# 12-MONTH PROGRESS REPORT AND GRANT RESUBMISSION

## (The 2nd Report: October 2005 - March 2006)

**For:** National Center for Genetic Engineering and Biotechnology (BIOTEC)
Biodiversity Research and Training Program (BRT)

**Project Title:** Molecular taxonomy of selected genera of coelomycetes and their biodiversity in Thailand

**BRT Project Code:** BRT R_148006

**Principal Investigator:** Prof. E. B. Gareth Jones

**Co Investigators:** Dr. Jariya Sakayaroj
Dr. Narumol Plaingam
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**This report is in three parts:**



**Progress Report Period:** October 2005 - March 2006

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# PART A: TWELVE MONTH REPORT

## 1. SUMMARY OF REPORT

Coelomycetes are anamorphic fungi producing asexual spores (conidia) (Sutton, 1980; Nag Raj, 1993) and play an important role in terrestrial ecosystems: as saprobes, or parasites of higher plants, fungi, lichens and vertebrates and may occur as endophytes (Kirk *et al.*, 2001). Some 200 coelomycete species have been documented for Thailand (Plaingam, *et al.*, 2004; Giatgong, 1980). Little or no work at the molecular level has been carried out on the phylogeny of coelomycetes, other than those of economic importance (in plant pathology). Therefore this project will make a major contribution to the classification of this anamorphic group of fungi. For the past 12 months, we have been collecting coelomycetes from samples of living and decaying plants from streams, river, ponds; from leaf litter in terrestrial habitats; and from mangrove areas for the presence of saprobic fungi. Thirty coelomycetes have been isolated into axenic culture, grown in solid culture for conservation in the BIOTEC Culture Collection over the last 6 months (6-12 months). Sixty-one coelomycetes were isolated over a one year period. Nine coelomycetes: *Chaetospermum camelliae, Guilia tenuis, Infundibulomyces cupulata, Infundibulomyces* sp. SFC 981, *Mycotribulus mirabilis, Robillarda sessilis, Satchmopsis brasiliensis, Pseudorobillarda siamensis* and *Xepiculopsis graminea* have been sequenced. Therefore, 9 coelomycetes and 21 PCR reactions have been performed within one year. Data analysis of the sequences obtained is in progress and phylogenetic trees for the nine ceolomycetes have been constructed. Preliminary results for all coelomycetes studied are presented in this report. Data assembled during this year have been integrated into a poster presentation.

## 2. PROJECT OBJECTIVES:

A). To document the diversity of coelomycetes in Thailand and to isolate strains for conservation in the BIOTEC Culture Collection. (Output: Addition of 50 coelomycetes per year to the BIOTEC Culture Collection)

B). To construct phylogenetic trees to examine the inter-relationships of coelomycete genera and species.

## 3. BIODIVERSITY OF COELOMYCETES IN THAILAND:

### 3.1. Collection, examination and isolation of fungi

**a) Collecting sites**

1. Bangkok
2. Khao Yai National Park, Nakhon Rashasima
4. Pathum Thani

**b) Substrata**

1. Decaying leaves
2. Twigs

**c) Materials and methods for examination and isolation.**

See the 6-month progress report

### 3.2 RESULTS

Coelomycetes have been collected from a variety of substrata and locations. Thirty strains have been isolated over the last 6 month (No. 32-61) and deposited in the BIOTEC Culture Collection (Table1). Therefore, a total of 61 species have been isolated within one year.

**Table 1. List of coelomycetes strains isolated over one year**

| No. | Original Code | Species | Substrate | Location | Collection Date | Isolation Date |
|---|---|---|---|---|---|---|
| 1 | NP00061 | *Chaetomella raphigera* | decaying leaves | Chatuchak, Bangkok | 23/7/2005 | 5/8/2005 |
| 2 | NP00062 | *Pestalotiopsis* sp. | decaying leaves | Chatuchak, Bangkok | 23/7/2005 | 5/8/2005 |
| 3 | NP00063 | Unidentified fungus | *decaying leaves* | Chatuchak, Bangkok | 23/7/2005 | 5/8/2005 |
| 4 | NP00064 | *Chaetospermum camelliae* | decaying leaves | Khao Yai National Park, Nakhon Rashasima | 23/7/2005 | 5/8/2005 |
| 5 | NP00065 | *Pestalotiopsis* sp. | decaying leaves | Lamlukka, Pathum Thani | 23/7/2005 | 5/8/2005 |
| 6 | NP00066 | *Chaetospermum artocarpi* | decaying leaves | Khao Yai National Park, Nakhon Rashasima | 23/7/2005 | 5/8/2005 |
| 7 | NP00067 | *Pseudorobillarda sojae* | decaying leaves | Khao Yai National Park, Nakhon Rashasima | 23/7/2005 | 5/8/2005 |
| 8 | NP00068 | *Coniella castaneicola* | decaying leaves | Chatuchak, Bangkok | 23/7/2005 | 5/8/2005 |
| 9 | NP00069 | *Phoma* sp. | decaying leaves | Chatuchak, Bangkok | 23/7/2005 | 5/8/2005 |
| 10 | SFC1909 | *Chaetospermum camelliae* | Dead leaf: *Typha angustifolia* | Lam Luk Ka, Pathum Thani | 1/6/2005 | 6/7/2005 |
| 11 | SFC1913 | *Coniella castaneicola* | Dead leaf: *Eucalyptus* sp. | Kasetsart University, Bangkok | 9/7/2005 | 15/7/2005 |
| 12 | SFC1915 | *Pestalotiopsis* sp. | Dead leaf: *Eucalyptus* sp. | Kasetsart University, Bangkok | 9/7/2005 | 15/7/2005 |
| 13 | SFC1917 | *Bartalinia robillardoides* | Dead leaf: *Lagerstroemia loudonii* | Kasetsart University, Bangkok | 9/7/2005 | 25/7/2005 |
| 14 | SFC1918.1 | *Phoma* sp. | Dead leaf: *Eucalyptus* sp. | Kasetsart University, Bangkok | 9/7/2005 | 25/7/2005 |
| 15 | SFC1918.2 | *Phoma* sp. | Dead leaf: *Eucalyptus* sp. | Kasetsart University, Bangkok | 9/7/2005 | 25/7/2005 |
| 16 | SFC1919 | *Chaetomella raphigera* | Dead leaf: *Eucalyptus* sp. | Kasetsart University, Bangkok | 9/7/2005 | 25/7/2005 |
| 17 | SFC1920 | Unidentified (pycnidia) | Dead leaf: *Eucalyptus* sp. | Kasetsart University, Bangkok | 9/7/2005 | 25/7/2005 |
| 18 | SFC1921 | *Bartalinia robillardoides* | Dead leaf: *Lagerstroemia loudonii* | Kasetsart University, Bangkok | 9/7/2005 | 17/8/2005 |
| 19 | SFC1922 | *Mycotribulus mirabilis* | Dead leaf: *Lagerstroemia loudonii* | Kasetsart University, Bangkok | 9/7/2005 | 17/8/2005 |
| 20 | SFC1923 | *Robillarda* sp. | Dead leaf: *Lagerstroemia loudonii* | Kasetsart University, Bangkok | 9/7/2005 | 17/8/2005 |
| 21 | SFC1924 | *Pestalotiopsis* sp. | Dead leaf: *Lagerstroemia loudonii* | Kasetsart University, Bangkok | 9/7/2005 | 17/8/2005 |
| 22 | SFC1925 | *Chaetospermum camelliae* | Dead leaf: *Lagerstroemia loudonii* | Kasetsart University, Bangkok | 9/7/2005 | 17/8/2005 |
| 23 | SFC1926 | *Chaetospermum camelliae* | Dead leaf: *Lagerstroemia speciosa* | Kasetsart University, Bangkok | 9/7/2005 | 17/8/2005 |
| 24 | SFC1927 | *Wiesneriomyces conjunctosporus* | Dead leaf: *Lagerstroemia speciosa* | Kasetsart University, Bangkok | 9/7/2005 | 17/8/2005 |
| 25 | SFC1928 | *Xepicular* sp. | Dead leaf: *Lagerstroemia speciosa* | Kasetsart University, Bangkok | 9/7/2005 | 17/8/2005 |
| 26 | SFC1929 | *Wiesneriomyces* sp. | Dead leaf: *Lagerstroemia speciosa* | Kasetsart University, Bangkok | 9/7/2005 | 18/8/2005 |
| 27 | SFC1930 | *Wiesneriomyces laurinus* | Dead leaf: *Lagerstroemia speciosa* | Kasetsart University, Bangkok | 9/7/2005 | 18/8/2005 |

| No. | OriginalCode | Species | Substrate | Location | CollectionDate | IsolationDate |
|---|---|---|---|---|---|---|
| 28 | SFC1931 | *Pestalotiopsis* sp. | Dead leaf: *Lagerstroemia speciosa* | Kasetsart University, Bangkok | 9/7/2005 | 18/8/2005 |
| 29 | SFC1932 | *Lasiodiplodia theobromae* | Dead leaf: *Lagerstroemia speciosa* | Kasetsart University, Bangkok | 9/7/2005 | 18/8/2005 |
| 30 | SFC1933 | *Coniella castaneicola* | Dead leaf: *Lagerstroemia speciosa* | Kasetsart University, Bangkok | 9/7/2005 | 18/8/2005 |
| 31 | SFC1934 | *Pseudorobillarda sojae* | Dead leaf: *Lagerstroemia speciosa* | Kasetsart University, Bangkok | 9/7/2005 | 18/8/2005 |
| 32 | SFC 01164 | *Satchmopsis brasiliensis* | Dead leaf | Khao Yai National Park | 15/06/2005 | 06/07/2005 |
| 33 | SFC 01165 | *Pseudorobillarda sojae* | Dead leaf | Khao Yai National Park | 15/06/2005 | 06/07/2005 |
| 34 | SFC 01166 | *Chaetospermum artocarpi* | Dead leaf | Khao Yai National Park | 15/06/2005 | 06/07/2005 |
| 35 | SFC 01167 | *Diplodia* sp. | Twig | Khao Yai National Park | 15/06/2005 | 06/07/2005 |
| 36 | SFC 01168 | *Chaetospermum camelliae* | Dead leaf | Khao Yai National Park | 15/06/2005 | 06/07/2005 |
| 37 | SFC 01169 | *Pestalotiopsis* sp. | Dead leaf | Pathum Thani | 20/04/2005 | 06/07/2005 |
| 38 | SFC 01170 | *Chaetospermum camelliae* | Dead leaf | Pathum Thani | 20/04/2005 | 06/07/2005 |
| 39 | SFC 01171 | *Chaetospermum camelliae* | Dead leaf | Khao Yai National Park | 15/06/2005 | 06/07/2005 |
| 40 | SFC 01172 | Unidentified | Dead leaf | Khao Yai National Park | 15/06/2005 | 15/07/2005 |
| 41 | SFC 01173 | *Coniella castaneicola* | Dead leaf | Khao Yai National Park | 09/07/2005 | 15/07/2005 |
| 42 | SFC 01174 | *Pestalotiopsis* sp. | Dead leaf | Bangkok | 09/07/2005 | 15/07/2005 |
| 43 | SFC 01176 | *Chaetomella raphigera* | Dead leaf | Bangkok | 09/07/2005 | 25/07/2005 |
| 44 | SFC 01177 | Unidentified | Dead leaf | Bangkok | 09/07/2005 | 25/07/2005 |
| 45 | SFC 01190 | *Chaetospermum camelliae* | Dead leaf | Khao Yai National Park | 27/09/2005 | 04/11/2005 |
| 46 | SFC 01191 | *Chaetospermum camelliae* | Dead leaf | Khao Yai National Park | 14/09/2005 | 06/10/2005 |
| 47 | SFC 01197 | *Satchmopsis brasiliensis* | Dead leaf | Khao Yai National Park | 13/09/2005 | 20/10/2005 |
| 48 | SFC 01198 | *Dinemasporium* sp. | Dead leaf | Khao Yai National Park | 14/09/2005 | 07/10/2005 |
| 49 | SFC 01199 | *Ceuthuspora* sp. | Dead leaf | Khao Yai National Park | 13/09/2005 | 20/10/2005 |
| 50 | SFC 01200 | *Pseudorobillarda sojae* | Dead leaf | Khao Yai National Park | 14/09/2005 | 19/10/2005 |
| 51 | SFC 01208 | *Pseudorobillarda sojae* | Dead leaf | Khao Yai National Park | 13/09/2005 | 14/12/2005 |
| 52 | GR 268 | Coelomycetes | Nypa (frond) | Trang | | 15/11/2005 |
| 53 | GR 269 | Coelomycetes | Nypa (frond) | Trang | | 15/11/2005 |
| 54 | GR 282 | Coelomycetes | Nypa (frond) | Trang | | 15/11/2005 |
| 55 | GR 283 | Coelomycetes | Nypa (frond) | Trang | | 15/11/2005 |
| 56 | GR 309 | Coelomycetes cf Rhabdospora rhizophorae | Sonneratia wood | Morib, Malasia | | 8/12/2005 |

| No. | OriginalCode | Species | Substrate | Location | CollectionDate | IsolationDate |
|---|---|---|---|---|---|---|
| 57 | GR 310 | Coelomycetes cf Rhabdospora rhizophorae | Sonneratia wood | Morib, Malasia | | 8/12/2005 |
| 58 | GR 311 | Coelomycetes cf Rhabdospora rhizophorae | Sonneratia wood | Morib, Malasia | | 8/12/2005 |
| 59 | GR 316 | Coelomycetes Yellow | Sonneratia wood | Morib, Malasia | | 7/12/2005 |
| 60 | GR 349 | *Phoma* sp. | Trang | Pine Wood | | 29/1/2006 |
| 61 | GR 350 | *Phoma* sp. | Trang | Pine Wood | | 29/1/2006 |

## 4. THE PHYLOGENETIC RELATIONSHIPS OF SELECTED COELOMYCETE GENERA:

### 4.1 Molecular methods

See the 6-month progress report

### 4.2 Sequenced species

Over the last 6 months, DNA of 9 coelomycetes have been sequenced for the ITS regions. Eight coelomycetes: *Chaetospermum camelliae, Guilia tenuis, Infundibulomyces cupulata, Mycotribulus mirabilis, Robillarda sessilis, Satchmopsis brasiliensis* and *Xepiculopsis graminea* have been sequenced by using primers NS5 and ITS4, while for *Infundibulomyces* sp. (SFC 981) primers used were ITS4 and ITS5 (White *et al.*, 1990; Landvik, 1996) and are listed in Table 2. The ITS region of DNA gene of coelomycetes were sequence by Macrogen, Korea.

