Final Report

(June 2008 – December 2008)

For: National Center for Genetic Engineering and Biotechnology (BIOTEC)

Biodiversity Research and Training Program (BRT)

Project Title: The Phylogenetic Relationships of Selected Coelomycete Genera (Year 3)

BRT Project Code: BRT R_251004

Principal Investigator: Prof. E. B. Gareth Jones

Co Investigators: Dr. Jariya Sakayaroj

Dr. Sayanh Somrithipol

Assistant Researcher Mr. Nattawut Rungjindamai

This report consists of two parts:

A: Final report of research undertaken (Page 2)

B: Appendices

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2) Draft manuscript entitled "Phylogeny of fusiform conidia coelomycetes: *Robillarda, Pseudorobillarda* and *Xepiculopsis* based on nuclear ribosomal DNA sequences"

3) Poster presented at BRT Annual Conference 2008, Surat Thani

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Part A: 12-month progress report

1. Summary of report

Coelomycetes are anamorphic fungi producing asexual spores (conidia) and play an important role in terrestrial ecosystems: as saprobes, or parasites of higher plants, fungi, lichens and vertebrates and may occur as endophytes. Over the past 12 months, a paper on cupulate-conidiomata coelomycete was accepted and published online in Mycologia. The rDNA of coelomycetes with fusiform-conidia comprising *Robillarda sessilis*, *Pseudorobillarda siamensis*, *P. sojae, P. texana* and *Xepiculopsis graminea*, was examined to determine their taxonomic position, and a draft manuscript has been prepared for publication in an international journal (Persoonia). Analyses of new coelomycete isolates are in progress. The result demonstrate that these marine coelomycetes grouped within the Pleosporales (Dothideomycetes), while a number of unidentified coelomycetes could be referred to the Dothideomycetes and Sordariomycetes, but their lower taxonomic level could not be resolved. Selected coelomycetes, *Falcocladium* and *Wiesneriomyces,* were studied and their taxonomic placement is discussed in this report. The analyses of other coelomycetes are in progress.

2. Project objectives

1) To continue the analyses of entire rDNA sequences (SSU, LSU and ITS regions) of selected coelomycetes: *Robillarda sessilis, Pseudorobillarda siamensis, P. sojae, P. texana* and *Xepiculopsis graminea.*
2) To examine the phylogenetic relationships of selected marine coelomycetes
3) To identify problematic unidentified coelomycetes using rDNA sequence analysis, and,

4) To evaluate of the taxonomic placement of selected coelomycetes at the generic, ordinal or familial levels.

3. Research methods

3.1 Growth of fungi:

The fungi were maintained on freshwater potato dextrose agar. Fungi were grown in liquid broth on a rotary shaker at 200 rpm at a temperature of 25 °C. The fungal biomass was harvested by filtration and washed with sterile distilled water. The biomass was then frozen at -20 ° C overnight.

3.2 Genomic DNA extraction: (Applied from O'Donnell *et al.*, 1997)

A fine powder of fungal mycelium of 50-100 mg was placed into 400 µl lysis buffer and incubated at 65 °C for 1 hour. Then an equal volume of phenol-chloroform-isoamyl alcohol was added. The upper liquid phase was transferred to a new microtube containing chilled absolute ethanol and 7.5 M ammonium acetate. The mixture was kept at –20 °C for at least 30 minutes, or until the DNA precipitated out, and centrifuged at 14K, 4 °C, for 10 minutes. The DNA pellet was washed twice with chilled 75 % ethanol and air dried. Finally, DNA was resuspended in 50 µl nanopure water and checked for the quantity and quality in a 1% agarose gel electrophoresis.

3.3 PCR amplification:

rDNA was amplified with Taq DNA polymerase from DyNAzyme™ II DNA Polymerase Kit, FINNZYMES, Finland. Different regions of ribosomal DNA were amplified using PCR in a MJ Research DNA Engine DYAD ALD 1244 thermal cycler. Primers used for amplification the rRNA gene follows White *et al.* (1990) and Landvik (1996).

3.4 PCR product purification:

The PCR product was purified directly following the manufacturer's instructions of NucleoSpin^{R} Extract (MACHEREY-NAGEL). The purified PCR product was used directly for DNA sequencing.

3.5 DNA Sequencing:

PCR products were directly sequenced by Macrogen Inc. (Korea) using primers NS1, NS3, NS5, NS6, NS8, JS1, JS5, JS8, LROR, LR7, NL3, NL4, NL4R, ITS1, ITS4 and ITS5 (White *et al.*, 1990; Landvik, 1996).

3.6 Phylogenetic analysis:

The sequences were aligned along with selected sequences obtained from the GenBank database and with suitable outgroup taxa. Sequences was aligned in Clustal W 1.6 program (Thompson, *et al.*, 1994) and refined visually in BioEdit version 6.0.7 (Hall, 2004). Alignment was entered into PAUP, version 4.0b10 (Swofford, 2002). Phylogenetic trees were generated using maximum parsimony using PAUP version 4.0b10. The statistical support, the maximum parsimony, was calculated to evaluate the robustness of the phylogenetic trees.

4. Results

4.1 Phylogenetic relationship of selected coelomycetes for the third year project

In order to analyze the phylogenetic relationships, the 15 coelomycetes were divided into three groups comprising: 1. cupulate-conidiomata, 2. basidiomycetous coelomycetes and 3. fusiform-conidia based on the morphological and sequence data of these fungi (Table 1).

The first group includes coelomycetes that produce funnel-shaped cupulate-conidiomata that are superficial on the substratum. Basidiomycetes with coelomycete anamorphs are rare. The final group comprises coelomycetes with fusiform to ellipsoidal conidia. The progress of this analysis is shown in Table 1. The phylogenetic analysis was performed for all the selected taxa and papers for each group of coelomycetes are in draft form. Two papers on basidiomycete coelomycetes and cupulate conidiomata are published in international mycological journals with an impact factor: Mycological Research and Mycologia, respectively. The last paper for coelomycetes with fusiform conidia is being prepared and will be submitted to Persoonia shortly.

Table 1. The updated status of DNA extraction, PCR amplification and rDNA sequencing of coelomycetes studied over the last six months.

Group of fungi	No	Coelomycetes names	Original SFC code	BCC code	DNA extracted	DNA sequencing	Phylogenetic analysis	Paper
Cupulate-conidiomata fungi	1	*Infundibulomyces cupulata*	0943	11929	/			
	2	*Infundibulomyces siamensis*	0981	13400	/	Completed	Completed	Published
	3	*Satchmopsis brasiliensis*	1901	18579	/			
Basidiomycetous fungi	4	*Chaetospermum camelliae*	1625	13401	/			
	5	*Chaetospermum camilliae*	1909	18582	/			
	6	*Chaetospermum camilliae*	1925	18604	/			
	7	*Chaetospermum artocarpi*	1904	18581	/	Completed	Completed	Published
	8	*Mycotribulus mirabilis*	0852	13341	/			
	9	*Mycotribulus mirabilis*	1922	18601	/			
	10	*Guilia tenuis*	0865	13066	/			
Fusiform-conidia fungi	11*	*Pseudorobillarda siamensis*	0795	12531	/			
	12	*Pseudorobillarda sojae*	1947	20495	/			
	13	*Pseudorobillarda texana*	0866	12535	/	Completed	Completed	Drafting
	14	*Robillarda sessilis*	0858	13393	/			
	15	*Xepiculopsis graminea*	-	-	/			

Summary of the phylogenetic relationships of selected coelomycetes

Phylogeny of coelomycetes with fusiform-conidia*: Robillarda sessilis, Pseudorobillarda siamensis* and *Xepiculopsis graminea* based on nuclear rDNA sequences.

These coelomycetes, share a common character of fusiform to ellipsoidal shaped conidia with polar appendages. A phylogenetic study was conducted in order to determine if there was any correlation between their morphology and phylogeny. The entire rDNA sequence was analyzed and a phylogenetic tree constructed. The analysis of phylogenetic relationship is almost finished. This manuscript is being prepared and will be submitted to Persoonia. See in Appendix 1.

4.2 Preliminary result of the phylogenetic relationship selected coelomycetes

The preliminary phylogenetic study of selected coelomycetes was performed using BLAST search tool from GenBank database for comparison with the coelomycete sequences. Our isolates were divided into three groups, consisting of 1. marine coelomycetes, 2. unidentified coelomycetes and 3. selected coelomycetes (Table 2). Genomic DNA of these isolates were extracted. Ribosomal DNA sequence was successfully amplified through PCR amplification. However some of them can not be amplified, therefore our experiment will be repeated with new pair of primers or new PCR conditions. Some of the consensus sequences were used for a BLAST search.

The result demonstrates that our marine coelomycetes have a phylogenetic affinity with several orders and families within the Dothideomycetes. Unidentified coelomycetes were compared with taxa from GenBank database. Based on SSU and LSU data, our isolates show relationship with diverse genera from the Sordariomycetes and Dothideomycetes. ITS regions will be used to clarify the lower taxonomic rank of these coelomycetes. Finally, most of the selected coelomycetes are well distributed within the Dothideomycetes, although their lower

taxonomic positions are not consistent and further evaluation is required. The analysis of entire or combined sequences, in order to clarify lower level of taxonomy of our coelomycetes, is in progress. Selected examples are presented in this report. All of the coelomycetes investigated have no known teleomorphs, so the study will greatly enhance our knowledge of the phylogenetic relationships of this group.

Table 2. The updated status of DNA extraction and rDNA sequencing of coelomycetes studied over the last 12 months.

Group of fungi	No	Coelomycetes names	Original code	BCC code	Culture	DNA	rDNA sequences			BLAST	Alignment	Preliminary study
							SSU	LSU	ITS			
Marine coelomycete	1	Marine coelomycete	KH0087	25065	/	/	/	/	/	/	/	
	2	Marine coelomycete	KH0088	25066	/	/	/	/	/	/	/	
	3	Marine coelomycete	KH0089	25067	/	/	/	/	/	/	/	Dothideomycetes
	4	Marine coelomycete	KH0090	25068	/	/	/	/	/	/	/	
	5	Marine coelomycete	KH0091	25069	/	/	-	-	-	-	-	
	6	Marine coelomycete	KH0092	25070	/	/	-	-	-	-	-	
Unidentified coelomycete	7	Unidentified sp. 1	SFC1912	18583	/	/	/	/	/	/	/	Valsa, Diaporthales
	8	Unidentified sp. 2	SFC1920	18586	/	/	/	/	/	/	/	Alternaria/Pleosporales
	9	Unidentified sp. 3	SFC1940*	-	-	-	-	-	-	-	-	
	10	Unidentified sp. 4	SFC1941	20494	/	/	/	/	/	/	/	Rhizopycnis/Pleosporales
	11	Unidentified sp. 5	SFC1946	20812	/	/	/	/	/	/	/	Lophiostoma/Pleosporales
	12	Unidentified sp. 6	SFC2109	21373	/	/	/	/	/	/	/	Volutella/Hypocreales

* = Culture is in preparation for BCC deposition. / = Completed - = in process

Table 2 (Continue)

Group of fungi	No	Coelomycetes names	Original code	BCC code	Culture	DNA	rDNA sequences			BLAST	Alignment	Preliminary study
							SSU	LSU	ITS			
Selected coelomycete	13	*Vermiculariopsiella* sp.	SFC2075	22244	/	/	/	/	/	/	/	Helotiales
	14	*Lauriomyces* sp. 1	SFC1649	18576	/	/	/	/	/	/	/	Helotiales
	15	*Lauriomyces* sp. 1	SFC1649	18577	/	/	/	/	/	/	/	Helotiales
	16	*Wiesnesriomyces* sp. 2	SFC1689*	-	-	-	-	-	-	-	-	
	17	*Wiesneriomyces* sp. 1	SFC1929	18608	/	/	/	/	/	/	/	
	18	*Wiesneriomyces laurinus*	SFC1930	18609	/	/	/	/	/	/	/	Dothideomycetes
	19	*Wiesn. conjunctosporus*	SFC00425	4027	/	?	-	/	-	/	/	*Incertae sedis*
	20	*Wiesn. conjunctosporus*	SFC01927	18606	/	/	/	/	/	/	/	
	21	*Wiesn. laurinus*	SFC00151	3922	/	/	/	/	/	/	/	
	22	*Wiesn. laurinus*	SFC01549	9453	/	?	-	-	-	-	/	
	23	*Falcocladium* sp. 1	SFC2101	22055	/	/	-	/	/	/	/	
	24	*F. thailandicum*	CBS121717		/	-	-	/	/	/	/	Hypocreomycetidae
	25	*F. multivesiculatum*	CBS120386		?	-	-	/	/	/	/	*Incertae sedis*
	26	*F. sphaeropedunculatum*	CBS111292		/	-	-	/	/	/	/	

* = Culture is in preparation for BCC deposition. / = Completed - = in process

(I) Marine coelomycete

Six coelomycetes were isolated from mangrove wood collected from Had Kanom Mu Ko Tale Tai, Nakhon Si Thammarat. The morphology of these are illustrated in Figure 1. Two marine coelomycetes (KH87 and KH88) produced dark brown striated conidia when released from the conidiomata (Figure 1, a and b). Coelomycete isolates, KH89 and KH90 produced septate branched conidiophores with ellipsoidal conidia (Figure 1, c and d). Variable shaped conidia were produced from the marine coelomycete isolates KH91 and KH92 (Figure 1, e-g).

Total DNA of the six fungi were extracted. Genomic DNA of first four isolates (KH87, KH88, KH89 and KH90) was extracted and rDNA was amplified. The preliminary result of these four isolates are presented herein. However, DNA of two isolates (KH91 and 92) could not be extracted, even when alternative extraction protocols were applied. These must be reeaxmined and amplified with new primers.

In order to characterize and identify the unknown marine coelomycetes, molecular methods and phylgenetic analysis were performed. Firstly, the dataset of SSU and LSU were analyzed independently but they yielded the same tree topology and phylogeny. Therefore the two regions were combined and analyzed in the same dataset in order to increase the robustness of the phylogenetic tree. The tree is shown in Figure 2.

Six major classes within the Ascomycota, consisting of the Eurotiomycetes, Lecanoromycetes, Leotiomycetes, Orbiliomycetes, Pezizomycetes and Sordariomycete, were alighned with members of the Taphrinomycetes as the outgroup. Our phylogenetic investigation showed that four marine coelomycete isolates could be referred to the Dothideomycetes. Therefore eight major orders within the Dothideomycetes (Acrospermales, Botryosphaeriales, Capnodiales, Dothideales, Jahnulales, Hysteriales, Myrangiales and Pleosporales) were analyzed with our four coelomycete isolates. The marine isolates formed a clade with various genera

within the Pleosporales with high statistical support (98 %BS), however, the order is polyphyletic and resolution at the family level difficult. Isolates KH87 and KH88 grouped together with a long branch length, while isolate KH89 showed a close relationship with isolate KH90 supported by high bootstrap support (100 %BS) for all internal node of this subclade.

