12- MONTH PROGRESS REPORT

(January 2007 – June 2007)

For	**National Center for Genetic Engineering and Biotechnology** **Biodiversity Research and Training Program (BRT)**
Project Title	**Phylogenetic Relationships of Marine Dothideomycetes (Ascomycota), and Related Taxa**
BRT Project Code	**BRT R_249001**
Principal Researcher	**Prof. E. B. Gareth Jones**
Co-investigator	**Dr. Jariya Sakayaroj**
Assistant Researcher	**Miss Satinee Seutrong**

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PART A: 12-Month Report

1. SUMMARY OF REPORT:

The Ascomycota are the largest group of filamentous fungi, but their classification is the least developed with many taxa not assigned to either a family or order. Likewise their phylogenetic relationship is poorly researched, especially the subclass Dothideomycetes or Dothideomycetidae (bitunicate ascomycetes). The objective of this project is to examine the evolution and phylogeny of a group of bitunicate marine Dothideomycetes, especially those referred to *Incertae sedis* ascomycetes. Since their classification has not been possible based on morphological features, we are studying them at the molecular level, in order to resolve their phylogeny. For the past 6 months, we have been collecting marine ascomycetes along the coastal areas of Thailand, especially mangrove habitats. These fungi have been isolated into axenic culture, grown in liquid culture and genomic DNA extracted. From the last 6 months we have been re-sequencing 22 marine bitunicate ascomycetes and 22 PCR reactions of different rRNA regions of new strains have been amplified (highlighted). Data analysis of the sequences obtained is in progress and phylogenetic trees for the genera *Biatriospora marina*, *Decaisnella formosa* and *Platystomum scabridisporum* are presented in this report.

2. PROJECT OBJECTIVES:

A). To acquire and isolate strains of selected genera and species for the molecular study, and for deposition in the BIOTEC Culture Collection where they will be available for biological screening for new chemical structures and bioactive compounds.

B). Examination of the molecular phylogeny of selected genera and species of bitunicate marine Dothideomycetes, whose ordinal status is currently not known.

C). To sequence rDNA genes of selected genera which have an uncertain taxonomic position (*Incertae sedis*).

D). To examine some closely related freshwater and terrestrial genera.

3. FUNGI STUDIED: See Table 1.

Table 1. Bitunicate marine fungi which have been isolated and sequenced over the past 6 months. The highlighted areas are new strains.

No.	Original code	Scienfific Name	Substrate	Location	Comment
1	GR 165	*Aigialus grandis*	*Sonneratia* wood	Morib, Malaysia	-
2	GR 166	*Aigialus grandis*	*Sonneratia* wood	Morib, Malaysia	-
3	JS 268	*Aigialus mangrovei* -like	Mangrove wood	Hat Khanom - Mu Ko Thale Tai National Park	-
4	SAT 395	*Aigialus mangrovei* -like	Mangrove wood	Pak Phanang coast, Nakhon Si Thammarat	-
5	GR 46	*Aigialus mangrovei*	Mangrove wood	Florida Keys, USA	-
6	GR 133	*Aigialus parvus*	*Sonneratia* wood	Morib, Malaysia	-
7	GR 134	*Aigialus parvus*	*Sonneratia* wood	Morib, Malaysia	-
8	PGI 323	*Ascocratera manglicola*	Mangrove wood	Laem Son National Park (Ranong)	-
9	JS 282	*Ascocratera manglicola*	Mangrove wood	Hat Khanom - Mu Ko Thale Tai National Park	-
10	1228	*Biatriospora marina*	Mangrove wood	Hong Kong	-
11	PGI 152	*Caryosporella rhizophorae*	Wood	Unknown	-
12	JS 97	*Dactylospora haliotrepha*	Drift Wood	Unknown	-
13	GL 00334	*Dactylospora haliotrepha*	Wood	Unknown	-
14	SAT 95	*Decasinella formosa*	Drift wood	Rye, Australia	-
15	SAT 96	*Decasinella formosa*	Drift wood	Rye, Australia	-
16	PF 8	*Jahnula appendiculata*	Petiole (Eleiodoxa conferta)	Sirindhorn Research and Nature Study Center (Narathiwat)	-
17	SS 44	*Jahnula bipolaris*	Soft wood	Khao Yai National Park (NakhonNayok)	-
18	SAT 53	*Lautospora gigantea*	Wood	Thailand	-
19	JS 76	*Lineolata rhizophorae*	Drift wood	Tak Bai, Narathiwat	-
20	GR 84	*Lineolata rhizophorae*	*Rhizophora* wood	Koh Chang	
21	GR 249	*Manglicola guatemalensis*	Nypa	Koh Chang	-
22	GR 250	*Manglicola guatemalensis*	Nypa	Koh Chang	-
23	SAT 179	*Manglicola guatemalensis*	Nypa	Trang	-
24	SAT 180	*Manglicola guatemalensis*	Nypa	Trang	-
25	GR 21	*Massarina thalassiae*	Mangrove wood	Florida Keys, USA	Misidentification

Table 1. Bitunicate marine fungi which have been isolated and sequenced over the past 6 months. The highlighted areas are new strains (Cont).

No.	Original code	Scienfific Name	Substrate	Location	Comment
26	GR 22	*Massarina thalassiae*	Mangrove wood	Florida Keys, USA	Misidentification
27	GR 31	*Massarina velataspora*	Wood	Florida Keys, USA	-
28	GR 32	*Massarina velataspora*	Wood	Florida Keys, USA	-
29	GR 79	*Massarina* sp.	On submerged coconut trunks	Koh Chang	Misidentification
30	GR 80	*Massarina* sp.	On submerged coconut trunks	Koh Chang	Misidentification
31	GR 135	*Massarina ramunculicola*	*Sonneratia* wood	Morib, Malaysia	Misidentification
32	GR 136	*Massarina ramunculicola*	*Sonneratia* wood	Morib, Malaysia	Misidentification
33	GR 147	*Massarina ramunculicola*	*Rhizophora* wood	Koh Chang	Misidentification
34	GR 148	*Massarina ramunculicola*	*Rhizophora* wood	Koh Chang	Misidentification
35	GL 07474	*Melaspilea mangrovei*	Mangrove wood	Unknown	-
36	GL 07478	*Melaspilea mangrovei*	Mangrove wood	Unknown	-
37	SAT 146	*Patellaria sp*	Mangrove wood	Kung kra Baen Bay, Trat	-
38	SAT 147	*Patellaria sp*	Mangrove wood	Kung kra Baen Bay, Trat	-
39	GR 171	*Julella aviceniae*	*Avicennia* wood	Koh Chang	-
40	SAT 238	*Platystomum scabridisporum*	Drift wood	Rye, Australia	-
41	SAT 239	*Platystomum scabridisporum*	Drift wood	Rye, Australia	-
42	GR 93	*Platystomum scabridisporum*	Drift wood	Rye, Australia	-
43	GR 94	*Platystomum scabridisporum*	Drift wood	Rye, Australia	-
44	-	*Pontoporeia biturbinata*	Seaweed	Cyprus	-
45	SAT 628	*Pontoporeia biturbinata* - like	Coconut	Had Ban Krut, Prachuap Khiri Kgan	-
46	SAT 629	*Pontoporeia biturbinata* - like	Coconut	Had Ban Krut, Prachuap Khiri Kgan	-
47	GR 48	*Pyrenographa xylographoides*	*Rhizophora* wood	Florida Keys, USA	-
48	JS 290	*Pyrenographa xylographoides*	*Rhizophora* wood	Hat Khanom - Mu Ko Thale Tai Nation Park	-
49	GR 49	*Pyrenographa xylographoides*	*Rhizophora* wood	Florida Keys, USA	-
50	GL 508	*Pyrenographa xylographoides*	Mangrove wood	Unknown	-
51	PGI 00442	*Quintaria* sp.	Wood	Bala Hala Wildlife Sanctuary (Narathiwat)	-
52	GR 25	*Quintaria lignatilis*	Mangrove wood	Florida Keys, USA	-

Table 1. Bitunicate marine fungi which have been isolated and sequenced over the past 6 months. The highlighted areas are new strains (Cont).

No.	Original code	Scienfific Name	Substrate	Location	Comment
53	PGI 50	*Quintaria lignatilis*	Mangrove wood	Laem Son National Park (Ranong)	-
54	JS 00178	*Saccardoella rhizophorae*	Mangrove wood	Ranong	-
55	PGI 00484	*Saccardoella* sp.	Mangrove wood	Panwa (Phuket)	-
56	SSK 20-1	*Saccardoella* sp.	Unknown	Unknown	-
57	SAT 633	*Sporomia* sp.	Mangrove wood	Nakhonn Sri Thammarat	
58	SAT 634	*Sporomia* sp.	Mangrove wood	Nakhonn Sri Thammarat	
55	GR 57	*Verruculina enalia*	Mangrove wood	Florida Keys, USA	-
56	GR 88	*Verruculina enalia*	Marine wood	Koh Chang	-
57	GR 129	*Verruculina enalia*	*Sonneratia* wood	Morib, Malaysia	-

4. MOLECULAR METHODS

A) Genomic DNA extraction:

Genomic DNA extraction using home made buffer (Applied from O'Donnell *et al.*, 1997)

A fine powder of fungal mycelia of 50-100 mg was placed into 400 µl Lysis buffer. The tube was then be incubated at 70 °C for 30 minutes. Then equal volume of phenol-chloroform (PIERCE) was added. The upper liquid phase was transferred to a new microtube containing chilled absolute ethanol and 7.5 M ammonium acetate. The mixture, kept at –20 °C for at least 30 minutes, or until required for DNA precipitation, then centrifuged at 14K, 4 °C, for 15 minutes. The DNA pellet was washed twice with chilled 75 % ethanol and air dried. Finally, DNA was resuspended in 50 µl TE buffer and checked for the quantity and quality on a 1% agarose gel electrophoresis.