**Table 2. Primers used for PCR and DNA sequencing of ITS regions**

| Primers | Sequence (5' – 3') |
|---|---|
| NS1 | GTA GTC ATA TGC TTG TCT C |
| ITS4 | TCC TCC GCT TAT TGA TAT GC |
| ITS5 | GGA AGT AAA AGT CGT AAC AAG G |

Details of consensus sequences and list of PCR products of coelomycetes have been updated from the last 6 months in Table 3.

**Table 3. Detail of consensus sequence of coelomycetes that have been performed and data update from the last 6 months (highlighted area)**

| No. | Coelomycetes | BCC code | Sequenced | | | BLAST searched | Sequenced alignment |
|---|---|---|---|---|---|---|---|
| | | | 18S | 28S | ITS | | |
| 1 | *Chaetospermum camelliae* | 13401 | ✓ | ✓ | ✓ | ✓ | ✓ (18S, 28S) |
| 2 | *Guilia tenuis* | 13066 | ✓ | ✓ | ✓ | ✓ | ✓ (18S, 28S) |
| 3 | *Infundibulomyces cupulata* | 13400 | ✓ | ✓ | ✓ | ✓ | ✓ (18S, 28S) |
| 4 | *Infundibulomyces* sp. SFC 981 | - | ✓ | ✓ | ✓ | ✓ | ✓ (18S, 28S) |
| 5 | *Mycotribulus mirabilis* | 13341 | ✓ | ✓ | ✓ | ✓ | ✓ (18S, 28S) |
| 6 | *Pseudorobillarda siamensis* | 12531 | ✓ | ✓ | ✓ | ✓ | ✓ (18S, 28S) |
| 7 | *Robillarda sessilis* | 13393 | ✓ | ✓ | ✓ | ✓ | ✓ (18S, 28S) |
| 8 | *Satchmopsis brasiliensis* | 18579 | ✓ | ✓ | ✓ | ✓ | ✓ (18S, 28S) |
| 9 | *Xepiculopsis graminea* | - | ✓ | ✓ | ✓ | ✓ | ✓ (18S, 28S) |

### 4.3. Phylogenetic analysis:

The DNA of nine coelomycete species has been extracted. All of the 18S, 28S regions were amplified and sequenced. All the ITS regions have been also amplified and sequenced but have not yet been analysed (Table 4). The 18S and 28S genes were sequenced as they contain highly conserved regions suitable for analysis of their familial and ordinal position, the primary objective of this study.

The sequences were analysed along with other sequences obtained from the Genbank Database with suitable outgroup taxa. Sequences are 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 4.0b8 (Swofford, 2002). Phylogenetic trees are generated using maximum parsimony criteria. Followed by a heuristic search with a stepwise starting tree, a random stepwise addition on 10 replicates and tree-bisection-reconnection branch-swapping algorithm. Bootstrap analysis is performed for testing the robustness of the final trees.

**Table 4. List of coelomycetes sequenced by Macrogen**

| No. | Coelomycetes | DNA extracted | 18S (primers: NS1-NS23UCBR) | | 28S (primers: LR7-LROR) | | ITS1-5.8S-ITS2 (primers: NS5-ITS4) | |
|---|---|---|---|---|---|---|---|---|
| | | | PCR | Sequenced | PCR | Sequenced | PCR | Sequenced |
| 1 | *Chaetospermum camelliae* | + | + | + | + | + | + | + |
| 2 | *Guilia tenuis* | + | + | + | + | + | + | + |
| 3 | *Infundibulomyces cupulata* | + | + | + | + | + | + | + |
| 4 | *Infundibulomyces* sp. SFC 981 | + | + | + | + | + | + | + |
| 5 | *Mycotribulus mirabilis* | + | + | + | + | + | + | + |
| 6 | *Pseudorobillarda siamensis* | + | + | + | + | + | + | + |
| 7 | *Robillarda sessilis* | + | + | + | + | + | + | + |
| 8 | *Satchmopsis brasiliensis* | + | + | + | + | + | + | + |
| 9 | *Xepiculopsis graminea* | + | + | + | + | + | + | + |

+ = have been made

**4.4 Analysis of SSU data for all nine coelomycetes studied.**

According to the BLAST searches, we have been analysing the SSU sequences of nine coelomycetes: *Infundibulomyces cupulata, Infundibulomyces* sp. SFC 981, *Satchmopsis brasiliensis, Pseudorobillarda siamensis, Robillarda sessils* and *Xepiculopsis graminea, Chaetospermum camelliae, Guilia tenuis* and *Mycotribulus mirabilis.* Figures 1 and 2 show a neighbor-joining tree obtained from total 59 taxa analysed (the Xylariales, Diaporthales, Ophiostomatales, Sordariales, Hypocreales, Halosphaeriales, Microascales, Helotiales, Pezizales and Hypocreales were chosen for the analysis, using the Yeasts (*Candida albicans* and *Pichia* sp.) as the outgroup). They fall into many different orders, including the Hypocreales, Sordariales, Xylariales and Helotiales (Figures 1, 2).

*Xepiculopsis graminea* grouped within the Hypocreales within a clade comprising *Myrothecium* and *Didymostilbe* (68 % bootstrap support, Figure 2). More taxa from the Hypocreales were incorporated and analysed in more detail and results are presented in section 4.5B.

*Infundibulomyces cupulata* and *Infundibulomyces* sp. SFC 981 are monophyletic and were placed consistently in the Sordariales. These two species were compared with *Satchmopsis brasiliensis,* which has the same type of nidulariaceous-like conidiomata (Sutton, 1975). Data matrix of these three species has been analysed and results presented in section 4.5C.

Analysis of sequence data shows that *Robillarda sessilis* and *Pseudorobillarda siamensis* nestled in the Xylariales with moderate bootstrap support (Figures 1, 2).

*Satchmopsis brasiliensis* is well placed within the Helotiales but with low bootstrap support (< 50 %, Figure 2). From this analysis there is no closely related taxon for *S. brasiliensis*. More taxa within the Helotiales will be further incorporated into the analysis.

Moreover the last three genera: *Chaetospermum camelliae*, *Guilia* sp. and *Mycotribulus mirabilis*, were analysed along with the same data matrix. From the molecular results, these three genera grouped together as a basal clade. However, BLAST searches indicate that these three genera showed affinities with some Basidiomycota. We did the analysis based on SSU along with many basidiomycetes and the trees shown in section 4.5D.

In conclusion from this dataset, the overall view for the teleomorphs of all nine ceolomycetes may be found in many different orders, including the Hypocreales, Sordariales, Xylariales, Helotiales and possibly within the Basidiomycota.

