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An additional fungal lineage in the *Hypocreomycetidae* (*Falcocladium* species) and the taxonomic re-evaluation of *Chaetosphaeria chaetosa* and *Swampomyces* species, based on morphology, ecology and phylogeny

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Abstract – The taxonomic position of the marine fungi referred to the TBM clade is re-evaluated along with the marine species *Chaetosphaeria chaetosa*, and the terrestrial asexual genus *Falcocladium*. Phylogenetic analyses of DNA sequences of two ribosomal nuclear loci of the above taxa and those previous recognized as the TBM clade suggest that they form a distinct clade amongst the Hypocreales, Microascales, Savoryellales, Coronophorales and Melanosporales in the Hypocreomycetidae. Four well-supported subclades in the "TBM clade" are discerned including: 1) the *Juncigena* subclade, 2) the *Etheirophora* and *Swampomyces s. s.* subclade, 3) the *Falcocladium* subclade and 4) the *Torpedospora* subclade. *Chaetosphaeria chaetosa* does not group in the Chaetosphaeriales but together with *Swampomyces aegyptiacus* and *S. clavatispora* they group in the *Juncigena* subclade, while *Falcocladium* forms a sister group to the *Etheirophora* and *Swampomyces s. s.* subclade. *Swampomyces aegyptiacus* and *S. clavatispora* share some morphological and ecological characteristics with *Juncigena,* but they are not monophyletic, and a new genus is introduced to accommodate them (*Fulvocentrum*). *Chaetosphaeria chaetosa* however, differs significantly from other *Chaetosphaeria* and *Juncigena* species and a new genus *Marinokulati* is proposed to accommodate it. The taxonomic significance of the phylogenetic data is discussed and new families are proposed for the four clades highlighted in this paper: Juncigenaceae, Etheirophoraceae, Falcocladiaceae and Torpedosporaceae, which differ from all other families in the Hypocreomycetidae.

DNA phylogeny / *Falcocladium* / *Fulvocentrum* gen. nov. / *Marinokulati* gen. nov. / new ascomycete lineage / new families *Juncigenaceae* / *Etheirophoraceae* / *Falcocladiaceae* / *Torpedosporaceae* / systematics

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INTRODUCTION

Schoch *et al.* (2007) reported on a new marine fungal lineage in the Hypocreomycetidae and named it the TBM clade (*Torpedospora/Bertia/ Melansopora*), comprising genera in the Coronophorales and Melanosporales and marine ascomycete genera not assigned to the Halosphaeriaceae. This clade comprised three marine fungal subclades: *Torpedospora*; *Juncigena/ Swampomyces*; and *Swampomyces s. s./Etheirophora* subclades, which grouped with taxa from the orders Coronophorales and Melanosporales and was well supported statistically. In that analysis the genus *Swampomyces* was polyphyletic grouping in the different subclades, and Schoch *et al.* (2007) suggested that *S. aegyptiacus* and *S. clavatispora*, and possibly *S. triseptatus*, belonged to a different genus. However, no formal taxonomic changes were made. Subsequently other lineages in the Hypocreomycetidae have been documented: Lulworthiales (Kohlmeyer *et al.*, 2000), Koralionastetales (Campbell *et al.*, 2009), Savoryellales (Boonyuen *et al.*, 2011). In an ongoing investigation of the phylogeny of tropical marine and freshwater fungi (Somrithipol *et al.*, 2006a, b, 2007; Pinnoi *et al.*, 2007; Rungjindamai *et al.*, 2008; Aveskamp *et al.*, 2008), we have employed molecular data to determine their phylogenetic relationships.

Chaetosphaeria chaetosa was described by Kohlmeyer (1963) and referred to the Sphaeriaceae. However, its placement in *Chaetosphaeria* was questioned by Jones *et al.* (1983) as it differs from other species in the genus, primarily in ascospores with both polar and equatorial appendages, formed by fragmentation of an exosporic sheath, while no asexual stage has been reported for *Ch. chaetosa* and its marine occurrence. Fresh collections of the marine ascomycete *Chaetosphaeria chaetosa* have been made and this enabled assessment of its phylogenetic relationship with other *Chaetosphaeria* species.