B) PCR amplification:

DNA was amplified with Taq DNA polymerase from DyNAzyme^{TM} II DNA Polymerase Kit, FINNZYMES, Finland and FERMENTAS Taq DNA polymerase (recombinant). Different regions of ribosomal DNA will be amplified using PCR Model MJ Research DYAD ALD. Primers used for amplification include the small subunit (18S), large subunit (28S) and the Internal Transcribed Spacers (ITS) of rDNA (White *et al.*, 1990; Landvik, 1996) and are listed in Table 2, with the DNA mixtures listed in Table 3.

Table 2. Primers used for PCR and DNA sequencing.

Primers	Sequence (5' – 3')
Small subunit (18s)	
NS1	GTA GTC ATA TGC TTG TCT C
NS2	GGC TGC TGG CAC CAG ACT TGC
NS3	GCA AGT CTG GTG CCA GCA GCC
NS4	CTT CCG TCA ATT CCT TTA AG
NS5	AAC TTA AAG GAA TTG ACG GAA G
NS6	GCA TCA CAG ACC TGT TAT TGC CTC
NS8	TCC GCA GGT TCA CCT ACG GA
Large subunit (28s)	
JS1	CGC TGA ACT TAA GCA TAT
JS5	TCT TGA AAC ACG GAC CAA
JS8	CAT CCA TTT TCA GGG CTA
LR7	TAC TAC CAC CAA GAT CT
LROR	ACC CGC TGA ACT TAA GC
NL3	AGA TGA AAA GAA CTT TGA AAA GAG AG
NL4	GGT CCG TGT TTC AAG ACG G
NL4R	CCG TCT TGA AAC ACG GAC C
Internal Transcribed Spacers (ITS)	
ITS1	TCC GTA GGT GAA CCT GCG G
ITS3	GCA TCG ATG AAG AAC GCA GC
ITS4	TCC TCC GCT TAT TGA TAT GC
ITS5	GGA AGT AAA AGT CGT AAC AAG G

Table 3. PCR mixtures (FINNZYMES, DyNAzyme™ II DNA Polymerase Kit and FERMENTAS).

Total volume of 25 ul	**Volume**	
	FINNZYMES	**FERMENTAS**
Nanopure water	19 ul	17.9 ul
10 x PCR buffer	2.5 ul	2.5 ul
$MgCl_2$	1.25 ul	2.5 ul
10 mM dNTPs	0.5 ul	0.5 ul
10 uM forward primer	0.5 ul	0.5 ul
10 uM reverse primer	0.5 ul	0.5 ul
Taq polymerase	0.25 ul	0.1 ul
DNA template	0.5 ul	0.5 ul

PCR profiles: The PCR profile for primers NS1/NS8, ITS5/LR7, JS1/JS8, LROR/LR7, NS5/ITS4, NS1/NS6, NS5/NS6 and ITS1/ITS4 are listed in Table 4.

Table 4. PCR profile for primer primers NS1/NS8, ITS5/LR7, JS1/JS8, LROR/LR7, NS5/ITS4, NS1/NS6, NS5/NS6 and ITS1/ITS4.

Primer	Cycle	Temperature	Time
NS1/NS8, ITS5/LR7, JS1/JS8, LROR/LR7 and NS5/ITS4.	35	94 °C	2 minute
		94 °C	1 minute
		55 °C	1.5 minutes
		72 °C	2.5 minutes
		72 °C	10 minutes
NS1/NS6 and NS5/NS6	35	94 °C	2 minute
		94 °C	1 minute
		55 °C	1 minutes
		72 °C	1.5 minutes
		72 °C	5 minutes
ITS1/ITS4.	35	94 °C	2 minute
		94 °C	1 minute
		55 °C	1 minutes
		72 °C	2 minutes
		72 °C	10 minutes

C) PCR product purification:

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

D) DNA Sequencing:

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

5. RESULTS:

5.1). Marine bitunicate ascomycetes have been collected, isolated and sequenced.

Marine ascomycetes have been collected along the coastal areas of Thailand and isolated into axenic culture. Over 30 samples have been collected over the last 6 months, with 40 strains isolated into axenic culture and deposited in BIOTEC Culture Collection. Collections from subtropical or temperate countries for study were made, in order to obtain fungi for the molecular study. The 18S, 28S, ITS region of rDNA gene of selected Ascomycota were sequenced by Macrogen, Inc., Korea.

5.2). Molecular phylogeny of *Biatriospora marina, Decaisnella formosa* and *Platystomum scabridisporum* (Part B).

6. OUTOUT FROM THE LAST 6 MONTHS.

6.1 A paper entitled "Morphological and molecular characteristics of a poorly known marine ascomycete, *Manglicola guatemalensis* (Jahnulales: Pezizomycotina; Dothideomycetes, *Incertae sedis*): A new lineage of marine ascomycetes" has been submitted for Mycologia (August 7th, 2007).

6.2 433 cultures deposited in BIOTEC Culture Collection (BCC).

6.3 Poster Presentation in NSTDA Annual Conference 2007: 28-30 March 2007, Convention Center, Thailand Science Park, Thailand.
-S. Suetrong, J. Sakayaroj, S. Phongpaichit and E.B.G. Jones. "Morphological and molecular characteristics of a poorly known marine ascomycete, *Manglicola guatemalensis*".

6.4 A presentation entitled "Classification of Marine Ascomycota" is in progress.

6.5 Attended the 2nd Annual Meeting of Thai Mycological Association (TMA) and Mycology Conference in Thailand: 23 June 2007, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand.

7. FUTURE WORK

7.1. Over the next 6 months further fieldwork will be undertaken to collect material from the intertidal zone at different coastal areas in Thailand. Additional collections will be made in other parts of the world.

7.2. Fungi collected so far will be identified to species level as time permits.

7.3. DNA extraction, sequencing, construction of trees, statistical analysis of the data.

7.4. Number of manuscripts will be in preparation and continuation.

7.5 A poster presentstion entitled "Morphological and molecular characteristics of a poorly known marine ascomycete, *Manglicola guatemalensis* (Jahnulales: Pezizomycotina; Dothideomycetes, *Incertae sedis*): A new lineage of marine

ascomycetes" wiil be presented at BRT Annual Meeting at Udonthani 15-17 October, 2007.

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PART B

Molecular phylogeny of *Biatriospora marina*, *Platystomum scabridisporum* and *Decaisnella formosa*

ABSTRACT

Biatriospora marina was isolated from mangrove wood collected in Singapore. *Decaisnella formosa* and *Platystomum scabridisporum* were collected from driftwood material collected at Rye, on the Mornington Peninsula National Park, Victoria, Australia.

The molecular phylogeny of the *Biatriospora marina*, *Decaisnella formosa* and *Platystomum scabridisporum* were investigated using nucleotide sequences of combine SSU and LSU rDNA and ITS sequences. 111 Sequences of these regions from 66 species from GenBank were aligned, with *Hypocrea lutea* and *Xylaria hypoxylon* as the outgroup. A combined data set was analysed phylogenetically using maximum parsimony, maximum likelihood and Bayesian analyses.

The result shows that *Biatriospora marina, Decaisnella formosa* and *Platystomum scabridisporum* grouped within the Pleosporales in all analyses supported by 99% bootstrap value and 100% posterior probabilities. Sequence analyses indicated that *Decaisnella formosa* and *Platystomum scabridisporum* are monophyletic and cluster within the Lophiostomataceae, grouping with 79% bootstrap value and 99% posterior probabilities with *Lophiostoma arundinis*, *L. caulium and L. crenatum*.

Key words: *Biatriospora*, *Decaisnella formosa*, Lophiostomstaceae, LSU rDNA, Melanommataceae, *Platystomum scabridisporum*, Pleosporales, SSU rDNA.

INTRODUCTION

Luttrell (1955) erected the subclass Loculoascomycetidae to accommodate ascomycetes with ascolocular type of ontogeny and asci with two functional wall layers (bitunicate asci). The Pleosporales is the largest order and accommodates members which produce pseudoparaphyses, as well as setose or glabrous, mostly unilocular pseudothecia. The families Lophiostomataceae, Massarinaceae and Melanommataceae are defined mainly based on morphology of the ascomata and centrum. The morphology of the pseudoparaphyses within the centrum is the key feature used in their higher level classification. The ascospores are frequently phragmosporous or dictyosporous. Pseudoparaphyses in the Dothideales are lacking, even though remnants of interthecial tissue can be found in some genera (Sivanesan, 1984), generating most of the confusion about the taxonomic placement of several fungi, whether in the Pleosporales or Dothideales.

