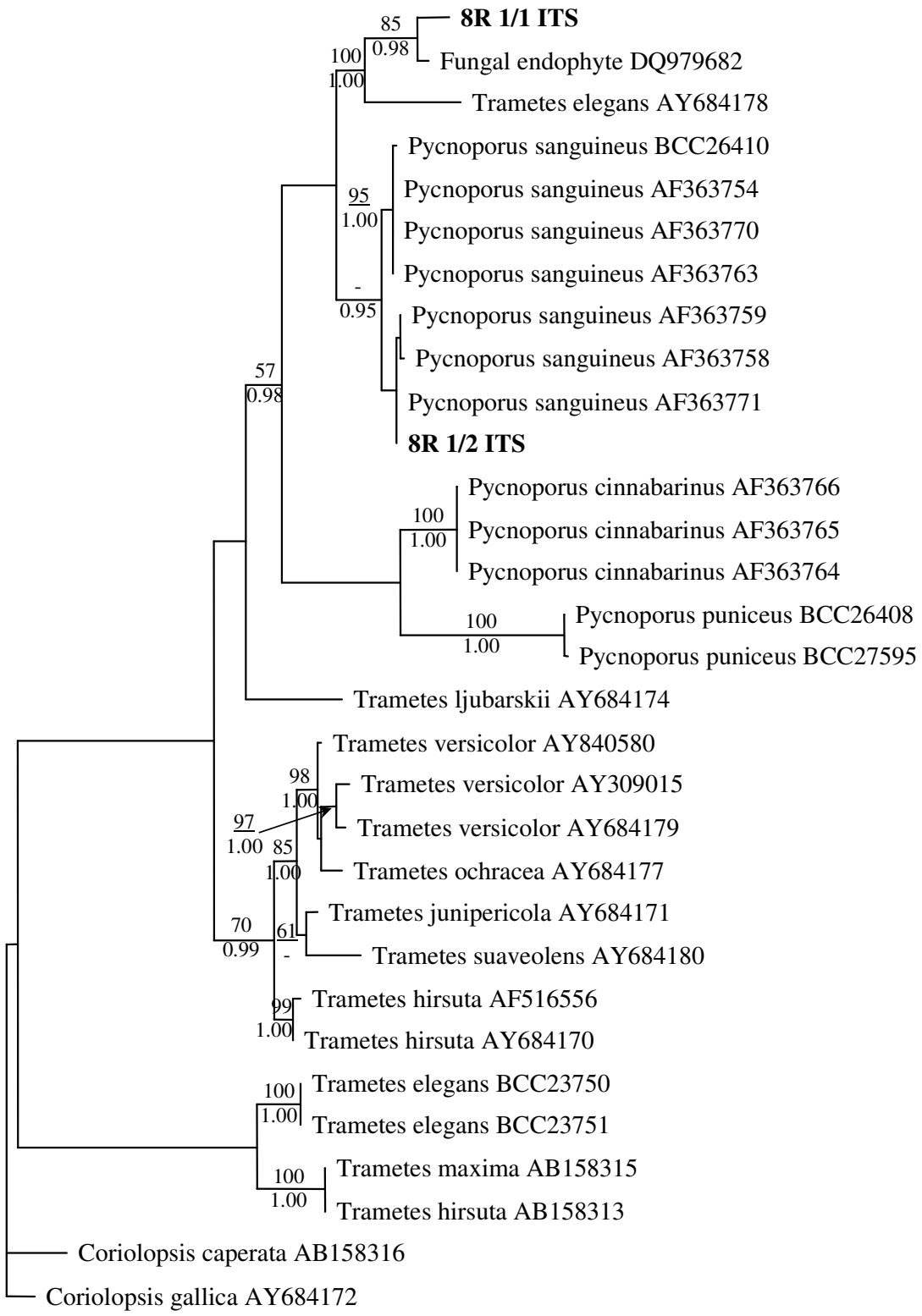

Fig. 4. One of 62 MPTs inferred from ITS sequenes of two isolates of the *Polyporaceae* isolated from *Elaeis guineensis*. The MP value (≥50%) and Bayesian PP (≥0.95) are shown above and below the branches, respectively (tree length= 335 steps, CI = 716, RI=0.884). Basidiomycete endophytes sequenced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