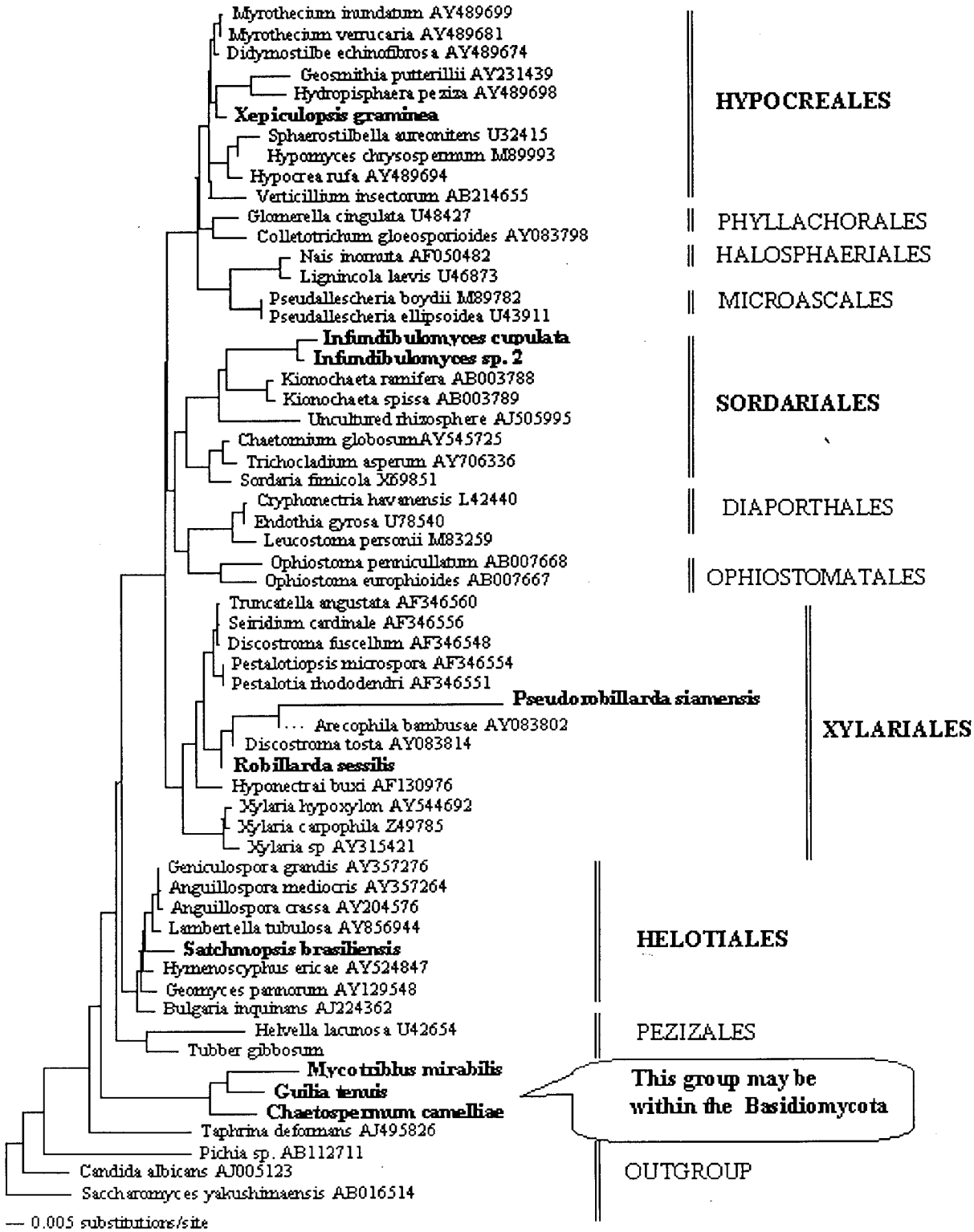


**Figure 1.** Neighbor-joining tree obtained from SSU sequences analysis of all nine coelomycetes studied.

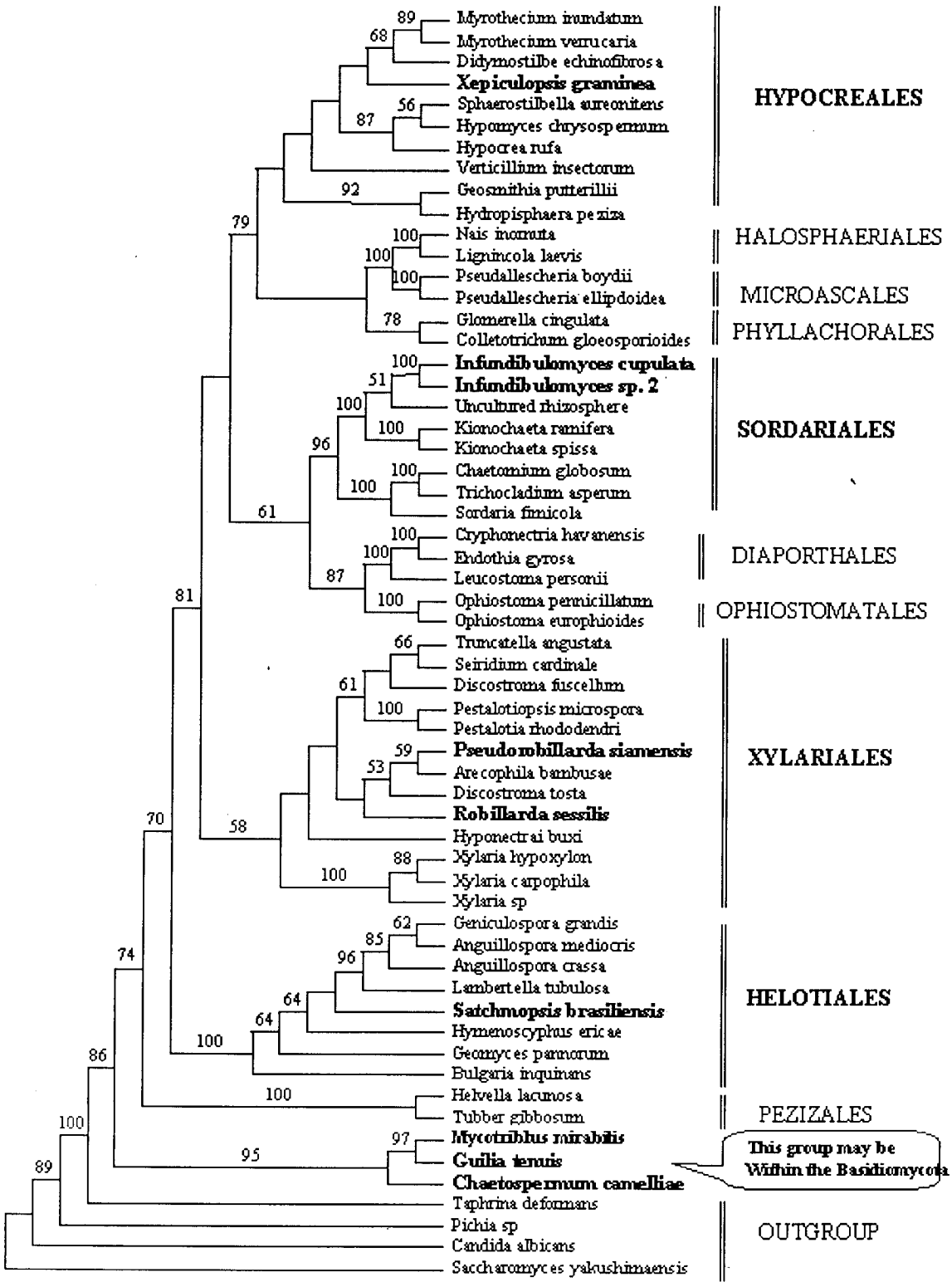


**Figure 2.** A bootstrap tree from neighbor-joining analysis obtained from SSU sequences of all nine coelomycetes studied. Bootstrap values higher than 50 % are shown above the clades.

### 4.5. Analysis of SSU data for each species aligned:

### 4.5A. Phylogenetic study of *Robillarda sessilis*

*Robillarda sessilis* was isolated from a *Eucalyptus camaldulensis* leaf, and produced fusiform, one septate conidia, bearing 3-4 branched, tubular appendages (Figure 3).

The SSU sequence of *Robillarda sessilis* was 1,102 bp long. Figure 4 shows one of the most parsimonious trees (Tree length = 808 steps, CI = 0.633, RI = 0.788). Total 38 taxa from the Xylariales, Diaporthales, Ophiostomatales, Sordariales, Hypocreales, Halosphaeriales, Microascales were chosen for the analysis, using the Lulworthiales as the outgroup. The placement for all orders in this analysis are supported by high bootstrap values.

*Robillarda sessilis* is well placed within the Amphisphaeriaceae, Xylariales. The most closely related taxon for *R. sessilis*, based on the phylogenetic analysis, is *Discostroma fuscellum*. They group together with the genera *Seiridium, Truncatella* and *Discostroma* (clade A) with 88 % bootstrap values (Figure 4).

Our molecular data shows that *R. sessilis* groups within clade A, comprising mainly coelomycetes. It shares some common features with other genera in this clade: fusiform, septate conidia with truncate apical cells and tubular, branched polar appendages. Conidia of *R. sessilis* are hyaline, whereas the median cells of related taxa are brown.

Figure 4 gives a preliminary result for the phylogenetic position of *R. sessilis*, while the LSU and ITS1-5.8S-ITS2 data await analysis.

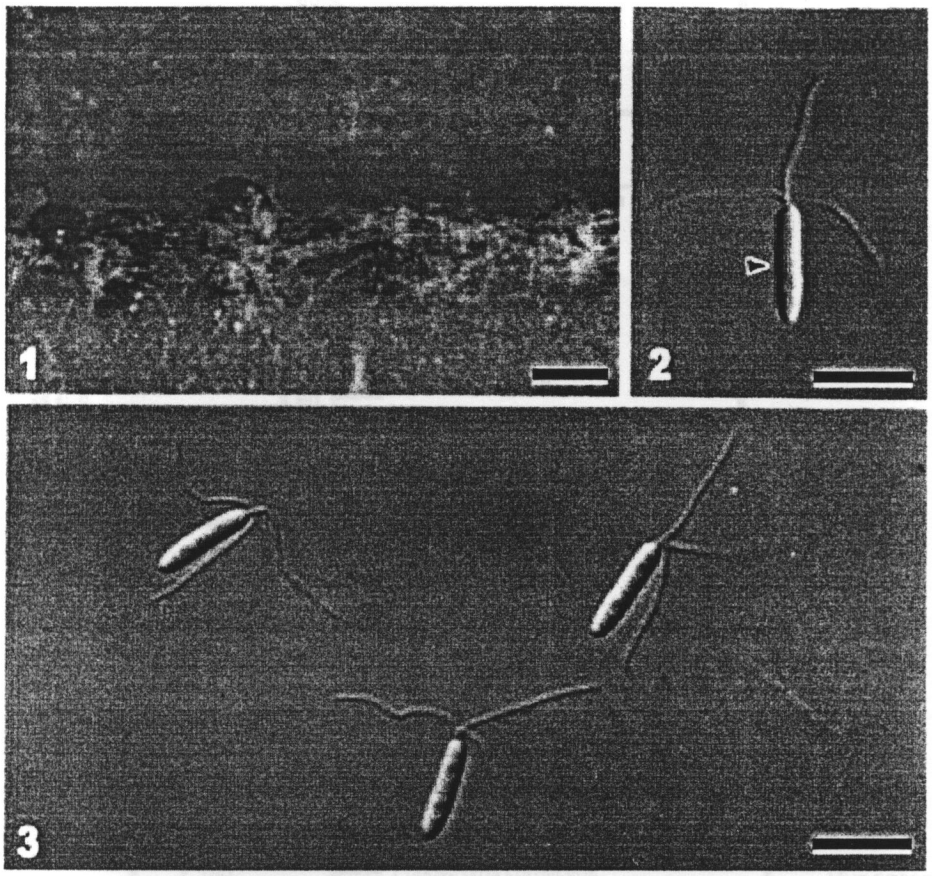


**Figure 3:** 1-3). *Robillarda sessilis* Light micrographs. 1). Pycnidia, immersed, unilocular, globose. Scale bar = 200 μm . 2-3). Mature conidia, fusiform straight or slightly curved, 1-septate (arrowed), colourless. Conidia bearing attenuated appendages. Scale bar = 10 μm.

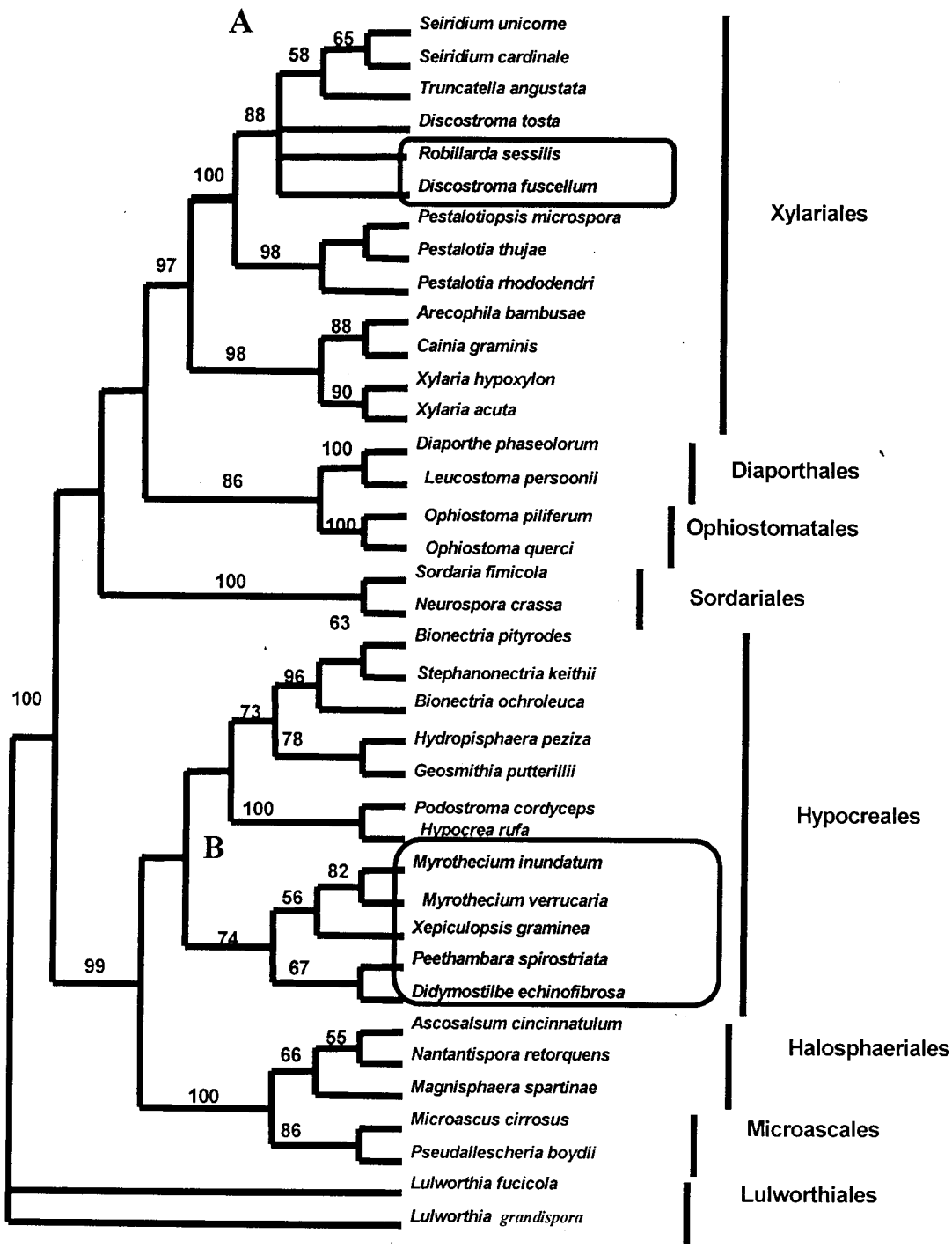


**Figure 4.** One of the most parsimonious trees of the SSU rDNA analysis of *Robillarda sessilis* and *Xepiculopsis graminea*. Bootstrap values higher than 50 % are shown above the clades.

**4.5B. Phylogenetic study of *Xepiculopsis graminea***

*Xepiculopsis graminea* is a saprophyte on grass leaves, forming fusiform to ellipsoidal conidia with an acute apex and a narrow truncate base, with apical mucoid appendages (Figure 5).

From the same analysis of *Robillarda sessilis*, the SSU sequence of *Xepiculopsis graminea* (1,107 bp long) was analysed (Figure 4) and shown to be well placed within the Hypocreales. It shows phylogenetic affinity with members of the Bionectriaceae, Hypocreales, especially the genus *Myrothecium* (anamorphic ascomycete), but with low bootstrap support of 56 % (clade B, Figure 4).

*Xepiculopsis graminea* and *Myrothecium* share some morphological characters such as: fusiform to ellipsoidal conidia with an acute apex and a narrow truncate base, with apical mucoid appendages. Our current molecular data based on SSU sequences does not totally resolved the phylogeny of *X. graminea*, and a further LSU region and ITS1-5.8S-ITS2 data remains to be analysed.