These fungi commonly occur on plant materials, such as dead leaves, herbaceous stems, branches, wood, as well as on green leaves and stems.

The type species of *Biatriospora marina* was originally collected from mangrove roots of *Sonneratia alba* from Anse Boileau mangrove stand, Seychelles, during January 1984. Subsequent collections were made on intertidal wood of *A. alba* from Revidanda, Maharashtra; on mangrove wood from Kampong Doatogangdi, Brunei (Hyde and Borse 1986). *Biatriospora marina* belongs to the Ascomycotina, Loculoascomycetes, Melanommatales (Barr 1983), Hyde and Borse (1986) classified it in the Dothideales, while Eriksson et al. (2001) listed as family *Incertae sedis* in the Ascomycota. Thus its phylogenetic position is not clear.

The eleven *Decaisnella* species have been reported from Europe and North America (mainly temperate), and all are terrestrial (Barr 1990). Saccardo (1883) relegated the ascomycete *Decaisnella spectabilis* Fabre as a synonym of the genus *Teichospora*. Barr (1979a, b) however, in her classification of Loculoascomycetes, was of the opinion that the differences between *Decaisnella* and *Teichospora* were sufficient to warrant their placement in different orders. Barr (1986) re-established the genus *Decaisnella* to accommodate taxa with dictyosporus ascospores and large ascomata, a wide peridium, a reflective apical ring surrounding an ocular chamber in the ascus, and distoseptation in immature ascospores. *Decaisnella* replaced *Titanella*, which is a lichenized taxon and is synonymized with *Anthracothecium*, the latter genera belonging to the Pyrenulaceae. *Decasinella* species may have a slight or well-developed clypeus with medium sized to large ascomata, while the asci may contain two, four, or eight ascospores (Barr 1986). *Decaisnella* species can be divided into two groups based on ascospores morphology: those with oblong ascospores with obtuse or rounded ends (*Decaisnella spectabilis* Fabre, *Decaisnella macrospora*), or those with fusoid-ellipsoid ascospores with tapered ends (*Decaisnella amelanchieris* Fabre) (Barr 1986). First collections of *Decaisnella formosa* were made on intertidal wood from a beach on the Mornington Peninsula, National Park, Victoria, Australia (Adbel-Wahab and Jones 2003).

The first collection of *Platystomum scabridisporum* weas made on intertidal wood from a beach on the Mornington Peninsula National Park, Victoria, Australia (Adbel-Wahab and Jones 2000), who assigned it to the genus *Platystomum* Trev., Platystomaceae, Melanommataceae (Barr 1990) in the *P. compressum* (Pers.: Fr.) Trev. group.. The type species of this genus, and family is *P. compressum*, which has usually been treated as a *Lophiostoma* species (Holm and Holm 1988). Eriksson and Hawksworth (1991) suggested that *Platystomum* be kept as a synonym of *Lophiostoma*, and in accordance with Holm and Holm (1988) the Platystomaceae is treated as a synonym of the Lophiostomataceae.

18S rDNA sequence data has provided support for the Pleosporales as a monophyletic group characterized by its pseudoparaphyses (Barbee 1996, Liew et al 2000, Silva-Hanlin and Hanlin 1999, Winka et al 1998). However published RPB2 data does not resolve its taxonomic position although *Sporomiella minima*, the single member of the Melanommatales included in that study clustered within the Pleosporales (Liu et al 1999). The Dothideales are a monophyletic group in the based on 18s rDNA analyses (Berbee 1996, Winka et al. 1998), but with weak bootstrap support.

Eriksson (2000) accepted seven orders in the Dothideomycetes, including Capnodiales, Dothideales, Hysteriales, Jahnulales, Myriangiales, Pleosporales and Patellariales.

The phylogenetic relationship of the selected marine fungi (*Biatriospora marina, Platystomum scabridisporum* and *Decaisnella formosa*) have been investigated using combined SSU and LSU rDNA and ITS rDNA sequences.

MATERIAL AND METHODS

Collection of fungi. — Driftwood weras collected from a sand dune at Rye, on the Mornington Peninsula, National Park, Victoria, Australia. Material placed in clean plastic bags and returned to the laboratory. After washing with freshwater to remove sediments, examination of the samples for fungi was carried out. Samples were kept moist by spraying with sterilized distilled water. Sporulating fungi were examined, identified, illustrated and single spore isolations made.

Fungal isolates and culture characteristics. Single ascospore isolations of *Decaisnella formosa* and *Platstomum scabridiosporum* were made on corn meal seawater agar (CMA/SW) with added antibiotics (streptomycin sulfate 0.5g/L, penicillin G 0.5 g/L) and allowed to germinate overnight. Germinating spores were transferred to a fresh agar plate and incubated for 2 w at 25 C and deposited in BIOTEC Culture Collection (BCC).

A fungal culture of *Biatriospora marina* was obtained from City University of Hong Kong Culture Collection (CY) (TABLE I). This is repetative and has been deleted.

DNA extraction, amplification and sequencing.— Fungal biomass was harvested through cheesecloth, washed several times with sterile distilled water, frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. Fifty to 100 mg ground fungal mycelium was placed into 400 µL lysis buffer (O'Donnell et al 1997) and DNA extracted as follows: the tube was incubated at 70 C for 30 m, and a equal volume of phenol-chloroform (PIERCE) 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 30 m, or until the DNA had precipitated, and then centrifuged at 14K, 4 C, for 15 m. The DNA pellet was washed twice with chilled 75 % ethanol and air dried. The DNA was resuspended in 50 µl TE buffer and checked for quantity and quality in a 1% agarose gel electrophoresis.

The rDNA was amplified with Taq DNA polymerase from FERMENTAS Taq DNA polymerase (recombinant) using PCR Model MJ Research DYAD ALD. Primers used for amplification include the small subunit (SSU) and large subunit (LSU) of rDNA (White et al 1990, Bunyard et al 1994).The PCR products were purified using NucleoSpin^{R} Extract Kit (Macherey-Nagel, Germany), following the manufacturer's instructions. PCR products were directly sequenced by Macrogen Inc., Korea. Forward and reverse primers: NS1, NS6, ITS1, ITS4 LROR, and LR7 were used for the sequencing reactions (White et al 1990, Bunyard et al 1994). Each sequence was checked for ambiguous bases and assembled using BioEdit 6.0.7 (Hall 2004).

Sequence alignment and phylogenetic analyses.—The consensus sequences for each DNA region were multiple aligned by Clustal W 1.6 (Thompson et al 1994) along with

other sequences obtained from the GenBank database. Accession numbers for selected taxa obtained from GenBank are listed in TABLE II. The dataset was refined visually in BioEdit 6.0.7 (Hall 2004). *Hypocrea lutea* and *Xylaria hypoxylon* were chosen as the outgroup for combine SSU and LSU rDNA sequences. *Pyrenophora tritici-repentis* was chosen for ITS rDNA sequences

The phylogenetic analyses of the combined SSU and LSU rDNA sequence and ITS rDNA data were performed using PAUP*4.0b 10 (Swofford 2002). Gaps were treated as missing data.

(i) Unweighted Maximum parsimony analyses: 100 replicates of random stepwise addition of sequence and tree-bisection reconnection (TBR) branch-swapping algorithm. All characters were given equal weight.
The consistency indices (CI), retention indices (RI) and rescaled consistency indices (RC) were calculated for each tree generated. Nucleotide transformation based on the transition: transversion (ti:tv) ratio, was estimated using maximum likelihood score in PAUP*4.0b 10 (Swofford 2002).The rate ratio of transition: transversion was 2.0.

(ii) Weighted (step matrix) parsimony was performed to weight transversion 2.0 times over transition; 100 replicates of random stepwise addition of sequence and tree-bisection reconnection (TBR) branch-swapping algorithm.

(iii) Reweighted parsimony: characters were reweighted according to their RC: 100 replicates of random stepwise addition of sequence and TBR branch-swapping algorithm.Tree topologies from different parsimony analyses were tested with the Kashino-Hasegawa (K-H) maximum likelihood test (Kashino and Hasegawa 1989) to find the most likelihood tree. Bootstrap supports (Felsenstein 1985) were calculated for all parsimony analyses by 1000 bootstrap replicates (full heuristic searches, stepwise addition of sequence, simple sequence addition and TBR branch-swapping algorithm).

(iv)Maximum likelihood analyses: The model of Substitution used for Maximum Likelihood (ML) was chosen using the program Mrmodeltest 2.2 (Nylander 2004). Tree was inferred with PAUP* using the heuristic search option starting branch length using Rogers-Swofford approximation method, asis stepwise addition of sequence, TBR.

(v) The model of substitution used for Bayesian was chosen with Mrmodeltest 2.2 (Nylander 2004). Independent Bayesian phylogenetic analysis was performed in MrBayes 3.0.b4 (Huelsenbeck and Ronquist 2001)

In combine SSU and LSU rDNA sequences using a uniform SYM+I+G model, as selected by hLRT in Mrmodeltest 2.2 ([SYM+I+G]) lset nst = 6 rates = invgamma; prset statefreqpr = fixed(equal). Four Markov chains were run from random starting tree for 2000000 generations and sampled every 100 generations. The First 2000 generations were discarded as burn-in of the chain. A 50% majority rule consensus tree of all remaining trees, as well as the posterior probabilities (PP), was calculated.

