Cryptogamie, Mycologie, 2011, 32 (4): 343-358

Trematosphaeriaceae fam. nov. (Dothideomycetes, Ascomycota)

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Abstract — Trematosphaeriaceae fam. nov. is introduced to accommodate the genera *Falciformispora*, *Halomassarina* and *Trematosphaeria*. The main distinguishing characters of the family are medium-sized rounded ascomata with a papillate ostiole, a relatively wide, coriaceous peridium, cellular pseudoparaphyses and cylindro-clavate asci. The ascospores are two-celled or many celled, hyaline or brown. Phylogenetic analysis inferred from combined nuclear SSU and LSU rRNA and translation elongation factor 1-alpha (TEF-1-alpha) and second largest subunit of RNA polymerase (RPB2) datasets show that these genera form a strongly supported cluster within the *Pleosporales*. The type species of each genus is illustrated and briefly discussed. *Asteromassaria pulchra* has been included in the family in previous publications; however, since the type of the genus (*A. macrospora*) was not included in the phylogenetic analysis, the familial placement cannot be confirmed. Furthermore *Asteromassaria pulchra* did not cluster in *Trematosphaeriaceae* in the analysis presented in this paper.

LSU rDNA / Pleosporales / RPB2 / SSU rDNA / Taxonomy / TEF-1-alpha

INTRODUCTION

The taxonomic placement of genera in families and orders of the Dothideomycetes previously relied heavily on morphological data (e.g. Barr 1987). More recently molecular data has been applied to resolve the higher level taxonomy of this class (Zhang *et al.*, 2009a, 2009b; Schoch *et al.*, 2009; Suetrong *et al.*, 2009 Tanaka *et al.*, 2009). One major finding resulting from this molecular phylogenetic study was that many morphological characters, such as form of ascomata (perithecioid or cleistothecioid), pseudoparaphyses (presence or absence, cellular or trabeculate), ascospore morphology (septation and pigmentation) had evolved on more than one occasion and are not taxonomically informative at the ordinal level (Liew *et al.*, 2000).

The results published in *Studies in Mycology*, *Volume 64* (Schoch *et al.*, 2009) on the phylogeny of Dothideomycetes, brought together a wide range of taxa previously not sequenced and from diverse ecological habitats: bambusicolous fungi,

freshwater, marine, and rock inhabiting fungi, resulting in a number of taxonomic changes, especially at the generic and familial level. New families proposed included: Dissoconiaceae (Capnodiales, Crous *et al.*, 2009); Amniculicolaceae, Lentitheciaceae (Pleosporales, Zhang *et al.*, 2009a); Lindgomycetaceae Pleosporales, Shearer *et al.*, 2009); Aigialaceae, Morosphaeriaceae (Pleosporales, Suetrong *et al.*, 2009) and Tetraplosphaeriaceae (Pleosporales, Tanaka *et al.*, 2009). Many of the taxa referred to these new families were *Lophiostoma* and *Massarina* species. Three studies (Schoch *et al.*, 2009; Suetrong *et al.*, 2009a; Zhang *et al.*, 2009b) also referred to the Trematosphaeriaceae in their phylogenetic trees as a family to represent a well-supported clade. The family name however, was not formally introduced and presently is a *nomen nudem* (Lumbsch & Huhndorf, 2010). Taxa assigned to the Trematosphaeriaceae include: *Falciformispora lignatilis*, *Halomassarina thalassiae*, *Trematosphaeria pertusa* and *Asteromassarina pulchra* (Schoch *et al.*, 2009). Zhang *et al.* (2009a) did not include *Halomassarina thalassiae* in their analysis, while Suetrong *et al.* (2009) did not include *A. pulchra*. All three studies showed that the family was well supported. *Falciformis* and *Halomassarina* are marine species, while *Trematosphaeria pertusa* and *Asteromassarina pulchra* are known from terrestrial wood.

The purpose of this paper is to formally introduce this family and detail the genera included with descriptions and illustrations.

MATERIAL AND METHODS

Collection of fungi: Drift and attached wood, culms and leaves of marsh plants were collected from a variety of habitats and geographical locations, placed in clean plastic bags and returned to the laboratory. After washing with freshwater to remove sediments, the samples were examined for fungi. 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: A selection of specimens were isolated by cutting the top of an ascoma with a sterilized razor blade, removing the contents of the centrum to make a spore suspension and then streaking the spores on antibiotic seawater agar (Kohlmeyer & Kohlmeyer 1979; Schoch *et al.*, 2007) and picking up germinating spores. Other single ascospore isolations were made on cornmeal 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 relevant culture collections (Table 1, see online supplementary material).

DNA extraction, amplification and sequencing: Fungi were grown in potato dextrose broth with seawater at a temperature of 25°C for 2 w or until enough mycelium for DNA extraction were obtained. Fungal biomass was harvested for a different set of isolates by filtering through cheesecloth, and washed several times with sterile distilled water. The harvested mycelium was stored at –20°C and ground to a fine powder with a mortar and pestle. Fifty to 100 mg ground fungal mycelium was placed into 400 ml lysis buffer (O'Donnell *et al.*, 1997) and DNA extracted as follows: the tube was incubated at 70°C for 30 min, and an equal volume of phenol-chloroform (PIERCE) added. The upper

liquid phase was transferred to a new micro tube containing chilled absolute ethanol and 7.5 M ammonium acetate. The mixture was kept at –20°C for 30 min, or until the DNA had precipitated, and then centrifuged at 14000 rpm, 4°C, for 15 min. The DNA pellet was washed twice with chilled 75% ethanol and air dried.

***Sequence alignment and phylogenetic analyses*:** Sequence data were generated from four loci: partial nuclear SSU and LSU ribosomal RNA gene, the translation elongation factor 1-alpha (TEF-1-alpha) and second largest subunit of RNA polymerase (RPB2), bounded by primers NS1, NS3, NS4 and NS6 for SSU rDNA (White *et al.*, 1990), bounded by primers JS1, JS8, LR7 and LROR for LSU rDNA (Bunyard *et al.*, 1994; Landvik, 1996), bounded by primers 983F, 2218R, CEFR2, CEFF2, 1577F and 1567R (Rehner, 2001) and bounded by primers 5F1, 5F2, 7cR and 7R (Liu *et al.*, 1999). Protocols and primer sequences for amplification and sequencing have been described (White *et al.*, 1990; Bunyard *et al.*, 1994; Landvik, 1996; Liu *et al.*, 1999). DNA sequencing was performed using the primers mentioned above in an Applied Biosystem 3730XL DNA Analyzer at Macrogen, Inc., Korea. Each sequence was checked for ambiguous bases and assembled using BioEdit 6.0.7 (Hall, 2004). Sequences of this study have been deposited in GenBank. Sequence homologies were also analyzed using BLAST search engine at the National Centre for Biotechnology Information (NCBI) to facilitate the selection of other fungal sequences to be used in the analyses. Sequences with the highest alignment score were selected for phylogenetic analyses. Alignments were checked and manually optimized along with other sequences obtained from GenBank nucleotide database. The consensus sequences for each DNA region were initially aligned with ClustalW v. 1.6 (Thompson *et al.*, 1994).