**Figure 5.** Mature conidia of *Xepiculopsis graminea* with funnel-shaped, apical mucoid appendage. Scale bar = 5 μm.

**4.5C: Phylogenetic study of *Infundibulomyces cupulata, Infundibulomyces* sp. (SFC981) and *Satchmopsis brasiliensis***

*Infundibulomyces cupulata* (Figures 6-8) was isolated from fallen leaves of *Lagerstroemia* sp. while *Infundibulomyces* sp. SFC 981 (Figure 9) was isolated from an angiosperm leaf. *Infundibulomyces cupulata* and *Infundibulomyces* sp. SFC 981 produced cylindrical conidia with an obtuse apex and obtuse but protuberant base, bearing two

appendages at each end: appendages are tubular, filiform and flexuous. Conidia of *Infundibulomyces cupulata* measure 6-10 x 1-1.5 μm length while conidia of *Infundibulomyces* sp. SFC 981 are shorter (Plaingam, *et al.*, 2003; Plaingam, 2002).

*Infundibulomyces* resembles *Satchmopsis brasiliensis* in its nidulariaceous-like conidiomata but differs in having holoblastic conidiogenesis and appendage conidia. *Satchmopsis brasiliensis* possess enteroblastic conidogenesis and conidia without appendages and is common on leaf litter (Sutton, 1975; Plaingam, *et al.*, 2003).

*Satchmopsis brasiliensis* was isolated from a dead leaf, and produced cylindrical conidia without an appendage in nidulariaceous-like conidiomata (Figure 10).

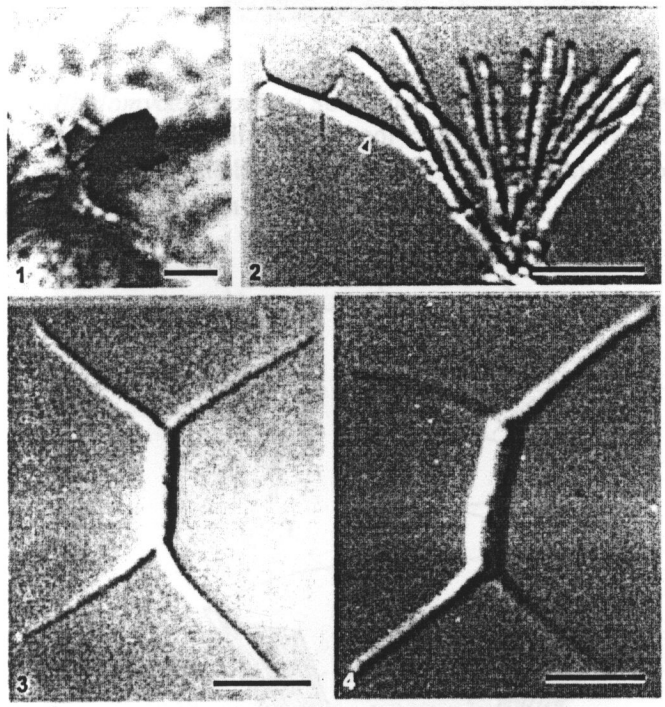


**Figure 6 :** **1-4.** *Infundibulomyces cupulata* Light micrographs (After Plaingam *et al.*, 2003). 1. Stromatic conidiomata on leaf of Lagerstroemia sp. Scale bar = 100 μm. 2. Cylindrical conidiogenous cell (arrowed) with conidia at different stages of development. 3-4. Mature conidia bearing two sub-polar appendages at both ends. Scale bar = 10 μm

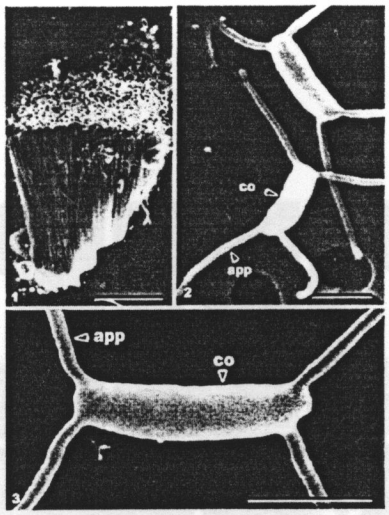


**Figure 7 :** 1-3 *Infundibulomyces cupulata* Scanning electron micrographs (After Plaingam *et al.*, 2003). 1.Stromatic conidioma on a polycarbonate membrane, funnel-shaped (arrowed) and lacking setae. Scale bar = 50 μm. 2-3. Mature condia (co) bearing two sub-polar appendages (app) at both ends. Scale bar = 5 μm.

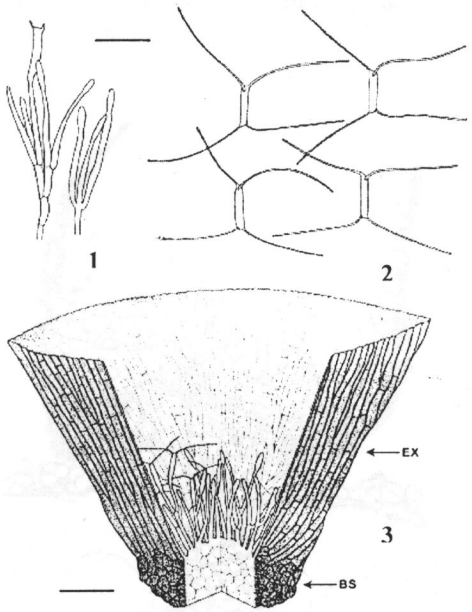


**Figure 8:** **1-3** *Infundibulomyces cupulata* Line drawings (After Plaingam *et al.*, 2003).
1. Conidiophores, conidiogenous cells and developing conidia invested in mucus. 2. Mature conidia with a drop of mucilage at the tip of the appendages. Scale bar = 20 μm. 3. Vertical section of a conidioma on natural substrate, comprises textura angularis for the basal stromata (BS) and textura prismatica for the excipular (EX); the upper cells of basal stromata give rise to branched conidiophores. Scale bar =10 μm.

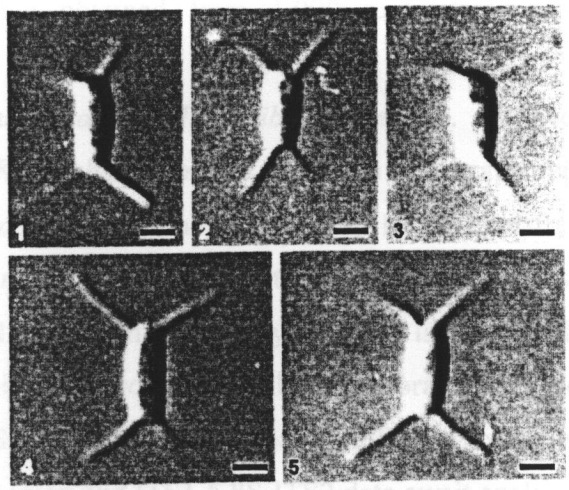


**Figure 9:** 1-5 *Infundibulomyces* sp. SFC981 Light micrographs. Mature conidia with two appendages at each end. Scale bar = 5 μm.

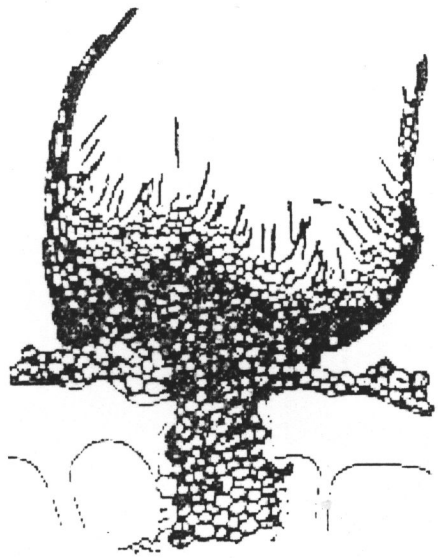

**Figure 10:** *Satchmopsis brasiliensis* Line drawing. Conidia were produced within nidulariaceous-like conidiomata (After Nag Raj, 1993).

The SSU sequences of *Infundibulomyces cupulata, Infundibulomyces* sp. and *Satchmopsis brasiliensis* were 1,807 long. Total 23 taxa (from the Sordariales, Xylariales and Helotiales) resulting from the preliminary analysis from section 4.4 were chosen for the analysis, using *Candida albicans* and *Pichia* sp. as the outgroup. Figure 11 shows a 50% majority rule consensus tree obtained from 1,000 replicates bootstrap from maximum parsimony analysis.

*Infundibulomyces cupulata* and *Infundibulomyces* sp. are well placed within the Sordariales in a clade with *Kionochaeta* species with high bootstrap support. *Infundibulomyces cupulata* and *Infundibulomyces* sp. are monophyletic with 100 % bootstrap values (Figure 11).

*Satchmopsis brasiliensis* is well placed within the Helotiales (Discomycetes) with 54 % bootstrap support (Figure 11). From this analysis there is no closely related taxon to *S. brasiliensis*. More taxa within the Helotiales will be incorporated into the analysis. Our current molecular data, based on SSU sequences, may not be sufficient to resolve the phylogeny of *S. brasiliensis*. A further LSU region and ITS1-5.8S-ITS2 data await analysis.

From the data, nidulariaceous-like conidiomata have arisen more than once as *Infundibulomyces* and *Satchmopsis* are distantly placed and share few other common morphological features.

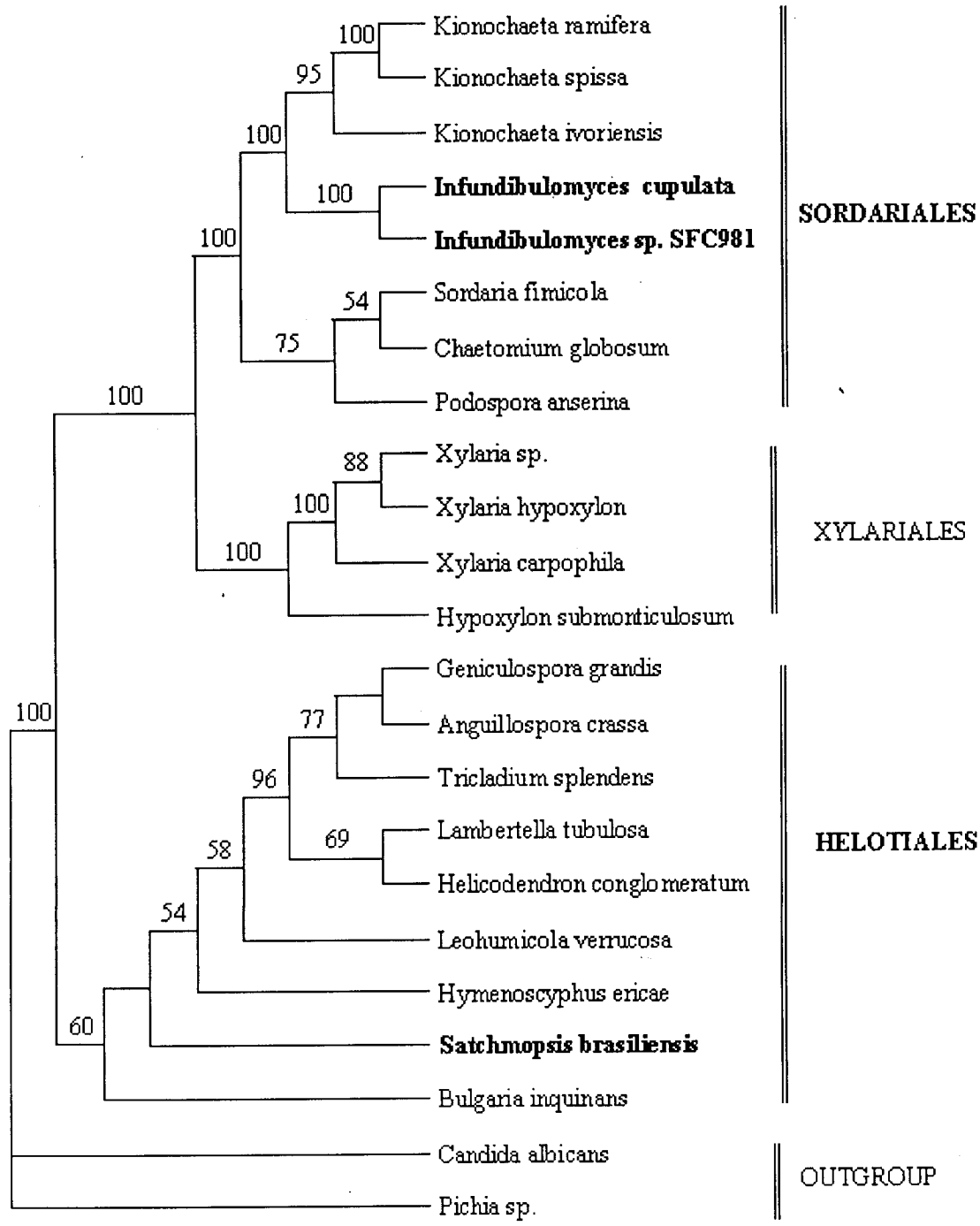


**Figure 11.** A 50% majority rule consensus tree of the SSU rDNA analysis of *Infundibulomyces cupulata, Infundibulomyces* sp. (SFC981) and *Satchmopsis brasiliensis.* Bootstrap values higher than 50 % are shown above the clades.