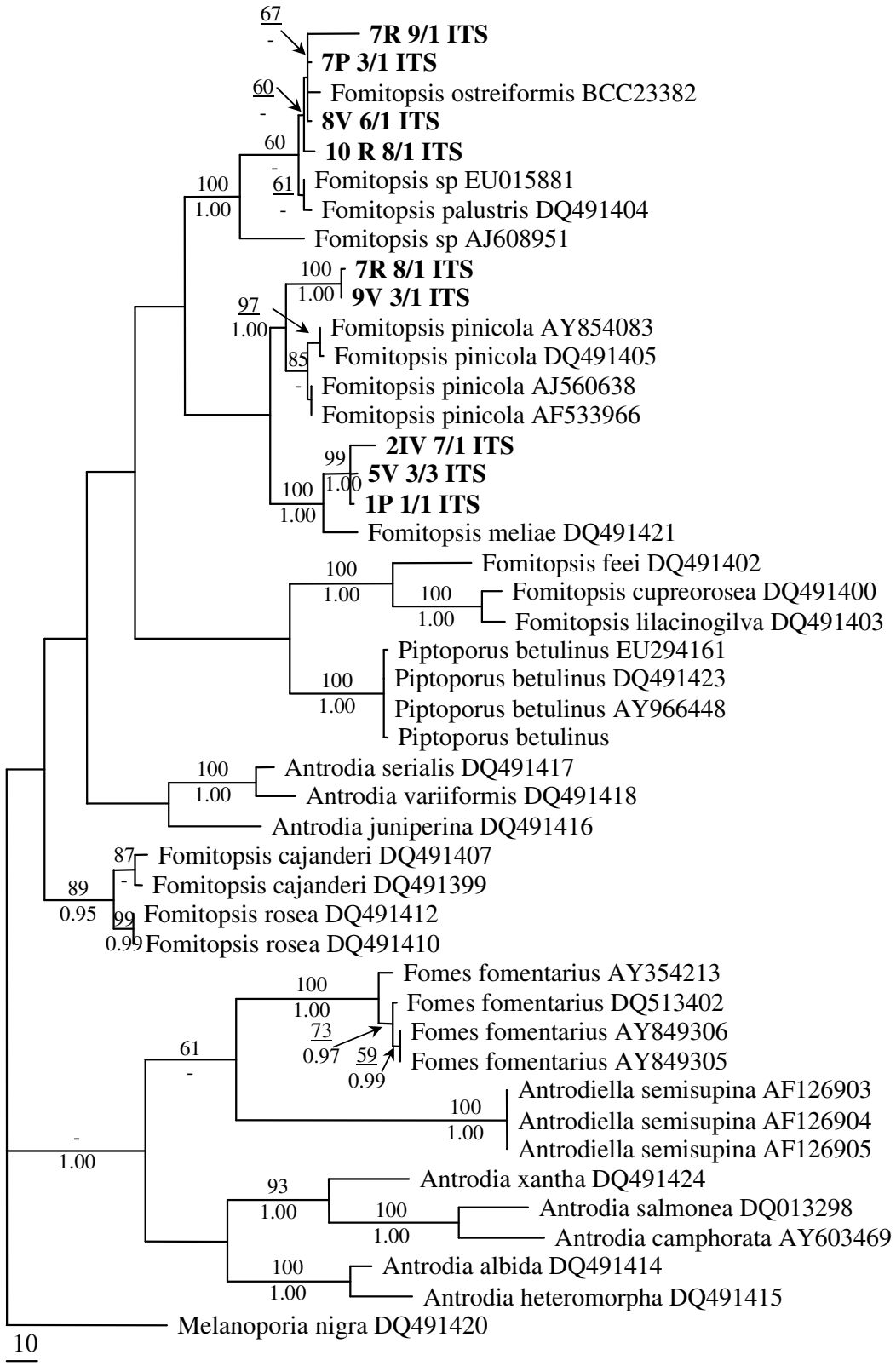

Fig. 5. One of 84 MPTs inferred from ITS sequenes of nine isolates of the *Fomitopsisdaceae* isolated from *Elaeis guineensis*. The MP value (≥50%) and Bayesian PP (≥0.95) are shown above and below the branches, respectively (tree length= 1,004 steps, CI = 0.574, RI=0.790). Basidiomycete endophytes sequenced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