Currently, only circa 40% of asexual fungi have been linked to their sexual state, either at the genus, family or order level, while for some 1,728 (60%) genera no sexual state link has been established (Hyde *et al.*, 2011). With the advent of molecular studies, a number of asexual genera have been linked to families, orders or classes (Rungjindamai *et al.*, 2008; Abdel-Wahab *et al.*, 2010; Diederich *et al.*, 2012). Traditionally, asexual fungi have been linked to their sexual states when observed growing together e.g. *Lecythothecium duriligni* and its asexual state *Sporidesmium* (Reblova & Winka 2001); or by culture techniques e.g. *Nereiospora cristata* and *Monodictys pelagica* (Mouzouras & Jones 1985). For this study we have selected the asexual genus *Falcocladium*, with no known sexual morph, for investigation (Hyde *et al.*, 2011).

Falcocladium was erected by Crous *et al.* (1994) with *F. multivesiculatum* as the type species. Subsequently three other species were described: *F. sphaeropedunculatum* (Crous *et al.*, 1997), *F. thailandicum* (Crous *et al.*, 2007) and *F. turbinatum* (Somrithipol *et al.*, 2007). This genus occurs on a wide range of substrata including *Eucalyptus grandis*, *E. camaldulensis* leaves, and leaf litter collected from tropical forests (Somrithipol *et al.*, 2007). The genus is characterized by white sporodochia, thick-walled, non-septate stipe extensions that terminate in thin-walled vesicles, and conidia that are hyaline, 0-1 septate, falcate, with short apical and basal appendages (Crous *et al.*, 1994; Somrithipol *et al.*, 2007). The four species are distinguished by the morphology of the terminal vesicle, conidial measurements and septation (Somrithipol *et al.*, 2007). Some species of *Falcocladium* were studied at the molecular level, but taxonomic placement of these asexual taxa was not resolved.

The aims of this study were 1) to clarify the phylogenetic position of *Falcocladium* species, 2) to determine the phylogenetic position of the marine ascomycete *Chaetosphaeria chaetosa*, and 3) to re-evaluate the taxonomic placement of *Etheirophora, Juncigena, Swampomyces* and *Torpedospora.* This was achieved by analyzing partial sequences of the nuclear SSU and LSU ribosomal DNA.

MATERIAL AND METHODS

Fungal cultures and maintenance: Four *Falcocladium* species were studied in this paper: *Falcocladium thailandicum, F. turbinatum*, collected from Thailand, and *F. multivesiculatum* and *F. sphaeropedunculatum* isolated from Brazil (Table 1). *Falcocladium turbinatum* was isolated by Somrithipol *et al*. (2007) and a culture is deposited in the BIOTEC Culture Collection (BCC), Thailand. Three other *Falcocladium* species, all associated with leaves of *Eucalyptus* trees, were isolated by Crous *et al*. (1994, 1997) and deposited at Centraalbureau voor Schimmelculture (CBS), The Netherlands. All strains of *Falcocladium* were cultured and sequenced in this study except two strains of *F. sphaeropedunculatum* (CBS111293 and CBS111294) as they were not available for study, but the DNA sequences of these two strains were obtained from the GenBank database. Fresh collections of *Chaetosphaeria chaetosa* were made in Turkey and UK, isolated into axenic cultures and deposited in Bioresource Collection and Research Center, Hsinchu, Taiwan.