Multiple alignments were carried out on the sequences generated with other sequences obtained from GenBank in Clustal W 1.6 and refined manually in BioEdit 6.0.7 (Hall 2004). Manual gap adjustments were made to improve the alignment. Ambiguously aligned regions were excluded. Missing data at the 5'- and 3'-end of partial sequences were coded by '?'. The tree construction procedure was performed in PAUP* 4.0b10 (Swofford, 2002) on Window versions and a Power Macintosh G4 (Apple Computer, Inc., Cupertio, California, USA). Phylogenetic trees were visualized using the program Treeview (Page, 1996). The phylogenetic analyses of different datasets were performed using maximum parsimony, Bayesian and maximum likelihood algorithms. Two members of the *Arthoniomycetes*, namely *Opegrapha dolomitica is* and, *Roccella fuciformis* were chosen as outgroup sequences based on their placement as sister group to the Dothideomycetes (Schoch *et al.*, 2009).

i) Maximum parsimony analyses were performed using PAUP v. 4.0b10 (Swofford, 2002), with gaps were treated as missing data. Trees were generated using 100 replicates of random stepwise addition of sequence and tree-bisection reconnection (TBR) branch-swapping algorithm, with all characters given equal weight. Branch support for all parsimony analyses was estimated by performing 1000 bootstrap replicates (Felsenstein, 1985) with a heuristic search of 10 random-addition replicates for each bootstrap replicate. The consistency indices (CI; Kluge & Farris, 1969), retention indices (RI; Farris, 1989) and rescaled consistency indices (RC; Farris, 1989) were calculated for each tree generated. Tree topologies from parsimony analyses were tested with the KashinoHasegawa (K-H) maximum likelihood test (Kishino & Hasegawa, 1989) to find the most likely tree. Confident branch support is defined as bootstrap values (BSMP) equal or more than 50%.

ii) Bayesian analyses: The model of substitution used for Bayesian was chosen using the program Mrmodeltest 2.2 (Nylander, 2004). Independent Bayesian phylogenetic analysis was performed in MrBayes 3.0b4 (Huelsenbeck & Ronquist, 2001) using a uniform [GTR+I+G] model, Iset 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 trees, which represented the burn-in phase of the analysis, were discarded, with 18000 trees used for calculating posterior probabilities (BYPP) in the consensus tree. Posterior probabilities were obtained for each clade. Confident branch support is defined as Bayesian posterior probabilities equal or more than 0.95.

iii) Maximum likelihood analyses (ML) were conducted in RAxML v. 7.2.2 (Stamatakis, 2006). The dataset was partitioned according to each gene and separated codons (eight partitions). A general time reversible (GTR+I+G model) plus invariant sites plus gamma distributed model A tree was obtained by simultaneously running a fast bootstrap search of 1000 pseudo replicates followed by a search for the most likely tree under functional setting "a". Maximum likelihood bootstrap value (BSML) equal or greater than 50% are given above each node.

Maximum parsimony (BSMP, Left) and likelihood (BSML, right) bootstrap values greater than 50% above the node. Bayesian posterior probabilities greater than 0.95, are given below each node (BYPP). The internodes that are highly supported by all bootstrap proportions (100%) and posterior probabilities (1.00) are shown as a thicker line.

RESULTS

The combined SSU and LSU and TEF-1-alpha and RPB2 data set consisted of 169 taxa, with *Opegrapha dolomitica* and *Roccella fuciformis* as the outgroup. Major insertions for each data set were excluded as in the individual data set analyses. The phylogenetic analyses of combined SSU and LSU and TEF-1-alpha and RPB2 sequence data were performed, along with various orders of the Dothideomycetes from the GenBank (Botryosphaeriales, Capnodiales, Dothideales, Hysteriales, Jahnulales Myriangiales, Mytilindiales, Patellariales and Pleosporales). Sequences were aligned and analyzed separately by maximum parsimony, Bayesian inference and maximum likelihood algorithms, and the resulting trees compared. The maximum parsimony dataset consists of 4126 total characters, 2076 (50.31%) characters are constant, 446 (10.81%) characters are parsimony informative and 1604 (38.88%) characters are parsimony uninformative. Heuristic searches run for 100 replicates of random stepwise addition of sequence that treated gaps as missing data. Independent Bayesian phylogenetic analysis was performed using a uniform GTR+I+G model, as selected by hLRT in Mrmodeltest 2.2: [GTR+I+G] Prset statefreqpr = dirichlet (1,1,1,1), Lset nst = 6 rates = invgamma. The unweighted parsimony resulted three MPTs in a length of 15705 steps (CI = 0.224, RI = 0.607, RC = 0.136). A single MPT is shown as a phylogram, representing the best topology with the best K-H-likelihood scores (Fig. 1). Phylogenetic trees obtained from maximum likelihood, Bayesian and maximum parsimony analyses yielded trees with similar overall

topology at subclass, order and family relationship in agreement with previous work based on maximum likelihood (Schoch *et al.*, 2006, 2009; Shearer *et al.*, 2009; Suetrong *et al.*, 2009a; Zhang *et al.*, 2009b). However, the internal node relationships of some taxa were resolved differently between the maximum likelihood, Bayesian and Maximum parsimony trees (Fig. 1).

Trematosphaeriaceae consisted of the reference strain *Trematosphaeria pertusa*, (neotype species of the genus *Trematosphaeria*) and *H. thalassiae*, with strong support (94%BSMP, 99%BSML and 1.00 BYPP), and two *F. lignatilis* strains forming a sister group with high support (95%BSMP, 99%BSML and 1.00 BYPP). This clade clusters weakly with the Montagnulaceae, Massarinaceae and Lentitheciaceae (55%BSML) (Fig. 1).