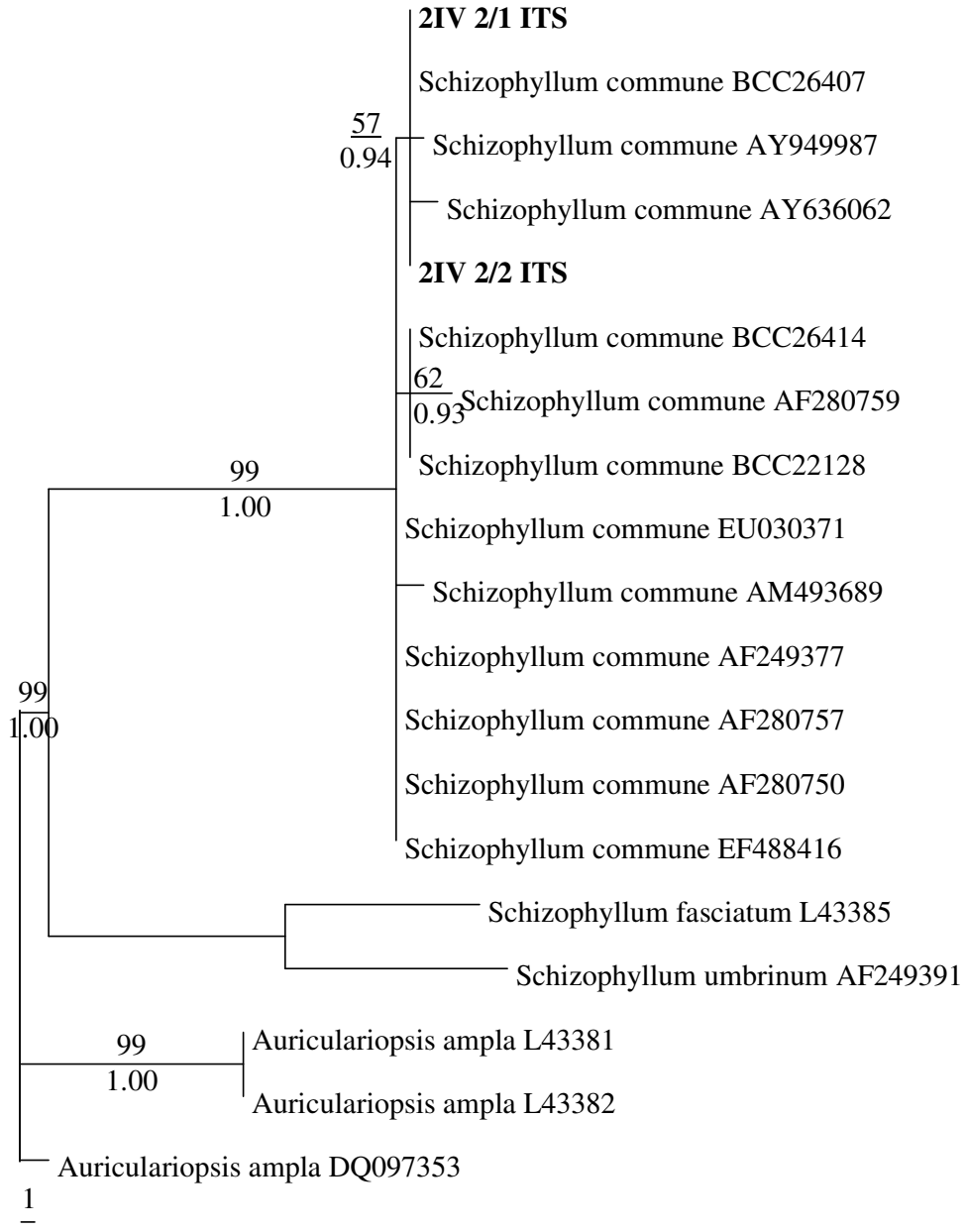

Fig. 6. One of 72 MPTs inferred from ITS sequenes of two isolates of the *Schizophyllaceae* isolated from *Elaeis guineensis*. The MP value (≥50%) and Bayesian PP (≥0.95) are shown above and below the branches, respectively (tree length= 84 steps, CI = 0.942, RI=0.924). Basidiomycete endophytes sequenced 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 s*pecies 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.

Acknowledgements

This project was supported by Thailand Research Fund (TRF) and National Center for Genetic Engineering and Biotechnology (BIOTEC) under special program for Biodiversity Research and Training Program (Grant numbers: BRT_R150004 and BRT_R650001). We thank Dr. Harry Evans for useful discussions on basidiomycetous endophytes and a preprint of a paper on endophytes; Prof. Jeff Duckett for updating the section on liverwort endophytes in Table 2. Special thanks to Prof. David Hibbett and Dr. Manfred Binder for their comments on taxon sampling for the molecular study. Anupong Klaysuban is thanked for technical support. Satinee Suetrong and Prasert Srikitikulchai are thanked for photographic assistance (Figs. 2 a-b). We thank Drs. Kanyawim Kirtikara and Morakot Tanticharoen, BIOTEC, for their constant interest and support.

References

Abdullah, F. (2000). Spatial and sequential mapping of the incidence of basal stem rot of oil palms (*Elaeis guineensis*) on a former coconut (*Cocus nucifera*) plantation. In: *Ganoderma Diseases of Perennial Crops* (eds. J. Flood, P.D. Bridge and

M. Holderness). CABI Publishing, Wallingford: 183-194.

Altschul, S.F., Gish, W., Miller, W., Myer, E.W. and Lipman, D.J. (1990). Basic local alignment search tool. Journal of Molecular Biology 215: 403-410.

Ariffin, D., Idris, A.S. and Singh, G. (2000). Status of *Ganoderma* in oil palm. In: *Ganoderma Diseases of Perennial Crops* (eds. J. Flood, P.D. Bridge and M. Holderness). CAB International, Wallingford: 49-70.