The ITS regions of all marine isolates were analysed in order to clarify their lower taxonomic position. ITS regions were amplified and sequenced with good result. Although ITS sequences of marine coelomycetes were blast searched with the GenBank database, the DNA similarity between ours and related sequences was quite low. Therefore the phylogenetic tree is not presented here. However DNA similarity of ITS sequence was calculated. Percentage similarity of isolates KH87 and KH88 showed 100% homology. This two isolates showed sequence similarity ranging from 67.0-71.4 % to *Preussia* sp. FJ377749, *Presussaria minima* AY943054, *Sporormia subticinensis* AY943051 and *Sporormiella tenerifae* AY943047, all members of the Sporormiaceae (Plesporales). However, this result confirms that our two marine coelomycetes had a relationship with the Pleosporales, although their lower taxonomic level can not be resolved at this time. ITS sequences of isolates KH89 and 90 showed a relationships with various unknown sequences from GenBank, consisting of fungal endophyte EU625406, ascomycete EF672295, uncultured root associated fungi EU144592, EU144594, EU144600 and *Rhizopycnis* sp. DQ682600. ITS sequence similarity ranged from 64.7-70.6%. Therefore the identification at the generic level of these two isolates can not be resolved with confidence at this time.

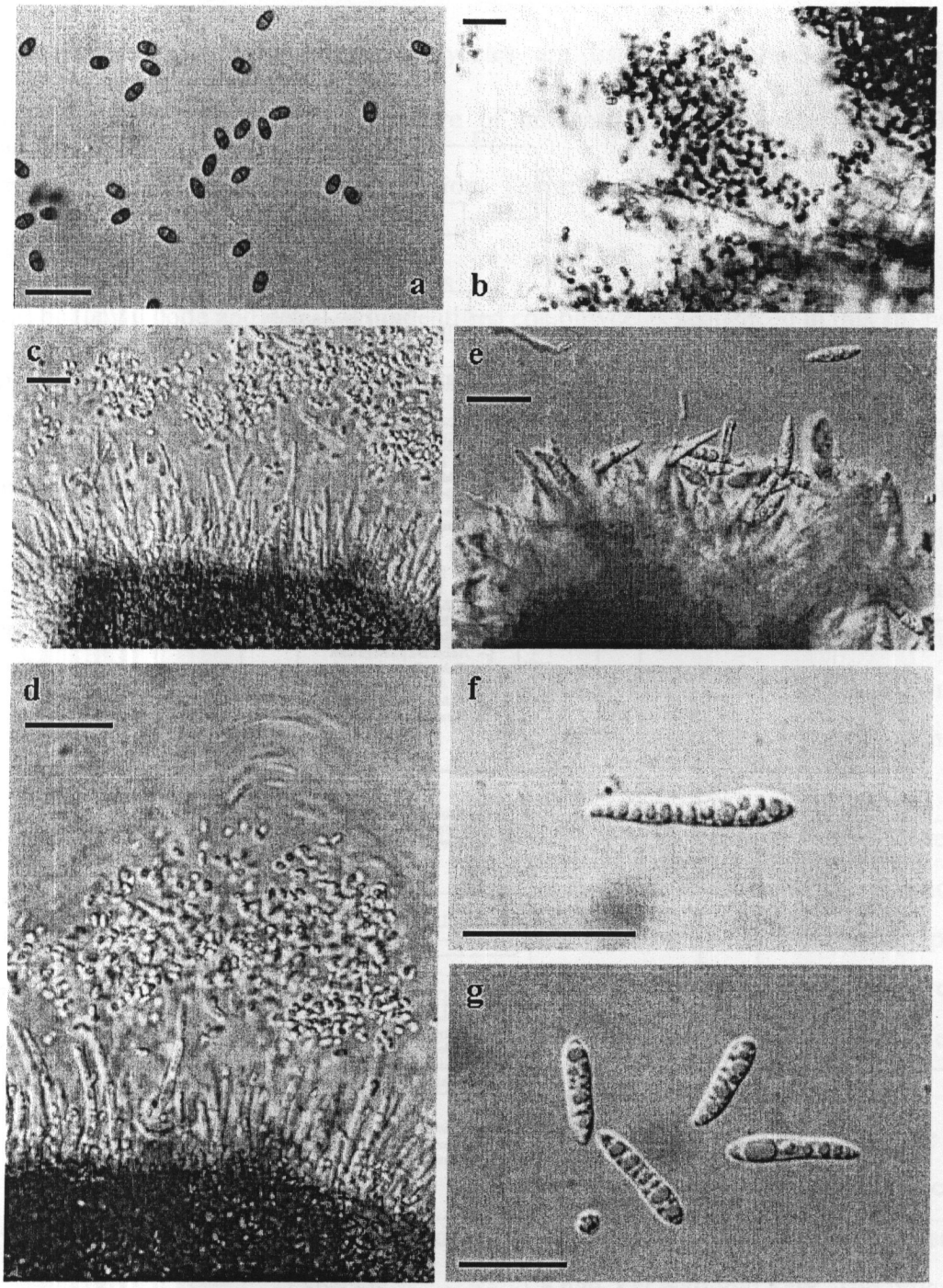

Figure 1. Marine coelomycetes collected from mangrove wood. (a) Dark brown striated conidia of isolates KH87 and KH88, (b) dark conidia exuded from the conidioma of isolates KH87 and KH88. (c) ellipsoidal conidia and (d) septate branched conidiophores of isolates KH89 and KH90. (e) conidia exuded from conidioma, (f, g) variable fusiform conidial shape of KH91 and KH92 isolates. Scale bar a = 25 µm, b = 50 µm, c = 50 µm, d = 50 µm, e-g = 25 µm.

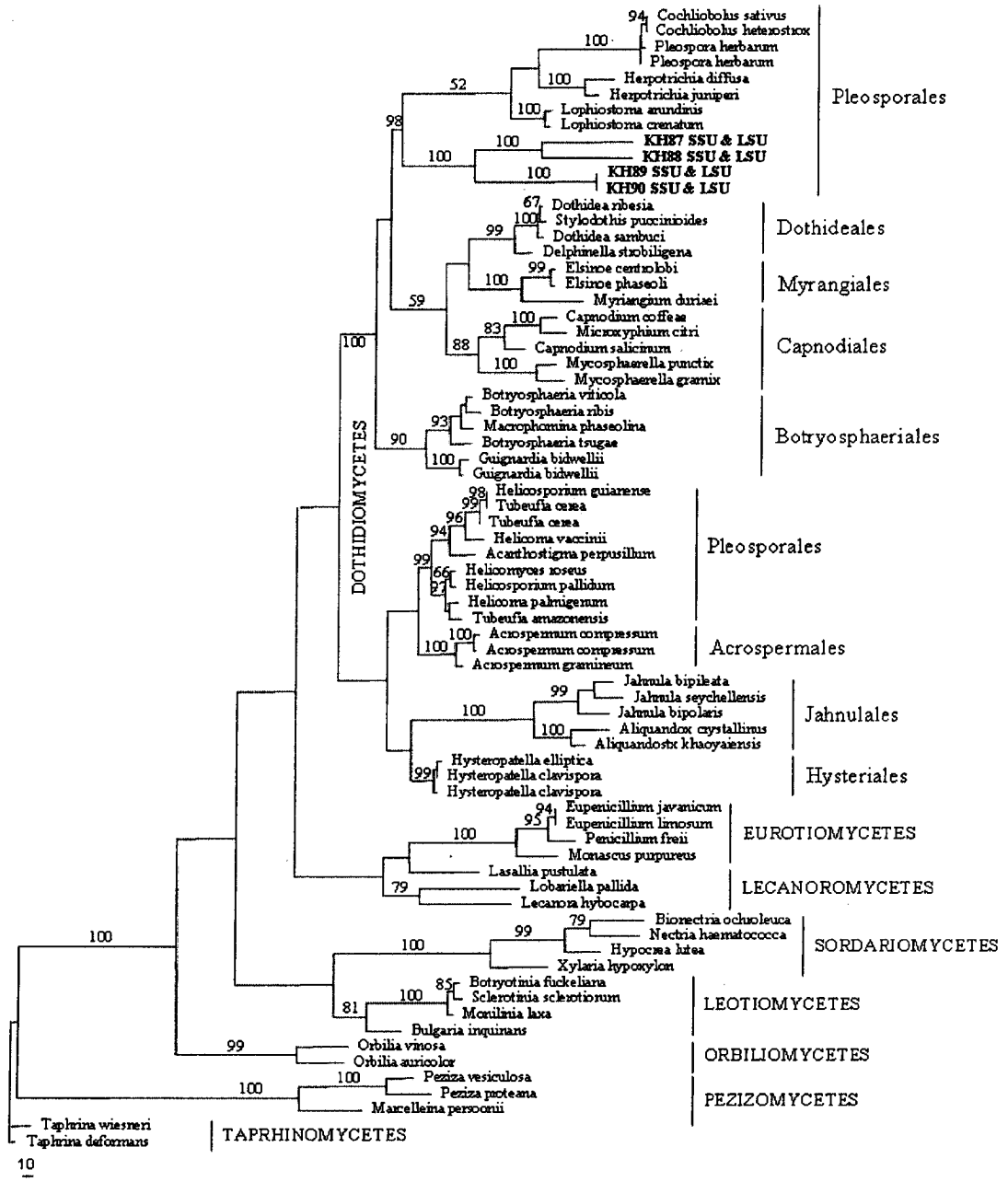

Figure 2. Phylogenetic tree inferred from combined dataset of SSU and LSU regions of four isolates of marine coelomycetes isolated from mangrove wood. The bootstrap value (>50%) is shown above the branches. Bar = number of changes per nucleotide position.

(II) Unidentified coelomycetes from leaf litter.

Six isolates of unidentified coelomycetes are listed in Table 3 along with their morphological features. However the culture of unidentified coelomycete SFC1940 is not available from the BIOTEC culture collection. Therefore the phylogenetic analysis of five isolates was performed.

Table 3. The fungal code and detail of unidentified coelomycetes sequenced in this study.

Original code	BCC code	Species	Biodata
SFC 1912	BCC 18583	Unidentified species	The fungus colonized decaying leaves of *Lagerstroemia* in Khao Yai National Park. Morphology of this fungus includes globose pycnidia and hyaline, 1-celled conidia.
SFC 1920	BCC18586	Unidentified species	The fungus colonized decaying leaves of *Eucalyptus* in from Kasetsart University. The fungus has globose pycnidia with setae around the ostiole.
SFC 1941	BCC 20494	Unidentified species	The fungus colonized dead leaves from Khao Yai National Park. The morphology of this fungus includes submersed pycnidia and cylindrical, hyaline, 1-celled conidia with mucilaginous appendages at each end.
SFC 1946	BCC 20812	Unidentified species	The fungus colonized decaying leaves, from Khao Yai National Park. The fungus has globose pycnidia with ellipsoidal conidia.
SFC 2109	BCC 21373	Unidentified species	The fungus colonized decaying leaves from Khao Yai National Park. The fungus has globose pycnidia with short-cylindrical conidia.

Phylogenetic relationships

The SSU dataset was analysed in order to sort out their higher taxonomic placement. Three major classes, representing the Arthoniomycetes, Eurotiomycetes and Lecanoromycetes were aligned along with our data with the Taphrinomycetes as the outgroup (Figure 3). A BLAST search of the unidentified coelomycete isolates placed them in the Sordariomycetes (2 isolates) and Dothideomycetes (3 isolates). Consequently, major orders of these two classes were sampled and aligned with our unidentified coelomycetes. Two isolates, BCC18583 and BCC21373, clustered within the Sordariomycetes with 98% BS and in the orders Diaporthales and Hypocreales, respectively (Figure 3). Isolate BCC21373 formed a clade with two *Volutella ciliata* strains with moderate support (54 % BS), located as a basal clade of the Hypocreomycetidae. Isolate BCC18583 grouped with various genera within the Diaporthales with good statistical support (100% BS) (Figure 3). Three isolates (BCC18586, BCC20494 and BCC20812) had a phylogenetic affinity with members of the Pleosporales (Dothideomycetes) with good support (98 %BS).

To determine the lower taxnomic level of the unidentified coelomycetes, ITS sequence were used and compared with the reference taxa from the GenBank. Isolate BCC21373 grouped within the Nectriaceae (Hypocreales) (Figure 4). Various genera i.e. *Cosmospora, Nectria, Neonectria, Fusarium, Gibberella* and *Volutella* were aligned along with our sequence. Isolate BCC21373 formed a clade with a leaf litter ascomycete (AF502638) as the nearest taxon with 100 % BS. It also grouped with three sequences of *Volutella*. ITS sequence analysis showed that our isolate shared 100% homology to leaf litter ascomycete AF502638. Sequence similarity to the *Volutella* species ranged from 90.6-94.6%, while similarity to *Cosmospora* and *Nectria* species ranged from 81.2-82.0%, 72.2-81.2%, respectively. Therefore isolate BCC21372 could be identified as a *Volutella* species.

Isolate BCC18583 clustered with *Cytospora* species (Figure 5), the closest similarity was with a *Cytospora* sp. (EU330636) with 90 %BS, while other *Cytospora* species formed a sister group. Sequence similarity of these *Cytospora* species ranged from 93.6-99.2%. Therefore isolate BCC18583 can be identified as a *Cytospora* species.

Isolate BCC20812 had an affinity with various species of *Lophiostoma* within the Pleosporales, but ITS sequence similarity between our isolate and related taxa was low (66.3-89.7%), so the phylogenetic trees is not shown here. Isolates BCC18586 and BCC20494 showed a relationship with an uncultured root fungi, fungal endophytes and an ascomycete sequence. Sequence similarity ranged from 72.1-89.2%. Therefore the classification of these three coelomycetes at lower taxonomic level could not be determined at the molecular level using ITS sequence analysis.

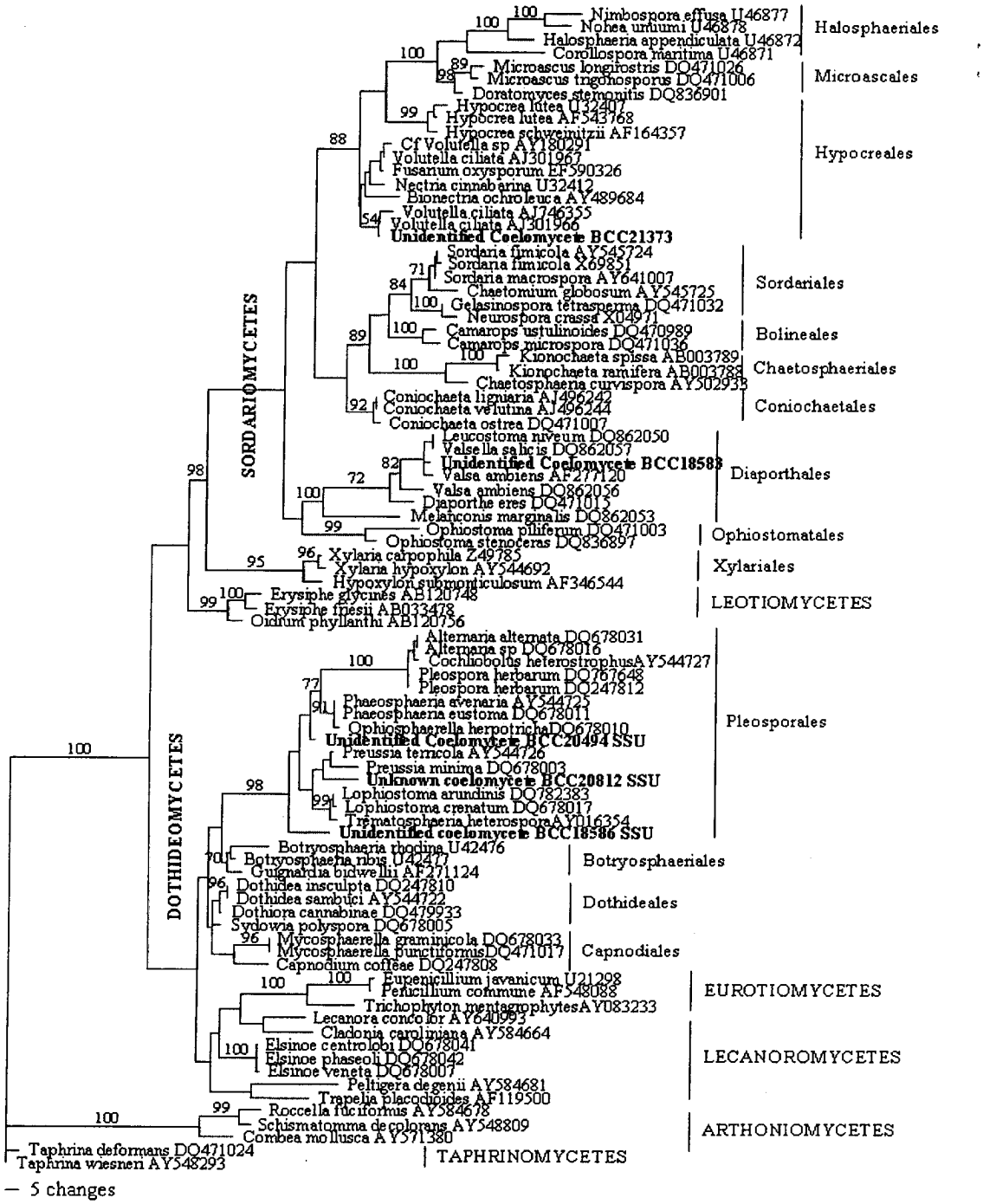

Figure 3. Phylogenetic tree inferred from SSU region of five unidentified coelomycetes isolated from leave litter. The bootstrap value (>50%) is shown above the branches. Bar = number of changes per nucleotide position.