In ITS rDNA sequences using a uniform SYM+I+G model, as selected by hLRT in Mrmodeltest 2.2 ([GTR+I+G]) lset nst = 6 rates = invgamma; prset statefreqpr = dirichlet(1,1,1,1). Four Markov chains were run from random starting tree for 2000000 generations and sampled every 100 generations. The First 2000 generations were discarded as burn-in of the chain. A 50% majority rule consensus tree of all remaining trees, as well as the posterior probabilities (PP), was calculated.

TABLE I. Fungal isolates sequenced for this study.

Fungus	Code	Location	GenBank accession No.		
			SSU	LSU	ITS
Biatriospora marina	CY1228	Singapore	***	***	***
Decaisnella formosa	BCC25616	Victoria, Australia	***	***	***
Decaisnella formosa	BCC25617	Victoria, Australia	***	***	***
Platstomum scabridiosporum	BCC22835	Victoria, Australia	***	***	***
Platstomum scabridiosporum	BCC22836	Victoria, Australia	***	***	***

[a] CY is from City University of Hong Kong Culture Collection, , BCC is from BIOTEC Culture Collection.

TABLE II. Accession numbers of DNA sequences obtained from GenBank and used in this study.

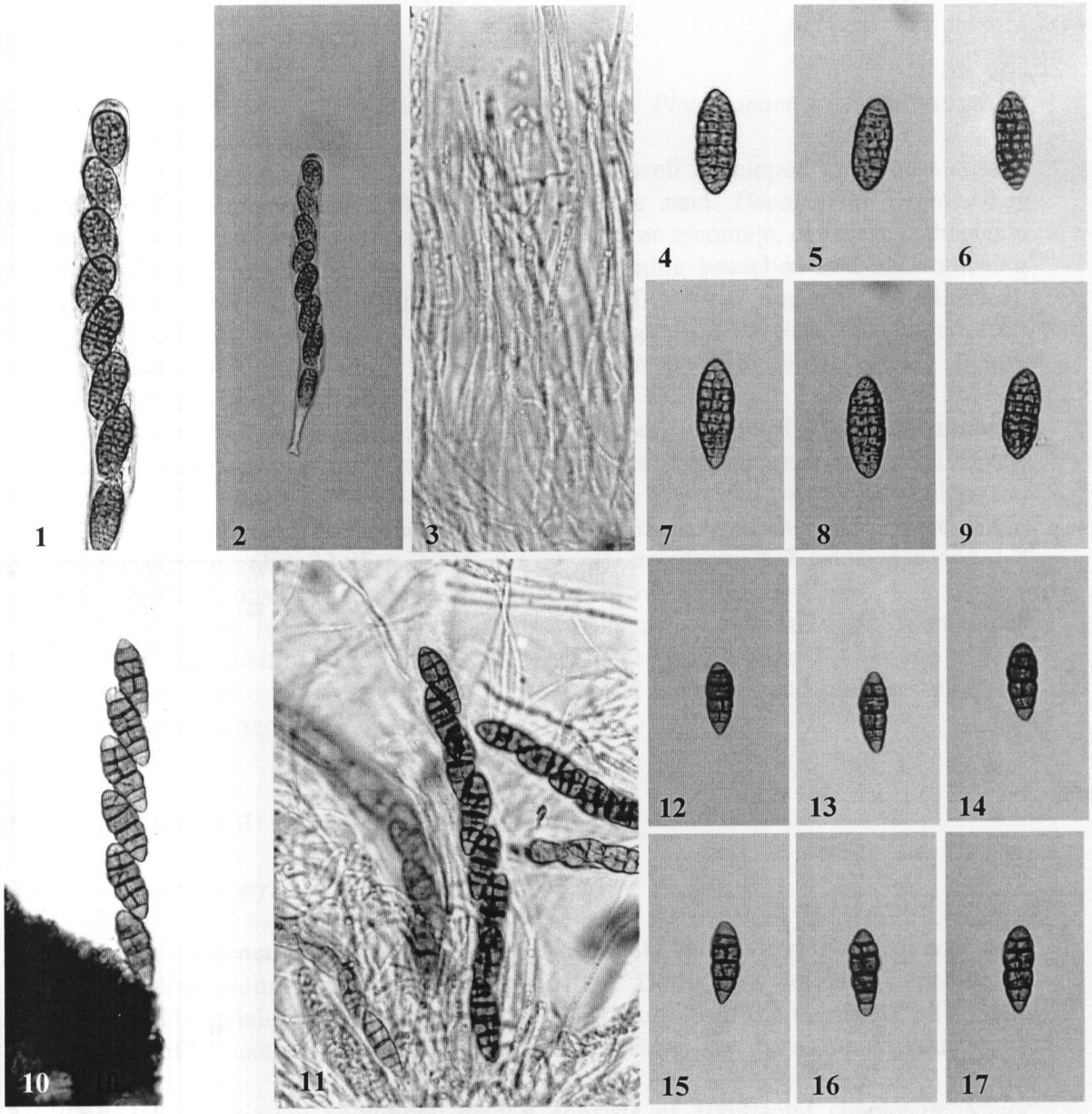
Fungi	Classification	GenBank Accession no.		
		SSU	LSU	ITS
Bimuria novae-zelandiae D. Hawksw., Chea & Sheridan	Dothideomycetes, Pleosporales, Melanommataceae	AY016338	AY016356	N/A
Byssothecium circinans Fuckel	Dothideomycetes, Pleosporales, Teichosporaceae	AY016339	AY016357	N/A
Capnodium citri Berk. & Desm	Dothideomycetes, Capnodiales	AY016340	AY004337	N/A
Capnodium coffeae Pat	Dothideomycetes, Capnodiales	DQ247808	DQ247800	N/A
Capronia mansonii (Schol-Schwarz) E. Müll., Petrini, P.J. Fisher, Samuels & Rossman	Chaetothyriomycetidae, Chaetothyriales	X79318	AY004338	N/A
Ceramothyrium carniolicum (Rehm) Petr	Chaetothyriomycetidae, Chaetothyriales	AF346418	AY004339	N/A
Cochliobolus heterostrophus (Drechsler) Drechsler	Dothideomycetes, Pleosporales, Pleosporaceae	AY544727	AY544645	N/A

Cochliobolus sativus (S. Ito & Kurib.) Drechsler ex Dastur	Dothideomycetes, Pleosporales, Pleosporaceae	DQ677995	AY544645	N/A
Delitschia didyma Auersw	Dothideomycetes, Pleosporales, Delitschiaceae	AF242264	DQ384090	N/A
Delitschia winteri W. Phillips & Plowr.) Sacc	Dothideomycetes, Pleosporales, Delitschiaceae	AF164354	DQ384091	N/A
Delphinella strobiligena (Desm.) Sacc. ex E. Müll. & Arx	Dothideomycetes, Dothideales	AY016341	AY016358	N/A
Dothidea hippophaeos (Pass.) Fuckel	Dothideomycetes, Dothideales	U42475	DQ678048	N/A
Dothidea insculpta Wallr	Dothideomycetes, Dothideales	DQ247810	DQ247802	N/A
Elsinoe centrolobi Bitanc. & Jenkins	Dothideomycetes, Myriangiales	DQ678041	DQ678094	N/A
Elsinoe phaseoli Jenkins	Dothideomycetes, Myriangiales	DQ678042	DQ678095	N/A
Herpotrichia diffusa (Schwein.) Ellis & Everh	Dothideomycetes, Pleosporales, Lophiostomataceae	DQ678019	DQ678071	N/A
Herpotrichia juniperi (Duby) Petr.	Dothideomycetes, Pleosporales, Lophiostomataceae	DQ678029	DQ678080	N/A
Hypocrea lutea (Tode) Petch	Sordariomycetes, Hypocreales	D14407	AF543791	N/A
Karstenula rhodostoma (Alb. & Schwein.) Speg.	Dothideomycetes, Pleosporales, Melanommataceae	N/A	AY787933	N/A
Kirschsteiniothelia elaterascus Shearer	Dothideomycetes, Pleosporales, Pleosporaceae	N/A	AY787934	N/A
Lepidosphaeria nicotiae Parg.-Leduc	Dothideomycetes, Incertae sedis, Testudinaceae	DQ384068	DQ384106	N/A
Leptosphaeria doliolum (Pers.) Ces. & De Not.	Dothideomycetes, Pleosporales, Leptosphaeriaceae	U43447	U43474	N/A
Leptosphaeria macrospore (Fuckel) Thüm.	Dothideomycetes, Pleosporales, Leptosphaeriaceae	N/A	DQ384092	N/A
Lophiostoma arundinis (Pers.) Ces. & De Not.	Dothideomycetes, Pleosporales, Lophiostomataceae	DQ782383	DQ782384	N/A
Lophiostoma caulium (Fr.) Ces. & De Not.	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	DQ528763	N/A
Lophiostoma crenatum (Pers.) Fuckel	Dothideomycetes, Pleosporales, Lophiostomataceae	DQ678017	DQ678069	N/A
Lophiostoma macrostomum (Tode) Ces. & De Not.	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	DQ384094	N/A