TAXONOMY

Figure 1 shows the phylogenetic relationship of five families: Montagnulaceae, Massarinaceae, Lentitheciaceae, Trematosphaeriaceae and Morosphaeriaceae (Pleosporales), with many taxa based on *Massarina* and *Lophiostoma* species that do not group with the type species *Massarina eburnea*. The Trematosphaeriaceae clade comprises four strains of *H. thalassiae*, a common species on mangrove wood, with two strains of *T. pertusa* and as a sister group. *Falciformispora lignatilis* also groups within this clade with high support, and known from mangrove wood, freshwater and on the terrestrial oil palm. All three are type species of monotypic genera. The position of *Asteromassarina pulchra* is unresolved, grouping in the Trematosphaeriaceae (Schoch *et al.*, 2009, Zhang *et al.*, 2009a) but also in the Morosphaeriaceae (Suetrong *et al.*, 2009a).

Trematosphaeriaceae K.D. Hyde, Yin. Zhang, Suetrong and E.B.G. Jones, fam. nov. (Figs 2-4)

MycoBank MB 519506

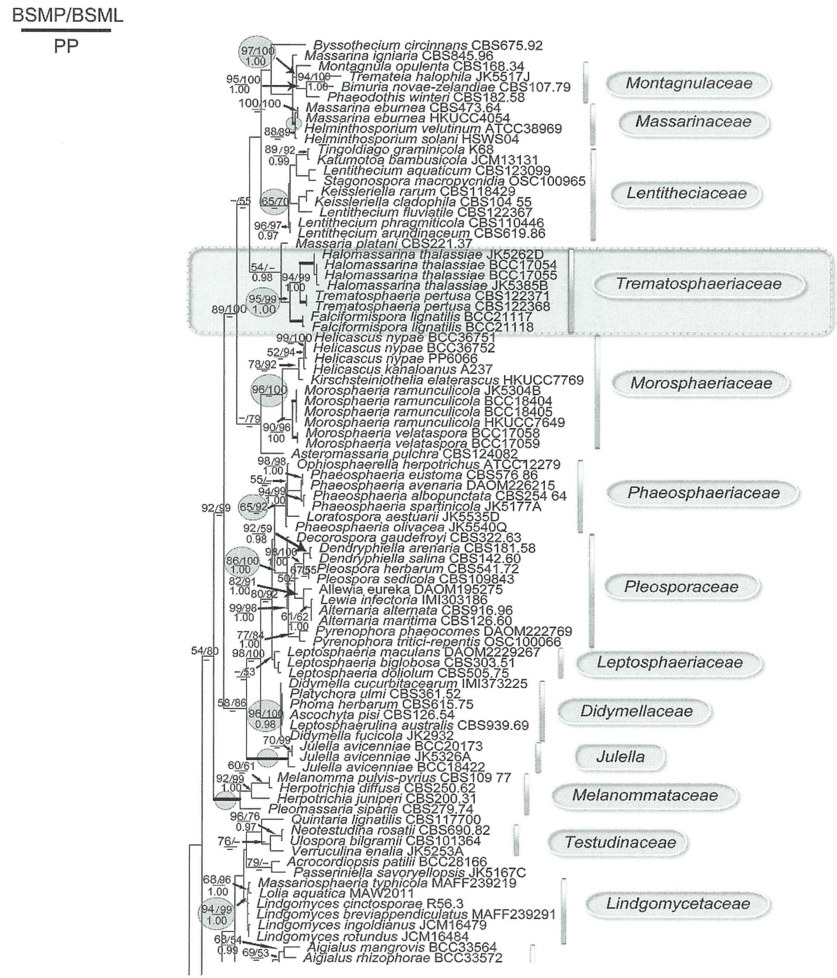
Ascomata solitaria, gregaria, immersa, semi-immersa vel erumpentes, subglobosa, nigra, breve papillata. Peridis coriacis. Hamathecis anastomosis, cellulae, dan gelatinosa. Asci bitunicati, fissitunicati, cylindro-clavati, pedicellate. Ascosporae fusiformes, hyalinae vel brunneae, trans-septatae.

Ascomata solitary, scattered, or in groups, initially immersed, becoming erumpent, to semi-immersed, subglobose, black; apex with a short papilla. ***Hamathecium*** relatively wide, embedded in mucilage, branching and anastomosing between and above the asci. ***Asci*** bitunicate, fissitunicate, cylindro-clavate, pedicellate, with an ocular chamber. ***Ascospores*** fusiform, hyaline or dark brown, trans-septate, and variously ornamented.

Type genus: *Trematosphaeria* Fuckel

Trematosphaeria Fuckel, *Jb. nassau. Ver. Naturk.* **23-24**: 161 (1870) [1869-70].

Ascomata solitary, scattered, or in groups, initially immersed, becoming erumpent, to semi-immersed, subglobose, black; apex with a short papilla. ***Peridium*** coriaceous, comprising small heavily pigmented thick-walled cells of *textura angularis*, with columns of *textura prismatica* orientated perpendicular to the ascomatal surface. ***Hamathecium*** of thick, septate, cellular pseudoparaphyses, embedded in mucilage, branching and anastomosing between and above the asci. ***Asci*** 8-spored, bitunicate, fissitunicate, clavate, with a short, thick, furcate pedicel