Arnold, A.E. (2007). Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. Fungal Biology Reviews 21: 51-66.

Arnold, A.E., Henk, D.A., Eells, R.L., Lutzoni, F. and Vilgalys, R. (2007). Diversity and phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing and enviormental PCR. Mycologia 99: 185-206.

Barazani, O., von Dahl, C.C. and Baldwin, I.T. (2007). *Sebacina vermifera* promotes the growth and fitness of *Nicotiana attenuata* by inhibiting ethylene signaling. Plant Physiology 144: 1223-1232.

Bernard, N. (1909). L'evolution dans la symbiose. Les orchidées et leurs champignons commensaux. Annales des Sciences Naturelles, Botanique, Paris 9: 1-196.

Bidartondo, M.I., Bruns, T.D., Weiss, M., Sergio, C. and Read, D.J. (2003). Specialized cheating of the ectomycorrhizal symbiosis by an epiparasitic liverwort. Proceedings of Royal Society of London: Biological Science 270: 835-842.

Binder, M., Hibbett, D.S., Larsson, K.H., Larsson, E., Langer, E. and Langer, G. (2005). The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (Homobasidiomycetes). Systematics and Biodiversity 3: 1-45.

Bougoure, J.J., Bougoure, D.S., Cairney, J.W.G. and Dearnaley, J.D.W. (2005). ITS-RFLP and sequence analysis of endophytes from *Acianthus, Caladenia* and *Pterostylis (Orchidaceae)* in southern Queensland. Mycological Research 109: 452-460.

Brundrett, M.C. (2002). Coevolution of roots and mycorrhizas of land plants. New Phytologist 154: 275-304.

Bunyard, B.A., Nicholson, M.S. and Royse, D.J. (1994). A systematic assessment of *Morchella* using RFLP analysis of the 28S ribosomal RNA gene. Mycologia 86: 762-722.

Carroll, G. (1988). Fungal endophytes in stems and leaves: from latent pathogen to mutalistic symbiont. Ecology 69: 2-9.

Chapela, I.H. and Boddy, L. (1988a). Fungal colonization of attached beech branches. I. Early stages of development of fungal communities. New Phytologist 110: 39-45.

Chapela, I.H. and Boddy, L. (1988b). Fungal colonization of attached beech branches. II. Spatial and temporal organization of communities arising from latent invaders in bark and functional sapwood, under different moisture regimes. New Phytologist 110: 47-57.

Corner, E.J.H. (1989). Ad Polyporaceae V. The genera *Albatrellus, Boletopsis, Coriopsis* (dimitic), *Cristelloporia, Diacanthodes, Elmerina, Fomitopsis* (dimitic), *Gloeoporus, Grifola, Hapalopilus, Heterobasidion, Hydnopolyporus, Ischnoderma, Loweporus, Parmastomyces, Perenniporia, Pyrofomes, Steccherícium, Trechispora, Truncospora* and *Tyromyces.* Beih. Nova Hedwigia 96: 1-218.

Crozier, J., Thomas, S.E., Aime, M.C., Evans, H.C. and Holmes, K.A. (2006) Molecular characterization of fungal endophytic morphospecies isolated from stems and pods of *Theobroma cacao*. Plant Pathology 55: 783-791.

Currah, R.S. and Sherburne, R. (1992). Septal ultrastructure of some fungal endophytes from boreal orchid mycorrhizas. Mycological Research 96: 583-587.

Duckett, J.G. and Ligrone, R. (2005). A comparative cytological analysis of fungal endophytes in the sporophyte rhizomes and vascularized gametophytes of *Tmesipteris* and *Psilotum*. Canadian Journal of Botany 83: 1443-1456.

Duckett, J.G. and Ligrone, R. (2008a). Endophytic liverwots in New Zealand liverworts. In: *The Liverwort Flora of New Zealand.* (eds. J. Engels and D. Glenny). Chicago University Press, Chicago.

Duckett, J.G. and Ligrone, R. (2008b). A cytological analysis of basidiomycetous endophytes in New Zealand Aneuraceae (simple thalloid liverworts, Metzgeriidae); confirmation of the derived status of *Verdoornia*. Canadian Journal of Botany 86: 346-358.

Duckett, J.G., Russell, J. and Ligrone, R. (2006). Basidiomycetous endophytes in jungermannialean (leafy) liverworts have novel cytology and species-specific host ranges: a cytological and experimental study. Canadian Journal of Botany 84: 1075-1093.

Duong, L.M., Jeewon, R., Lumyong, S. and Hyde, K.D. (2006). DGGE coupled with ribosomal DNA gene phylogenies reveal uncharacterized fungal phylotypes. Fungal Diversity 23: 121-138.