**4.5D: Phylogenetic study of *Chaetospermum camelliae*, *Guilia tenuis* and *Mycotribulus mirabilis.***

*Chaetospermum camelliae* was isolated from grass clumps, and produced unicellular, cylindrical conidia with obtuse ends, straight or slightly curved, smooth, hyaline and bearing 8 subpolar appendages in groups of two; appendages tubular, filiform, attenuated, unbranched, flexuous. (Figure 12)

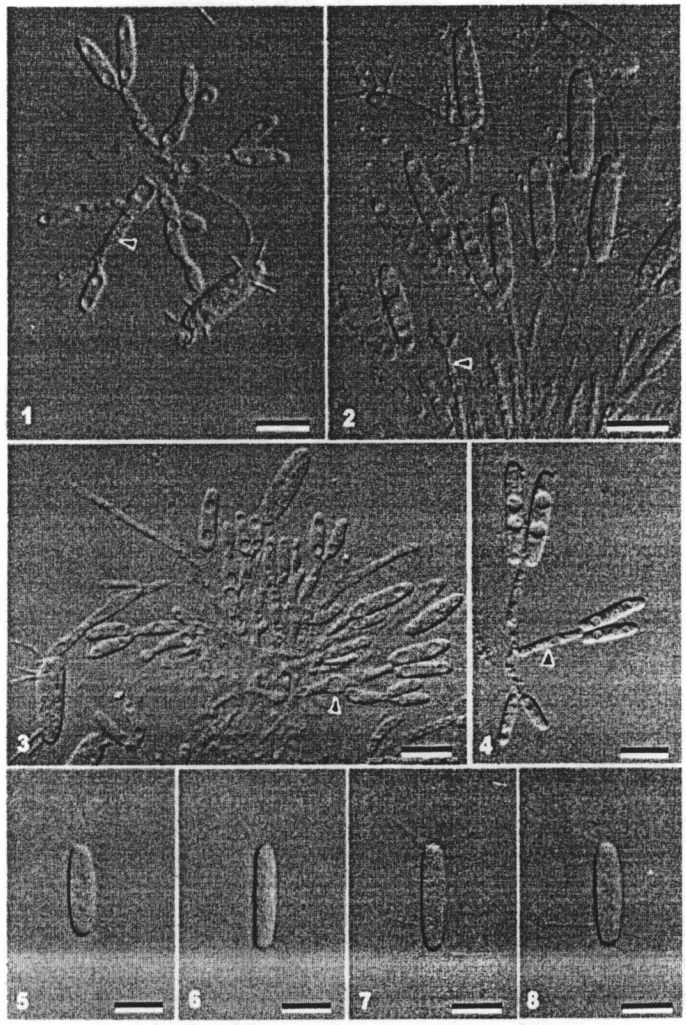


**Figure 12.** 1-8. *Chaetospermum camelliae* Light microscope micrographs.
1-4. Conidiogenous cells (arrowed) producing conidia at different times.
5-8. Mature conidia with 8 subpolar appendages in groups of two.
Scale bar = 10 μm

*Giulia tenuis* was isolated from a leaf of *Bambusa arundinacea*, and produced obclavated conidia, slightly tapered toward the narrow, truncate base and attenuated at the apex, unicellular, colourless, smooth, bearing a cap-like or irregular, mucilaginous appendages at the apex. (Figure 13)

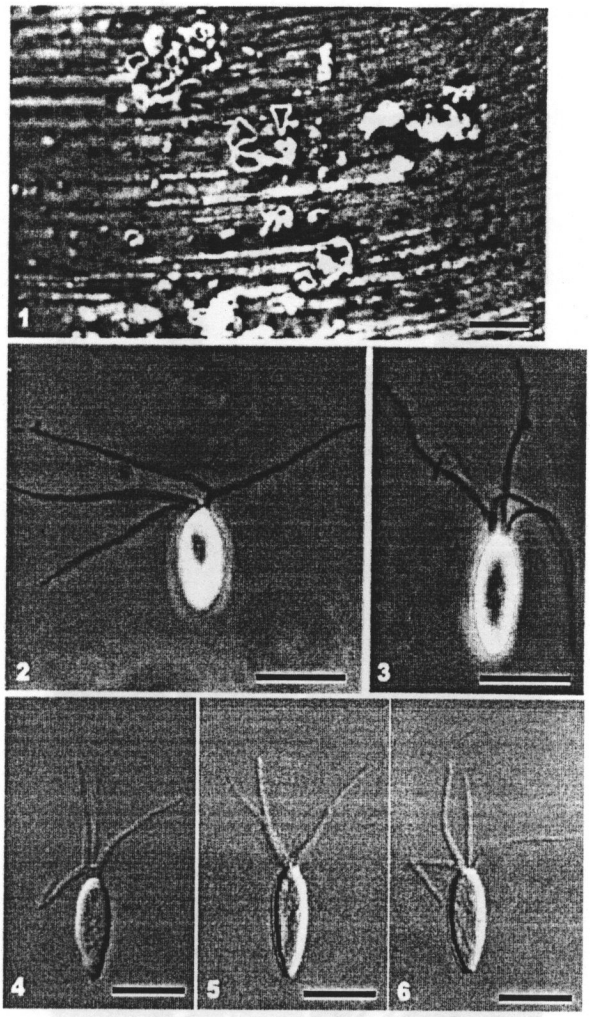


**Figure 13:** 1-6. *Giulia tenuis* Light micrographs. 1. Pycnidia on wood with a circus of released conidia (arrow = conidial mass). Scale bar = 200 μm 2-6. Mature conidia, cylindrical with an obtuse apex and truncate base, straight or curved, unicellular, colourless, bearing 4-7 apical extra-cellular appendages; filiform, flexuous. Scale bar = 20 μm.

*Mycotribulus mirabilis* was isolated from a leaf of *Eucalyptus camaldulensis*, and produced conidia naviculate to fusiform with and acute apex and a somewhat truncate base, unicellular, colourless, smooth, guttulate, bearing tubular, filiform, flexuous appendages at both ends; apical appendages single, polar, unbranched, straight or curved; basal appendage, inserted laterally slightly above the truncate base, unbranched, divergent, straight or often curved (Figure 14)

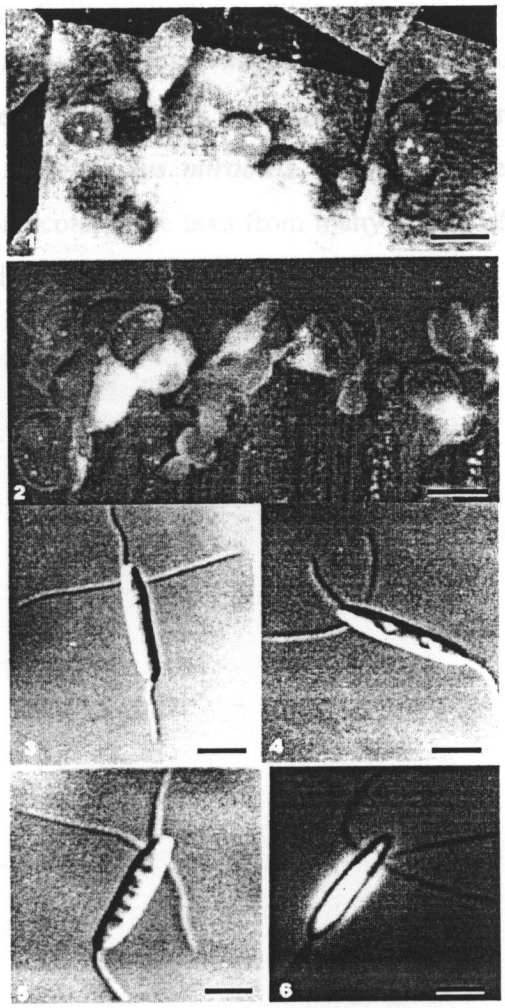

**Figure 14 :** 1-6. *Mycotribulus mirabilis* Light micrographs.1. Pycnidia on filter paper in culture. 2. Pycnidia on bamboo leaf in culture. Scale bar = 200 μm.
3-6. Mature conidia, fusiform with a truncate base and acute apex, unicellular, colourless, bearing 3 basal appendages and a single apical appendage. Scale bar = 5 μm.

The SSU sequences of these three genera were 1,779 long. A total 19 taxa (from the Agaricales, Ceratobasidiales and Aphyllophorales, Basidiomycota) resulting from the preliminary BLAST search were chosen for the analysis, using Urediniomycetes as the outgroup. Figures 15, 16 resulting from maximum parsimony analysis, showed that they are well positioned within the Basidiomycota. *Mycotribulus mirabilis* is well placed within the Agaricales with high support (86 %), with *Physalacria maipoensis* and *Athelia bombacina* as sister taxa. *Guilia tenuis* groups with *Tretopileus sphaerophorus* with 89 % bootstrap values within the Aphyllophorales. Finally, *Chaetospermum camelliae* formed a basal clade.

The data shown are preliminary results but with no related group for *Chaetospermum camelliae*, *Guilia tenuis* and *Mycotribulus mirabilis*. In order to resolve their phylogenetic relationship within the Basidiomycota, more taxa from many orders of the Basidiomycota need to be chosen as well as further DNA regions.

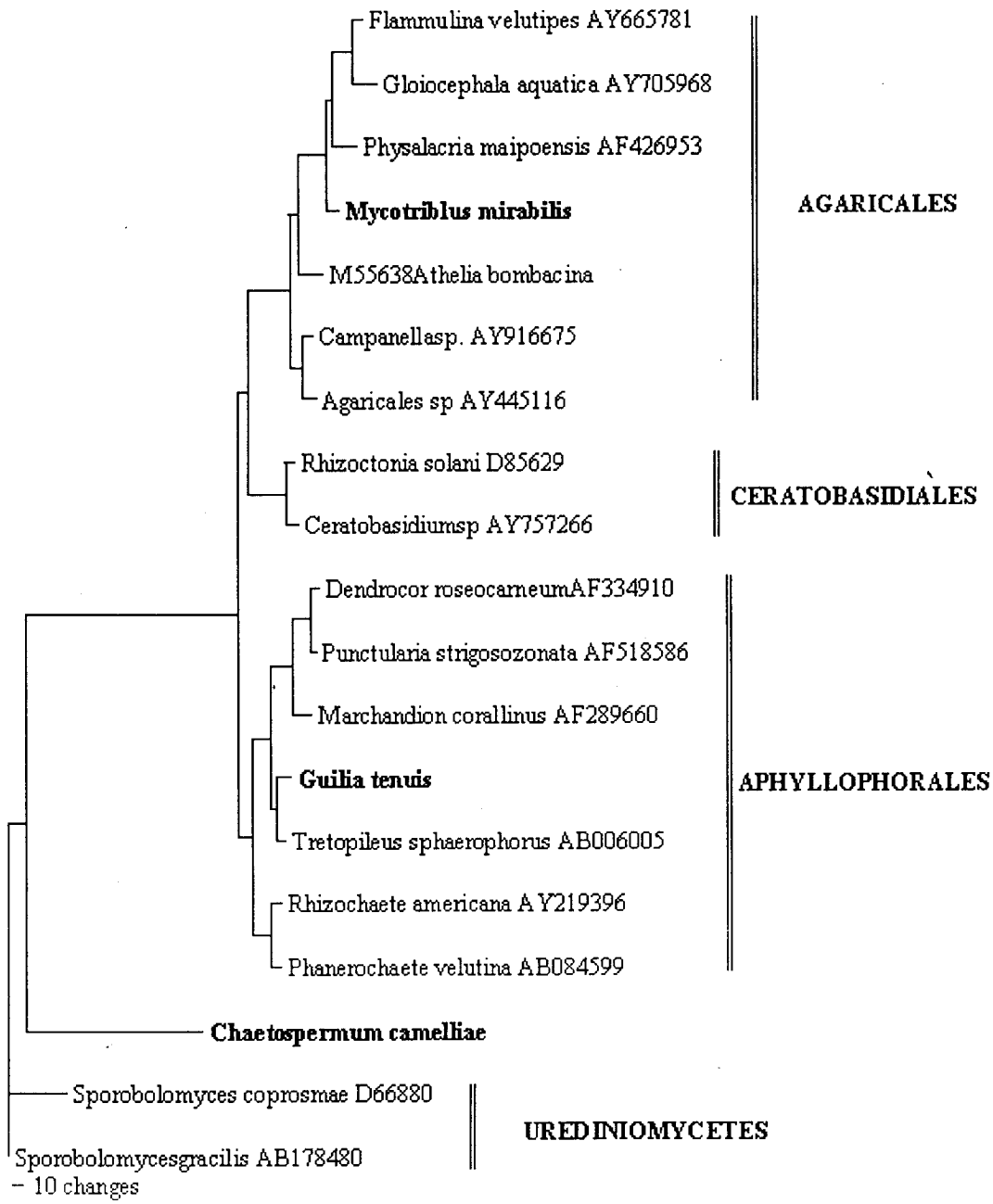


**Figure 15**. One of the most parsimonious trees of the SSU rDNA analysis of *Chaetospermum camelliae*, *Guilia* sp. and *Mycotribulus* sp.