Table 1. Sources, substratum, origin, date of isolation and GenBank accession number of the *Chaetosphaeria chaetosa* and *Falcocladium* species used in this study

Taxa	*Source*	*Substratum and geographical origin*	*Reference*	*Ribosomal DNA*	
				SSU	*LSU*
C. chaetosa	BCRC FU30271 (NTOU4048)	Driftwood, Turkey	Kohlmeyer (1963)	KJ866929	KJ866931
C. chaetosa	BCRC FU30272 (NTOU4060)	Driftwood, Cornwall, UK	Kohlmeyer (1963)	KJ866930	KJ866932
F. multivesiculatum	CBS120386	Leaf litter of *Eucalyptus grandis*, Brazil	Crous *et al*. (1997)	JF831928	JF831932
F. sphaeropedunculatum	CBS111292	Leaves of *Eucalyptus pellita*, Brazil	Crous *et al*. (1997)	JF831929	JF831933
F. sphaeropedunculatum	CBS111293*	N/A	Crous *et al*. (2007)	EU040219	EU040219
F. sphaeropedunculatum	CBS111294*	N/A	Crous *et al*. (2007)	EU040220	EU040220
F. thailandicum	CBS121717	Leaves of *Eucalyptus camaldulensis*, Thailand	Crous et al. (2007)	JF831930	JF831934
F. turbinatum	BCC22055	Dead leaves of evergreen tree in a tropical forest, Thailand	Somrithipol *et al*. (2007)	JF831931	JF831935

BCC = BIOTEC Culture Collection, CBS = Centraalbureau voor Schimmelculture, BCRC = Bioresource Collection and Research Center

* sequenced by Crous *et al*. (2007) and downloaded from the GenBank database.

Genomic DNA extraction and PCR amplification: Fungal strains of *Falcocladium* were grown on potato dextrose agar (PDA) and transferred to potato dextrose broth (PDB) under static conditions at 25ºC for 2 weeks for DNA extraction. Fungal mycelia were harvested, washed with sterilized distilled water, the biomass frozen at –20ºC overnight and ground into fine power with a sterilized mortar and pestle. DNA was extracted using CTAB lysis buffer (O'Donnell *et al*., 1997) and incubated at 65°C for 1 hour. The mixture was extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1). The tube was centrifuged at 14,000 rpm for 10 min. The upper liquid phase was precipitated with 7.5 M ammonium acetate and absolute ethanol and kept at –20ºC for at least 30 min. The tube was centrifuged at 14,000 rpm for 10 min and supernatant discarded. Extracted DNA was washed twice with 70% ethanol, air dried and the DNA resuspended in 50 µl TE buffer.

Partial nuclear small subunit (SSU) and large subunit (LSU) DNA sequences were amplified with primers: NS1, NS4, NS6 (for SSU) and LROR, LR6, LR7 (for LSU) (White *et al*., 1990; Bunyard *et al*., 1994) using Finnzymes, DyNAzyme™ II DNA polymerase kit (Cat No F-551S, Finnzymes, Espoo, Finland). The amplification cycles for SSU were performed following White *et al*. (1990), the PCR conditions consisted of initialisation at 95°C (5 min); 35 cycles of denaturation at 95°C (1 min), annealing at 55°C (1 min) and extension at 72°C (1 min 30 s); final extension at 72°C (10 min) and final hold at 4°C. The PCR conditions for LSU were performed following Bunyard *et al*. (1994) using 95°C (2 min); 35 cycles of 95°C (1 min), 55°C (1 min 30 s), 72 °C (2 min 30 s); 72°C (10 min) and final hold at 4°C using a DNA Engine DYAD ALD 1244 thermocycler (MJ Research, Inc, Waltham, MA). The PCR products were purified with NucleoSpin® Extract DNA purification kit (Cat. No. 740 609.50, Macherey-Nagel, Duren, Germany) following the manufacturer's instruction and then sequenced by Macrogen Inc. (Seoul, S. Korea) or Genomics BioSci. & Tech., Taiwan using the same primers as for amplification.