Myriangium duriaei Mont. & Berk.	Dothideomycetes, Myriangiales	AF242266	AY016365	N/A
Lophiostoma arundinis (Pers.) Ces. & De Not.	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	N/A	AF383952
Lophiostoma caulium (Fr.) Ces. & De Not.	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	N/A	AF383953
Lophiostoma vagabundum Sacc.	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	N/A	AF383954
Massarina armatispora K.D. Hyde, Vrijmoed, Chinnaraj & E.B.G. Jones	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	N/A	AF383955
Massarina bipolaris K.D. Hyde	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	N/A	AF383956
Massarina corticola (Fuckel) L. Holm	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	N/A	AF383957
Massarina eburnean 1 (Tul. & C. Tul.) Sacc.	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	N/A	AF383959
Massarina eburnean 2 (Tul. & C. Tul.) Sacc.	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	N/A	AF383958
Massarina frondisubmersum Liew Aptroot and K.D. Hyde	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	N/A	AF383960
Massarina papulosa (Durieu & Mont.) S.K. Bose	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	N/A	AF383961
Massarina ramunculicola K.D. Hyde	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	N/A	AF383962
Massarina rubi (Fuckel) Sacc.	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	N/A	AF383963
Massarina walkeri Shoemaker, C.E. Babc. & J.A.G. Irwin	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	N/A	AF383965
Melanomma pulvis-pyrius (Pers.) Fuckel	Dothideomycetes, Pleosporales, Melanommataceae	AF164369	DQ384095	N/A
Mycosphaerella fijiensis M. Morelet	Dothideomycetes, Capnodiales	DQ767652	DQ678098	N/A
Neotestudina rosatii Segretain & Destombes	Dothideomycetes, Incertae sedis, Testudinaceae	DQ384069	DQ384081	N/A
Pleomassaria siparia (Berk. & Broome) Sacc.	Dothideomycetes, Pleosporales, Pleomassariaceae	AF164373	AY004341	N/A
Phaeosphaeria avenaria (G.F. Weber) O.E. Erikss.	Dothideomycetes, Pleosporales, Phaeosphaeriaceae	AY544725	AY544684	N/A

Phaeosphaeria eustoma (Fuckel) L. Holm	Dothideomycetes, Pleosporales, Phaeosphaeriaceae	DQ678011	DQ678063	N/A
Pleospora herbarum (Pers.) Rabenh.	Dothideomycetes, Pleosporales, Pleosporaceae	DQ767648	DQ678049	AF071344
Pyrenophora phaeocomes (Rebent.) Fr.	Dothideomycetes, Pleosporales, Pleosporaceae	DQ499595	DQ499596	N/A
Pyrenophora tritici-repentis (Died.) Drechsler	Dothideomycetes, Pleosporales, Pleosporaceae	AY544716	AY544672	AF071348
Scorias spongiosa (Schwein.) Fr.	Dothideomycetes, Capnodiales	DQ678024	DQ678024	N/A
Stylodothis puccinioides (DC.) Arx & E. Müll.	Dothideomycetes, Dothideales	AY016353	AY004342	N/A
Trematosphaeria pertusa (Pers.) Fuckel	Dothideomycetes, Pleosporales, Melanommataceae	DQ678020	DQ678072	N/A
Wettsteinina dryadis (Rostr.) Petr.	Dothideomycetes, Pleosporales, Pleosporaceae	N/A	AY849968	N/A
Wettsteinina macrotheca (Rostr.) E. Müll.	Dothideomycetes, Pleosporales, Pleosporaceae	N/A	AY849969	N/A
Sydowia polyspora Bref. & Tavel) E. Müll.	Dothideomycetes, Dothideales	DQ678005	DQ678058	N/A
Ulospora bilgramii (D. Hawksw., C. Booth & Morgan-Jones) D. Hawksw., Malloch & Sivan.	Dothideomycetes, Incertae sedis, Testudinaceae	DQ384071	DQ384108	N/A
Vaginatispora aquatica K.D. Hyde	Dothideomycetes, Pleosporales, Lophiostomataceae	N/A	N/A	AF383968
Venturia chlorospora (Ces.) P. Karst.	Dothideomycetes, Pleosporales, Venturiaceae	N/A	DQ384101	N/A
Venturia inaequalis (Cooke) G. Winter	Dothideomycetes, Pleosporales, Venturiaceae	N/A	AY152606	N/A

N/A = not avaliable



FIGS 1-9. *Decaisnella formosa*. FIGS 1, 2. Mature asci with ascospores in asci. FIG 3. Hyaline trabeculate pseudoparaphyses. FIGS 4-9. Mature ascospores.

FIGS 10-17. *Platystomum scabridisporum.* FIG 10. Ascospores in asci. FIG 11. Hyaline trabeculate pseudoparaphyses. FIGS 12-17. Mature ascospores.

RESULTS AND DISCUSSION

The morphology *of Decaisnella formosa* and *Platystomum scabridisporum* is illustrated in FIGS 1-17.

The Mornington Peninsula Nature Park has a well developed sand dune system with a significant amount of driftwood buried in the sand. *Decaisnella formosa* has subglobose, immersed, black, cortiacieous and solitary or ascomata, ostioles. Trabeculate pseudoparaphyses, unbranched at the base and becoming branched and anastomosing above the asci. Asci 125-230 x 12.5-17.5 um (X= 180 x 14.33 um, n=30), cylindrical, fusiform to elongate, pedunculate, eight-spored. Ascospores 22.5-40 x 7.5-12.5 um (X= 30 x 10.3 um, n=50), muriform, 9-13 transverse and 1-6 longidinal septate, slightly constricted at the septum, brown (FIGS 1-9).

Platystomum scabridisporum has subglobose, immersed, black, erumpent, coriaceous soilary or gregarious, ostiolate. Trabeculate pseudoparaphyses. Asci 112.5-172.5 x 8.75-12.5 um (X= 144.67 x 11.67 um, n =50), cylindrical, fusiform to elongate, pedunculate, eight-spored, fissitunicate, with an apical ocular chamber. Ascospores 17.5-27.5 x 6.25-7.5 um (X= 22.05 x 7.375 um, n=50), muriform, 5-7 transverse and 1-2 longidinal septate, constricted at the central septum, brown (FIGS 10-17).

Biatriospora marina has immersed ascomata, cylindrical asci, filamentous pseudoparaphyses fusiform ascospores. In term of ascospore have 2-4 septate toward each end, non-septate in the centre, not constricted at the septa and globose refrective chamber or appendage.

I. The combined SSU and LSU rDNA sequence

Maximum parsimony:

The phylogenetic analyses of the combined SSU and LSU rDNA sequence data were performed, along with various orders of the Dothideomycetidae (Capnodiales, Dothideales, Hysteriales, Pleosporales and Myriangiales) from the GenBank. The data set consisted of 52 taxa, with *Hypocrea lutea* and *Xylaria hypoxylon* as an outgroup.

The Originally maximum parsimony dataset consists of 3068 total characters. Major insertions were present in the genes of five species (*Biatriospora marina, Delitschia didyma* SSU; *Delitschia didyma* LSU). Between 464-798 bp of SSU rDNA and 1214-1558 bp of LSU rDNA were included from the analyses. The unweighted parsimony maximum parsimony dataset consists of 3068 total characters, 2300 characters are constant, 485 characters are parsimony informative and 283 variable characters are parsimony uninformative. Maximum parsimony analyses on different settings (unweighted, weighted (step matrix) and reweighted) gave the same topology for all analyses.

This analysis resulted in a single MPT with 1906 steps long (CI = 0.536, RI = 0.760, RC = 0.407). The reweighted parsimony resulted in Single MPT with a tree length of 1020.75318 steps, CI = 0.707, RI = 0.826, RC = 0.584, which gave the same topology as

unweighted parsimony (trees not shown). The weighted step matrix parsimony yielded two MPTs with 2609 steps long, CI = 0.557, RI = 0.771 and RC = 0.429. Tree length from step matrix parsimony was longer than unweighted parsimony and had higher consistency and rescaled consistency indices. Morever, Bayesian inference also provided identical topology to other analyses. Bootstrap value (equal to or above 50%) based on 1000 replicates are shown on the upper branches (FIG 18).

Maximum likelihood and Bayesian analysis:

Maximum likelihood analyses yield a single tree of log likelihood –14159.42219. Estimated nucleotide frequencies were equal and shape parameter (alpha) was 0.5538. Maximum likelihood and Bayesian analyses yield tree that were slightly different in topology from those derived in Maximum parsimony. The posterior probabilities (PP) are represented on the branches (FIG 19).