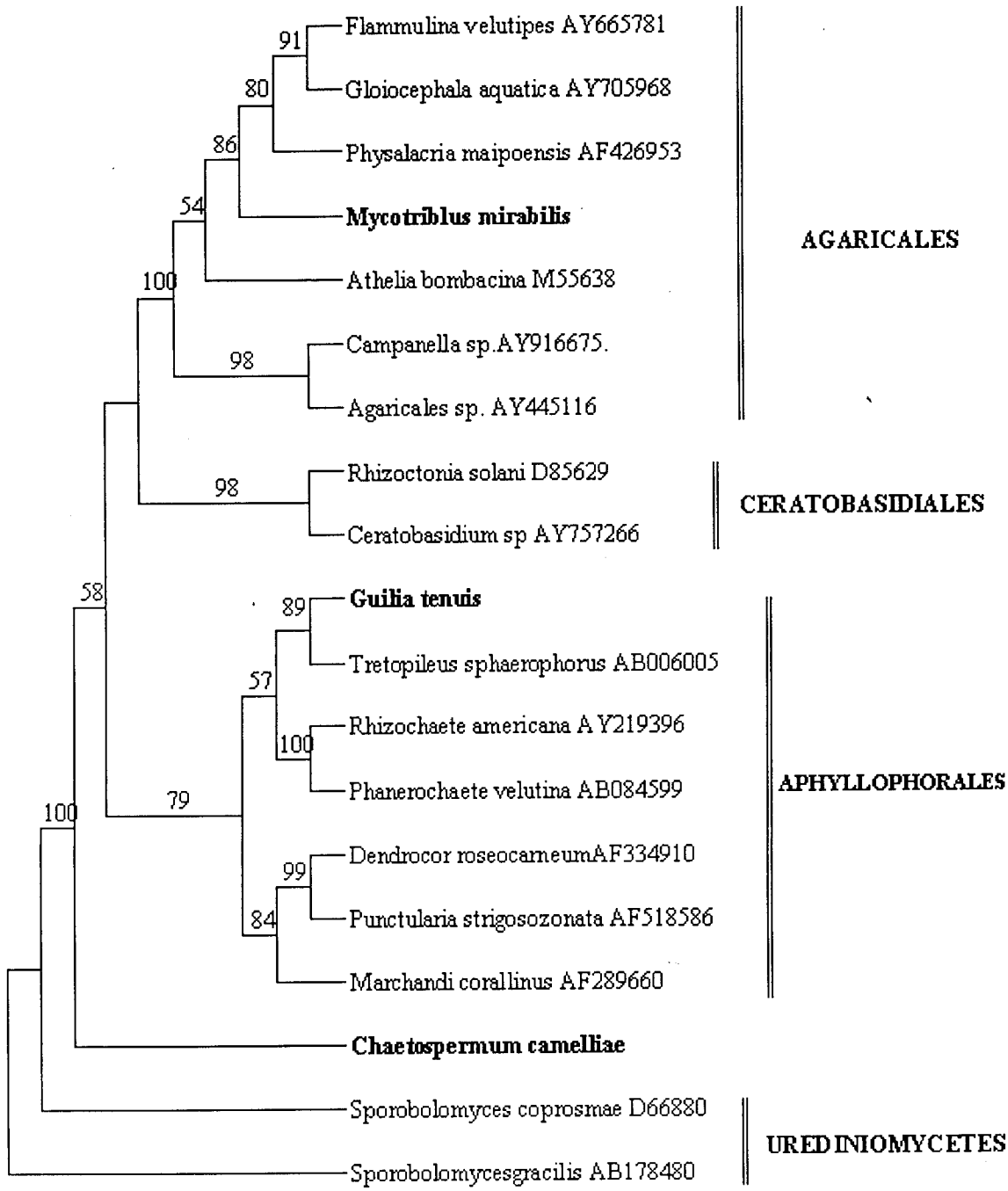


**Figure 16.** The 50% majority rule consensus tree of the SSU rDNA analysis of *Chaetospermum camelliae*, *Guilia* sp. and *Mycotribulus mirabilis* Bootstrap values higher than 50% are shown above clade.

## 5. CONCLUSIONS

- The teleomorph of *Robillarda sessilis* may be found in the Amphisphaeriaceae, Xylariales and is closely related to the anamorphic ascomycete *Discostroma*.
- The teleomorph of *Pseudorobillarda siamensis* may be found in the Xylariales.
- The teleomorph of *Xepiculopsis graminea* may be found in the Bionectriaceae, Hypocreales.
- The teleomorph of *Infundibulomyces cupulata* and *Infundibulomyces* sp. SFC 981 may be found in the Sordariales.
- The teleomorph of *Satchmopsis brasiliensis* may be found in the Helotiales.
- The teleomorph of *Chaetospermum camelliae*, *Guilia tenuis* and *Mycotribulus mirabilis*, may be found in the Basidiomycota.

## 6. FUTURE PLANS

1. The phylogenetic trees for LSU and the ITS1-5.8S-ITS2 regions of *Robillarda sessilis* and *Pseudorobillarda siamensis* data matrix will be constructed in order to complete the analysis.

2. The phylogenetic trees for LSU and the ITS1-5.8S-ITS2 regions of *Xepiculopsis graminea* will be constructed in order to complete the analysis.

3. The phylogenetic trees for LSU and the ITS1-5.8S-ITS2 regions of *Infundibulomyces cupulata, Infundibulomyces* sp. SFC 981 and *Satchmopsis brasiliensis* data matrix will be constructed in order to complete the analysis.

4. The phylogenetic trees for LSU and the ITS1-5.8S-ITS2 regions of *Chaetospermum camelliae*, *Guilia tenuis* and *Mycotribulus mirabilis*, data matrix will be analysed with more taxa in order to see the affinity within Basidiomycota.

## 7. OUTPUT

1. Poster presentation: Narumol Plaingam, Jariya Sakayaroj, Sayanh Somrithipol, and E. B. Gareth Jones "A phylogenetic study of *Robillarda sessilis* and *Xepiculopsis graminea*" the 9 th BRT conference on 10-13 October 2005, Khon Khen, Thailand.
2. 61 coelomycetes from various substrata collected in Thailand, were isolated and deposited in BCC.
3. Nine coelomycetes have been sequenced (SSU, LSU and ITS1-5.8S-ITS2 regions) and SSU phylogenetic trees constructed for *Robillarda sessilis, Xepiculopsis graminea, Infundibulomyces cupulata, Infundibulomyces* sp. SFC 981 and *Satchmopsis brasiliensis, Chaetospermum camelliae, Guilia tenuis* and *Mycotribulus mirabilis.*
4. A poster will be presented at the 8th Internal Mycological Congress in Cairne Australia, August 2006.

## ACKNOWLEDGEMENTS

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# PART B: REVISED GRANT APPLICATION: THE PHYLOGENETIC RELATIONSHIPS OF SELECTED COELOMYCETE GENERA

**For:** National Center for Genetic Engineering and Biotechnology
Biodiversity Research and Training Program (BRT)

**Old Project Title:** Molecular taxonomy of selected genera of coelomycetes and their biodiversity in Thailand

**BRT Project Code:** BRT R_148006

**Principal Investigator:** Prof. E. B. Gareth Jones

**Co Investigator:** Dr. Jariya Sakayaroj

**Assistant Researcher:** Mr. Nattawut Rungjindamai

**Revised Submission:** 14th March 2006

## A: Synopsis

The first year of the research grant focused on sequencing nine coelomycetes we had in culture from a previous Ph.D. study by Dr N. Plaingam. As anamorphic fungi, these could not be classified within the fungal kingdom by culturing techniques. Therefore this molecular study was embarked upon to resolve their taxonomic position. Currently some 60% of all coelomycetes cannot be assigned to any order/family.

With the resignation of Dr. Plaingam and the award of a separate research grant to Dr. Somrithipol, we have consequently redefined the scope of the project. In the revised application we will focus entirely on the molecular phylogeny of coelomycetes as Dr. Somrithipol under his research grant will be responsible for the documentation and isolation of Thai coelomycetes.

## B: Targets for years 2 and 3:

A. To complete the anlaysis and alignment of the 18S, 28S and ITS sequences for nine coelomycetes

B. To evaluate the phylogenetic relationship of a further 20 species per year. Three of these are marine species, others will be from Dr. Somrithipol's study, taxa isolated as endophytes of palms, and from the purchase of selected strains from international culture collections.

As the result of data from year 1 of the grant, we propose to write up three papers:

1. **Phylogeny of two coelomycete genera with cupulate conidiomata based on SSU sequence analysis: *Infundibulomyces cupulata* and *Satchmopsis brasiliensis.* (Narumol Plaingam, Jariya Sakayaroj, Sayanh Somrithipol, and E. B. Gareth Jones)**

Both these genera have cupulate conidiomata but differ in conidial development and morphology. *Infundibulomyces cupulata* has appendaged conidia, while they are lacking in *Satchmopsis brasiliensis.* However despite the similarity in conidiomata, they are distantly placed with *Infundibulomyces cupulata* positioned in the Sordariales with 100% bootstrap

support along with other anamorphic fungi e.g. *Kionochaeta* species. *Satchmopsis brasiliensis* is positioned in the discomycete order Helotiales, with moderate support. A second isolate of *Infundibulomyces* was also isolated from leaf material but differes from the type species in conidial measurements. *Infundibulomyces* species are monophyletic and group with 100% bootstrap support, but differ in conidial morphology, *I. cupulata* with longer narrower conidia (6-10 x 1-1.5μm), while the new taxon has shorter conidia (4-7 x 1-1.5μm).

**2. Phylogenetic relationship of the coelomycete species *Pseudorobillarda siamensis* and *Robillarda sessils*, evaluation of their conidial appendage development as stable criterion for generic delineation. (Narumol Plaingam, Jariya Sakayaroj, Sayanh Somrithipol, and E. B. Gareth Jones)**

Conidia of these species superficially resemble one another with attenuate polar appendages. Besides establishing their ordinal status in the ascomycetes it will test if the similarity in conidial morphology has support at the molecular level.

**3. Are the genera *Chaetospermum, Guilia* and *Mycotribulus* member of the Basidiomycota?**

Although preliminary molecular data suggests an affinity with the Basidiomycota, further evolution of more genes are required for a more definite resolution. Coelomycetes with Basidiomycete teleomoph are rare, especially as none of the species studied bear clamp connections. Thus, further sequences (from GeneBank) from different genes and more taxa (both Ascomycota and Basidiomycota) need to be aligned.

## C: Reasons for revised submission

1. Dr. Somrithipol has new BRT research grant for the collection and identification of Thai Coelomycetes
2. Dr. Narumol has resigned her position due to greater commitments at her University.
3. Appointment of new assistant researcher Mr. Nattawut Rungjindamai to take over the position relinquished by Dr. Narumol (see his CV in the appendix).

## D: Objectives

1. To complete the analysis of data gathered during the first year of the research grant.
2. To sequence other coelomycete species so as to determine their phylogenetic affinities and teleomorph connections. As outlined in the original application, some 60 % of coelomycetes have no known teleomorph (Table 5) and therefore cannot be classified based on their morphology.