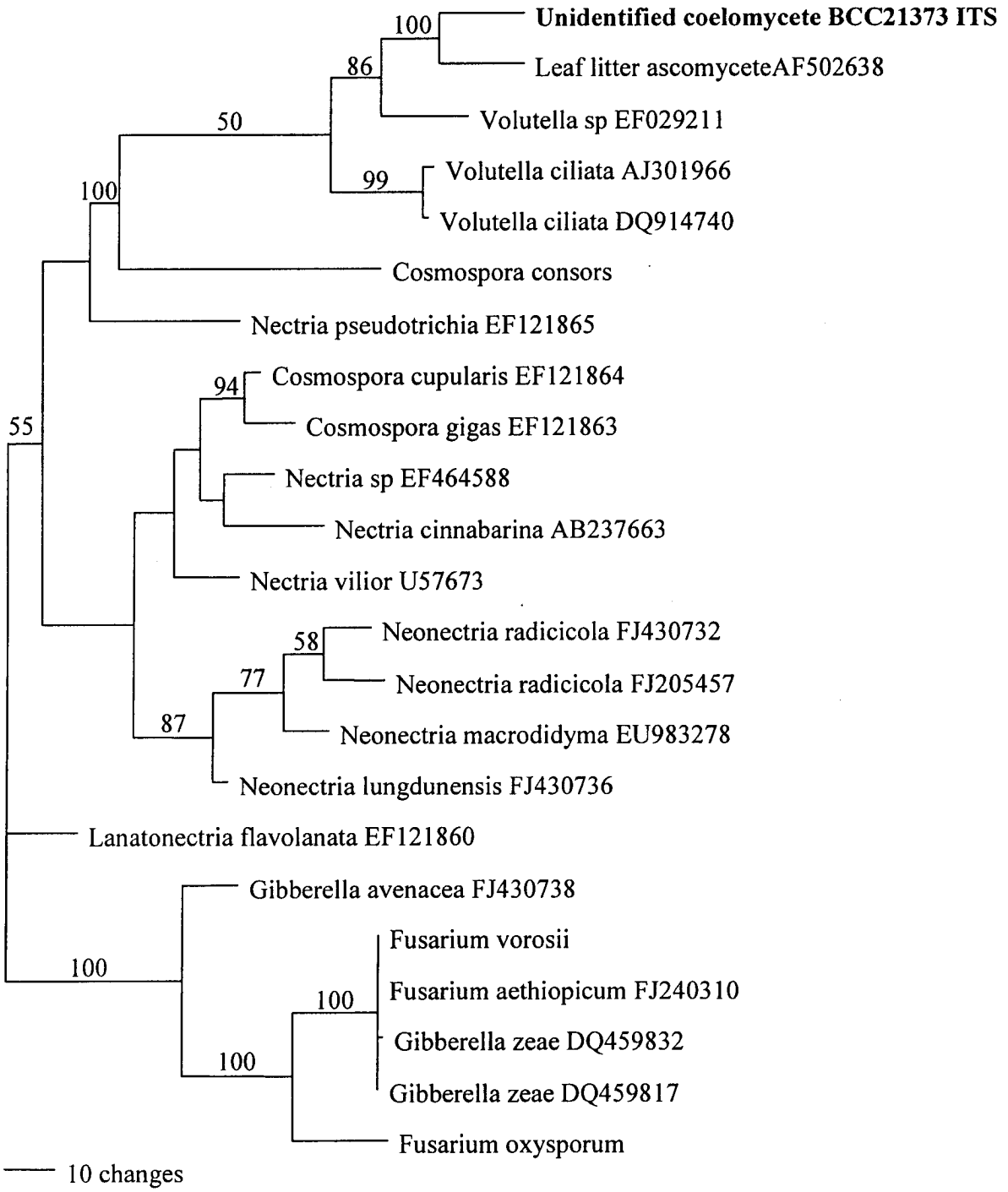

Figure 4. Phylogenetic tree inferred from ITS region of unidentified coelomycete BCC21373 isolated from leave litter. The bootstrap value (>50%) is shown above the branches. Bar = number of changes per nucleotide position.

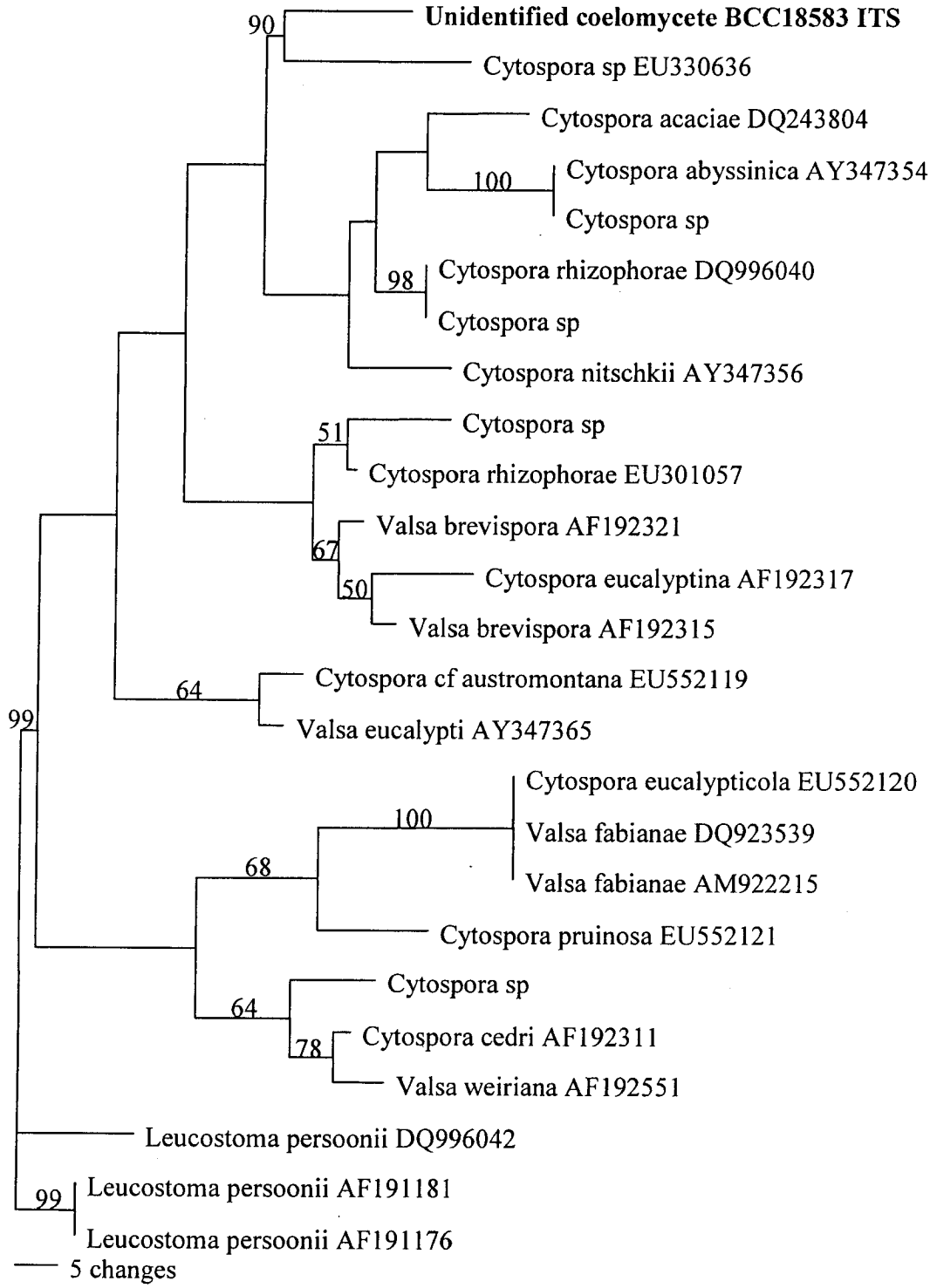

Figure 5. Phylogenetic tree inferred from ITS region of unidentified coelomycete BCC18583 isolated from leave litter. The bootstrap value (>50%) is shown above the branches. Bar = number of changes per nucleotide position.

(III) Selected coelomycetes

***Vermiculariopsiella* sp. and *Lauriomyces* sp.**

Vermiculariopsiella sp. colonized decaying leaves collected at Khao Yai National Park. Morphologically this fungus has flattened sporodochia with setae and hyaline, 1-celled conidia. *Lauriomyces* species are common saprobic fungi in Thai tropical forests and a new species has been described from decaying fruit (Figure 6). There is no reported teleomorphs for these two genera. Three regions consisting of SSU, LSU and ITS rDNA were amplified and sequenced. The preliminary results of a BLAST search suggested that *Vermiculariopsiella* and *Lauriomyces* species had a phylogenetic relationship with the Sordariomycetes and Leotiomycetes, respectively. Although a phylogenetic tree was constructed, the taxonomic placements of these two genera remains unresolved. The SSU and LSU sequences demonstrated that these two coelomycetes did not group with any order of the two classes (tree not shown). This is possibly due to the lack of sequences from the GenBank to enable a comparison to be made. Therefore, their taxonomy could not be resolved with any confidence. More gene regions and taxa sampling are needed to further determine their taxonomic position.

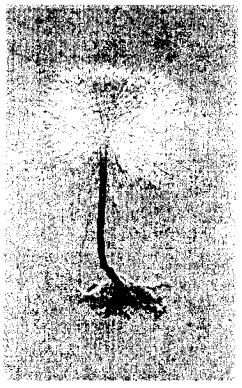

Figure 6. *Lauriomyces* species. The fungus possess conidiophores bearing branched chains of hyaline, 1-celled conidia.

***Wiesneriomyces* species**

The genus *Wiesneriomyces* was collected from different geographical regions in Java, India Panama, and Thailand. Isolates of *Wiesneriomyces* from leaf litter collected from Thai rain and evergreen forests were made. The genus possesses sporodochia and mature conidial chains, which are the key morphological characters of this coelomycete (Figure 7.). There is no report on the phylogenetic status of this genus and its taxonomic position remains unresolved. Therefore SSU and LSU sequences were analyzed to clarify its taxonomic placement.

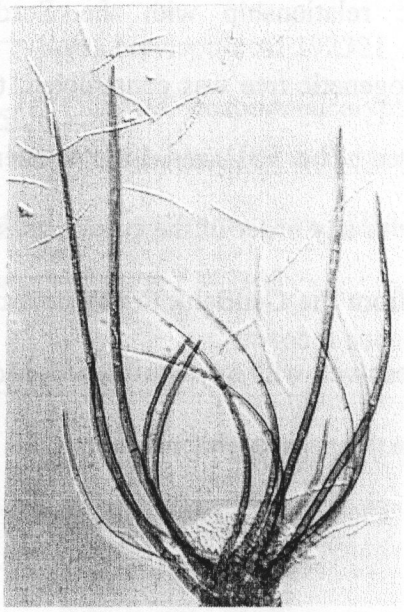

Figure 7. *Wiesneriomyces* species. The fungi possess sporodochia with incurved setae, and isthmo-conidia.

SSU and LSU phylogeny

Combined SSU and LSU rDNA sequence data were analysed to clarify its taxonomic position at the class and ordinal levels. A dataset "backbone" of major clades of the Ascomycota was performed based on published data (Spatafora *et al.*, 2006; Schoch *et al.*, 2006). Five isolates of *Wiesneriomyces* were incorporated with various taxa from the Ascomycota. Fifty two

taxa from 9 orders within the Dothideomycetes, representing Acrospermales, Botryosphaeriales, Capnodiales, Dothideales, Hysteriales, Jahnulales, Myrangiales, Patellariales and Pleosporales, were selected for the analysis (Figure 8). Twenty one sequences from 6 major classes, the Eurotiomycetes, Lecanomycetes, Leotiomycetes, Orbiomycetes, Pezizomycetes and Sordariomycetes were added into the dataset with the Taphrinomycetes as the outgroup.

The *Wiesneriomyces* isolates formed a monophyletic clade with good support (86-96 %BS). *Wiesneriomyces* sp. (BCC18608) clustered with two strains of *Wiesneriomyces conjunctosporus* (BCC18606 and BCC4027), and formed a clade with *Wieseriomyces laurinus* (BCC18609 and BCC3922).

Wiesneriomyces can be classified in the Dothideomycetes, while at the ordinal level the genus shows affinity with the Pleosporales and Aerospermales with low statistical support. A wider range of genes and genera within the Pleosporales need to be sequenced, in order to better clarify the phylogeny of the genus.