BSMP/BSML
PP
Byssothecium circinnans CBS675.92
Massarina igniaria CBS845.96
Montagnula opulenta CBS168.34
Trematela halophila JK5517J
Bimuria novae-zelandiae CBS107.79
Phaeodothis winteri CBS182.58
Massarina eburnea CBS473.64
Massarina eburnea HKUCC4054
Helminthosporium velutinum ATCC38969
Helminthosporium solani HSWS04
Tingoldiago graminicola K68
Katumotoa bambusicola JCM13131
Lentithecium aquaticum CBS123099
Stagonospora macropycnidia OSC100965
Keissleriella rarum CBS118429
Keissleriella cladophila CBS104 55
Lentithecium fluviatile CBS122367
Lentithecium phragmiticola CBS110446
Lentithecium arundinaceum CBS619.86
Massaria platani CBS221.37
Halomassarina thalassiae JK5262D
Halomassarina thalassiae BCC17054
Halomassarina thalassiae BCC17055
Halomassarina thalassiae JK5385B
Trematosphaeria pertusa CBS122371
Trematosphaeria pertusa CBS122368
Falciformispora lignatilis BCC21117
Falciformispora lignatilis BCC21118
Helicascus nypae BCC36751
Helicascus nypae BCC36752
Helicascus nypae PP6066
Helicascus kanaloanus A237
Kirschsteiniothelia elaterascus HKUCC7769
Morosphaeria ramunculicola JK5304B
Morosphaeria ramunculicola BCC18404
Morosphaeria ramunculicola BCC18405
Morosphaeria ramunculicola HKUCC7649
Morosphaeria velataspora BCC17058
Morosphaeria velataspora BCC17059
Asteromassaria pulchra CBS124082
Ophiosphaerella herpotrichus ATCC12279
Phaeosphaeria eustoma CBS576 86
Phaeosphaeria avenaria DAOM226215
Phaeosphaeria albopunctata CBS254 64
Phaeosphaeria spartinicola JK5177A
Loratospora aestuarii JK5535D
Phaeosphaeria olivacea JK5540Q
Decorospora gaudefroyi CBS322.63
Dendryphiella arenaria CBS181.58
Dendryphiella salina CBS142.60
Pleospora herbarum CBS541.72
Pleospora sedicola CBS109843
Allewia eureka DAOM195275
Lewia infectoria IMI303186
Alternaria alternata CBS916.96
Alternaria maritima CBS126.60
Pyrenophora phaeocomes DAOM222769
Pyrenophora tritici-repentis OSC100066
Leptosphaeria maculans DAOM2229267
Leptosphaeria biglobosa CBS303.51
Leptosphaeria doliolum CBS505.75
Didymella cucurbitacearum IMI373225
Platychora ulmi CBS361.52
Phoma herbarum CBS615.75
Ascochyta pisi CBS126.54
Leptosphaerulina australis CBS939.69
Didymella fucicola JK2932
Julella avicenniae BCC20173
Julella avicenniae JK5326A
Julella avicenniae BCC18422
Melanomma pulvis-pyrius CBS109 77
Herpotrichia diffusa CBS250.62
Herpotrichia juniperi CBS200.31
Pleomassaria siparia CBS279.74
Quintaria lignatilis CBS117700
Neotestudina rosatii CBS690.82
Ulospora bilgramii CBS101364
Verruculina enalia JK5253A
Acrocordiopsis patilii BCC28166
Passeriniella savoryellopsis JK5167C
Massariosphaeria typhicola MAFF239219
Lolia aquatica MAW2011
Lindgomyces cinctosporae R56.3
Lindgomyces breviappendiculatus MAFF239291
Lindgomyces ingoldianus JCM16479
Lindgomyces rotundus JCM16484
Aigialus mangrovis BCC33564
Aigialus rhizophorae BCC33572
Montagnulaceae
Massarinaceae
Lentitheciaceae
Trematosphaeriaceae
Morosphaeriaceae
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Julella
Melanommataceae
Testudinaceae
Lindgomycetaceae

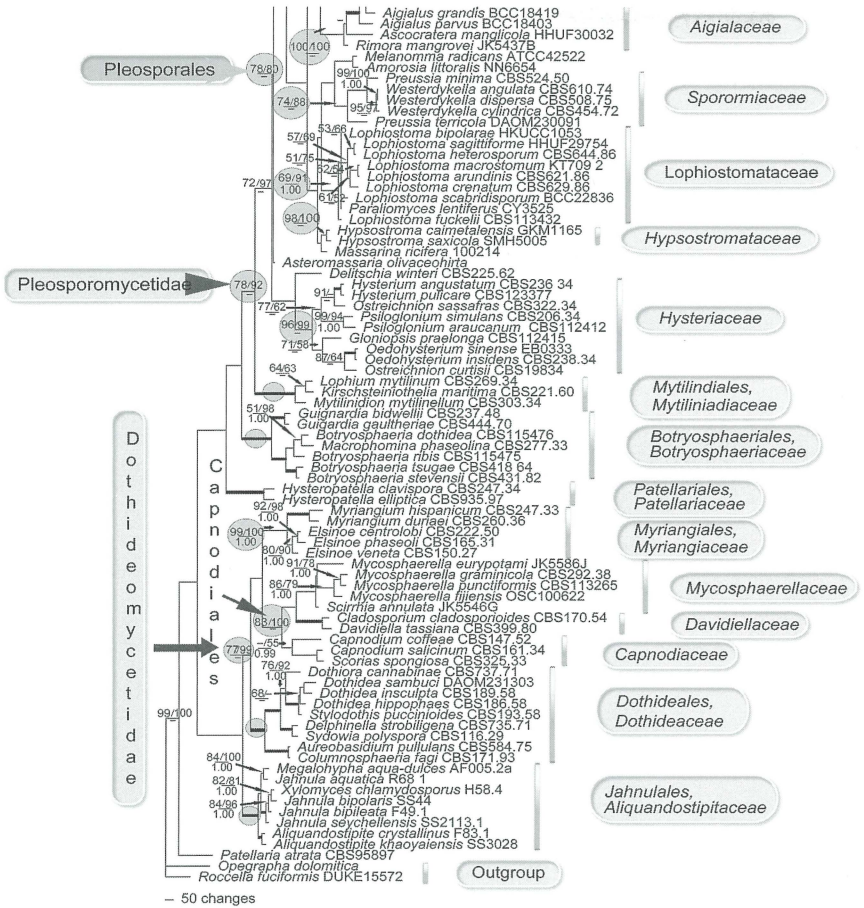
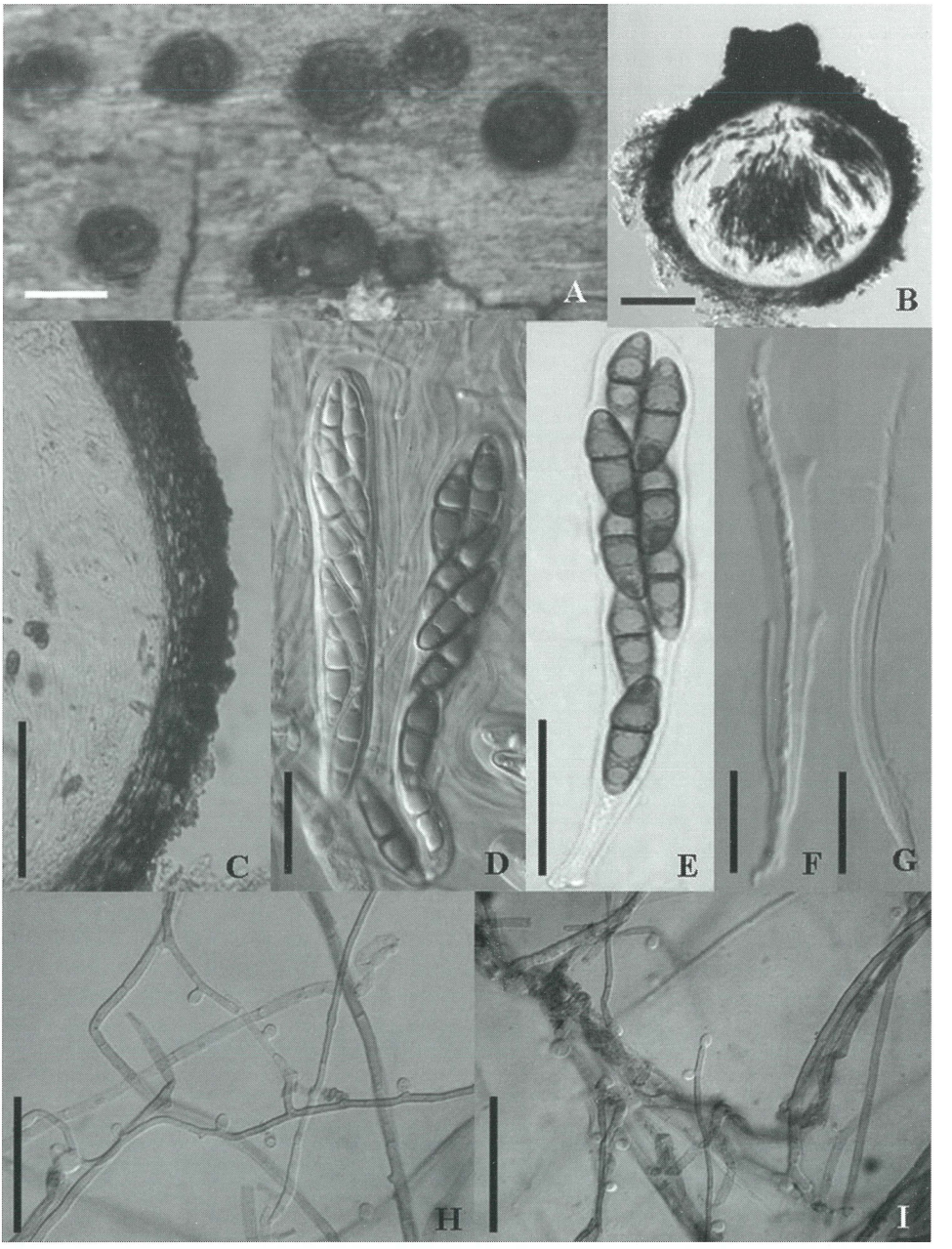