**Table 5 . List of selected genera of appendaged coelomycetes, whose teleomorphs has not been established (Nag Raj, 1993)**

| No. | Coelomycetes | Appendage type | Teleomorph | Holomorph |
|---|---|---|---|---|
| 1 | *Abropelta fusarioides* | A | Unknown | |
| 2 | *Acarosporium sympodiale* | A | | *Pycnopeziza* |
| 3 | *Acrocalymma medicaginis* | H | | ***Massarina*** |
| 4 | *Ajrekarell aplychaetriae* | A | Unknown | |
| 5 | *Allantophomopsis cytisporea* | C | | *Phacidium* |
| 6 | *Alloneottiosporina carolinensis* | H | Unknown | |
| 7 | *Amarenographium metableticum* | H | | *Amarenomyces* |
| 8 | *Annellotacinia dinemasporioides* | A | Unknown | |
| 9 | *Aphanofalx mali* | A | Unknown | |
| 10 | *Bartalinia robillarodoides** | A | Unknown | |
| 11 | *Bellulicauda dialii* | A2 | Unknown | |
| 12 | *Bleptosporium pleurochaetum* | A | | *Amphisphaeria* |
| 13 | *Brencklea sisyrinchii* | A | Unknown | |
| 14 | *Brycekendrickia indica* | A | Unknown | |
| 15 | *Callistospora gaubae* | H | Unknown | |
| 16 | *Catenophoropsis eucalypticola* | A | Unknown | |
| 17 | *Cenangiomyces luteus* | A | Unknown Basidiomycets | |
| 18 | *Ceuthospora lauri* | C | | *Phacidium* |
| 19 | *Chaetoconis polygone* | A | | *Ceriospora* |
| 20 | *Chaetospermum chaetosporum*** *Chaetospermum camelliae** *Chaetospermum artocarpi** | A | Unknown | |
| 21 | *Chithramia elegantissima* | I | Unknown | |
| 22 | *Choanatiara lunata* | C | Unknown | |
| 23 | *Ciliochora longiseta* | A | | *Physalospora* |
| 24 | *Ciliocharella mangiferae* | A | Unknown | |
| 25 | *Ciliophora cryptica* | A | Unkonwn | |
| 26 | *Ciliosporella selenospora* | A | Unknown | |
| 27 | *Colletotrichum dematium* | A | | *Glomerella* |
| 28 | *Coma circularis* | A | | *Ascocoma* |
| 29 | *Comatospora suttonii* | D | Unknown | |

| No. | Coelomycetes | Appendage type | Teleomorph | Holomorph |
|---|---|---|---|---|
| 30 | *Conicomyces transvaalensis* | A | Unknown | |
| 31 | *Coniella fragariae* | F | | *Schizoparme* |
| 32 | *Cornutispora limaciformis* | A | Unknown | |
| 33 | *Crinitospora pulchra* | A | Unknown | |
| 34 | *Crucellisporiopsis gelatinosa* | A | Unknown discomycete | |
| 35 | *Crucellisporium selaginellae* | A | Unknown | |
| 36 | *Diachorella onobrychidis* | A | | *Diachora* |
| 37 | *Diarimella setulosa* ** *Diarimella* sp* | A | Unknown | |
| 38 | *Dilophospora alopecuri* | A | | *Lidophia* |
| 39 | *Dimastigosporium musimonum* | A2 | Unknown | |
| 40 | *Dinemasporium strigosum* | A | | *Phomatospora* |
| 41 | *Diploceras hypericinum* | A | | *Discostromopsis* |
| 42 | *Discosia strobilina*** *Discosia* sp.* | A | Unknown | |
| 43 | *Discosiella cylindrospora* | F | | *Strigula* |
| 44 | *Doliomyces senegalensis* | A | Unknown | |
| 45 | *Dwayalomella vaccinii* | A | Unknown | |
| 46 | *Ebollia valdiviensis* | A | Unknown | |
| 47 | *Eleutheromyces subulatus* | A2 | Unknown | |
| 48 | *Ellula guaduae* | A | Unknown | |
| 49 | *Entomosporium mespili* | A | | *Diplocarpon* |
| 50 | *Eriosporella calame* | A | | *Lachmum* |
| 51 | *Fibulocoela indica* | A | Unknown basidiomycetes | |
| 52 | *Furcaspora pinicola* | A | Unknown | |
| 53 | *Gampsonema exile* | A | Unknown | |
| 54 | *Giulia tenuis*** *Giulia* sp.* | D | Unknown | |
| 55 | *Groveolopsis pandane* | H | | ***Didymella*** |
| 56 | *Harknessia eucalypti* | A1 | | *Wuestneia* |
| 57 | *Heteropatella lacera* | A | | *Heterosphaeria* |
| 58 | *Hoehneliella perplexa* | A | Unknown | |
| 59 | *Hyalotiella transvalensis* | A | Unknown | |
| 60 | *Hyalotiopsis subramanianii* | A | | *Elurema* |
| 61 | *Hymenopsis trochiloides* | C | Unknown | |
| 62 | *Idiocercus pirozynskii* | F | Unknown | |
| 63 | *Japonia quercus* | A | Unknown | |
| 64 | *Kellermania yucciqena* | A1 | Unknown | |
| 65 | *Koorchaloma maclreeya*** *Koorchaloma bambusae** | C | | *Kananascus* |
| 66 | *Labridella cornu-cervae* | A | | ***Griphosphaerioma*** |
| 67 | *Lasmeniella guaranitica* | A2 | Unknown | |
| 68 | *Libartania laserpiti* | A | | *Phragmiticola* |
| 69 | *Linochorella striiformis* | C | Unknown | |
| 70 | *Mastigonema bruchianum* | A | Unknown | |
| 71 | *Mastigosporella hyaline* | A1 | | *Wuestneiopsis* |
| 72 | *Mirimyces pulcher* | H | Unknown | |

| No. | Coelomycetes | Appendage type | Teleomorph | Holomorph |
|---|---|---|---|---|
| 73 | *Monochaetia monochaeta*** *Monochaetia* sp.* | A | Unknown | |
| 74 | *Monochaetiella hyparrheviae* | A | Unknown | |
| 75 | *Monochaetiellopisis themedae* | A | | *Hypnotheca* |
| 76 | *Monochaetinula terminaliae* | A | Unknown | |
| 77 | *Monodia elegans* | A | Unknown | |
| 78 | *Mycohypallage congesta* | A | | *Plagiostigme* |
| 79 | *Mycotriculs mirabilis** | A | Unknown | |
| 80 | *Nagrajomyces dictyosporus* | A2 | Unknown | |
| 81 | *Neobarchaya primria* | A | Unknown | |
| 82 | *Neochaetospora quezelii* | A | Unknown | |
| 83 | *Neoheteroceras flageoletii* | A | Unknown | |
| 84 | *Neoplaconema napelli* | A | Unknown | |
| 85 | *Neottiospora caricina*** *Neottiospora* sp.* | C | Unknown | |
| 86 | *Neottiosporina apoda* | C | Unknown | |
| 87 | *Nothostrasseria dendritica* | A2 | Unknown | |
| 88 | *Obstipipilus malabaricus* | A | Unknown | |
| 89 | *Orthanocoela calamagrostidis* | A1 | Unknown | |
| 90 | *Parahyalotiopsis borassi* | A1 | Unknown | |
| 91 | *Pestalotia pezizoides* | A | Unknown | |
| 92 | *Pestalotiopsis guepinii* | A | | *Broomella* and ***Pestalosphaeria*** |
| 93 | *Pestalotiopsis subsessilis* | A | Unknown | |
| 94 | *Phyllosticta convallariae* | G | | ***Guignardia*** |
| 95 | *Placonema bambusacearum* | A | | *Phyllachora* |
| 96 | *Plectronidiopsis chilensis* | A | Unknown | |
| 97 | *Plectronidium sinense* | A | Unknown | |
| 98 | *Polynema ornatum* | A | Unknown | |
| 99 | *Proboscispora manihotis* | F | Unknown | |
| 100 | *Pseudobasidiospora caroliniana* | C | Unknown | |
| 101 | *Pseudolachnea ubakii* | A | Unknown | |
| 102 | *Pseudolachnella scolecospora* | A | Unknown | |
| 103 | *Pseudoneottiospora cunicularia* | A | Unknown | |
| 104 | *Pseudorobillarda phragmitis*** *Pseudorobillarda sojae** *Pseudorobillarda taxana** *Pseudorobillarda siamensis** | E | Unknown | |
| 105 | *Pullospora tetrachaeta* | A | Unknown | |
| 106 | *Pycnovellomyces foliicola* | A | Unknown basidiomycete | |
| 107 | *Rhabdogloeum pseudotsugae* | G | | *Rhabdocline* |
| 108 | *Rhodesiopsis gelatinosa* | C | Unknown | |
| 109 | *Rileya piceae* | A | Unknown | |
| 110 | *Robillarda sessilis** | A | Unknown | |
| 111 | *Sarcostroma berkeleyi* | A | Unknown | |
| 112 | *Scolecosporiella typhae* | A1 | | ***Phaeosphaeria*** |
| 113 | *Scopaphoma corioli* | A2 | Unknown | |
| 114 | *Seimatosporiopsis salvadorae* | A | Unknown | |

| No. | Coelomycetes | Appendage type | Teleomorph | Holomorph |
|---|---|---|---|---|
| 115 | *Seimatosporium rosae* | A | | *Discostroma* |
| 116 | *Seiridium marginatum* | A | | *Lepteutypa* and *Blogiascospora* |
| 117 | *Septoriella phragmitis* | H | | ***Phaeosphaeria*** |
| 118 | *Sphaerellopsis quercuum* | H | | *Eudarluca* |
| 119 | *Stauronema cruciferum* | A | Unknown | |
| 120 | *Stevensomula ciliate* | A | Unknown | |
| 121 | *Strasseria carpophila* | A2 | Unknown | |
| 122 | *Strasseriopsis tsugae* | A2 | Unknown | |
| 123 | *Tiarospora perforans* | H | | ***Phaeosphaeria*** |
| 124 | *Tiarosporella paludosa* | C | | *Darkera* |
| 125 | *Toxosporiopsis capitata* | B | Unknown | |
| 126 | *Tracylla spartinae* | A | Unknown | |
| 127 | *Truncatella truncate* | A | | *Broomella* |
| 128 | *Uniseta flagellifera* | A | | *Cryptodiaporthe* |
| 129 | *Urohendersonia platensis* | G | Unknown | |
| 130 | *Urohendersoniella mastigospora* | H | Unknown | |
| 131 | *Vasudevella sporoboli* | A1 | Unknown | |
| 132 | *Vermisporium walkeri* | A | Unknown | |
| 133 | *Xenidiocercus macarangae* | A2 | Unknown | |
| 134 | *Xepicula leucotricha** | C | Unknown | |
| 135 | *Xepiculopsis perpulchra*** *Xepiculopsis* sp.* | C | Unknown | |
| 136 | *Yalomyces pulcher* | C | Unknown | |
| 137 | *Zelandiocoela ambigua* | H | Unknown | |
| 138 | *Zelosatchmopsis sacciformis* | A | Unknown | |
| 139 | *Zetesimomyces setibicolor* | A | Unknown | |
| 140 | *Zetiasplozna unicolor* | A | Unknown | |
| 141 | *Zinzipegasa argentinensis* | A | Unknown | |
| 142 | *Zunura appendiculata* | A1 | Unknown | |

*** genera in culture to be sequenced**
**** genera to be acquired**
**Bold = Bitunicate Ascomycetes**

## E : Work plan for the next two years (Table 6)

1. To examine the phylogenetic relationships of selected coelomycetes from our own culture collection, isolated from marine, freshwater and terrestrial habitats. Also selected strains will be purchased from international culture collections.
2. To extract DNA, sequence, align sequences and construct trees.
3. To write 6 monthly reports and at least one paper per year.

## F: Materials and methods

These are fully outlined in the original application.

## G: Fungi to sequence

These are listed in Table 7.

## H. Budget (Table 8)

**Table 6. Work plan for years 2 and 3.**

| **Activities** | **Year 2** | | **Year 3** | | **Responsibility** |
|---|---|---|---|---|---|
| Months | 1-6 | 7-12 | 13-18 | 19-24 | |
| DNA extraction | ← | — | — | → | 1 |
| DNA Sequencing | | ← | — | → | 1 |
| Construction trees | | ← | — | → | 1,2 |
| Analysis of data | | ← | — | → | 1,2, 3 |
| Conference presentation | | ↔ | | ↔ | 1,2, 3 |
| Paper preparation | | ↔ | | ↔ | 1,2, 3 |
| Final report | | | | ↔ | 1,2, 3 |

**Duties: 1 = Mr. Nattawut Rungjindamai, 2 = Dr. Jariya Sakayaroj, 3 = Prof. E. B. Gareth Jones**

**Table 7. Coelomycetes to be sequenced during the 2nd year of the research grant**

| Species | Habitat | Rational for selection |
|---|---|---|
| 1. *Chaetospermum carneum* | Terrestrial | To compare with *Chaetospermum camelliae* |
| 2. *Bartalinia robillaroides* | Terrestrial | |
| 3. *Discosia aquatic* | Freshwater | |
| 4. *D. artocreas* | Terrestrial | |
| 5. *Hyphodiscosia jaipunensis* | Terrestrial | To compare with Discosia spp. |
| 6. *Pseudorobillarda phragmitis* | Aquatic | To compare with *Pseudorobillarda siamensis* |
| 7. *Pestalotiopsis* species | Terrestrial | To determine if they all belong in the genus Pestalotia |
| 8. *Monochaetia dimorphospora* | Terrestrial | To determine of all Monochaetia species are monophyletic |
| 9. *Monochaetia lutea* | Terrestrial | |
| 10. *Monochaetia saceaerdoi* | Terrestrial | |
| 11. *Pestalotia matilolae* | Terrestrial | To test if all species belong in the teleomorphic genus Broomella |
| 12. *Pestalotia populi-nigrae* | Terrestrial | |
| 13. *Coniella castaneicola* | Terrestrial | |
| 14. *Chaetomella raphigera* | Terrestrial | |
| 15. *Wiesneriomyces laurinus* | Terrestrial | Taxonomic position |
| 16. Unidentied coelomycete from Nypa | Marine | |
| 17. Unidentifide from Nypa | Marine | |
| 18. Yellow coelomycete from *Sonneratia pneumatophores* | Marine | |
| 19. *Cytospora rhizophorae* | Marine | |
| 20. *Rhabospora rhizophorae* | Marine | |

# PART C: APPENDIX

**A: Curriculum Vitae of Mr. Nattawut Rungjindamai**

**B: Publications of principal investigators for 2005.**

**C: Poster print out presented at the in BRT meeting 2005: 10-13 October 2005 Narumol Plaingam, Jariya Sakayaroj, Sayanh Somrithipol, and E. B. Gareth Jones "A phylogenetic study of *Robillarda sessilis* and *Xepiculopsis graminea*"**

## A: Curriculum Vitae

**Name** Mr. Nattawut Rungjindamai

**Birth Date** June 11, 1980

**Birth Place** Ratchaburee, Thailand

**E-mail address** nathawuthr@yahoo.com

**Education Attainment**

| Degree | Name of Insitute | Year of Graduation |
|---|---|---|
| B.Sc. (Microbiology) | Prince of Songkla University | 2001 |
| M.Sc. (Microbiology) | Prince of Songkla Univerisity | |

**Scholarships Award during Enrollment**

Thailand Graduate Institute of Science and Technology (TGIST), National Science and Technology Development Agency (NSTDA) (2005-2006)

**Working experience**

| Company/Academic Place | Location | Department | Responsibilities | Position |
|---|---|---|---|---|
| 1. Prince of Songkla University | Songkhla | Department of Microbiology | Susceptibility test of plant crude extracts | Research assistance |
| 2. TFI Green Biotech company under regime of Thai Fermentation Industry company (Rachachurose co., ltd.) | Ratchaburee | Department of Quality Control | Biocontrol Production | Head of Production |

## B: Publications of principal investigators for 2005.

## PAPERS PUBLISHED IN 2005

1. SAKAYAROJ, J., PHONGPAICHIT, S. and JONES, E.B.G. (2005). Viability and biodiversity of freshwater hyphomycetes in foam at Ton-Nga Chang Wildlife Sanctuary, Songkla, southern Thailand. Fungal Diversity 18: 135-145

2. PLAINGAM, N., SOMRITHIPOL, S. and JONES, E.B.G. (2005). *Pseudorobillarda siamensis* sp. nov. and notes on *P. sojae* and P. *taxana* from Thailand. Nova Hedwigia 80: 335-348.