Evans, H.C. (2008). The endophyte-enemy release hypothesis: implications for classical biological control and plant invasions. (in press)

Evans, H.C., Holmes, K.A and Thomas, S.E. (2003). Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gileri*, in Ecuador and preliminary assessment of their potential as biocontrol agents of cocoa diseases. Mycological Progress 2: 149-160.

Ezra, D., Hess, W.M. and Strobel, G.A. (2004). New endophytic isolates of *Muscodor albus*, a volatile antibiotic producing fungus. Microbiology 150: 4023-4031.

Felenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

Flood, J., Bridge, P.D. and Holderness, M. (2000). Preface. *Ganoderma Diseases of Perennial Crops*. CABI Publishing, Wallingford: 288.

Flood, J., Keenan, L., Wayne, S. and Hasan, Y. (2005). Studies on oil palm trunks as sources of infecton in the field. Mycopathologia 159: 101-107.

Fröhlich, J., Hyde, K.D. and Petrini, O. (2000). Endophytic fungi associated with palms. Mycological Research 104: 1202-1212.

Gomez-Vidal, S., Lopez-Llorca, L.V., Jansson, H.B. and Salinas, J. (2006). Endophytic colonization of date palm (*Phoenix dactylifera* L.) leaves by entomopathogenic fungi. Micron 37: 624-632.

Gonthier, P., Gennaro, M., and Nicolotti, G. (2006). Effects of water stress on the endophytic mycota of *Quercus robur*. Fungal Diversity 21: 69-80.

Guo, L.D., Hyde, K.D. and Liew, E.C.Y. (2001). Detection and taxonomic placement of endophytic fungi within frond tissues of *Livistona chinensis* based on rDNA sequence. Molecular Phylogenetics and Evolution 20: 1-13.

Hadley, G. (1975). Organization and fine structure of orchid mycorrhizas. In: *Endomycorrhizas.* (eds. F.E. Sanders, B. Mosse and P.B. Tinker). Academic Press, London: 335-351.

Hall, T. (2006). BioEdit. Version 7.5.0.3. Department of Microbiology, North Carolina State University.

Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf, S., James, T., Kirk, P.M., Lucking, R., Thorsten Lumbsch, H., Lutzoni, F., Matheny, P.B., McLaughlin, D.J., Powell, M.J., Redhead, S, Schoch, C.L., Spatafora, J.W., Stalpers, J.A., Vilgalys, R., Aime, M.C., Aptroot, A., Bauer, R., Begerow, D., Benny, G.L., Castlebury, L.A., Crous, P.W., Dai, Y.C., Gams, W., Geiser, D.M., Griffith, G.W., Gueidan, C., Hawksworth, D.L., Hestmark, G., Hosaka, K., Humber, R.A., Hyde, K.D., Ironside, J.E., Koljalg, U., Kurtzman, C.P., Larsson, K.H., Lichtwardt, R., Longcore, J., Miadlikowska, J., Miller, A., Moncalvo, J.M., Mozley-Standridge, S., Oberwinkler, F., Parmasto, E., Reeb, V., Rogers, J.D., Roux, C., Ryvarden, L., Sampaio, J.P., Schussler, A., Sugiyama, J., Thorn, R.G., Tibell, L., Untereiner, W.A., Walker, C., Wang, Z., Weir, A., Weiss, M., White, M.M., Winka, K., Yao, Y.J. and Zhang, N. (2007). A higher-level phylogenetic classification of the Fungi. Mycological Research 111: 509-547.

Hjortstam, K. and Ryvarden, L. (1982). Aphyllophorales from Nothern Thailand. Nordic Journal of Botany 2: 273-281.

Huelsenbeck, J.P. and Ronquist, F. (2001). Mr.Bayes: Bayesian inference of phylogenetic diversity. Bioinformatics (Oxford) 17: 754-755.

Hyde, K.D. (2001). Where are the missing fungi? Does Hong Kong have the answers? Mycological Research 105: 1514-1518.

James, T.Y., Moncalvo, J.M., Li, S. and Vilgalys, R. (2001). Polymorphism at the ribosomal DNA spacers and its relation to breeding structure of the widespread mushroom *Schizophyllum commune*. Genetics 157: 149-161.

Kim, S., Shin, D.S., Lee, T. and Oh, K.B. (2004). Periconicins, two new fusicoccane diterpenes produced by endophytic fungus *Perciconia* sp. with antibacterial activity. Journal of Natural Products 67: 448-450.

Kim, K.M., Yoon, Y.G. and Jung H.S. (2005). Evalution of the monophyly of *Fomitopsis* using parsimony and MCMC methods. Mycologia 97: 812-822.

Kim, K.M, Lee, J.S. and Jung, H.S. (2007). *Fomitopsis incarnatus* sp. nov. based on generic evaluation of *Fomitopsis* and *Rhodofomes*. Mycologia 99: 833-841.

Kishino, H. and Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in the *Homioidea*. Journal of Molecular Evolution 29: 170-179.