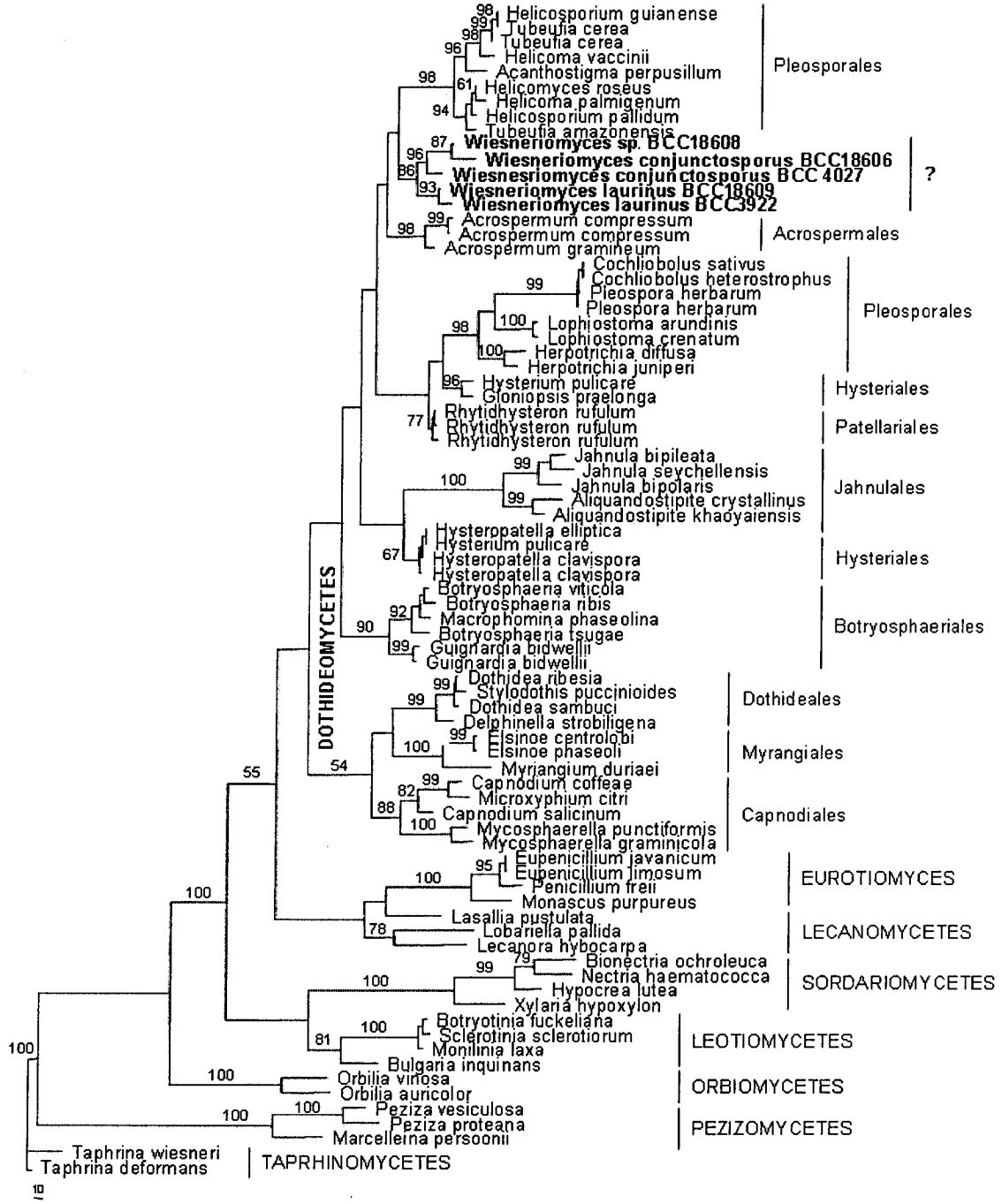

Figure 8. Phylogenetic tree inferred from combined dataset of SSU and LSU regions of five isolates of *Wiesneriomyces conjunctosporus, Wiesneriomyces laurianus* and *Wiesneriomyces* species. The bootstrap value (>50%) is shown above the branches. Bar = number of changes per nucleotide position.

***Falcocladium* species**

New species of *Falcocladium* were collected and reported from Thailand (Figure 9) and isolated from leaf litter. The genus is characterized by "having thick-walled, non-septate stipe extensions that terminate in a vesicle and falcate appendaged conidia". In order to expand the basic knowledge and sort out the taxonomic placement of this genus, SSU and LSU regions were selected for study.

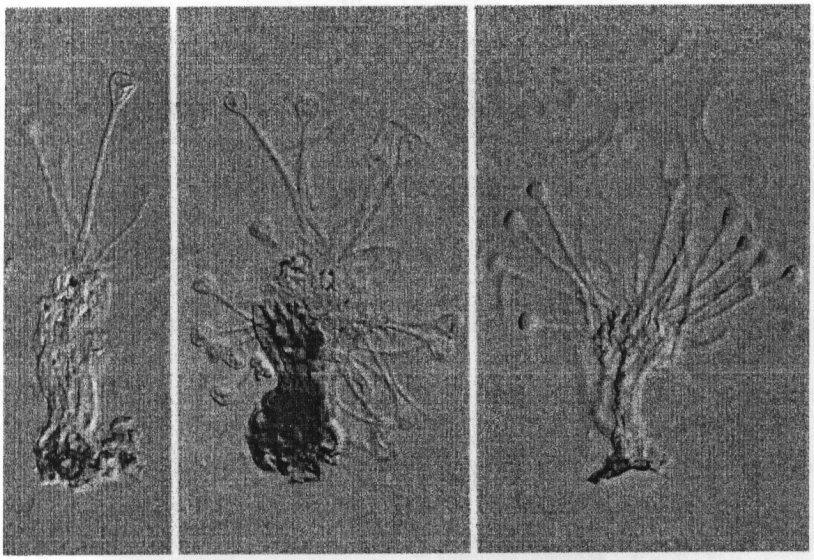

Figure 9. *Falcocladium* species.The morphology of this genus includes sporodochia with conidiophores terminating into vesicles, and falcate, 1-celled conidia.

SSU and LSU phylogeny

The phylogenetic investigation based on the combined SSU and LSU dataset was performed under maximum parsimony criteria. A dataset was established based on three subclasses of the Sordariomycetes (Ascomycota): Sordariomycetidae included the orders: Chaetosphaeriales, Diaporthales, Ophiostomatales and Sordariales; Hypocreomycetidae with the orders Cornophorales, Halosphaeriales, Hypocreales, Melasporales and Microascales, and a

taxonomic group of uf uncertainaffinity (TMBA group deep hypha project) *Torpedospora, Swampomyces*, *Juncigena* and *Etheiophora*, were aligned along with our coelomycete isolates, with the Xylariales (Xylariomycetidae) as the outgroup (Figure 10). The *Falcocladium* strains formed a monophyletic group with high statistical support (100 % BS) and can be referred to the Hypocreomycetidae. *Falcocladium* isolate BCC22055 formed a clade with *Falcocladium thailandicum* with moderate support (69 %BS), while *Falcocladium multivesiculatum* was in a sister group. The three *Falcocladium sphaeropedunculatum* isolates formed a monophyletic group with 92 % BS, as a subclade to *F. thailandicum* and the *Falcocladium* species.

Falcocladium species formed a sister clade to the orders Cornophorales and Melasporales with no support and with a long branch length. Moreover, four species of the uncertain taxonomic group (*Etheirophora, Juncigena, Swampomyces* and *Torpedospora*, Hypocreomycetidae, *Incertae sedis*) did not share the ancestoral node with our coelomycete. Therefore, the molecular classification of *Falcocladium* species could only be placed within the Hypocreomycetidae, while its ordinal position remains unresolved. More DNA regions and taxon sampling are required to further clarify its taxonomic position at the ordinal level. .

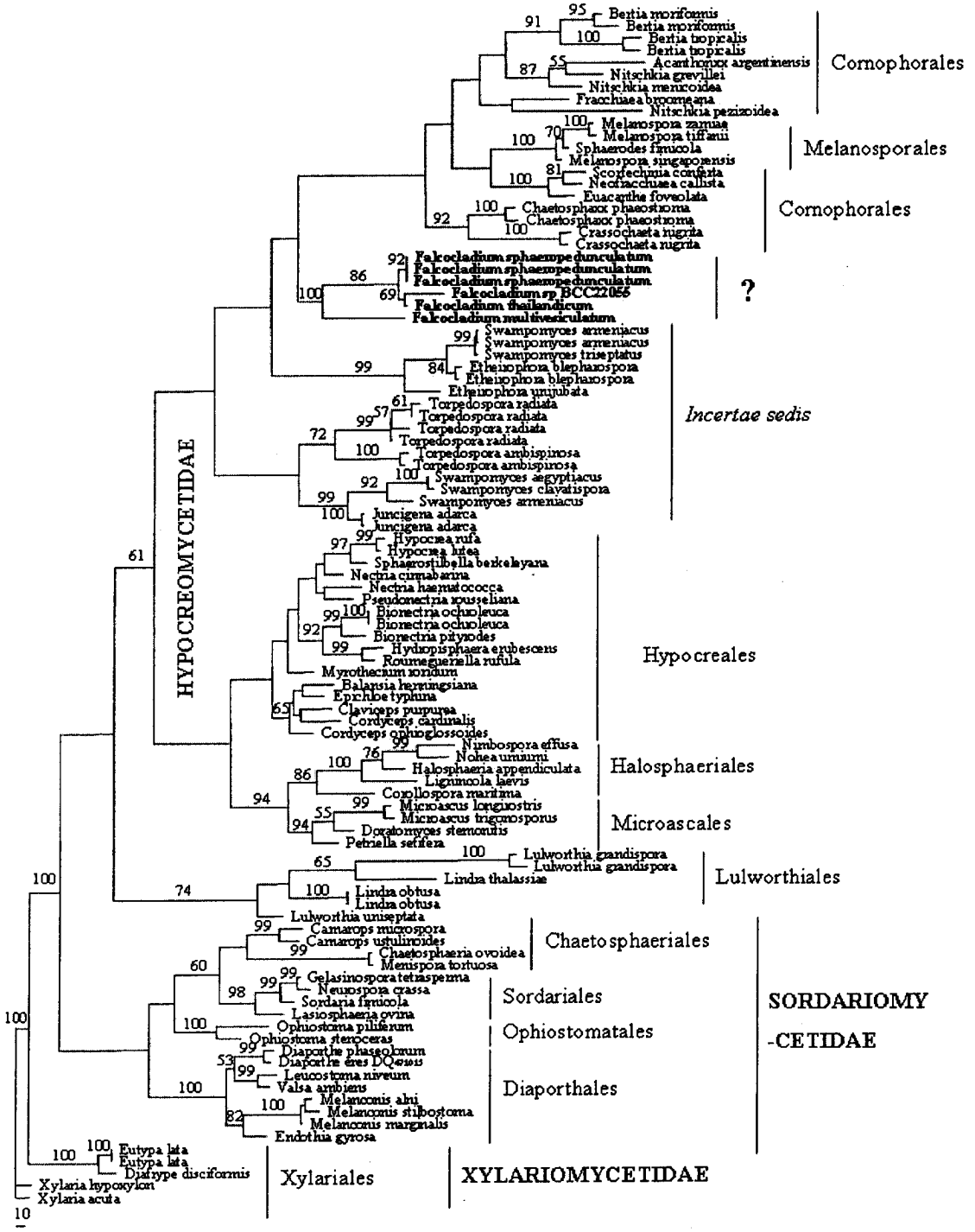

Figure 10. Phylogenetic tree inferred from combined dataset of SSU and LSU regions of four isolates of *Falcocladium* species. The bootstrap value (>50%) is shown above the branches. Bar = number of changes per nucleotide position.

5. Conclusions

1. The teleomorph of coelomycetes with fusiform-conidia, consisting of *Robillarda sesillis*, and *Xepiculopsis graminea*, are located in the Xylariales and Hypocreales, respectively.

2. The taxonomic position of *Pseudorobillarda* species is not resolved because they did not show a close relationship within any order, although well placed in the Dothideomycetes.

3. Four marine coelomycete isolates grouped within the Pleosporales (Pleosporomycetidae, Dothideomycetes), although their familial level remains unresolved.

4. Five unidentified coelomycetes are well placed within the Ascomycota. Two and three isolates grouped with the Sordariomycetes and Dothideomycetes, respectively.

5. Coelomycete isolates, BCC18583 and BCC21373, could be identified as *Cytospora* and *Volutella* species, respectively, while the taxonomic position of three isolates (BCC18586, BCC20494 and BCC20812) remain unresolved, but phylogenetically they can be referred to the Pleosporales.

6. *Wiesneriomyces* and *Falcocladium* species are monophyletic and well placed within the Dothideomycetes and Hypocreomycetidae (Sordariomycetes), respectively, although their ordinal and familial levels remain unresolved.

6. Future Plans

1. A manuscript on the phylogenetic observations of coelomycetes with fusiform-conidia will be submitted shortly to Persoonia, a journal published by the Centraalbureau voor Schimmelcultures (CBS).

2. The phylogeny of the Thai coelomycetes outlined in this report will be published in a series of papers: a) marine; b). Unidentified litter coelomycetes; c). *Vermiculariopsiella* sp. and

Lauriomyces sp.; d). *Wiesneriomyces*; e). *Falcocladium*; and f). *Xepiculopsis graminea.* However, further taxa may need to be sequenced to resolve the ordinal status of these taxa.

7. Outputs

1) Reprint of paper

Somrithipol, S., Sakayaroj, J., Plaingam, N. Rungjindamai, N., and Jones, E.B.G. 2008. Phylogenetic relationship of the coelomycete genus *Infundibulomyces* based on nuclear rDNA data. Mycologia. 100: 735-741.

2) Poster presentation

Rungjindamai, N., Sakayaroj, J., Somrithipol, S., Plaingam, N. and Jones, E.B.G. 2008. Molecular phylogeny of selected coelomycetes with fusiform conidia, *Robillarda, Pseudorobillarda* and *Xepiculopsis*, based on nuclear ribosomal DNA sequences. Abstracts: Research and Thesis 2008. 12th BRT Annual Conference 2008. 10-13 October 2008. Diamond Plaza, Surat Thani.

Acknowledgements

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White, T.J., Bruns, T., Lee, S and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocol: A guide to methods and applications (eds. M.A.Innis, D.H.Gelfand, J.S. Sninsky amd T.J.White), Academic Press, San Diego, pp. 315-322.

Part B: Appendix

Appendix 1

1) Published paper entitled:

"Phylogenetic relationship of the coelomycete genus *Infundibulomyces* based on nuclear rDNA data" published in Mycologia. 100: 735-741.

Mycologia, 100(5), 2008, pp. 735–741. DOI: 10.3852/07-040

Phylogenetic relationship of the coelomycete genus *Infundibulomyces* based on nuclear rDNA data

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Abstract: The phylogenetic relationship of the coelomycete genus *Infundibulomyces* with cupulate conidiomata was assessed by ribosomal DNA sequences of partial small subunit (SSU) and partial large subunit (LSU) regions using maximum parsimony and Bayesian analysis. The genus has no known teleomorph. A new species, *Infundibulomyces oblongisporus*, is described from collections on a senescent angiosperm leaf from Thailand based on morphological and phylogenetic evidence. Both *Infundibulomyces* species form a monophyletic group in the Chaetosphaeriaceae (Chaetosphaeriales, Sordariomycetidae) with *Dictyochaeta simplex* as a sister clade. Chaetosphaeriaceae with a coelomycete anamorph has not been reported previously.

Key words: Chaetosphaeriales, coelomycetes, *Infundibulomyces*, molecular phylogeny, systematics

INTRODUCTION

Coelomycetes are anamorphic fungi producing asexual spores (conidia) in a pycnidium, acervulus or stroma (Sutton 1980, Nag Raj 1993). Currently 1000 genera comprising 7000 species have been described (Kirk et al 2001). Despite the large number of coelomycetes documented worldwide, little is known on their teleomorphic counterparts and hence systematic position. However with the advent of molecular techniques one can link anamorphic fungi to their putative teleomorphs. As part of a continuing study of Thai coelomycetes we have examined a selected number of genera at the molecular level (Plaingam et al 2004).