Fig. 1. One of 3 MPTs inferred from combined SSU, LSU rRNA sequences, TEF-1-alpha and RPB2 sequences, generated with maximum parsimony criterion. Maximum parsimony (BSMP, Left) and likelihood (BSML, right) bootstrap value greater than 50% above the node. Bayesian posterior probabilities greater than 0.95 are given below each node (BYPP). The internodes that are high supported by all bootstrap proportions (100%) and posterior probabilities (1.00) are shown as a thicker line..



Figs 2 A-I. *Trematosphaeria pertusa* (in water). **A**, **D**, **F-I**. from epitype. **B-C**, **E**. from neotype. **A**. Ascomata on the host surface. **B**. Section of an ascoma. **C**. Section of the peridium. **D**. Asci amongst pseudoparaphyses. **E**. Ascus with pedicle. **F-G**. Dehiscent ascus. **H-I**. Hyphopodia-like structures produced on agar. Scale bars: A = 0.5 mm, B, C = 100 µm, D-I = 20 µm.

and ocular chamber. ***Ascospores*** fusiform with broadly to narrowly rounded ends, dark brown, trans-septate, secondary septum forming late or often absent, deeply constricted at the median septum, the upper cell often shorter and broader than the lower one, smooth to finely verruculose.

Anamorphs: Hyphopodia-like structures (or conidia).

Type species: *Trematosphaeria pertusa* (Pers.) Fuckel

Mode of life: saprobic.

Substrata: terrestrial wood.

Trematosphaeria pertusa (Pers.) Fuckel , *Jb. nassau. Ver. Naturk.* **23-24**: 161 (1870) [1869-70] (Figs 2A-K).

≡ *Sphaeria pertusa* Pers., Synopsis Methodica Fungorum (Göttingen) 1: 83 (1801).

(for other synonyms see Index Fungorum).

Ascomata 350-550 µm high × 320-480 µm diam., solitary, scattered, or in groups, initially immersed, becoming erumpent, to semi-immersed, subglobose, black; apex with a short ostiole usually slightly conical and widely porate, to 100 µm high (Figs 2A-B). ***Peridium*** 48-55 µm wide laterally, to 80 µm at the apex, thinner at the base, 30-40 µm thick, coriaceous, comprising a single cell type of small heavily pigmented thick-walled cells of *textura angularis*, cells 4-8 µm diam., cell wall 1.5-3 µm thick in places with columns of *textura prismatica* orientated perpendicular to the ascomatal surface, apex cells smaller and walls thicker, forming thick-walled cells of *textura pseudoparenchymata*, and larger, paler cells of mixture of *textura epidermoidea* and *textura angularis* at the base, 10-25 µm (Figs 2B-C, H). ***Hamathecium*** of 1.5-2.5 µm broad, septate, cellular pseudoparaphyses, embedded in mucilage, branching and anastomosing between and above the asci, (Figs 2D-F). ***Asci*** 100-145 × 15-17 µm ($\bar{x}$ = 118 × 15.5 µm, n = 10), 8-spored, bitunicate, dehiscence fissitunicate, clavate, with a short, thick, furcate pedicel which is 12-30 µm long, with a truncate ocular chamber (Figs 2D-G, I). ***Ascospores*** 27.5-32.5 × 7.5-8.5 µm ($\bar{x}$ = 29.5 × 8 µm, n = 10), biseriate to uniseriate near the base, fusiform with broadly to narrowly rounded ends, dark-brown, 1-3-septate, secondary septum forming late or often absent, deeply constricted at the median septum, the upper cell often shorter and broader than the lower one, smooth to finely verruculose, containing refractive globules (Figs 2J-K).