Kristiansen, K.A., Fredenstein, J.V., Rasmussen, F.N. and Rasmussen, H.N. (2004) Molecular identification of mycorhizal fungi in *Neuwiedia veratrifolia* (Orchidaceae). Molecular Phylogenetics and Evolution 33: 251-258.

Kotlaba, F. and Pouzar, Z. (1990). Type studies of Polypores described by A. Pilat-III. Ceska Mykololy 44:228-237.

Kottke, I., Beiter, A., Weiss, M., Haug, I., Oberwinkler, F. and Nebel, M. (2003). *Heterobasidiomycetes* form symbiotic associations with hepatics: *Jungermanniales* have sebacinoid mycobionts while *Aneura pinguis (Metzgeriales)* is associalted with a *Tulasnella* species. Mycological Research 107: 957-968.

Kupka, J. Anke, T., Steglich, W. and Zechlin, L. (1981) Antibiotics from Basidiomycetes. XI The biological activity of siccayne, isolated from marine fungus *Halocyphina villosa* J. & E. Kohlmeyer. The Journal of Antibiotics 3: 298-304.

Ligrone, R., Pocock, K. and Duckett, J.G. (1993). A comparative ultrastructural study of endophytic basidiomycetes in the parasitic achlorophyllous hepatic *Crytothallus mirabilis* and the closely allied photosynthetic species *Aneura pinguis* (Metzgeriales). Canadian Journal of Botany 71: 666-679.

Likhitekaraj, S. and Tummakate, A. (2000). Basal stem rot of oil palm in Thailand caused by *Ganoderma*. In: *Ganoderma Diseases of Perennial Crops.* (eds. J. Flood, Bridge P.D. and M.

Holderness) CAB Internaltional, Wallingford: 69-70.

Lomascolo, A., Cayol, J.L., Roche, M., Guo, L., Robert, J.L., Record, E., Lesage-Meessen, L., Ollivier, B., Sigoillot, J.C. and Asther, M. (2002). Molecular clustering of *Pycnoporus* strains from various geographic origins and isolation of monokaryotic strains for laccase hyperproduction. Mycological Research 106: 1193-1203.

Maria, G.L., Sridhar, K.R. and Raviraja, N.S. (2005). Antimicrobial and enzyme activity of mangrove endophytic fungi of southwest coast of India. Journal of Agricultural Technology 1: 67-80.

Márquez, S.S., Bills, G.F. and Zabalgogeazcoa, I. (2007). The endophytic mycobiota of the grass *Dactylis glomerata.* Fungal Diversity 27: 171-195.

Moncalvo, J.M., Vilgalys, R., Redhead, S.A., Johnson, S.E., James, T.Y., Aime, M.C., Hofstetter, V., Verduin, S., Larsson, E., Baroni, T.J., Thorn, R.G., Jacobsson, S., Clemencon, H. and Miller, O.K. (2002). One-hundred and seventeen clades of eugarics. Molecular Phylogenetics and Evolution 23: 357-400.

Munusamy, U., Sabaratnam, V., Muniandy, S., Abdullah, N., Pandey, A. and Jones, E.B.G. (2008). Characterisation of laccase from *Pycnoporus sanguineus* KUM60953 and KUM60954. Journal of Biological Sciences 8:866-873.

Nebel, M., Kreier, H.P., Preussing, M., Weiss, M. and Kottke, I. (2004). Symbiotic fungal associations of liverworts are the possible ancestors of mycorrhizae. In: *Frontiers in Basidiomycote Mycology.* (eds. R. Agerer, M. Piepenbring and P. Blanz). IHW-Verlag, Eching: 339-360.

O'Donnell, K., Cigelnik, E., Weber, N.S. and Trappe, J.M. (1997). Phylogenetic relationship among ascomycetous truffles and the true and false morels inferred from 18S and 28S ribosomal DNA sequence analysis. Mycologia 89: 48-65.

Oses, R., Valenzuela, S., Freer, J., Baesa, J. and Rodriguez, J. (2006). Evaluation of fungal endophytes for lignocellulolytic enzyme production and wood biodegradation. International Biodeterioration and Biodegradation 57: 129-135.

Paterson, R.R.M. (2007). *Ganoderma* disease of oil palm- A white rot perspective necessary for integrated control. Crop Protection 26: 1369-1376.

Petrini, O. (1986). Taxonomy of endophytic fungi of aerial plant tissues. In: *Microbiology of the Phyllosphere.* (eds. N.J. Fokkema and J. van den Heuvel). Cambridge University Press, Cambridge: 175-187.

Petrini, O., Fisher, P.J. and Petrini, L.E. (1992). Fungal endophytes of bracken (*Pteridium aquilinum*) with some reflections on their use in biological control. Sydowia 44: 282-293.

Phanichapol, D. (1968). Check-list of fungi in the forest herbarium. *Report of Forest Products Research Division.* Royal Forest Department. Bangkok, Thailand: 263-269.