Cupulate conidiomata (Plaingam et al 2003) are common in coelomycetes, but those with superficial, funnel-shape conidiomata (nidulariaceous-like) are unique and limited to few genera and include *Ojibwara*, *Satchmopsis*, *Shawiella* and *Stevensonula*, all classified in family Parasphaeropsidaceae (Sutton 1975) and the recently described *Infundibulomyces* (Plaingam et al 2003). The new coelomycete genus *Infundibulomyces* is characterized by a single peripheral wall layer of textura prismatica and a basal stroma of textura angularis with holoblastic conidiogenous cells and appendaged conidia.

Infundibulomyces cupulata (the type species) was collected from leaves of *Lagerstroemia* species, has cylindrical conidia with an obtuse apex and an obtuse but protuberant base, bearing two appendages at each end, which are tubular, filiform and flexuous (Plaingam et al 2003). The new taxon *Infundibulomyces oblongisporus* was isolated from decaying angiosperm leaves at Khao Yai National Park, Thailand, and differs from *I. cupulata* in conidial morphology. The objective of this study was to clarify the ordinal and familial placement of *Infundibulomyces*. In this study sequence data from the nuclear small subunit (SSU), large subunit (LSU) ribosomal DNA (rDNA) were analyzed.

MATERIALS AND METHODS

Collection and isolation.—*Infundibulomyces cupulata* (BCC11929, type culture) and *Infundibulomyces oblongisporus* (BCC13400, type culture) were isolated from leaf litter collected from Thailand (Plaingam et al 2003) and deposited in the BIOTEC Culture Collection (BCC) (TABLE I).

Fungal isolates and culture conditions.—Fungi were maintained on potato dextrose agar (PDA) and grown in potato dextrose broth (PDB) under static conditions at 25 C for 2 wk for DNA extraction. Fungal mycelium was harvested, washed with sterilized distilled water, the biomass frozen at −80 C for 1–2 h and ground into fine powder with a sterilized mortar and pestle.

Genomic DNA extraction and PCR amplification.—DNA was extracted with CTAB lysis buffer (applied from O'Donnell et al 1997) and incubated at 65 C for 1 h. The mixture was extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1). The upper liquid phase was precipitated with 7.5 M ammonium acetate and absolute ethanol and kept at −20 C at least 30 min or overnight. The extracted DNA was washed twice with 70% ethanol and air dried. The DNA then was resuspended in TE buffer.

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TABLE I. List of source, locality, substrate and GenBank accession number of *Infundibulomyces cupulata* and *Infundibulomyces oblongisporus* isolates used in this study

Taxa	Source[1]	Locality and substrate	GenBank accession number	
			LSU	SSU
Infundibulomyces cupalata	BCC11929	Thailand, on leaves of *Lagerstroemia* sp.	EF113979	EF113982
Infundibulomyces oblongisporus	BCC13400	Thailand, on decayed angiosperm leaves	EF113980	EF113983

[1] BCC = BIOTEC Culture Collection, Pathum Thani, Thailand

Partial SSU and LSU regions of rDNA were amplified with gene specific primers NS1, NS4, LROR and LR7 (White et al 1990, Bunyard et al 1994, Landvik 1996) using FINNZYMES, DyNAzyme™ II DNA polymerase kit (Cat. No. F-551S, Finnzymes, Finland). The amplification cycles were performed following White et al (1990) and Landvik (1996) with a DNA Engine DYAD ALD 1244 thermo cycler (MJ Research Inc). PCR products were purified with NucleoSpin® Extract DNA purification kit (Cat. No. 740 609.50, Macherey-Nagel, Germany) following the manufacturer's instruction and sequenced by Macrogen Inc. (Korea) with the same primers.

Sequence alignments and phylogenetic analyses.—A BLAST analysis was used to find closest matched sequences in the GenBank database (Altschul et al 1990). SSU, LSU and ITS rDNA sequences were multiple aligned along with other related sequences obtained from GenBank with Clustal W 1.6 (Thompson et al 1994) and adjusted manually where necessary with BioEdit 7.5.0.3 (Hall 2006).

The aligned dataset was analyzed with maximum parsimony in PAUP* 4.0b10 (Swofford 2002) for the most parsimonious trees (MPT). Heuristic searches algorithm with tree bisection reconnection (TBR) branch swapping, 1000 replicates of random stepwise sequence addition, were performed. Gaps were treated as missing data and were given equal weight. The tree length, consistency indices (CI) and retention indices (RI) were calculated for each tree generated. Statistical support for the internal branches was estimated by bootstrapping analysis (Felsenstein 1985) with 1000 replications (10 replicates of random stepwise sequence addition, TBR branch swapping).

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 2 000 000 generations and sampled every 100 generations. The first 100 000 generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. The statistical supportive data of posterior probabilities were performed.

The maximum parsimony bootstrap values (PB $\geq$ 50%) and Bayesian posterior probabilities (PP $\geq$ 0.95) were shown respectively above and below tree branches. The rDNA sequences, consisting of SSU and LSU, were submitted to GenBank (TABLE I). The accession numbers for all sequences derived from the GenBank database are included the phylogenetic trees. The new sequences generated for *Infundibulomyces* are provided (TABLE I). The alignments were deposited in TreeBase: S2120.

RESULTS

Phylogenetic analyses.—SSU phylogeny. To determine the taxonomic placement of our *Infundibulomyces* species at the class level the SSU rDNA sequences of *I. cupulata* and *I. oblongisporus* were incorporated with 59 taxa from eight representative classes within the Ascomycota. The analyzed SSU dataset comprises various taxa sampled from the Arthoniomycetes, Dothideomycetes, Eurotiomycetes, Lecanoromycetes, Leotiomycetes, Lichinomycetes and Sordariomycetes, whereas two taxa of the Taphrinomycetes were used as outgroup (FIG. 1). Maximum parsimony and Bayesian analysis generated similar tree topologies, therefore only the maximum parsimonious trees (MPT) have been shown in this investigation. Maximum parsimony analysis resulted in 129 MPT with tree length, CI and RI of 1092 steps, 0.523 and 0.800 respectively. Our two isolates of *Infundibulomyces* were aligned along with 34 taxa from eight major orders within the Sordariomycetes, consisting of Boliniales, Chaetosphaeriales, Coniochaetales, Diaporthales, Hypocreales, Ophiostomatales, Sordariales and Xylariales. *Infundibulomyeces cupulata* and *I. oblongisporus* formed a clade with good support (100% MP and 1.00 PP) but with a long branch length. Closely related taxa of the Chaetosphaeriales include the Boliniales and Sordariales, which in our analysis form sister clades with high support (97% MP and 1.00 PP). While the *Coniochaeta* species (Coniocheatales) form a weak relationship with these three orders.

Phylogenetic analyses.—LSU phylogeny. The LSU rDNA sequences of two *Infundibulomyces* species was compared with 38 taxa within the Chaetosphaeriales, six and four taxa of the Boliniales and Coniochaetales, respectively, whereas the Sordariales was chosen as outgroup (FIG. 2). Maximum parsimony resulted in six MPT with tree length, CI and RI of 912 steps, 0.432 and 0.735 respectively. *Infundibulomyces* species are well placed in the Chaetosphaeriaceae, a single family

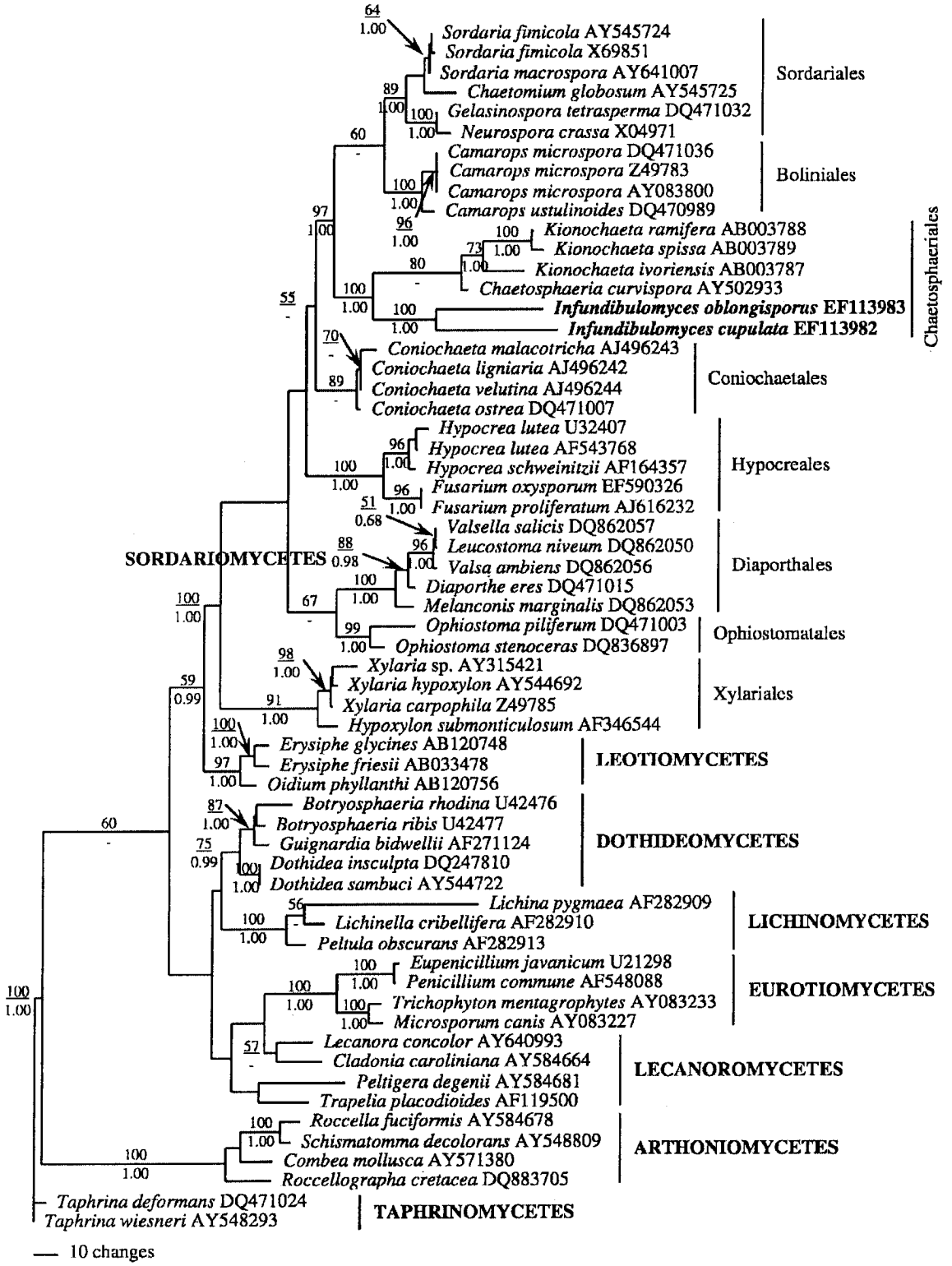


FIG. 1. One of 129 the most parsimonious trees inferred from SSU rDNA sequences. Maximum parsimony bootstrap values (MP) and Bayesian posterior probabilities (PP) are indicated respectively above and below the branches.

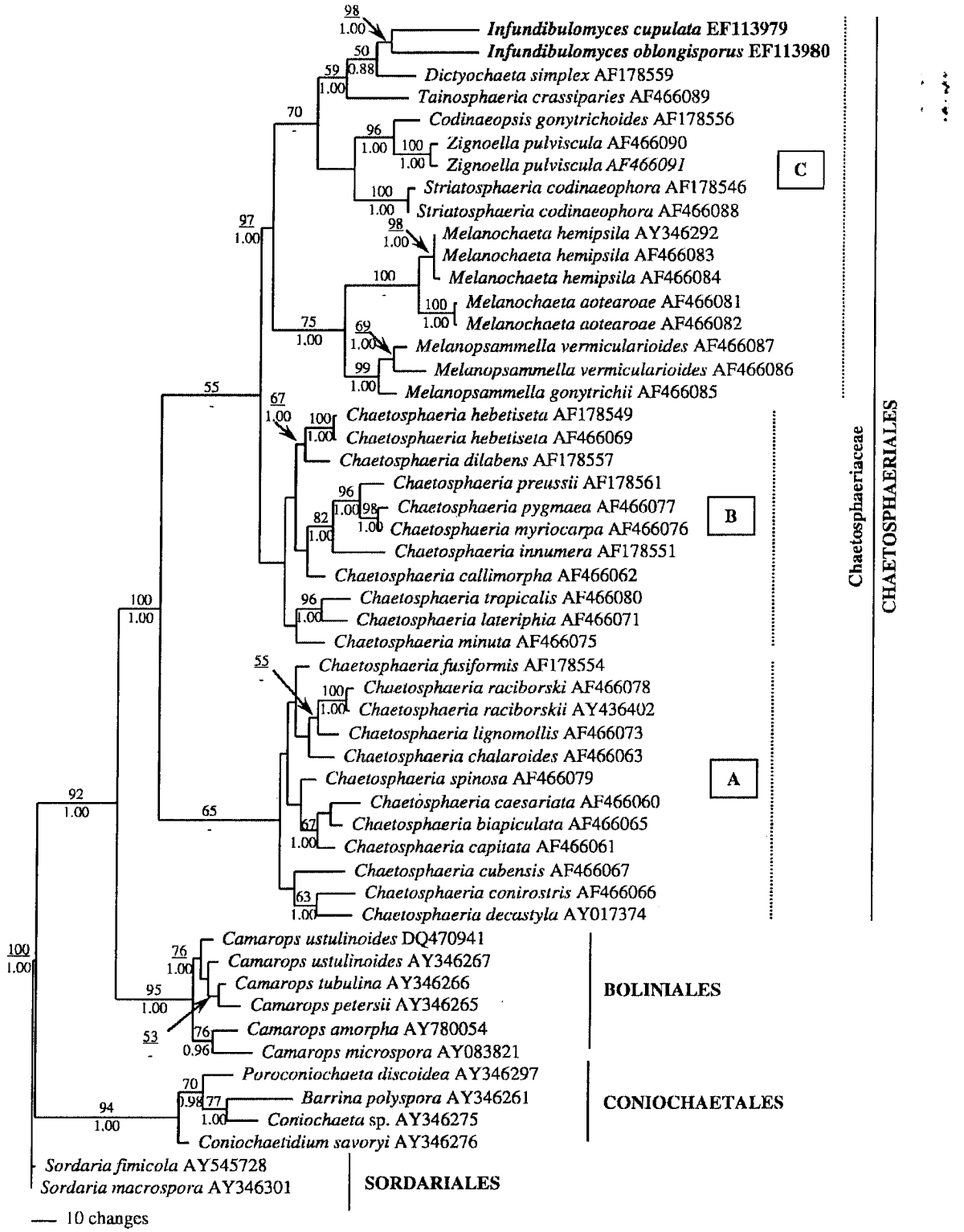


FIG. 2. One of six most parsimonious trees inferred from LSU rDNA sequences. Maximum parsimony bootstrap values (MP) and Bayesian posterior probabilities (PP) are indicated respectively above and below the branches.

within the Chaetosphaeriales with good support (100% MP and 1.00 PP). The Boliniales and Coniochaetales formed separate clades of the Chaetosphaeriales with good support. In this phylogenetic analysis, members of the Chaetosphaeriales separated into three subgroups. The fist two subclades (A and B) included genus *Chaetosphaeria* while the third clade (subclade C) comprised genera *Codinaeopsis*, *Dictyochaeta*, *Tainosphaeria*, *Striatosphaeria* and *Zignoella*, all regarded as members of the Chaetosphaeriales. *Infundibulomyces* falls within the third subgroup with 97% MP and 1.00 PP. *Infundibulomyces cupulata* and *I. oblongisporus* form a clade with high support (98% MP and 1.00 PP) but with long branch lengths. *Infundibulomyces* species form a sister group with *Dictyochaeta simplex* and *Tainosphaeria crassiparies* as the most closely related taxa (FIG. 2). Genera *Zignoella* and *Striatosphaeria* formed a sister group as did *Melanochaeta* and *Melanopsammella* with 97% MP and 1.00 PP.