3. SOMTOTHIPOL, s. and JONES, E.B.G. (2005). An addition to the hyphomycete genus *Melanographium* from Thailand. Fungal Diversity 19: 137-144.

4. *SAKAYAROJ, J., PANG, K.L., PHONGPAICHIT, S. and JONES, E.B.G. (2005). A phylogenetic study of the genus *Haligena* (Halosphaeriales, Ascomycota). Mycologia 97: 804-811.

5. *PILANTANAPAK, A., JONES, E.B.G. and EATON, R.E. (2005). Marine fungi on *Nypa fruticans* in Thailand. Bot. Mar. 48: 365-378.

6. HUANG, H.-R., FENG, X.-L., SHE, Z.-G. LIN, Y.-C., VRIJMOED, L.L.P. and JONES, E.B.G. (2005). 1-(2,6-Dihydroxyphenyl) butanone. Acta. Cyst. E61: o282-o283.

7. YIN, W.Q., ZOU, J.M., SHE, Z.G. VRIJMOED, L.L.P., JONES, E.B.G. and LIN, Y.-C. (2005). Two cyclic peptides produced by the endophytic fungus #2221 from *Castaniopsis fissa* on the South China Sea Coast. Chinese Chem. Letter 16: 219-222.

8. *SAKAYAROJ, J., PANG, K.L., JONES, E.B.G., PHONGPAICHIT, S. VRIJMOED, L.L.P. and ABDEL-WAHAB, M.A. (2005). A systemtatic reassessment of the marine ascomycetes *Torpedospora* and *Swampomyces*. Bot. Mar. 48: 395-406.

9. JONES, E.B.G. and ABDEL-WAHAB, M.A. (2005). Marine fungi from the Bahamas Islands. Bot. Mar. 48: 356=364.

10. *JONES, E.B.G. (2005). 5th Asia Mycological Congress and 9th International Marine and Freshwater Mycology Symposium, Chiang Mai, Thailand, 14-19th November 2004. Bot. Mar. 48: 331.

## PAPERS NOT CITED IN THE 2004 LIST

1. LI, H.J., LIN, Y.-C., VRIJMOED, L.L.P. and JONES, E.B.G. (2004). A new cyto-toxic sterol produced by an endophytic fungus from *Castaniopsis fissa* at the South China Sea Coast. Chinese Chem. Letter 15: 419-122.

2. LI, J.-J., LIN, Y.-C., YAO, J.-H., VRIJMOED, L.L.P. and JONES, E.B.G. (2004). Two new metabolites from the mangrove endophytic fungus No. 1514. J. Asian Nat. Prods. Res. 6: 185-191.

3. HUANG, H.-R., XIA, X.-K., SHE, Z.-G. LIN, Y.-C., VRIJMOED, L.L.P. and JONES, E.B.G. (2005). 1-(2,6-Dihydroxyphenyl) ethanone. Acta. Cyst. E60: o2509-o2510.

## PAPERS PUBLISHED IN 2006

- VIVKINESWARY,S., ABDU LLAH, N., RENUVATHANI, M., SEKARAN, M., PANDEY, A. and **JONES, E.B.G. (2006)**. Productivity of laccase in solid substrate fermentation of selected agro-residues by *Pycnoporus sanguineus*. Bioresource Technology 97: 171-177.

## PAPERS IN PRESS

# Accepted for publication
* BRT funding in part

1. FAN, K.W., VRIJMOED, L.L.P. and **JONES, E.B.G.** Zoospore motility of mangrove thraustochytrids. Mycologia.

2. KWONG, T., **JONES, E.B.G.,** HYWEL-JONES. N.L., KONG, R.Y.C. and VRIJMOED, L.L.P. Molecular phylogenetic relationship of *Metarhizium* species based on host group and geographical origin.

*3. #PINNOI, A., 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 21:

4. SOMRITHIPOL, S., PLAINGAM, N., and **JONES, E.B.G.** A preliminary survey of the Coelomycetes of Thailand. Mycotaxon

*5. #**JONES, E.B.G.,** PILANTYANAPAK, A., CHATMALA. I. SAKAYAROJ, J., PHONGPAICHIT, S. and CHOEYKLIN, R. Thai marine fungal diversity.

*6. #SOMRITHIPOL, S., KOSOL, S. and **JONES, E.B.G**. *Lauriomyces sakaeratensis* sp. nov., a new Hyphomycete on decaying *Diptercarpus costatus* fruits from Sakaerat Biosphere

reserve, Thailand. Nova Hedwigia.

*7. #**JONES, E.B.G.**, CHATMALA, I. and PANG, K.L. (2006). Two new genera of the Halosphaeriales isolated from marine habitats in Thailand: *Pseudosigmoidea* and *Thalespora.*

*8. #PANG, K.L., **JONES, E.B.G.** CHIANG, W.L. and VRIJMOED, L.L.P. (2006). Ascospores ultrastructure of *Halosarpheia fibrosa* Kohlm. and E. Kohlm. (Halosphaeriales, Ascomycota).

*9. #**JONES, E.B.G.** (2006). Form and function of fungal spores with special reference to ascospore appendages in aquatic ascomycetes. Mycoscience

*10. #**JONES, E.B.G.** and PUGLISI, M.P. (2006) Marine fungi of Florida. Florida Scientist

*11. PINNOI, A., JEEWON, R., SAKAYAROJ, J., HYDE, K.D. and **JONES, E.B.G.** *Berkleasmium crunisia* sp. nov. and its teleomorphic affinities to the Pleosporales based on 18S, 28S and ITS-5.8S rDNA sequence analyses. Mycologia

*12. KOCH, J., PANG, K.L. and **JONES, E.B.G.** (2006). *Lulworthia* G.K. Sutherl. revisited and *Rostrupiella* gen. nov. in the Lulworthiales, from Denmark. Bot. Mar.

*13. SOMRITHIPON, S. and JONES E.B.G. *Calcarisporium phaeopodium* sp. nov., a new hyphomycete from Thailand. Sydowia.

**C: Poster presentation in BRT meeting 2005: 10-13 October 2005 Narumol Plaingam, Jariya Sakayaroj, Sayanh Somrithipol, and E. B. Gareth Jones "A phylogenetic study of *Robillarda sessilis* and *Xepiculopsis graminea*"**

# A phylogenetic study of selected freshwater coelomycetes in Thailand

Narumol Plaingam[1,2], Jariya Sakayaroj[1], Sayanh Somrithipol[1], and E. B. Gareth Jones[1]

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## Introduction

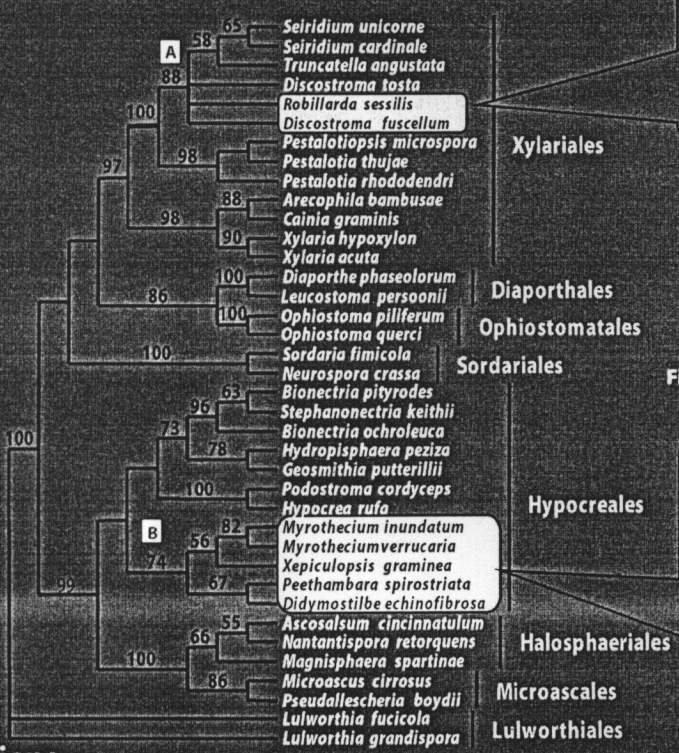
Coelomycetes are anamorphic fungi producing mitotic spores (conidia) in a pynidium, acervulus or stomatum (Sutton, 1980; Nag Raj, 1993). Currently, 1,000 genera comprising 7,000 species have been described. Despite this large number of coelomycetes documented worldwide (Sutton, 1973; Nag Raj, 1993), little is known on their teleomorphic counterparts and hence, systematic positions.

With the advent of molecular techniques, one can link anamorphic fungi to their teleomorphs. This study was initiated to examine the phylogeny of *Robillarda sessilis* and *Xepiculopsis graminea* (commonly isolated in streams of Thailand), using sequence analysis of the partial nuclear small subunit (SSU) ribosomal RNA gene.

*Robillarda sessilis* was isolated from an *Eucalyptus camaldulensis* leaf, and produced fusiform, one septate conidia, bearing 3-4 branched, tubular appendages (Fig.1 ). *Xepiculopsis graminea* is a saprophyte on grass leaves, forming fusiform to ellipsoidal conidia with an acute apex and a narrow truncate base, with apical mucoid appendages (Fig. 2).

## Materials and Methods

Genomic DNA was extracted using a modified procedure of O'Donnell et al. (1997). SSU rDNA was amplified by PCR using primers NS1/NS6 and sequenced. Maximum parsimony and bootstrapping analyses were executed in PAUP* 4.0b10 (Swofford, 2002). A consensus tree was generated from six most parsimonious trees.




The SSU sequence of *Robillarda sessilis* was 1,102 bp long and identical to the same region of *Discostroma fuscellum* They all belong to the Amphisphaeriaceae, Xylariales. Our molecular data show that *R. sessilis* groups within clade A, comprising mainly coelomycetes. It shares some common features with the genera in thisclade : fusiform, septate conidia with truncate apical cells and tubular, branched polar appendages. Conidia of *R. sessilis* are hyaline, whereas the median cells of related taxa are brown.

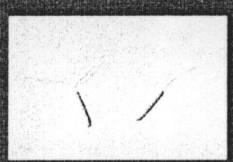


Figure 1 : *Robillarda sessilis*

Figure 2 : *Xepiculopsis graminea*

The SSU sequence of *Xepiculopsis graminea* was 1,107 bp long. The sequences in this group shares > 99% similarity. *Xepiculopsis graminea* shows phylogenetic affinity with members of the Bionectriaceae, Hypocreales, especially the genus *Myrothecium* (Hyphomycetes). They share some morphological characters fusiform to ellipsoidal conidia with an acute apex and a narrow truncate base, with apical mucoid appendages. No clear phylogenetic relationshis can be advanced for *X. graminea*, further DNA region (LSU, ITS) need to be sequenced.

## Conclusions

. Teleomorph of *Robillarda sessilis* belongs in the Amphisphaeriaceae, Xylariales and could be in the genus, Discostroma.
. Teleomorph of *Xepiculopsis graminea* belongs in the Bionectriaceae, Hypocreales.

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Swofford, D.L. (2002). PAUP: Phylogenetic Analysis Using Parsimony, version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

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