Phongpaichit, S., Nikom, J., Rungjindamai, N., Sakayaroj, J., Hutadilok-Towatana, N., Rukachaisirikul, V. and Kirtikara, K. (2007). Biological activities of extracts from endophytic fungi isolated from *Garcinia* plants. FEMS Immunology and Medical Microbiology 51: 517-525.

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

Pinruan, U., Hyde, K.D., Lumyong, S., McKenzie, E.H.C. and E.B.G. Jones (2007). Occurrence of fungi on tissues of the peat swamp palm *Licuala longicalycata*. Fungal Diversity 25: 157-173.

Pointing, S.B., Jones, E.B.G. and Vrijmoed, L.L.P. (2000). Optimisation of laccase production by *Pycnoporus sanguineus* in submerged liquid culture. Mycologia 92: 139-144.

Pongcharoen, V., Rukachaisirikul, V., Phongpaichit, S., Kuhn, T., Pelzing, M., Sakayaroj, J. and Taylor, W.C. (2008). Metabolites from the endophytic fungus *Xylaria* sp. PSU-D15. Phytochemistry 69: 1900-1902.

Puri, S.C., Asiya, N., Raman, C., Rajesh, A., Hasan, R., Touseef, A., Bilal, A., Vijeshwar, V., Shinkha, S., Ravinder, S., Ashok, S. Raj, K. Sharma, R.K. and Qazi, G.N. (2006). The endophytic fungus *Trametes hirsuta* as a novel alternative source of podophyllotoxin and related aryl tetralin lignans. Journal of Biotechnology 122: 494-510.

Rodrigues, K.F. (1994). The foliar fungal endophytes of the Amazonian palm *Euterpe oleracea*. Mycologia 83: 376-385.

Rogers, J.D. (2000). Thoughts and musings on tropical *Xylariaceae*. Mycological Research 104: 1412-1420.

Rosa, L.H., Machado, K.M.G., Jacob, C.C., Capelari, M., Rosa, C.A. and Zani, C.L. (2003). Screening of Brazilian basidiomycetes for antimicrobial activity. Memorias do Instituto Oswaldo Cruz 98: 967-974.

Russell, J. and Bulman, S. (2005). The liverwort *Marchantia foliacea* forms a specialized symbiosis with arbuscular mychorrhizal fungi in the genus *Glomus*. New Phytologist 165: 567-579.

Ryvarden, L. and Johansen, I. (1980). *A Preliminary Polypore Flora of East Africa.* Fungiflora, Oslo.

Ryvarden, L. and Gilbertson, R.L. (1993). *European Polypores*. Part I. Fungiflora, Oslo.

Sánchez Márquez, S., Bills, G.F. and Zabalgogeazcoa, I. (2007). The endophytic mycobiota of the grass *Dactylis glomerata*. Fungal Diversity 27: 171-195.

Schulz, B. and Boyle, C. (2005). The endophytic continuum. Mycological Research 109: 661-686.

Schulz, B., Boyle, C., Draeger, S., Rommert, A.K. and Krohn, K. (2002). Endophytic fungi: a source of novel biologically active secondary metabolites. Mycological Research 106: 996-1004.

Schulz, B., Draegerm S., dela Cruz, T.E., Rheinheimer, J., Siems, K., Loesgen, S., Bitzer, J., Schloerke, O., Zeeck, A., Kock, I., Hussain, H., Dai, J. and Krohn, K. (2008). Screening strategies for obtaining novel, biologically active, fungal secondary metabolites from marine habitats. Botanica Marina 51: 219-234.

Photita, W., Lumyong, S., Lumyong, P. and Hyde, K.D. (2001). Endophytic fungi of wild banana (*Musa acuminata*) at Doi Suthep Pui National Park, Thailand. Mycological Research 105: 1508-1513.

Sieber, T.N., Sieber-Canavesi, F. and Dorworth, C.E. (1991). Endophytic fungi of red alder (*Alnus rubra*) leaves and twigs in British Columbia. Canadian Journal of Botany 69: 407-411.

Southcott, K.A. and Johnson, J.A. (1997). Isolation of endophytes from two species of palm from Bermuda. Canadian Journal of Microbiology 43: 789-792.

Sridhar, K.R. and Raviraja, N.S. (1995). Endophytes-A crucial issue. Current Science 69: 570-571.

Stevenson, T. (2006). Malaysia targets alternative fuels market. Telegraph. 22 December 2006.

Strobel, G.A. (2002). Microbial gifts from the rainforest. Canadian Journal of Phytopathology 24: 14-20.

Strobel, G.A., Dirksie, E., Sears, J. and Markworth, C. (2001). Volatile antimicrobials from *Mucodor albus*, novel endophytic fungi. Microbiology 147: 2943-2950.