TAXONOMY

Infundibulomyces oblongisporus Somrithipol sp. nov. FIGS. 3–8

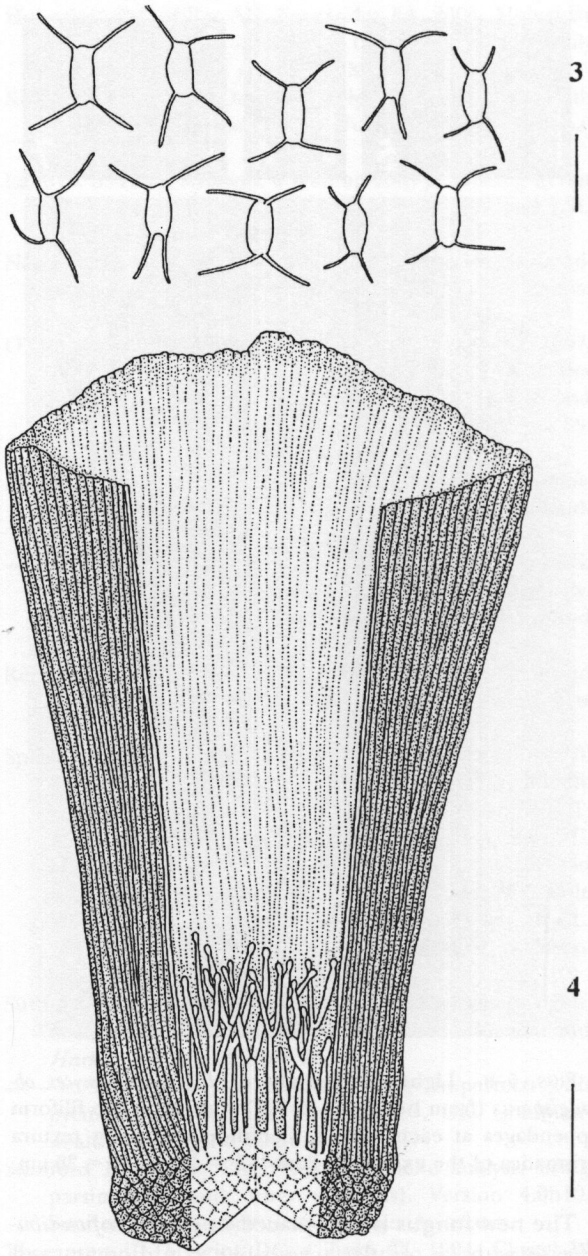
Conidiomata stromatica, superficiales, cupulata, ad basim 60–100 µm lata, ad apicem 100–150 µm lata, 150–200 µm altus cum stroma basale e textura anguleris et excipulum lateralis latitudine unicellula e textura prismatica, primo cylindricus, dein late cupulatus, postremo nidulariaceus; setae conidiomatum absentia. Conidiophora e cellulis summis stromatis basalis, in muco involuta, irregulatim ramosa, hyalinae, laevia. Cellulae conidiogenae cylindraceae vel subcylindraceae, hyalinae, laevia, 8–16 × 1–2 µm. Conidiogenesis: ontogenea conidiorum holoblastica; maturitus diffusa; delimitato per septum duplex; liberatio schizolytica. Conidia oblongae, apicibus et basibus obtusis, unicellaria, laevia, guttulata, 3–6 × 1–1.5 µm ($\bar{x}$ = 4.6 × 1.5 µm, n = 50), apicales et basales duobus appendices ferrentia; appendix tubulares, filiforme, flexuosi, 4–10 µm longae. Ratione conidii long./lat. = 3/1 (n = 50).

Holotypus: THAILAND, Khao Yai National Park, Khao Yai Golf Course, in mortuo folio, Oct. 2002, S. Somrithipol (SFC 1615 in BBH).

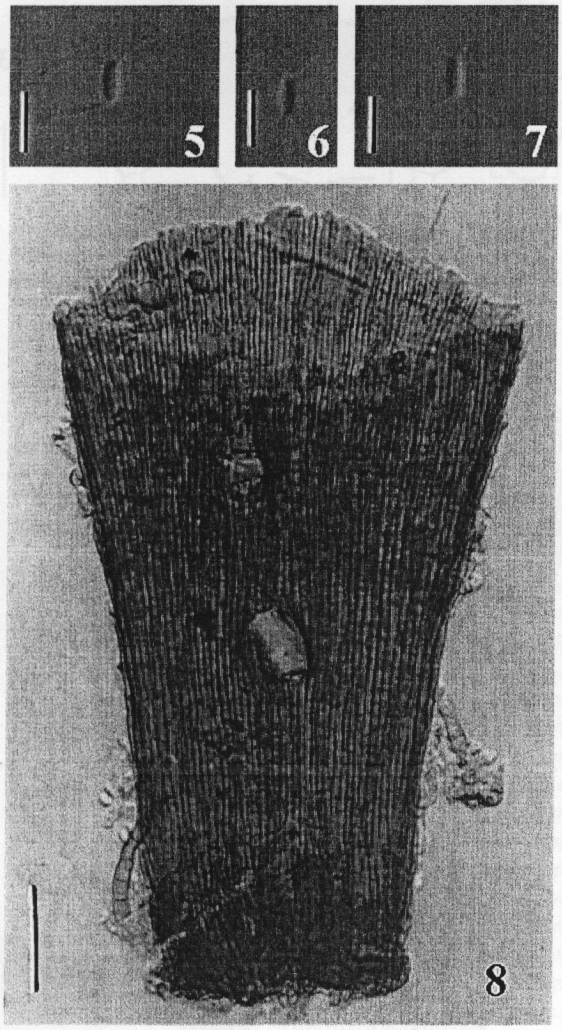
Etymology: refers to the conidial shape

Cultura ex-typus. BCC 13400.

Conidiomata stromatic, superficial, cupulate, 60–100 µm wide at the base, 100–150 µm wide at the apex, 150–200 µm high, with a basal stroma of textura angularis and a lateral excipulum of 1-cell thick textura prismatica (FIGS. 4, 8), initially cylindrical, becoming cupulate and finally appearing nidulariaceous; conidiomatal setae absent. Conidiophores arising from the upper cells of the basal stroma, invested in mucus, irregularly branched, hyaline, smooth. Conidiogenous cells cylindrical to subcylindrical, hyaline, smooth, 8–16 × 1–2 µm. Conidiogenesis: ontogeny holoblastic by apical wall building; maturation by diffuse wall-building; delimitation by a double septum; secession schizolytic. Conidia oblong with an obtuse ends, nonseptate, smooth-walled, guttulate, 3–6 × 1–1.5 µm ($\bar{x}$ = 4.6 × 1.5 µm, n = 50), bearing two appendages at each end; appendages tubular, filiform, flexuous, 4–10 µm long (FIGS. 3, 5–7). Mean conidium length/width ratio = 3/1 (n = 50).



FIGS. 3–4. Line drawings of *Infundibulomyces oblongisporus*. 3. Conidia with two filiform appendages at each end. 4. Conidioma showing textura prismatica of the excipular. Bars: 3 = 5 µm, 4 = 25 µm.



FIGS. 5–8. Light micrographs of *Infundibulomyces oblongisporus* (from holotype). 5–7. Conidia with two filiform appendages at each end. 8. Conidioma showing textura prismatica of the excipular. Bars 1–3 = 10 μm, 4 = 35 μm.

The new fungus is best placed in genus *Infundibulomyces* with its nidulariaceous-like conidiomata and conidia with two unicellular appendages at each end. However it markedly differs from *I. cupulata*, the monotypic species of the genus, in its conidial shape and size. The type species possesses cylindrical conidia while the new species has oblong conidia (FIGS. 3, 5–7). Conidial length of the new species (3–6 μm) is shorter than that of the type species (6–10 μm). Shape and size of the conidium are important morphological characteristics for species separation of the coelomycetes, and this fungus therefore is described as a new species, *Infundibulomyces oblongisporus*.

Other minor differences between *I. oblongisporus* and *I. cupulata* include conidial septation and appendages. In *I. oblongisporus* all conidia are nonseptate while in *I. cupulata* 1-septate conidia sometimes could be found. Conidial appendages of *I. oblongisporus* (4–10 μm) also are shorter than those of *I. cupulata*. (12–25 μm).

DISCUSSION

Phylogenetic relationship of Infundibulomyces.—In this discussion the classification proposed by Spatafora et al (2006) is followed, along with the study by Zhang et al (2006). Genus *Infundibulomyces* is well placed in the Chaetosphaeriaceae, Chaetosphaeriales, Sordariomycetidae, Sordariomycetes (FIGS. 1, 2). The Chaetosphaeriaceae comprise nine genera with ca. 90 species (Réblová and Winka 2000, Kirk et al 2001, Huhndorf et al 2004, Fernández and Huhndorf 2005); most are saprobic on wood, bark and leaves with hyphomycete anamorphs. The anamorphs have hyaline to brown conidia, percurrent proliferating conidiogenous cells, usually with wide fluted collarettes. Those with endoblastic conidiogenesis include *Catenularia*, *Chaetopsis*, *Chalara*, *Chloridium*, *Codinaea*, *Dictyochaeta*, *Exserticlava*, *Gonytrichum*, *Kylindria*, *Menispora*, *Phialophora*, *Stanjehughesia* and *Zanclospora*. However conidiogenesis in *Ellisembia*, *Sporidesmuium* and *Infundibulomyces* is holoblastic. No coelomycete has been reported as the anamorph of any genera in the Chaetosphaeriaceae.

Fernández et al (2006) evaluated the systematics of *Chaetosphaeria* (29 species) and allied genera (five) with partial sequences of the nuclear LSU rDNA and β-tubulin genes. Two major lineages were discerned: *Chaetosphaeria*, a well supported monophyletic clade of 13 species and eight paraphyletic taxa; the second lineage included *Melanochaeta* (anamorph: *Sporochisma*), *Melanopsammella* (anamorphs: *Chloridium*, *Gonytrichum*), *Striatosphaeria* (anamorph: *Codinaeopsis*), *Zigonella* (anamorph: *Menispora*) and *Tainosphaeria* (anamorph: *Dictyochaeta*). In our analysis four monophyletic groups were identified: (i) *Melanochaeta*, (ii) *Melanopsammella*, (iii) *Zigonella*, *Codinaeopsis* and *Striatosphaeria* and (iv) *Infundibulomyces*, *Dictyochaeta* and *Tainosphaeria*. While *Striatosphaeria codinaephora* formed a sister group of *Zigonella* species, as reported by Fernández et al (2006), *Tainosphaeria crassiparies* (and its *Dictyochaeta* anamorph) showed no affinity with these two genera. Various authors have suggested that anamorphs should be used as a primary character in the separation of *Chaetosphaeria* species (Gams and Holubová-Jechová 1976, Réblová and Winka 2000). In our study *Infundibulomyces* with holoblastic conidiogenesis differs from all other taxa in the second

lineage reported by Fernández et al (2006) because they possess enteroblastic conidiogenous cells with wide collarettes. Plaingam et al (2003) have illustrated holoblastic development in *Infundibulomyces cupulata* with scanning electron microscopy with no collarette. To resolve the position of *Infundibulomyces* species within the Chaetosphaeriaceae other taxa need to be studied, including further coelomycetes. Two *Infundibulomyces* species are recognized and differ in conidial morphology supported by molecular evidence. The rDNA sequence of *I. cupulata* are different from *I. oblongisporus* by 19, 35 and 60 nucleotide substitutions and insertions in the SSU, LSU and ITS rDNA regions respectively. Species of *infundibulomyces* are known presently only from the tropics and are considered saprobic fungi. *Infundibulomyces cupulata* was recorded on dead leaves of the *Lagerstroemia* tree (Plaingam et al 2003) while the new species, *I. oblongisporus*, has been collected on dead leaves of an evergreen broadleaf tree.

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3) Draft manuscript entitled "Phylogeny of fusiform conidia coelomycetes: *Robillarda*, *Pseudorobillarda* and *Xepiculopsis* based on nuclear ribosomal DNA sequences" will be submitted in Persoonia

PERSOONIA

Phylogeny of fusiform conidia coelomycetes: *Pseudorobillarda, Robillarda* and *Xepiculopsis* based on nuclear ribosomal DNA sequences.

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ABSTRACT

A molecular study of selected species of the genera *Pseudorobillarda, Robillarda* and *Xepiculopsis* was undertaken to determine phylogenetic relationship and their putative teleomorphs. These three genera share many morphological features, in particular the procession of ellipsoid appendaged conidia, but are they phylogenetically related? Xepiculopsis graminea nestles in the Hypocreales, but can not be assigned to any know family, grouping with other anamorphic fungi: Didymostilbe, Peethambara, Myrothecium with Stachybotrys species in the sister clade. Robillarda sesillis clusters with member of the Amphisphaeriaceae (Xylriales) but shows no affinity with any other coelomycete genera. The Pseudorobillarda species formed a monophyletic group with Dothideomycetes, but did not show an affinity with any family or order. The Pseudorobillarda species formed a basal clade to the Mytilindriaceae (Hysteriales), Lophiostomataceae and Pleosporaceae (Pleosporales) and their taxonomic position remain unresolved.

INTRODUCTION

Many coelomycetes have fusiform-conidia belonging to diverse genera and species. Conidial appendages and conidioginensis are key characters in their identification at the generic and species level. The genera *Robillarda, Pseudorobillarda* and *Xepiculopsis* posses ellipsoidal conidia with appendages and differ in conidial appendage ontogeny (Plaingam et al. 2005). Conidia of *Robillarda* species are formed holoblastically, always 1-septate while septation of *Pseudorobillarda* species (phialospore) ranges from 0 (non-septate) to 4-septate. Moreover, conidial appendages in *Robillarda* are always apical while those of *Pseudorobillarda* can be apical or basal (Plaingam et al. 2005). No teleomorphs are known for *Pseduorobillarda* and *Xepiculopsis* while theputative teleomorph of *Robillarda* is a member of the Amphisphaeriaceae (Xylariales, Sordariomycetes, Ascomycota) (Jeewon et al. 200x). Thirty five *Robillarda* species have been described with six referred to *xxx* (Index Fungorum) while 16 are listed for *Pseudorobillarda.* These two genera are often confused as they both posses septate conidia with polar and or basal appendages, with many species described as *Robillarda* transferred to *Pseudorobillarda* (e.g. *Robillarda agrostidis, R. jaczewskii, R. muehlenberiae*). The genus *Xepiculopsis* was described by Naj Raj (1993) with *X. perpulchra* as the type species. He also transferred *Myrothecium gramineum* to this new genus, but with reservation, as there was some confusion in the material deposited by Libert (1837). The type material contains more than one fungus. Because of the lack of clarity in identifying Robillarda and *Pseudorobillarda*, a taxonomic study was undertaken to resolve their familial and ordinal status. An ultrastructural study of conidial appendage development in these three genera showed significant differences (Plaingam, 2002). Need to add a few sentences here describing the different observations made by Noi, will do that when I have her thesis at hand. The aim of this study was to identify their putative teleomorphs and to clarify their phylogenetic relationship based on ribosomal DNA sequences.