Sequence alignment and phylogenetic analysis: SSU and LSU DNA sequences of *Falcocladium* spp. were compared to sequences deposited in the GenBank Database using the BLAST search tool to obtain the closest matched sequences (Altschul *et al*., 1990). Additional representative taxa from the Hypocreomycetidae and Sordariomycetidae (the Sordariomycetes) appeared in previously published papers were added into the dataset (Zhang *et al*., 2006; Schoch *et al*., 2007; Huhndorf *et al*., 2004). The SSU and LSU sequences were multiple aligned using Clustal W 1.6 (Thompson *et al*., 1994) and adjusted manually where necessary using BioEdit 7.5.0.3 (Hall 2006) and MUSCLE (Edgar 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 final alignment was again optimised by eye and manually corrected using Se-Al v. 2.0a8 (Rambaut 1996). 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.

i) Maximum parsimony analyses were performed using PAUP v. 4.0b10 (Swofford 2002), with gaps 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 1,000 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 Kashino-Hasegawa (K-H) maximum likelihood test (Kishino & Hasegawa 1989) to find the most likely tree.

ii) Bayesian analyses: The model of substitution used for Bayesian analyses 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 2,000,000 generations and sampled every 100 generations. The first 2,000 trees, which represented the burn-in phase of the analysis, were discarded, with 18,000 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. 7.2.6 (Stamatakis 2006). The dataset was partitioned according to each gene and separated codons (two partitions). The best-scoring ML tree was estimated using a general time reversible (GTR) + I^{-} model of sequence evolution with 1,000 pseudoreplicates. Maximum parsimony (BSMP, left) and likelihood (BSML, right) bootstrap value greater than 50% are given 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. The rDNA sequences, consisting of SSU and LSU, were submitted into the GenBank database and the new sequences generated for this investigation are listed in Table 2.

Table 2. Taxa, isolate or culture numbers and GenBank accession numbers of sequences used in the phylogenetic analyses

Taxon	*Voucher/culture*	*SSU*	*LSU*
Subclass Hypocreomycetidae			
Coronophorales			
Bertia moniformis	SMH4320	N/A	AY695260
Bertia tropicalis	SMH1707	N/A	AY695262
Bertia tropicalis	SMH3513	N/A	AY695263
Crassochaeta nigrita	SMH1667	N/A	AY695265
Crassochaeta nigrita	SMH2931	N/A	AY695266
Chaetosphaerella phaeostroma	SMH4585	N/A	AY346274
Chaetosphaerella phaeostroma	SMH2931	N/A	AY695264
Fracchiaea broomeana	SMH2809	N/A	AY695268
Nitschkia calyculus	SMH918	N/A	FJ968983
Nitschkia grevillet	SMH4663	N/A	AY346294
Nitschkia menicoidea	SMH1523	N/A	AY695270
Nitschkia tetraspora	GKML148N	N/A	FJ968987
Nitschkia tetraspora	SMH2469	N/A	FJ968986
Hypocreales			
Bionectria ochroleuca	GJS90.227	AY489684	AY489716

Table 2. Taxa, isolate or culture numbers and GenBank accession numbers of sequences used in the phylogenetic analyses *(continued)*