In the analysis the Dothideomycetidae form four major groups: Capnodiales, Dothideales, Pleosporales and Myriangiales. *Biatriospora marina, Platystomum scabridisporum* and *Decaisnella formosa* grouped within the Pleosporales in all analyses supported by 99% bootstrap value (FIG.18) and 100% posterior probabilities (FIG.19). The relationship of *Biatriospora marina* with other taxa in the Pleosporales can not be inferred from the current study as it occurred singly in all analyses. The morphology of *Biatriospora marina* is unique with subglobose to pyriform ascomata, wide ostioles, bitunicate asci, cylindrical, thick-walled, fusiform, 2-4 septate toward each end, non-septate in the centre, not constricted at the septum, brown to dark brown ascospores.

Sequence analyses indicated that *Decaisnella formosa* and *Platystomum scabridisporum* are monophyletic and cluster within the Lophiostomataceae, grouping with 79% bootstrap value and 99% posterior probabilities with the species *Lophiostoma arundinis, L. caulium and L. crenatum* (FIGS 18, 19). The families Melanommataceae, Lophiostomataceae, and Pleosporaceae are not monophyletic in our analysis. The Delitschiaceae, Pleomassariaceae and Teichosporaceae form separate monophyletic groups and are not closely related. The Leptosphaeriaceae and Phaeosphaeriaceae are closely related with 57% bootstrap value and 100% posterior probabilities. The main feature distinguishing these two families are the scleroplectenchymatous cells in ascomata of the Leptosphaeriaceae. These two families have not been resolved as separate taxa in earlier studies (Camara et al 2003, Rossman et al 2002, Cheng et al 2004). The Venturiaceae formed a strongly supported group (100% bootstrap value and 100% posterior probabilities), and falls outside of Pleosporales

The Lophiostomataceae Melanommataceae and Pleosporaceae are inferred as polyphyletic (FIGS 18, 19). The Lophiostomataceae can be divided into three clades, with one clade including three species of *Lophiostoma* (Lophiostomataceae 1). Clade Lophiostomataceae 1, with 79% bootstrap value and 99% posterior probabilities, includes *Decaisnella formosa, Lophiostoma arundis, L. caulium, L. crenatum and Platystomum scabridisporum. Platystomum scabridisporum* grouped with 100% bootstrap value and 100% posterior probabilities with Lophiostoma *arundis, L. caulium* and *L. crenatum.*

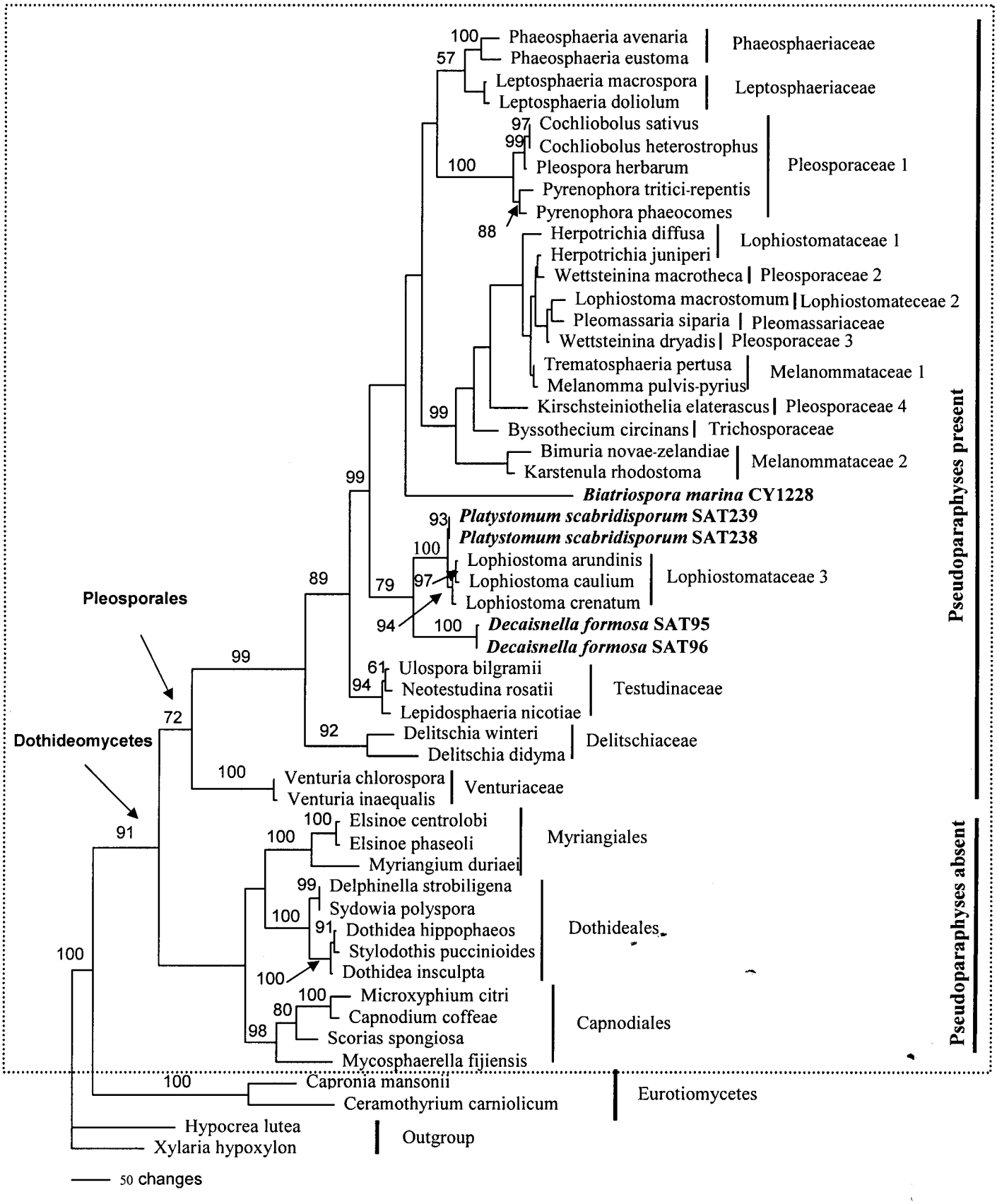


FIG 18. One of the most parsimony tree of weighted step matrix parsimony from combine SSU and LSU data set. Bootstrap value (equal to or above 50%) based on 1000 replicates are shown.

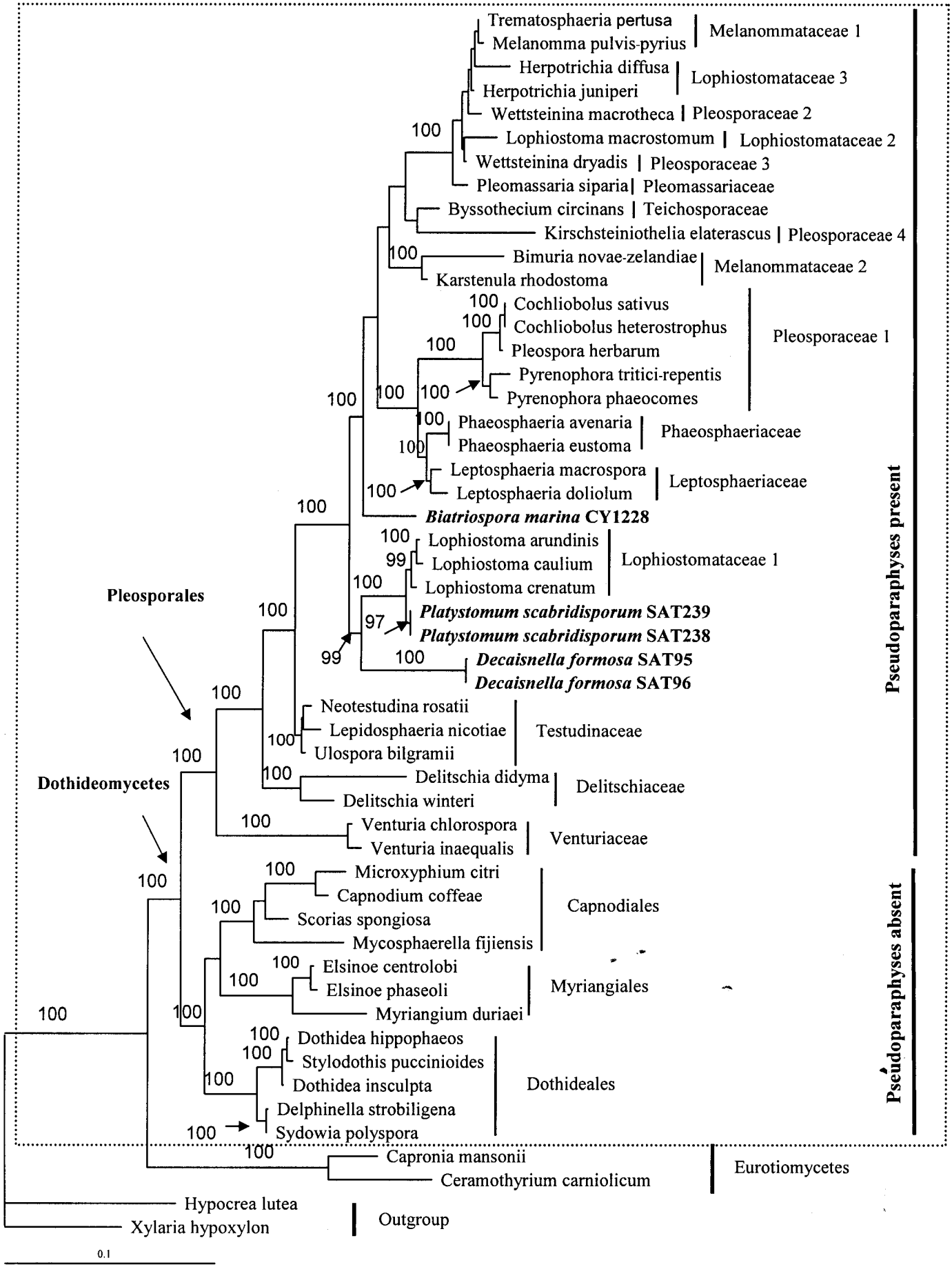


FIG 19. Maximum likelihood tree estimated from one of the most parsimonious trees from unweighted data set with gaps treated as missing data.The posterior probabilities (PP) are represented on the branches.