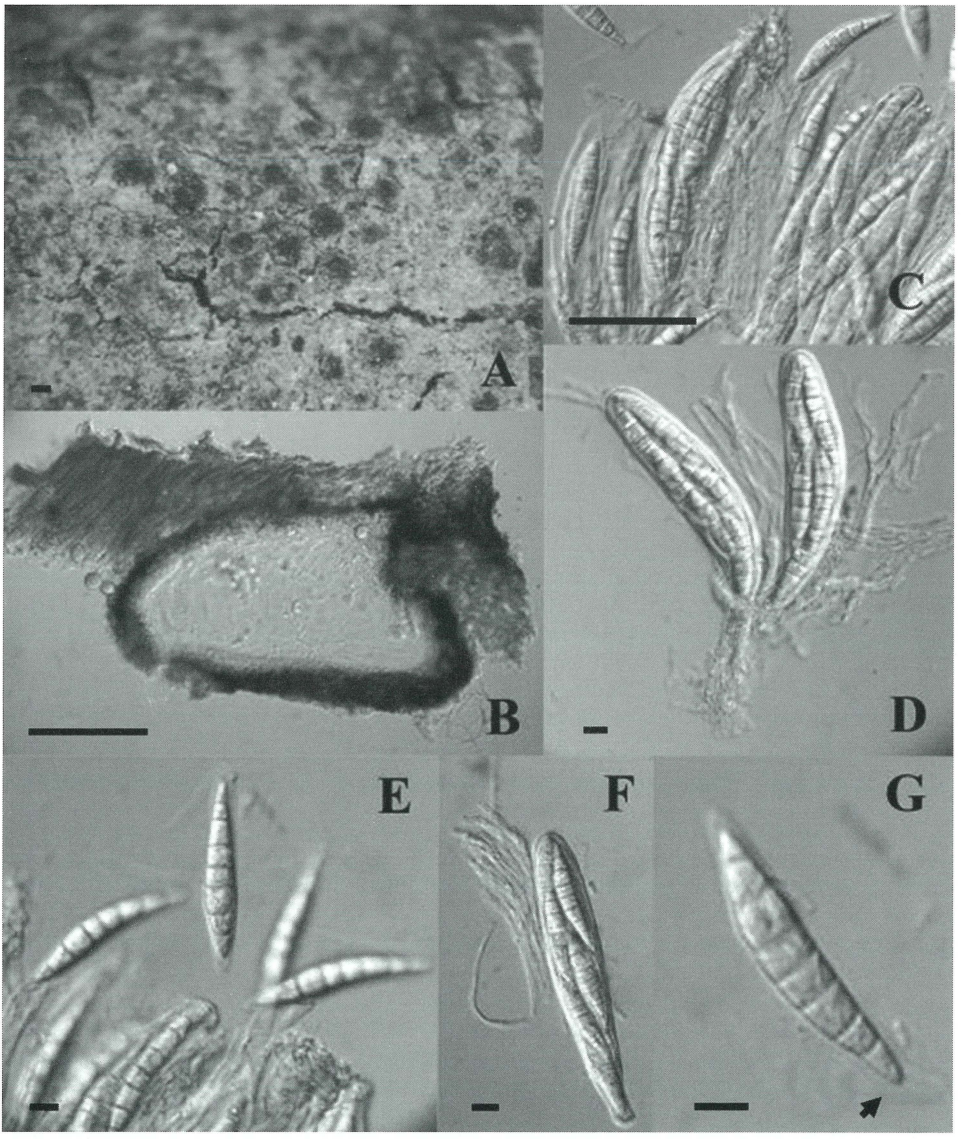
Anamorph: Hyphopodia-like structures (or conidia).

Colonies (of epitype) reaching 5 cm diam after 20 d growth on MEA at 25°C, raised, woolly, deep grey, with irregular to rhizoidal margin, reverse darkened. Hyphopodia-like structures (or conidia) produced after 6 months, hyaline to light-brown, lobed, 4-4.5 (-5) µm long and 3-3.5 µm diam.

Material examined: SWEDEN, Upsala, 1985, *J.R. Boise*, *L-Pers 910269-172* (neotype); FRANCE, Deux Sèvres, Sansais, Le Vanneau, Les Grandes Mottines, swamp, 25 April 2004, *J. Fournier, IFRD 2002*, epitype; FRANCE, Haute Garonne, Avignonet, Canal du Midi, 23 Nov. 2006, leg. *Michel Delpont, det. J. Fournier, IFRD2003.*

Known distribution: Sweden, France.

Notes: Trematosphaeria pertusa, the lectotype species of *Trematosphaeria* (Clements and Shear 1931), is characterized by semi-immersed to erumpent ascomata, cellular pseudoparaphyses, cylindric-clavate asci, and fusiform, one-septate reddish-brown to dark-brown ascospores (Boise, 1985; Zhang *et al.*, 2008). *Trematosphaeria pertusa* usually grows on the surface of decaying terrestrial wood, but also can survive within freshwater. *Trematosphaeria pertusa* forms a



Figs 3 A-G. *Falciformispora lignatilis* (holotype). **A.** Appearance of ascomata on host surface with small papilla. **B.** Section of immersed ascoma. **C-F.** Squash mounts showing asci with cellular pseudoparaphyses. The asci are cylindro-clavate. **F-G.** Ascospores. Note the scythe-like appendage at the base (arrowhead). Scale bars: **A-B.** = 100 μm, **C** = 50 μm; **D-F** = 10 μm.

robust phylogenetic clade with *Falciformispora lignatilis* and *Halomassarina thalassiae* (Fig. 1).

Falciformispora K.D. Hyde, *Mycol. Res.* 96(1): 26 (1992). (Fig. 3)

Ascomata solitary to gregarious, immersed and eventually superficial by sloughing off of the upper woody cells, subglobose to ovoid, coriaceous, black,

papillate, ostiolate. ***Peridium*** composed of outer thick-walled angular or rounded brown cells, and inner of hyaline cells of *textura prismatica*. ***Hamathecium*** of thick, septate, cellular pseudoparaphyses, surrounded by a gelatinous matrix. ***Asci*** 8-spored, bitunicate, fissitunicate, cylindro-clavate, with a short, thick pedicel, and ocular chamber. ***Ascospores*** 2-3-seriate, fusiform to clavate, hyaline, straight or slightly curved, trans-septate, third cell from apex largest, smooth-walled, surrounded by a thin mucilaginous sheath and a single scythe-like appendage at the base.

Anamorphs: none reported.

Type species: *Falciformispora lignatilis* K.D. Hyde

Mode of life: saprobic.