Taxon	*Voucher/culture*	*SSU*	*LSU*
Bionectria pityrodes	GJS95.26	AY489696	AY489728
Calonectria colombiensis	CBS112221	N/A	GQ280689
Cordyceps militaris	NRRL28021	AF049146	AF327374
Epichloe typhina	ATCC56429	U32405	U17396
Hypocrea lutea	ATCC208838	AF543768	AF543791
Nectria cinnabarina	G.J.S89.107	U32412	U00748
Nectria haematococca	GJS89.70	AY489697	AY489729
Pseudonectria rousseliana	AR 2716	AF543767	U17416
Sphaerosilibella berkeleyana	GJS82.274	AF543770	U00756
Melanosporales			
Melanospora singaporensis	ATCC38268	N/A	AY015629
Melanospora tiffanii	ATCC15515	AY015619.1	AY015630
Melanospora zamiae	ATCC12340	AY046578	AY046579
Microascales			
Custingophora cecropiae	CCF3568	AM267267	N/A
Custingophora olivacea	CBS 335.68	JX070460	AF178566
Doratomyces stemonitis	AFTOL1380	DQ836901	DQ836907
Gondwanamyces scolytodis	CCF 3569	AM267268	AM267262
Gondwanamyces capensis	AFTOL1907	FJ176834	FJ176888
Halosphaeria appendiculata	CBS197.60	U46872	U46885
Lignincola laevis	JK5180A	U46873	U46890
Microascus cirrosus	CBS217.31	EU984279	AF400860
Microascus longirostris	AFTOL1237	DQ471026	DQ471026
Nimbospora effusa	JK5104A	U46877	U46892
Nohea umiumi	JK5103F	U46878	U46893
Petriella setifera	AFTOL956	DQ471020	DQ470969
Savoryellales			
Ascotaiwania sawadae	SS00051	HQ446283	HQ446363
Savoryella lignicola	NFSR0001	HQ446300	HQ446378
Savoryella longispora	SAT00320	N/A	N/A
TBM			
Etheirophora blepharospora	JK5289	N/A	EF027724
Etheirophora blepharospora	JK5397A	EF027717	EF027723
Etheirophora unijubata	JK5443B	EF027718	EF027725
Fulvocentrum (*Swampomyces aegyptiacus*)	CY2973	AY858943	AY858950
Fulvocentrum (*Swampomyces*) *clavatispora*	LP83	AY858945	AY858952
Glomerulispora mangrovis	NBRC105264	GU252150	GU252149
Juncigena adarca	JK5235A	EF027719	EF027726
Juncigena adarca	JK5548A	EF027720	EF027727
Marinokulati (*Chaetosphaeria*) *chaetosa*	BCRC FU30271 (NTOU4048)	KJ8669290	KJ866931

Table 2. Taxa, isolate or culture numbers and GenBank accession numbers of sequences used in the phylogenetic analyses *(continued)*

Taxon	*Voucher/culture*	*SSU*	*LSU*
Marinokulati (*Chaetosphaeria*) *chaetosa*	BCRC FU30272 (NTOU4060)	KJ866930	KJ866932
Moheitospora fructicosae	EF14	GU252146	GU252145
Swampomyces armeniacus	JK5325A	N/A	EF027729
Swampomyces armeniacus	JK5059C	EF027721	EF027728
Swampomyces triseptatus	CY2802	AY858942	AY858953
Torpedospora ambispinosa	CY3386	AY858941	AY858946
Torpedospora radiata	AFTOL751	DQ470999	DQ470951
Torpedospora radiata	BCC11269	AY858938	AY858948
Torpedospora radiata	JK5095A	DQ470999	DQ470951
Torpedospora radiata	JK5252C	EF027722	EF027730
Torpedospora radiata	PP7763	AY858939	AY858947
Subclass Sordariomycetidae			
Boliniales			
Camarops microspora	AFTOL1361	DQ471036	N/A
Camarops ustulinoides	AFTOL72	DQ470989	DQ470941
Sordariales			
Gelasinospora tetrasperma	AFTOL1287	DQ471032	DQ470980
Neurospora crassa	MUCL19026	X04971	AF286411
Sordaria fimicola	15-6291	AY545724	AY545728
Sordaria macrospora	SMH5147	AY641007	AY346301
Chaetosphaeriales			
Chaetosphaeria ovoidea	SMH2605	N/A	AF064641
Chaetosphaeria ciliata	ICMP 18253	GU180614	GU180637
Chaetosphaeria curvispora	CBS 113644/ ICMP 18255	AY502933	GU180636
Outgroups			
Morchella esculenta	OSC100041	AY544708	AY544664
Scutellinia scutellata	AFTOL62	DQ247814	DQ247806

RESULTS

Molecular phylogeny of combined SSU and LSU DNA sequences: The aligned dataset comprised a total of 2,098 characters for 78 taxa, of which 793 were parsimony informative, 191 were parsimony uninformative and 1,114 constant. Tree length was 3921, CI = 0.403, RI = 0.718, RC = 0.289 and HI = 0.597. The maximum parsimony analysis resulted in twenty-one most parsimonious trees (MPTs). RAxML yielded a best scoring likely tree with a log likelihood –21936.145644, alpha: 0.585866, invar: 0.331890, Tree-Length: 5.100200, rate A <-> C: 0.946272, rate A <-> G: 2.670095, rate A <-> T: 1.256489, rate C <-> G: 1.303933, rate C <-> T: 6.475264, rate G <-> T: 1.000000, freq pi(A): 0.250234, freq pi(C): 0.224494, freq pi(G): 0.291704 and freq pi(T): 0.233568 (data not shown).