Decaisnella formosa are the sister group of the *Platystomum scabridisporum*. The second clade (Lophiostomataceae 2) consists of one species of *Lophiostoma macrostomum*, which was type species of *Lophiostoma*. The third clade (Lophiostomataceae 3) comprised of *Herpotrichia diffusa* and *H. juniperi*, it well known plant pathogens.

Taxa producing pseudoparaphyses (i.e., Pleosporales) form a monophyletic group with 72% bootstrap value (FIG.18) and 100% posterior probabilities (FIG.19). The aparaphysate species are the sister-group of the Pleosporales clade, this grouping has no bootstrap support but 100% posterior probabilities. Within this group, two sister-groups can be distinguished: the *Dothideales sensu* Barr (1979, 1987) with 100% bootstrap value (FIG.18) and 100% posterior probabilities (FIG.19), and the *Myriangiales sensu* Luttrell (1973) and Barr (1979) with the *Capnodiales sensu* Barr (1987) which have 100% bootstrap value (FIG.18). In weighted step matrix parsimony analysis, *Myriangiales* appear as paraphyletic and basal to the Dothideales with 100% bootstrap value (FIG.18), while in the Maximum likelihood and Bayeaian tree it appear as paraphyletic and basal to the Capnodiales 100% posterior probabilities (FIG.19).

II. The ITS rDNA sequence

The ITS rDNA dataset consisted of 20 taxa. *Pleospora herbarum* and *Pyrenophora tritici-repentis* were desidnated outgroups. Gaps were trested as missing data.

Maximum parsimony:

The phylogenetic analyses of the ITS rDNA sequence data were performed, along with different genera of the Lophiotomataceae from the GenBank.

The Orginally maximum parsimony dataset consists of 651 total characters. The unweighted parsimony maximum parsimony dataset consists of 651 total characters, 237 characters are constant, 284 characters are parsimony informative and 130 variable characters are parsimony uninformative. Maximum parsimony analyses on different settings (unweighted, and reweighted) gave the same topology for all analyses.

Unweighted maximum parsimony analysis of this data set yield two most parsimony trees of 1204 step in length, and with CI = 0.621, RI = 0.605, RC = 0.376 (FIG 20). The reweighted parsimony resulted in Single MPT with a tree length of 748.00001 steps, CI = 0.709, RI = 0.650, RC = 0.461, which gave the one of the most topology as unweighted parsimony (FIG 20). Bayesian inference also provided identical

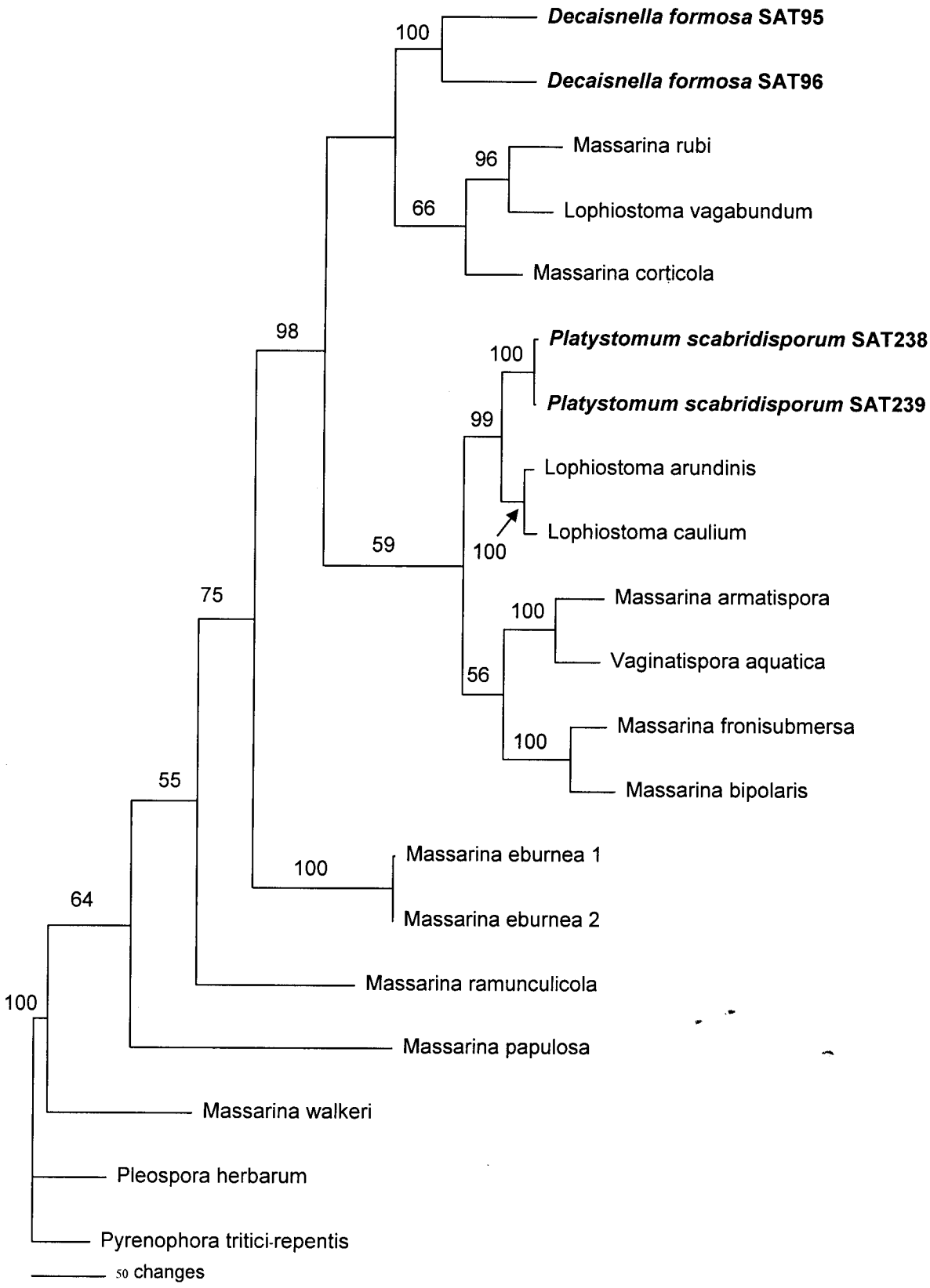


FIG 20. One of the most parsimonious tree obtained from ITS-5.8S data set. Bootstrap value (equal to or above 50%) based on 1000 replicates are shown on the branch.