Substrata: mangrove wood, rachis of the palm *Elaeis guineensis.*

Falciformispora lignatilis K.D. Hyde, *Mycol. Res.* 96(1): 27 (1992). (Figs 3A-F)

Ascomata 150-270 µm high × 240-360 µm diam., solitary to gregarious, erumpent and eventually superficial by sloughing off of the upper woody cells, subglobose to ovoid, coriaceous, black, papillate, ostiolate (Figs 3A-B). *Papilla* small, rounded. ***Peridium*** up to 36 µm wide, comprising two cells types, out layer composed of thick-walled angular or rounded brown cells, up to 8 µm diameter, cell wall up to 5 µm thick, inner layer composed of hyaline cells of *textura prismatica*, cells 12 × 3 µm diameter, cell wall 1-1.5 µm thick (Fig. 3 B). ***Hamathecium*** of thick, cellular pseudoparaphyses 2-3 µm broad, septate, surrounded by a gelatinous matrix. ***Asci*** 110-136 × 20-32 µm, 8-spored, bitunicate, fissitunicate, cylindro-clavate, with a short, thick pedicel, 8-15 µm long, with an ocular chamber (to 5 µm wide × 3 µm high), with no visible ring (Figs 3 C-F). ***Ascospores*** 42-50 × 7.5-10 µm, 2-3 seriate, fusiform to clavate, hyaline, straight or slightly curved, 6-8-septate, mostly 7-septate, slightly constricted at all septa, third cell from the top the largest, tapering to narrow but rounded ends, smooth-walled, surrounded by a thin mucilaginous sheath and a single scythe-like appendage at the base, sheath may spread in older specimens and is up to 20-30 µm long (Figs 3 E-F).

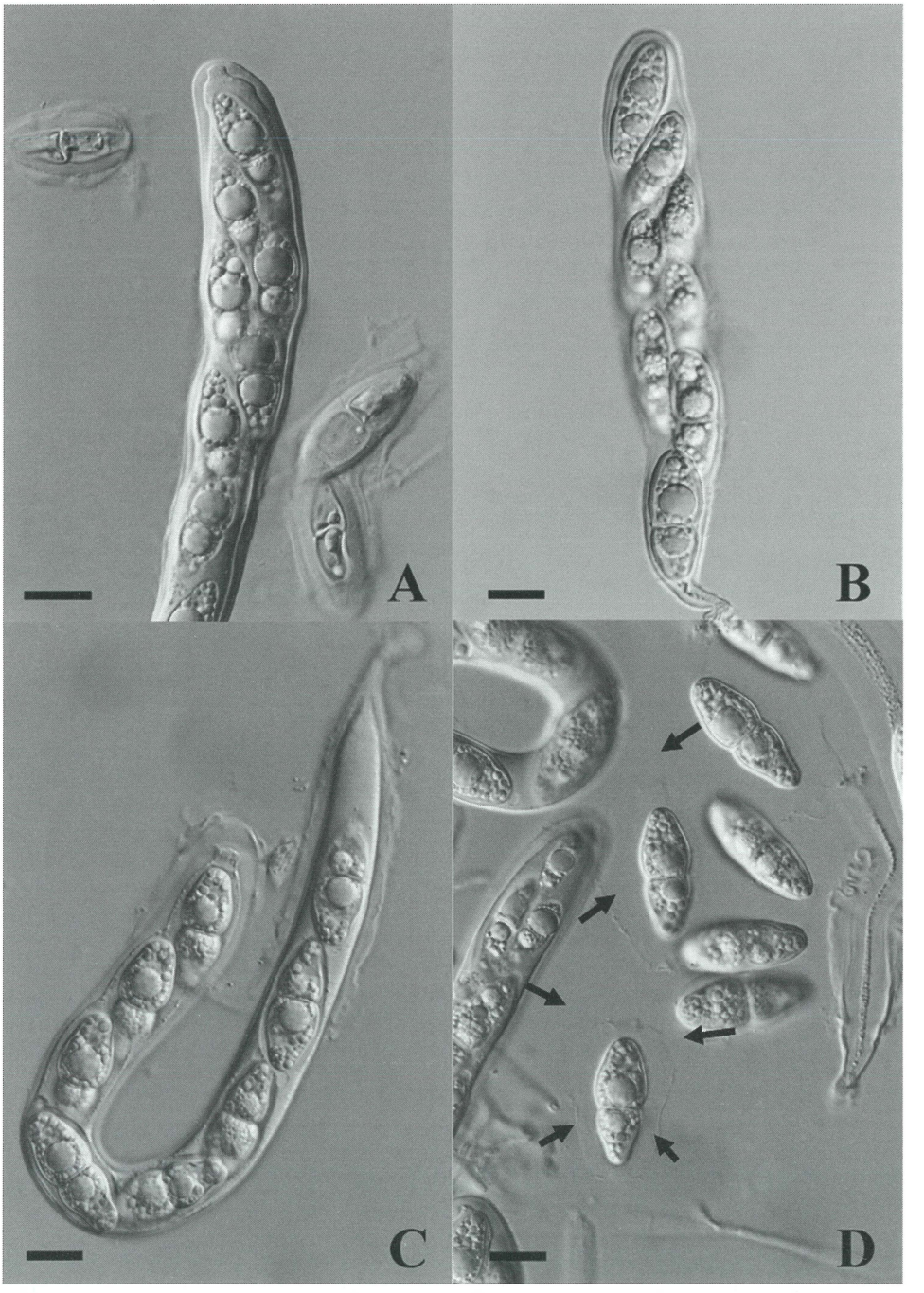
Material examined: MEXICO, Nova Hispania, mangrove near Boca de Pascuales, Mar 1988, *K.D. Hyde*, *BRIP 16972*; THAILAND, Trang Province, Huai Yot, Ban Sai Bo Village, NOV 2005 and 2008, *U. Pinruan.*

Known distribution: Mexico, Thailand, USA (Florida).

Notes: Falciformispora was formally established by Hyde (1992) as a monotypic genus and was assigned to the Pleosporaceae when compared with *Setosphaeria*, which has an *Exserohilum* anamorphic state and is exclusively parasitic on *Gramineae*. It was considered that the species was more closely related to *Chaetomastia* than *Setosphaeria*, and *Falciformispora* differed in having hyaline ascospores. Raja and Shearer (2008) collected this species from freshwater in Florida., while Suetrong *et al.* (2009a) reported it from the terrestrial oil palm (*Elaeis guineensis*) in Thailand. In phylogenetic analysis (Fig. 1) *Falciformispora* forms a well-supported clade with *Trematosphaeria pertusa* and *Halomassarina thalassiae* in Trematosphaeriaceae. The genus *Carinispora* is also similar and may be related (Hyde, 1992).