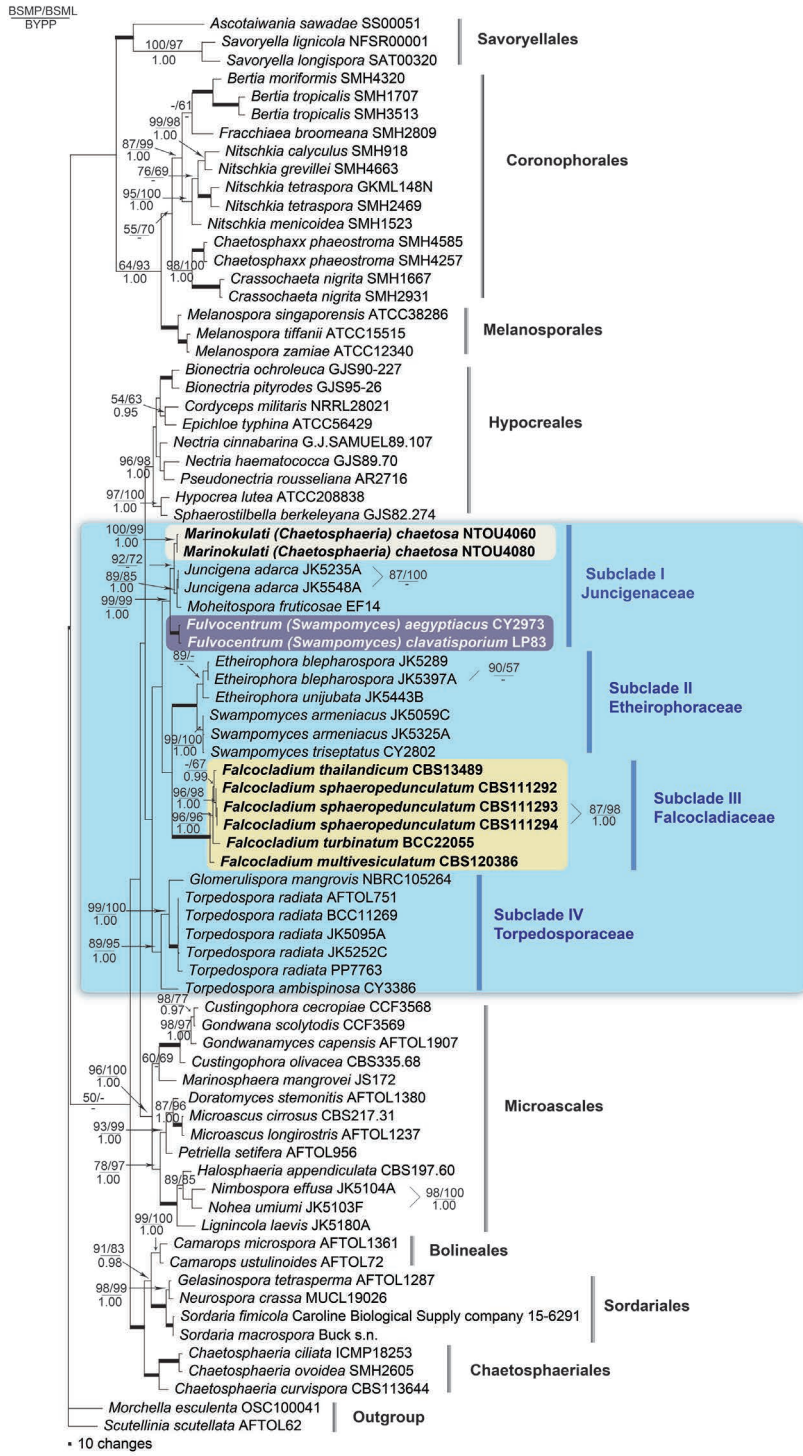
Bayesian phylogenetic analysis was performed using a uniform GTR + I + G model, as selected by hLRT in Mrmodeltest 2.2: [GTR + I + G] Prset statefreqpr = dirichlet (1,1,1,1), Lset nst = 6 rates = invgamma. The GenBank accession numbers of sequences used in the phylogenetic analysis are given in Table S1.