MATERIALS AND METHODS

Specimen collection, culture maintenance and fungal cultivation

Five coelomycetes collected in Thailand were selected for study: three species of *Pseudorobillarda* (*P. siamensis, P. sojae* and *P. texana*), *Robillarda sesillis* and *Xepiculopsis graminea*. Details of collection sites, substratum and isolate accession codes are listed in Table 1. All strains are deposited in the BIOTEC Culture Collection (BCC). The accession numbers of species from the GenBank used in the phylogenetic analysis are listed in Table 2.

Genomic extraction and PCR amplification

Our coelomycetes were grown on potato dextrose agar (PDA) and transferred into potato dextrose broth (PDB) and incubated without agitation at 25 °C for 2 weeks for DNA extraction. Mycelium was harvested, washed with warm sterilized distilled water, frozen at -80 °C for 1-2 hours, and the fungal biomass ground into fine powder with sterilized mortar and pestle. DNA was extracted using CTAB lysis buffer (O'Donnell *et al.* 1997) and incubated at 65 °C for 1 hour. The mixture was extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1). The upper liquid phase was precipitated with 7.5 M ammonium acetate and absolute ethanol and kept at -20 ° for at least 30 min. Extracted DNA was washed twice with 70% ethanol, air dried and the DNA resuspended in 50 µl TE buffer.

Partial nuclear small subunit (SSU) and nuclear large subunit (LSU) regions of rDNA were amplified with gene specific primers: NS1, NS2, NS3, NS4, NS5, NS6 and LROR, LR2, LR3, LR7, respectively (White et al. 1990, Bunyard et al. 1994) using FINNZYMES, DyNAzyme™ II DNA polymerase kit (Cat No F-551S, Finnzymes, Espoo, Finland). The amplification cycles were performed following White et al. (1990) and Bunyard et al. (1994)

with a DNA Engine DYAD ALD 1244 thermocycler (MJ Research, Inc, Waltham, MA). The PCR products were purified with NucleoSpin® Extract DNA purification kit (Cat. No. 740 609.50, Macherey-Nagel, Duren, Germany) following the manufacturer's instruction and then sequenced by Macrogen Inc. (Seoul, Korea) using the same primers as for amplification.

Sequence alignment and phylogenetic analysis

A BLAST search was employed to obtain the closest matched sequences in the GenBank database (Altschul et al. 1990). The SSU and LSU rDNA sequences were multiple aligned along with other related sequences obtained from GenBank using Clustal W 1.6 (Thompson et al. 1994) and adjusted manually where necessary using BioEdit 7.5.0.3 (Hall 2006).

The aligned dataset was subsequently analysed using maximum parsimony 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 & 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 & Ronquist 2001). Four Markov chains were run from random starting trees for 5 000 000 generations and sampled every 100 generations. The first 500 000 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 analysis (Felsenstein 1985) with 1000 replications (10 replicates of random stepwise sequence

addition, TBR branch swapping) and posterior probabilities were performed. The maximum parsimony bootstrap values (≥50%) and Bayesian posterior probabilities (≥0.95) are shown above and below the tree branches, respectively. The rDNA sequences, consisting of SSU and LSU were submitted into the GenBank database and the new sequences generated for this investigation are shown in Table 1. The alignments are deposited in TreeBase: xxxx, xxxx.

RESULTS

SSU and LSU phylogeny

A phylogenetic tree was constructed from a dataset consisting of nuclear small subunit (SSU) and nuclear large subunit (LSU) sequences. This alignment was combined from two fragments of 1,409 bps for SSU and 1,377 bps for LSU. The data set contained 107 sequences with *Peziza vesiculosa* and *Peziza proteana* (Pezizomycete) as the outgroup. A total of 2,786 characters, 793 are parsimony informative, 207 are parsimony uninformative and 1,786 are constant characters. The phylogenetic analysis based on maximum parsimony criterion and Bayesian inference results in the same tree topology for the major orders and classes of fungi, therefore the maximum parsimonious trees is shown in this paper (Fig. 1).

Within the two major classes *i.e.* the Sordariomycete and Dothideomycetes, only minor swapping was found in the tree resulting from the two analyses. In order to solve the taxonomic position of our five coelomycetes, various taxa from those classes were incorporated in this dataset. Within the Sordariomycetes, 60 representative taxa from all major orders: Chaetosphaeriales, Diaporthales, Sordariales (Sordariomycetidae); Halosphaeriales, Hypocreales, Microascales, Ophiostomatales (Hypocreomycetidae); and the Xylariales (Xylairiomycetidae), while 43 taxa from eight orders of the Dothideomycetes, comprising Botryosphariales, Capnodiales, Dothidales, Hysteriales, *Incertae sedis*, Myriangiales, and Pleosporales were analysed. Our isolates *i.e.* *Robillarda sesillis,*

Pseudoriballarda siamensis, P. sojae, P. texana and *Xepiculopsis graminea* were distributed in different orders and distant from one another *Robillarda sesillis* and *X. graminea* were placed within the Sordariomycete with 100 % BS, while the three *Pseudorobillarda* species were located in the Dothidiomycete with 94 % BS.

In this investigation, *Xepiculopsis* nestled within the Hypocreales with 97 % BS and formed a clade with *Peethambara spirostriata* and *Didymostilbe echinofibrosa* (Fig 1 subclade A). This subclade has phylogenetic affinity with several *Myrothecium* species. Furthermore, *Stachybotrys* species were closely allied in a sister clade with moderate support (73 % BS). Other families within the Hypocreales, (Bionectriaceae, Nectriaceae, Niessliaceae and Hypocreaceae) formed a lower clade, while the Halosphaeriales and Microascales were placed as basal group of the Hypocreomycetidae.

A single isolate of *Robillarda sesillis* falls within the Xylariales with 100 % BS (Fig. 1 subclade B). *Robillarda sesillis* formed a clade with the member of the Amphisphaeriaceae with 91% BS and associated with the Diatrypaceae and Xylariaceae. Although *R. sesillis* grouped with the Amphisphaeriaceae, it showed no affinity with the genera included in the analysis (*Amphisphaeria, Bartalinia, Discosia, Discostroma, Monochaetea, Pestalotiopsis, Seiridium* and *Truncatella*).

Three *Pseudorobillarda* species (*P. siamensis, P. sojae* and *P. texana*) were phylogenetically related with members of the Dothideomycetes with 94 % BS (Fig. 1 subclade C). Various orders within the Dothideomycete (Botryosphaeriales, Capnodiales, Dothideales, Hysteriales, Myriangiales and Pleosporales) were included in the analysis to determine their phylogenetic position. *P. siamensis*, *P. sojae* and *P. texana* formed a monophyletic clade with 100 % BS, although there was variation in branch length between them. They could not be assigned to any family or order in the Dothideomycetes.

Discussion

The identification of coelomycetes a group of anamorphic fungi comprising circa xx,xxx species, is based exclusively on morphological characters, which often overlap and may be due to convergent evolution. Identification of genera such as Pestalotiopsis and Pestalotia; Pseudorobillarda and Robillarda; Monochaeta and xxxxxx; are contentious with consequent transfer from one genus to the other. In these cases colour, degree of septation and possession and number of conidial appendages determine the genus. Apart from genera of economic importance, the phylogenetic affiliation of coelomycetes genera remains largely unresolved. It is import that these are studied at the molecular level as they may also help to resolve missing lineages of ascomycetes and basidiomycetes (Woody ref here).

In order to resolves the taxonomic position of selected coelomycetes collected in Thailand, we embarked on a molecular study of a selected group of genera with appendaged ellipsoid conidia (Somrithipol et al. 2008, Rungjindamai et al. 2008).

Our earlier study of the ultrastructure of selected coelomycetes we were able to show that conidial appendages development was significantly different for Pseudorobillarada and Robillarda. Will add a sentence here on the differences. While such studies enabled clarification of morphological characters in the delineation of genera, it did not resolve their phylogenetic relationship.

Our study demonstrated that Pseuorobillarda and Robillarda are not phylogenetically related, but are distantly placed from each other in the Sordariomycetes and Dothiediomycetes, respectively.

Acknowledgement

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Table 1. Collection site, substrata and GenBank accession number of *Robillarda sessilis, Pseudorobillarda siamensis, P. sojae, P. texana* and *Xepiculopsis graminea* sequenced in this study.

	Original code	Source*	Substratum and geographical origin	Date of isolation	GenBank accession number	
					SSU	LSU
larda sessilis (Saccardo) Saccardo)	SFC00858	BCC13393	Leaf of *Eucalyptus camaldulensis*, Kasetsart University, Bangkok	2 April 2003		
lorobillarda siamensis N. Plaingam, ithipol & E.B.G. Jones (2005)	SFC00795	BCC12531	Fallen dicotyledonous leaf, Khao Yai National Park, Nakon Nayok	19 July 2000		
ae Uecker & Kulik (1986)	SFC01947	BCC20495	Dead leaf, Khao Yai National Park, Nakhon Ratchasima	20 March 2006		
ana Nag Raj (1993)	SFC00866	BCC12535	Fallen dicotyledonous leaf, Khao Yai National Park, Prachin Buri	18 August 2000		
culopsis graminea (Libert) Nag Raj 3)	SFC00785	BCC9458 #	Grass leaf, Banphkong, Chachaensao	4 July 2001		

*BCC = BIOTEC Culture Collection, Pathum Thani, Thailand

Data in BCC is recorded as unidentified fungus.

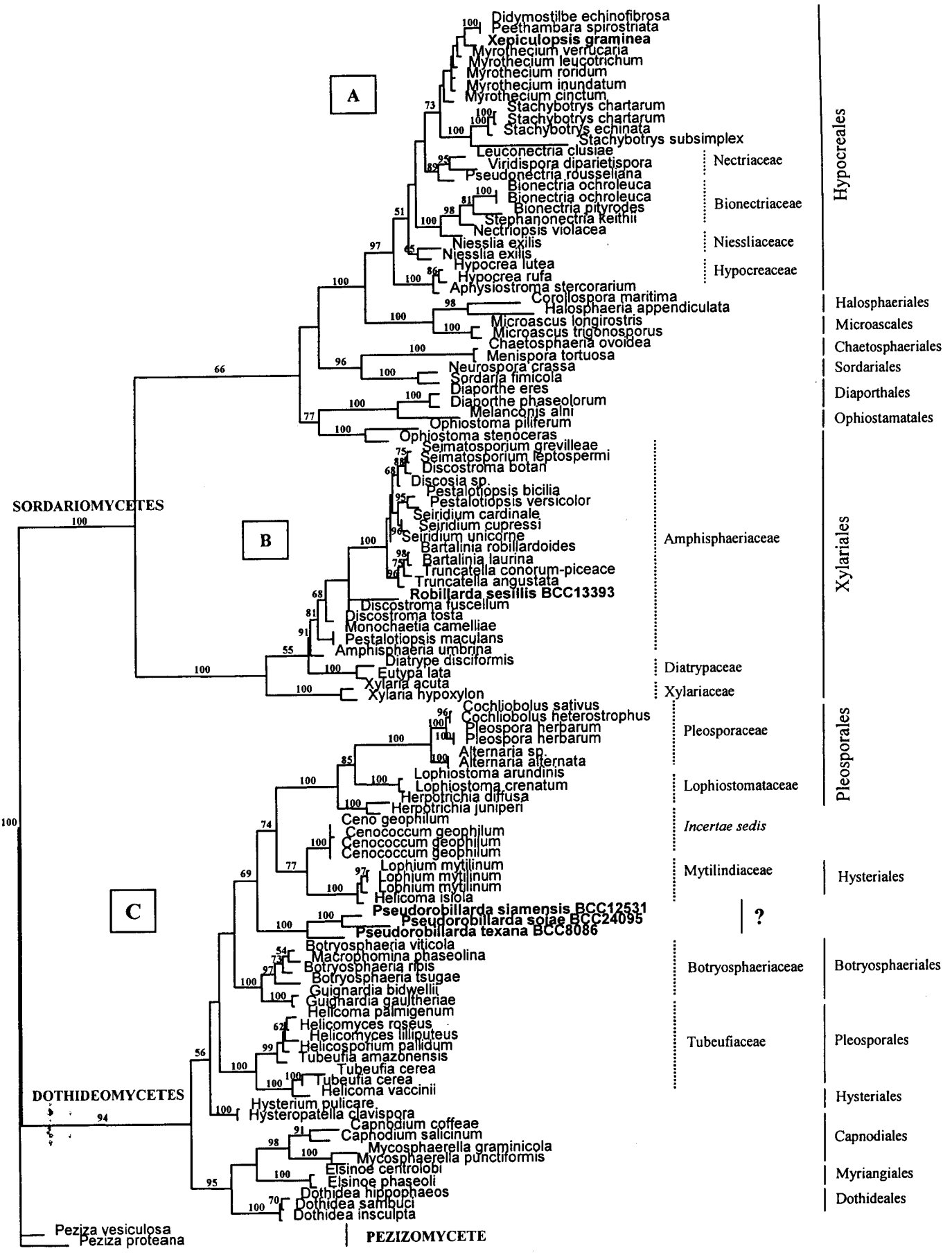
List of species, source of cultures and GenBank accession number in this study