Halomassarina Suetrong, Sakayaroj, E.B.G. Jones, Kohlm., Volkm.-Kohlm. & C.L. Schoch, *Stud. Mycol.* 64: 161 (2009). (Fig. 4)

Ascomata subglobose to pyriform, completely immersed in the substrata becoming erumpent, ostiolate, periphysate, epapillate or short papillate, clypeate, coriaceous, brown to dark-brown, solitary or gregarious. ***Hamathecium***,



Figs 4. A-D. Morphological features of *Halomassarina thalassiae*. **A.** Apical region of asci with ocular chamber, not bluing in IKI (I–). **B.** Cylindrical asci. **C.** Ascospores in mature ascus, ectoascus has split. **D.** Ascospores with a gelatinous sheaths, arrows Scale bars **A-D** = 25 μm.

pseudoparaphyses relatively wide, mostly simple, sparingly septate, rarely anastomosing. ***Asci*** 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short pedicellate, with an apical ocular chamber, but without apical apparatus and not bluing in IKI (I–), arising from a basal ascogenous tissue. ***Ascospores*** ellipsoidal, 1-3-septate, constricted at the central primary septum, hyaline and surrounded by a gelatinous sheath.

Anamorphs: none reported.

Type species: *Halomassarina thalassiae* (Kohlm. & Volkm.-Kohlm.) Suetrong, Sakay., E.B.G. Jones, Kohlm., Volkm.-Kohlm. & C.L. Schoch

Mode of life: saprobic.

Substrata: Various species of mangrove wood.

Halomassarina thalassiae (Kohlm. & Volkm.-Kohlm.) Suetrong, Sakay., E.B.G. Jones, Kohlm.,Volkm.-Kohlm. & C.L. Schoch, *Stud. Mycol.* 64: 161 (2009). (Figs 4A-D)

≡ *Massarina thalassiae* Kohlm. & Volkm.-Kohlm., *Can. J. Bot.* 65(3): 575 (1987)

Ascomata 150-400 µm high, 170-437.5 µm wide, subglobose to pyriform, completely immersed in the substrata or erumpent, ostiolate, periphysate, epapillate or short papillate, clypeate, coriaceous, brown to dark-brown, solitary or gregarious. ***Hamathecium*** pseudoparaphyses 1.25-2.5 µm in diameter, mostly simple, sparingly septate, rarely anastomosing. ***Asci*** 157.5-222.5 × 30-37.5 µm, 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short pedicellate, with an apical ocular chamber, but without apical apparatus and not bluing in IKI (I–), arising from a basal ascogenous tissue. ***Ascospores*** 35-47.5 × 10-16.25 µm, ellipsoidal, at first 1-septate and later 3-septate, constricted at the central primary septum, hyaline and surrounded by a gelatinous sheath.

Anamorph: None reported.

Colonies on potato dextrose seawater agar (PDA/SW) cottony; gray brown to dark-brown, producing a brown pigment. Colonies grow slowly on CMA/SW and PDA/SW reaching ca. 2.5-3 cm diameter in 30 d at room temperature (21-25 °C), mycelium 2.5 µm wide, superficial, hypha smooth-walled, septate.

Material examined: BELIZE, Carrie Bow Cay, 1 December 1985, *J. Kohlmeyer and B. Volkmann-Kohlmeyer, JK 4804a (holotype) and JK 4804b (isotype)*; THAILAND, Satun Province, Mueang Satun, Tammarang Pier, 1 September 2006, *R. Choeyklin, A. Pinnoi, U. Pinruan*; THAILAND, Satun Province, Mueang Satun, Tammarang Pier, 9 October 2008, *R. Choeyklin, U. Pinruan*; THAILAND, Chanthaburi Province, Tha Mai, Khung Kraben Bay Royal Development Study Center, 29 June 2008, *S. Suetrong, K.-L. Pang, N. Rungjindamai.*

Habitat: Mangrove wood: *Aegiceras corniculatum, Excoecaria agallocha, Rhizophora apiculata, R. mangle, R. mucronata, Sonneratia griffithii, Xylocarpus granatum.* Prop roots of *Rhizophora apiculata.* Submerged dead mangrove branches of *Avicennia germinans.* Dead mangrove root, intertidal branch or root: *Rhizophora mucronata.* Submerged intertidal log and wood from fishing vessel.

Known distribution: ALDABRA ISLANDS; BELIZE (Volkmann-Kohlmeyer & Kohlmeyer, 1993); BRUNEI (Hyde, 1988a, 1988b); HONG KONG (Vrijmoed *et al.*, 1994); INDIA (Patil & Borse, 2001; Sarma & Vittal, 2000, 2001); MALAYSIA (Alias & Jones, 2000); MEXICO (Baja California) (Kohlmeyer & Volkmann-Kohlmeyer, 1987) and THAILAND (Hyde *et al.*, 1990; Suetrong, 2010).

Other genera

Asteromassaria Höhn., *Sber. Akad. Wiss. Wien*, Math.-naturw. Kl., Abt. 1 126: 368 (1917).

Asteromassaria pulchra (Harkn.) Shoemaker & P.M. LeClair has been included in the family in previous phylogenetic publications (Schoch *et al.*, 2009; Zhang *et al.*, 2009b), however, since the type of the genus (*A. macrospora* (Desm.) Höhn.), was not included in the phylogenetic analysis the familial placement cannot be confirmed. Furthermore *Asteromassaria pulchra* did not cluster in the *Trematosphaeriaceae* in our analysis (Fig. 1).

Acknowledgements. K.D. Hyde thanks TRF/BIOTEC program Biodiversity Research and Training Grant for the award of a scholarship (BRT R_253012) to study Dothideomycetes. Satinee Suetrong thanks Jariya Sakayaroj for advice on phylogenetic analysis, Aom Pinnoi, Rattaket Choeyklin, Umpava Pinruan, Nattawut Rungjindamai for collecting samples and Anupong Klaysuban for laboratory assistance. Juntima Chanprasert is thanked for RAxML analysis. This work was supported by the TRF/BIOTEC program Biodiversity Research and Training Grant BRT R_251006 and R_325015 to study marine fungi of Thailand. For their continued interest and support we also thank BIOTEC; Morakot Tanticharoen; Kanyawim Kirtikara and Lily Eurwilaichitr. The Global Research Network for Fungal Biology and Kind Saud University and thanked for their support.

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