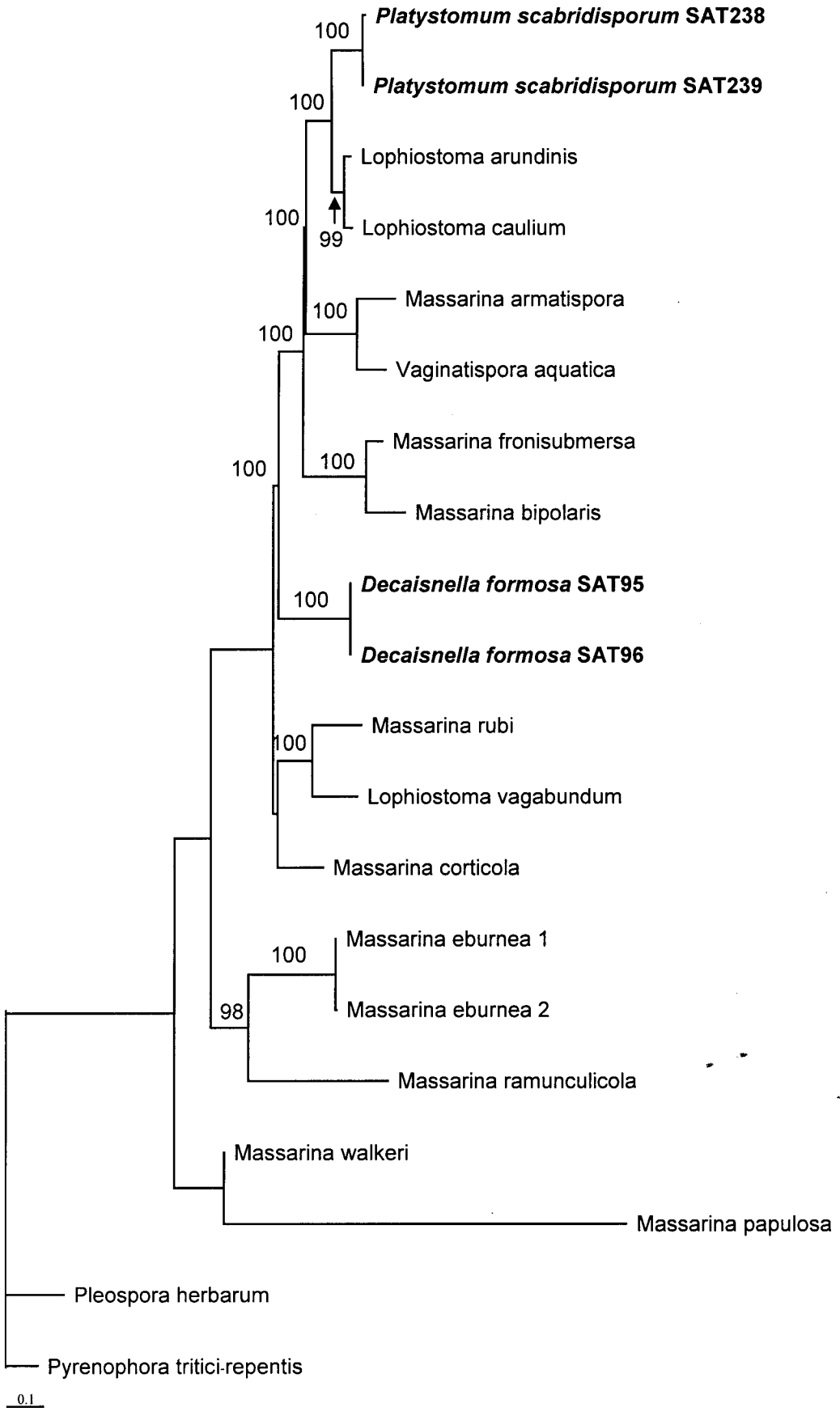


FIG 21. Maximum likelihood tree obtained from ITS-5.8S data set. The posterior probabilities (PP) are represented on the branches.

topology to other analyses. Bootstrap value (equal to or above 50%) based on 1000 replicates are shown on the upper branches.

Maximum likelihood and Bayesian analysis:

Maximum likelihood-ratio test in Mrmodeltest 2.2 suggested that the best-fit model of evolution for thus dataset was GTR+I+G. Maximum likelihood analyses yield a single tree of log likelihood –5472.65497 Estimated nucleotide freuencies were equal and shape parameter (alpha) was 0.9986. Maximum likelihood and Bayesian analyses yield tree that were slightly different in topology from those derived in Maximum parsimony. FIG 21 shows the tree with Maximum likelihood and posterior probabilities (PP) are represented on the upper branches.

Two strains of *Decaisnella formosa* grouped together with species of *Lophiostoma vagabundum, Massarina corticola* and *M. rubi* with low bootstrap support (FIG 20), while in Maximum likelihood tree it closely related with *Lophiostoma arundinis, L. caulium, Massarina bipolaris, M fronisubmersa, Platystomum scabridisporum and Vaginatispora aquatica* (FIG 21). *Platystomum scabridisporum* clustered with *Lophiostoma arundinis* and *L. caulium* with with 99% bootstrap value and 100% posterior probabilities.

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PART C

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Morphological and molecular characteristics of a poorly known marine ascomycete, *Manglicola guatemalensis*

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INTRODUCTION

Kohlmeyer (1984) was the first to report 15 marine fungi from Thailand in Chonburi Province. Pilantanapak *et al.*, (2005) reported 126 marine fungi on *Nypa fruticans*, while Jones *et al.*, (2006) documented 116 ascomycetes, 3 basidiomycete and 28 anamorphic fungi for Thailand, of 33 species which were new records for the country. *Manglicola guatemalensis* is one of these new record for Thailand.

Two collections of this poorly known ascomycete, *M. guatemalensis* were made on *N. fruticans*, Koh Chang National Park; Trat Province and Trang Province, Thailand.

OBJECTIVE

In order to determine the phylogenetic relationship of this fungus, SSU rDNA was sequenced and the results are presented in this poster.

MATERIAL AND METHODS

Nypa fruticans samples (leaves, rachids, petioles and bases) were collected from Koh Chang National Park; Trat Province (October 2005 & 2006) and Trang Province (November 2005). Cultures used for the molecular study were BCC 24217 and BCC 20156. DNA was extracted using CTAB lysis buffer (O'Donnell *et al.*, 1997). PCR products were directly sequenced by Macrogen.,Inc Korea. Forward and reverse primers: NS1 & NS6 were used for the sequencing reactions. The phylogenetic analyses were performed with PAUP* 4.0b10 (Swofford, 2002) using maximum parsimony analyses.

RESULT & DISCUSSION

M. guatemalensis (Fig. 1)

Ascomata 1100-1750 um height, 290-640 um in dia. around the center, 82.5-280 um in dia. around the base, 100-200 um in dia. around the apex; obtusely clavate to obtusely fusiform; stipitate, ascoma wall differentiated into several layers of polygonal.

Peridium 30-55 um thick, composed of three to five layers of cells.

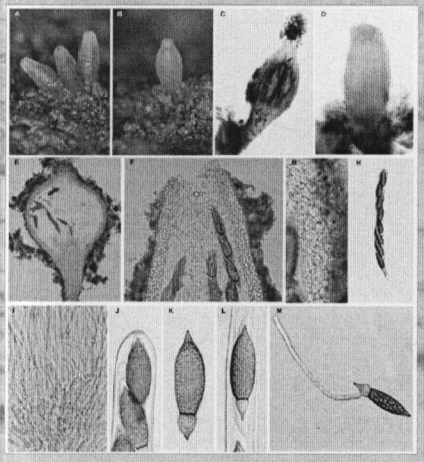

Fig. 1 Manglicola guatemalensis. A. Mature ascomata. B. Immature ascoma. C. Ascoma, dark ascospores of mature asci visible through the thin wall, some spores exuded at the ostiole. D. Immature ascoma strained in Lactophenol. E-F. Longitudinal section of ascoma. G. Longitudinal section of ascomal wall. H. Asci. I. Pseudoparaphyses. J. Apical pore in the endoascus. K-L. Ascospores with apical appendage. M. Germinating ascospore, with germ tube developing laterally from the small basal cell.

Pseudoparaphyses 1.25-2.5 um in diameter, numerous, septate and simple or reticulate.

Asci 440-640 X 30-50 um, eight-spored, cylindrical, bitunicate.

Ascospores from Koh Chang were 87.5-137.5 X 20-47.5 um and from Trang were 92.5-12.5 X 22.5-40 um, uniseriate, fusiform, unequally one-septate, constricted at the septum; apical cell larger, chestnut-brown; basal cell, turbinate, light brown; gelatinous appendages cover both apices; and cylindrical morphology.

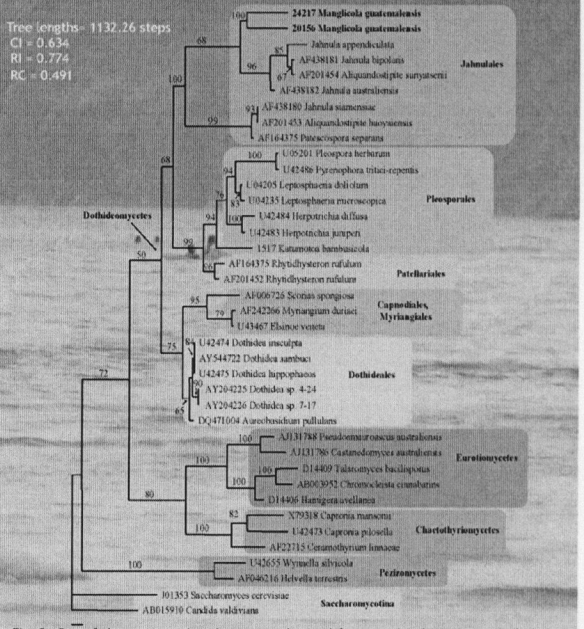

Fig. 2 One of the most parsimonious trees obtained from weighted parsimony analysis (step matrix), from partial SSU rDNA sequences. Bootstrap values higher than 50% from weighted parsimony (characters reweighted) are given above the branches. Bar indicates 10 character state changes

SSU sequences positioned *M. guatemalensis* in a sister clade to the Jahnulales clade, with high bootstrap support (100%) based on maximum parsimony analyses and distantly related to the Dothideales and Pleosporales (Fig. 2). However, it has a closer phylogenetic relationship with Pleosporales than the Dothideales. This supports the earlier views of Kohlmeyer and Kohlmeyer (1971) who noticed the close relationship of *M. guatemalensis* with the Pleosporaceae and Venturiaceae. An obvious morphological character that places *M. guatemalensis* in the Jahnulales. Huhndorf (1992, 1994) classified *Manglicola* in the Hypsostromataceae, order Incertae sedis, however, sequences of Hypsostroma species are not available for phylogenetic comparison.

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