Species	Source of culture*	GenBank accession number	
		SSU	LSU
Alternaria alternata	CBS 916.96	DQ678031	DQ678082
Alternaria sp. (as *'Clathrospora diplospora'*)	CBS 174.52	DQ678016	DQ678068
Amphisphaeria umbrina	HKUCC994	-	AY083811
Aphysiostroma sterocorarium	ATCC62321	AF543769	AF543792
Bartalinia robillardoides	BRIP 14180	-	AF382366
Bartalinia laurina	HKUCC 6537	-	AF382369
Bionectria ochroleuca	GJS 71-328	DQ862044	DQ862027
Bionectria ochroleuca	CBS 114056	AY489684	AY489176
Bionectria pityrodes	ATCC208842	AY489696	AY489728
Botryosphaeria ribis	CBS 115475	DQ678000	DQ678053
Botryosphaeria tsugae	CBS 418.64	AF271127	DQ767655
Botryosphaeria viticola	CBS 117009	DQ678036	DQ678087
Capnodium coffeae	CBS 147.52	DQ247808	DQ247800
Capnodium salicinum	CBS 131.34	DQ677997	DQ678050
Cenococcum geophilum	-	L76614	AY112935
Cenococcum geophilum	-	L76615	-
Cenococcum geophilum	-	L76616	-
Cenococcum geophilum	-	L76617	-
Cenococcum geophilum	-	L76618	-
Unculturable *C. geophilum*	N72*	-	DQ473308
Unlculturable ectomycorrhiza *(C. geophilum)*	NEU9*	-	AY748873
Chaetosphaeria ovoidea	SMH 2605		AF064641
Cochliobolus heterostrophus	CBS 134.39	AY544727	AY544645
Cochliobolus sativus	DAOM 226212	DQ677995	DQ678045
Corollospora maritima	JK 4834	U46871	U46884
Dendryphiopsis atra	DAOM 231155	DQ677996	DQ678046
Diaporthe eres	CBS 109767	DQ471015	AF408350
Diaporthe phaseolorum	NRRL 13736	L36985	U47830
Diatrype disciformis	CBS 197.49	DQ471012	DQ470964
Didymostilbe echinofibrosa	AR 2824	AY489674	AY489706
Discosia sp.	HKUCC 6626	-	AF382381
Discostroma botan	HHUF4642	DQ368660	DQ368629
Discotroma fuscellum	10071*	AF346548	
Discostroma tosta	HKUCC1004	AY083814	-
Dothidea hippophae¨s	DAOM 231303	U42475	DQ678048
Dothidea insculpta	CBS 189.58	DQ247810	DQ247802
Dothidea sambuci	DAOM 231303	AY544722	AY544681
Elsinoe¨ centrolobi	CBS222.50	DQ678041	DQ678094
Elsinoe¨ phaseoli	CBS 165.31	DQ678042	DQ678095
Eutypa lata	CBS 208.87	DQ836896	DQ836903
Guignardia bidwellii	CBS 237.48	DQ678034	DQ678085
Guignardia gaultheriae	CBS 447.70	-	DQ678089
Halosphaeria appendiculata	CBS 197.60	U46872	U46885
Helicoma isiola	UAMH1359	AY856935	AY856890
Helicoma paligenum	NBRC32663	AY856941	AY856898
Helicoma vaccinii	CBS216.90	AY856926	AY856879
Helicomyces lilliputeus	NBRC32664	AY856942	AY856899
Helicomyces roseus	CBS 283.51	DQ678032	DQ678083
Helicosporium pallidum	CBS962.69	AY856932	AY856886
Herpotrichia diffusa	CBS 250.62	DQ678019	DQ678071
Herpotrichia juniperi	CBS 200.31	DQ678029	DQ678080
Hypocrea lutea	ATCC 208838	AF543768	AF543791
Hypocrea rufa	CBS 114374	AY489694	AY489726
Hysterium pulicare	CBS 239.34	DQ678002	DQ678055

Hysteropatella clavispora	CBS 247.34	DQ678006	AY541493
Kirschsteiniothelia aethiops	CBS 109.53	AY016344	AY016361
Leuconectria clusiae/ Gliocephalotrichum bulbilium	ATCC 22228	AY489700	AY489732
Lophiostoma arundinis	CBS 269.34	DQ782383	DQ782384
Lophiostoma crenatum	CBS 629.86	DQ678017	DQ678069
Lophium mytilinum	AFTOL1069	-	DQ678081
Lophium mytilinum	CBS 114111	-	EF596819
Lophium mytilinum	CBS 269.34	-	EF596817
Macrophomina phaseolina	CBS 227.33	DQ678037	DQ678088
Melanconis alni	AR 3500	DQ862052	AF408371
Menispora tortuosa	DAOM 231154	AY544723	AY544682
Microascus longirostris	CBS 267.49	DQ471026	AF400865
Microascus trigonosporus	CBS 218.31	DQ471006	DQ470958
Monochaetia camelliae	152319*	AF346549	-
Mycosphaerella graminicola	CBS 292.38	DQ678033	DQ678084
Mycosphaerella punctiformis	CBS 113265	DQ471017	DQ470968
Myrothecium roridum	ATCC 16297	AY489676	AY489708
Myrothecium cinctum	ATCC 22270	AY489678	AY489710
Myrothecium inundatum	IMI 158855	AY489699	AY489731
Myrothecium leucotrichum	AR 3506	AY489675	AY489707
Myrothecium verrucaria	ATCC 9095	AY489681	AY489713
Nectriopsis violacea	CBS 424.64	AY489687	AY489719
Neurospora crassa	MUCL19026	X04971	AF286411
Niesslia exilis	CBS 357.70	AY489686	AY489718
Niesslia exilis	CBS 560.74	AY489688	AY489720
Ophiostoma piliferum	CBS 158.74	DQ471003	DQ470955
Ophiostoma stenoceras	CBS 139.51	DQ836897	DQ836904
Peethambara spirostriata	CBS 110115	AY489692	AY489724
Pestalotiopsis bicilia	HKUCC7893	-	AF382356
Pestalotiopsis maculans	CBS 361.61	AB220235	-
Pestalotiopsis versicolor	BRIP 14534	-	AF382357
Phaeotrichum benjaminii	CBS 541.72	AY016348	AY004340
Pleospora herbarum var. *herbarum*	CBS 714.68	DQ767648	DQ678049
Pleospora herbarum var. *herbarum*	CBS 541.72	DQ247812	DQ247804
Pseudonectria rousseliana	CBS114049	AF543767	U17416
Seimatosporium leptospermi	ICMP 11845		AF382373
Seimatosporium vaccinii	ICMP 7003		AF382374
Seiridium cardinale	ICMP 7323		AF382377
Seiridium cupressi	FABI, CMW 5596		AF382378
Seiridium unicorne	138068*	AF346557	
Sordaria fimicola	CBSC 15-5973	AY545728	AY545724
Stachybotrys chartarum	ATCC 66238 (=UAMH 6417, =CBS 25089)	AY489680	AY489712
Stachybotrys chartarum	ATCC 9182	AY489682	AY489714
Stachybotrys echinata (=*Memnoniella echinata*)	UAMH 6594	AY489704	AY489736
Stachybotrys subsimplex (=*M. subsimplex*)	ATCC 32888	AY489679	AY489711
Stephanonectria keithii	CBS 114057	AY489695	AY489727
Truncatella angustata	ICMP 7062	-	AF382383
Truncatella conorum-piceae	ICMP 11213	-	AF382384
Tubeufia amazonensis	ATCC42524	AY856951	AY856911
Tubeufia cerea	NBRC9014	AY856947	AY856903
Tubeufia cerea	CBS 254.75	DQ471034	DQ470982
Tyrannosorus pinicola	CBS 124.88	DQ471025	DQ470974
Xylaria acuta	ATCC 56487	AY544719	AY544676
Xylaria hypoxylon	OSC 100004	AY544692	AY544648
Viridispora diparietispora	ATCC MYA 627	AY489703	AY489735

Peziza proteana	AFTOL 71	AY544703	AY544659
Peziza vesiculosa	AFTOL507	DQ470995	AY470948

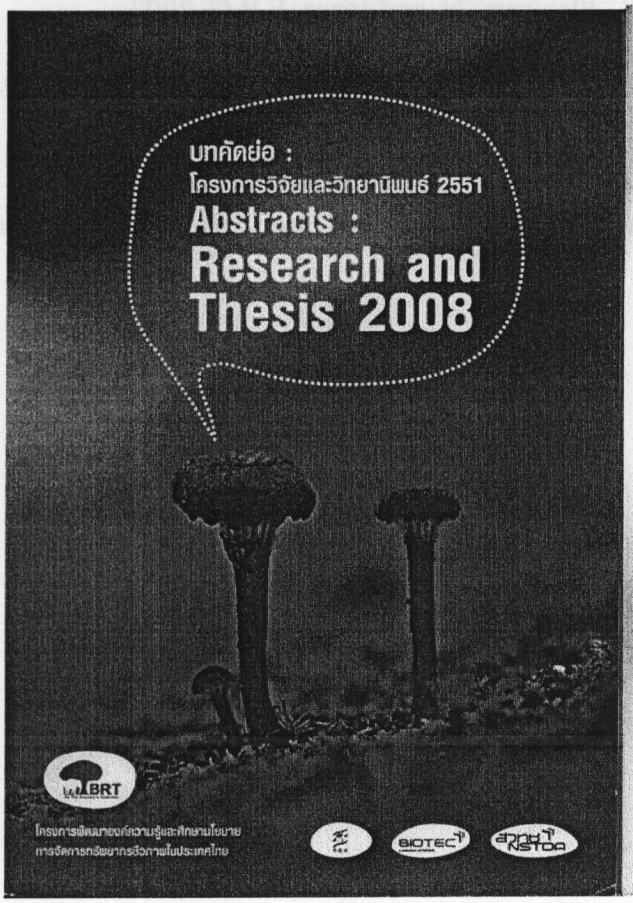
AFTOL, Assembling the Fungal Tree of Life, Duke University, Durham, North Carolina; ATCC, American Type Culture Collection, Manassas, Virginia; AR, Amy Y. Rossman personal collection; BRIP, Queensland Department of Primary Industries Plant Pathology Herbarium; Indooroopilly Queensland; CBS, Centraalbureau voor Schimmelcultures, Utrecht; DAOM, National Mycological Herbarium, Department of Agriculture, Ottawa, Ontario; FABI, Forestry and Agricultural Biotechnology Institute, Pretoria; GJS, Gary J. Samuels personal collection; HKUCC, The University of Hong Kong Culture Collection, Hong Kong; IMI, CAB International, Egham; ICMP, International Collection of Microorganisms from Plants, Auckland; JK, Jan Kohlmeyer personal collection; MUCL, Mycothèque de l'Universite catholique de Louvain, Ottignies, Belgium; NBRC: NITE Biological Resource Center, Tokyo; NRRL, ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, Illinois; OSC, Oregon State University Herbarium, Corvallis, Oregon; SMH, Sabine M. Huhndorf personal collection; UAMH, University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta; * Description from GenBank Database


A
B
C
SORDARIOMYCETES
DOTHIDEOMYCETES
PEZIZOMYCETE
Didymostilbe echinofibrosa
Peethambara spirostriata
Xepiculopsis graminea
Myrothecium verrucaria
Myrothecium leucotrichum
Myrothecium roridum
Myrothecium inundatum
Myrothecium cinctum
Stachybotrys chartarum
Stachybotrys chartarum
Stachybotrys echinata
Stachybotrys subsimplex
Leuconectria clusiae
Viridispora diparietispora
Pseudonectria rousseliana
Bionectria ochroleuca
Bionectria ochroleuca
Bionectria pityrodes
Stephanonectria keithii
Nectriopsis violacea
Niesslia exilis
Niesslia exilis
Hypocrea lutea
Hypocrea rufa
Aphysiostroma stercorarium
Corollospora maritima
Halosphaeria appendiculata
Microascus longirostris
Microascus trigonosporus
Chaetosphaeria ovoidea
Menispora tortuosa
Neurospora crassa
Sordaria fimicola
Diaporthe eres
Diaporthe phaseolorum
Melanconis alni
Ophiostoma piliferum
Ophiostoma stenoceras
Seimatosporium grevilleae
Seimatosporium leptospermi
Discostroma botan
Discosia sp.
Pestalotiopsis bicilia
Pestalotiopsis versicolor
Seiridium cardinale
Seiridium cupressi
Seiridium unicorne
Bartalinia robillardoides
Bartalinia laurina
Truncatella conorum-piceace
Truncatella angustata
Robillarda sesillis BCC13393
Discostroma fuscellum
Discostroma tosta
Monochaetia camelliae
Pestalotiopsis maculans
Amphisphaeria umbrina
Diatrype disciformis
Eutypa lata
Xylaria acuta
Xylaria hypoxylon
Cochliobolus sativus
Cochliobolus heterostrophus
Pleospora herbarum
Pleospora herbarum
Alternaria sp.
Alternaria alternata
Lophiostoma arundinis
Lophiostoma crenatum
Herpotrichia diffusa
Herpotrichia juniperi
Ceno geophilum
Cenococcum geophilum
Cenococcum geophilum
Cenococcum geophilum
Lophium mytilinum
Lophium mytilinum
Lophium mytilinum
Helicoma isiola
Pseudorobillarda siamensis BCC12531
Pseudorobillarda solae BCC24095
Pseudorobillarda texana BCC8086
Botryosphaeria viticola
Macrophomina phaseolina
Botryosphaeria ribis
Botryosphaeria tsugae
Guignardia bidwellii
Guignardia gaultheriae
Helicoma palmigenum
Helicomyces roseus
Helicomyces lilliputeus
Helicosporium pallidum
Tubeufia amazonensis
Tubeufia cerea
Tubeufia cerea
Helicoma vaccinii
Hysterium pulicare
Hysteropatella clavispora
Capnodium coffeae
Capnodium salicinum
Mycosphaerella graminicola
Mycosphaerella punctiformis
Elsinoe centrolobi
Elsinoe phaseoli
Dothidea hippophaeos
Dothidea sambuci
Dothidea insculpta
Peziza vesiculosa
Peziza proteana
Amphisphaeriaceae
Diatrypaceae
Xylariaceae
Pleosporaceae
Lophiostomataceae
Incertae sedis
Mytilindiaceae
?
Botryosphaeriaceae
Tubeufiaceae
Nectriaceae
Bionectriaceae
Niessliaceace
Hypocreaceae
Hypocreales
Halosphaeriales
Microascales
Chaetosphaeriales
Sordariales
Diaporthales
Ophiostamatales
Xylariales
Pleosporales
Hysteriales
Botryosphaeriales
Pleosporales
Hysteriales
Capnodiales
Myriangiales
Dothideales
10 changes

4) Poster presented in BRT Annual Conference 2008 at Surat Thani, Thailand.

Rungjindamai, N., Sakayaroj, J., Somrithipol, S., Plaingam, N. and Jones, E.B.G. 2008. Molecular phylogeny of selected coelomycetes with fusiform conidia, *Robillarda, Pseudorobillarda* and *Xepiculopsis*, based on nuclear ribosomal DNA sequences. Abstracts: Research and Thesis 2008. 12th BRT Annual Conference 2008. 10-13 October 2008. Diamond Plaza, Surat Thani.

Abstract printed in Abstract Book of 12th BRT Annual Conference 2008



Molecular phylogeny of selected coelomycetes with fusiform conidia, *Robillarda*, *Pseudorobillarda* and *Xepiculopsis*, based on nuclear ribosomal DNA sequences

Nattawut Rungjindamai, Jariya Sakayaroj, Sayanh Somrithipol, Narumol Plaingam and E. B. Gareth Jones
National Center for Genetic Engineering and Biotechnology, Pathum Thani, Thailand

Conidial appendages and conidiogenesis are key characters in the identification of coelomycete genera and species. The genera *Robillarda*, *Pseudorobillarda* and *Xepiculopsis* possess ellipsoidal conidia with appendages but differ in conidial appendage ontogeny. No teleomorphs are known for *Pseudorobillarda* and *Xepiculopsis*, while the putative teleomorph of *Robillarda* is a member of the Amphisphaeriaceae. *Robillarda* and *Pseudorobillarda* are often confused as they both possess septate conidia with polar and or basal appendages. Because of the lack of clarity in identifying *Robillarda*, *Pseudorobillarda* and *Xepiculopsis*, a taxonomic study was undertaken to resolve their familial and ordinal statuses using nuclear SSU and LSU rDNA sequences. The phylogenetic analysis demonstrated that these three coelomycetes fall into two distinct lineages. Firstly, *Robillarda* and *Xepiculopsis* are well placed within the Sordariomycetes, and the ordinal position of these two genera fall within the Hypocreales and Xylariales, respectively. Secondly, *Pseudorobillarda* species have a phylogenetic affinity with the Dothideomycetes, although their ordinal and familial levels remain unresolved. Although these three genera morphologically have similar fusiform conidia, they show no consistency in their phylogenetic relationships. This study has enabled the identification of the putative teleomorphs for these three coelomycete genera.

Poster presented at 12th BRT Annual Conference