A preliminary analysis of the phylogeny of the genus Falcocladium: In an initial study, various representative taxa from three sub-classes of the Sordariomycetes including the Hypocreomycetidae, Sordariomycetidae and Xylariomycetidae were aligned along with *Falcocladium* sequences with *Xylaria hypoxylon* and *X. acuta (*Xylariales*,* Xylariomycetidae), chosen as the outgroup. Six major orders (Boliniales, Chaetosphaeriales, Coniochaetales, Diaporthales, Ophiostomatales and Sordariales) within the Sordariomycetidae as well as five orders of the Hypocreomycetidae (Coronophorales, Hypocreales, Melanosporales, Microascales and Savoryellale*s*) were included in the dataset (data not shown). Initial results suggested that the *Falcocladium* species grouped in the Hypocreomycetidae with high support and closely related to the TBM clade (Schoch *et al*., 2007). Therefore the dataset was redefined with some taxa from these three sub-classes excluded, particularly the Sordariomycetidae, and other taxa from the Hypocreomycetidae included.

Phylogenetic analysis of SSU and LSU regions: The final dataset consists of five orders from the Hypocreomycetidae (Coronophorales, Hypocreales, Melanosporales, Microascales and Savoryellales) and three orders from the Sordariomycetidae (Bolineales, Sordariales and Chaetosphaeriales) with *Morchella esculenta* and *Scutelinia scutellenta* as the outgroup. There was a dichotomy between the Hypocreomycetidae and Sordariomycetidae (Figure 1). At the ordinal level, two different lineages within the Hypocreomycetidae were observed, the first lineage was a group of three orders the Coronophorales, Melanosporales and Savoryellales, and the second lineage comprised the Hypocreales, Microascales, *Falcocladium* spp. and taxa referred to as the TBM group. Within the second lineage, the Hypocreales and Microascales were clearly separated.

Four distinct and well-supported lineages were noticeable in the tree: (1) a lineage of diverse genera, (2) a lineage comprising *Etheirophora*, *Swampomyces sensu stricto*, (3) *Falcocladium* species, and (4) a lineage with *Glomerulispora* and *Torpedospora*. For the first lineage, two strains of *Chaetosphaeria chaetosa* (BCRC FU30271 (NTOU4048) and BCRC FU30272 (NTOU4060)) grouped together with high support (100 BSMP, 99 BSML and 1.00 BSPP) and formed a clade with *Juncigena adarca* and *Moheitospora fructicosae*. While the other three *Chaetosphaeria* species (*Ch. ciliata*, *Ch. ovoidea*, *Ch. curvispora*), were placed within the Chaetosphaeriales (Sordariomycetidae) in a basal clade with high statistical support. This clearly shows the polyphyletic nature of *Chaetosphaeria*. Therefore *Ch. chaetosa* cannot be accommodated in the

Fig. 1. One of the most parsimonious trees resulted from maximum parsimony analysis from the combined SSU and LSU rDNA sequences. Maximum parsimony (BSMP) and maximum likelihood (BSML) bootstrap value greater than 50% are given above the branches and Bayesian posterior probabilities (BYPP) greater than 0.95 are given below the branches. Hypen "–" indicates a value lower than 50% (BSMP and BSML) or 0.95 (BYPP). Thickened branches indicate parsimony and likelihood bootstrap and Bayesian posterior probabilities equal 100% and 1.00, respectively. The tree was rooted to *Morchella esculenta* and *Scutellinia scutellata*. Bar indicates 10 character state changes.