Swofford, D.L. (2002). PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods) Version 4.0b10. Sinauer Associates, Sunderland, MA.

Taylor, J.E., Hyde, K.D. and Jones, E.B.G. (1999). Endophytic fungi associated with the temperate palm, *Tranchycarpus fortunei*, within and outside its natural geographical range. New Phytologist 142: 335-346.

Tejesvi, M.V., Kini, K.R., Prakash, H.S., Ven Subbiah and Shetty, H.S. (2007). Genetic diversity and antifungal activity of species of *Pestalotiopsis* isolated as endophytes from medicinal plants. Fungal Diversity 24: 37-54.

Tao, G., Liu, Z.Y. and Hyde, K.D. (2008). Whole rDNA analysis reveals novel and endophytic fungi in *Bletilla ochraea* (Orchidaceae). Fungal Diversity 33: 101-122.

Thaithatgoon, P., Thaithatgoon, B., Boonkemthong, C., Tanticharoen, M. and Jones, E.B.G. (2004). Edible mushrooms. In: *Thai Fungal Diversity*. (eds. E.B.G. Jones, M. Tanticharoen and K.D. Hyde). National Center for Genetic Engineering and Biotechnology, Pathum Thani: 241-251.

Thomas, S.E., Crozier, J., Aime, M.C., Evans, H.C. and Holmes, K.A. (2008). Molecular characterization of fungal endophytic morpho-species associated with the indigeneous forest tree, *Theobroma gileri*, in Ecuador. Mycological Research 112: 852-860.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994). CLUSTAL W improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choise. Nucleic Acids Research 22: 4673-4680.

Tomšovsky, M., Kolarik, M., Pazoutova, S. and Homolka, L. (2006). Molecular phylogeny of European *Trametes* (Basidiomycetes, Polyporales) species based on LSU and ITS (nrDNA) sequences. Nova Hedwigia 82: 269-280.

Ujang, S., Jones, E.B.G. and Wong, A. (2007). Wood decay fungi. In: *Malaysian Fungal Diversity*. (eds. E.B.G. Jones, K.D. Hyde and S.Vikineswary). Mushroom Research Centre, Univeristy Malaya and Ministry of Natural Rsesources and Environment, Malayasia: 163-183.

Valdicia, C., Kettering, M., Anke, H., Thines, E. and Sterner, O. (2005). Diterpenoids from *Coprinus heptemerus*. Tetrahedron 61: 9527-9532.

Vikineswary, S., Abdullah, W., Ibrahim, W., Tan, Y.H., Daud, F. and Jones, E.B.G. (2007). Edible and medicinal mushrooms. In : *Malaysian Fungal Diversity.* (eds. E.B.G. Jones, K.D. Hyde and S. Vikisneswary). Mushroom Research Centre, University Malaya, Ministry of Natural Resouces and Environment, Malaysia: 287-305.

Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., Heier, T., Hückelhoven, R. Neumann, C., von Wettstein, D., Franken, P. and Kogel, K.H. (2005). The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proceedings of the National Academy of Sciences 102: 13386-13391.

Wang, Y., Guo, L.D. and Hyde, K.D. (2005). Taxonomic placement of sterile morphotypes of endophytic fungi from *Pinus tabulaeformis* (Pinaceae) in notheast China based on rDNA sequences. Fungal Diversity 20:235-260.

Warcup, J.H. (1971). Specificity of mycorrhizal association in some Australian terrestrial orchids. New Phytologist 70: 41-46.

Warcup, J.H. (1988). Mycorrhizal associations of isolates of *Sebacina vermifera*. New Phytologist 110: 227-231.

Warcup, J.H. (1991) The *Rhizoctonia* endophytes of *Rhizanthella* (Orchidaceae). Mycological Research 95: 656-641

Warcup, J.H. and Talbot, P.H.B. (1980). Perfect states of *Rhizoctonias* associated with orchids. III. New Phytologist 86: 267-272.

Weiss, M., Selosse, M.A., Rexer, K.H., Urban, A. and Oberwinkler, F. (2004). Sebacinales: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. Mycological Research 108: 1003-1010.

White, T.F., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA gene for phylogenetics. In: *PCR Protocal: A Guide to Methods and Applications.* (eds. M.A. Innis, D.H. Gelfand, F.S. Sninsky and T.T. White). Academic Press, San Diego: 315-322.

Wiyakrutta, S., Sriubolmass, N., Panphut, W., Thongon, N., Danwisetkanjana, K., Ruang-rungsi, N. and Meevootsom, V. (2004). Endophytic fungi with anti-microbial, anti-cancer and anti-malarial activities isolated from Thai medicinal plants. World Journal of Microbiology and Biotechnology 20: 265-272.

Zjawiony, J.K. (2004). Biologically active compounds from Aphyllophorales (Polypore) fungi. Journal of Natural Products 67: 300-310.