BSMP/BSML
BYPP
Ascotaiwania sawadae SS00051
Savoryella lignicola NFSR00001
Savoryella longispora SAT00320
Savoryellales
Bertia moriformis SMH4320
Bertia tropicalis SMH1707
Bertia tropicalis SMH3513
Fracchiaea broomeana SMH2809
Nitschkia calyculus SMH918
Nitschkia grevillei SMH4663
Nitschkia tetraspora GKML148N
Nitschkia tetraspora SMH2469
Nitschkia menicoidea SMH1523
Chaetosphaxx phaeostroma SMH4585
Chaetosphaxx phaeostroma SMH4257
Crassochaeta nigrita SMH1667
Crassochaeta nigrita SMH2931
Coronophorales
Melanospora singaporensis ATCC38286
Melanospora tiffanii ATCC15515
Melanospora zamiae ATCC12340
Melanosporales
Bionectria ochroleuca GJS90-227
Bionectria pityrodes GJS95-26
Cordyceps militaris NRRL28021
Epichloe typhina ATCC56429
Nectria cinnabarina G.J.SAMUEL89.107
Nectria haematococca GJS89.70
Pseudonectria rousseliana AR2716
Hypocrea lutea ATCC208838
Sphaerostilbella berkeleyana GJS82.274
Hypocreales
Marinokulati (Chaetosphaeria) chaetosa NTOU4060
Marinokulati (Chaetosphaeria) chaetosa NTOU4080
Juncigena adarca JK5235A
Juncigena adarca JK5548A
Moheitospora fruticosae EF14
Fulvocentrum (Swampomyces) aegyptiacus CY2973
Fulvocentrum (Swampomyces) clavatisporium LP83
Subclade I
Juncigenaceae
Etheirophora blepharospora JK5289
Etheirophora blepharospora JK5397A
Etheirophora unijubata JK5443B
Swampomyces armeniacus JK5059C
Swampomyces armeniacus JK5325A
Swampomyces triseptatus CY2802
Subclade II
Etheirophoraceae
Falcocladium thailandicum CBS13489
Falcocladium sphaeropedunculatum CBS111292
Falcocladium sphaeropedunculatum CBS111293
Falcocladium sphaeropedunculatum CBS111294
Falcocladium turbinatum BCC22055
Falcocladium multivesiculatum CBS120386
Subclade III
Falcocladiaceae
Glomerulispora mangrovis NBRC105264
Torpedospora radiata AFTOL751
Torpedospora radiata BCC11269
Torpedospora radiata JK5095A
Torpedospora radiata JK5252C
Torpedospora radiata PP7763
Torpedospora ambispinosa CY3386
Subclade IV
Torpedosporaceae
Custingophora cecropiae CCF3568
Gondwana scolytodis CCF3569
Gondwanamyces capensis AFTOL1907
Custingophora olivacea CBS335.68
Marinosphaera mangrovei JS172
Doratomyces stemonitis AFTOL1380
Microascus cirrosus CBS217.31
Microascus longirostris AFTOL1237
Petriella setifera AFTOL956
Halosphaeria appendiculata CBS197.60
Nimbospora effusa JK5104A
Nohea umiumi JK5103F
Lignincola laevis JK5180A
Microascales
Camarops microspora AFTOL1361
Camarops ustulinoides AFTOL72
Bolineales
Gelasinospora tetrasperma AFTOL1287
Neurospora crassa MUCL19026
Sordaria fimicola Caroline Biological Supply company 15-6291
Sordaria macrospora Buck s.n.
Sordariales
Chaetosphaeria ciliata ICMP18253
Chaetosphaeria ovoidea SMH2605
Chaetosphaeria curvispora CBS113644
Chaetosphaeriales
Morchella esculenta OSC100041
Scutellinia scutellata AFTOL62
Outgroup
10 changes

Chaetospheriales and a new combination is proposed (*Marinokulati chaetosa*). Meanwhile, *Swampomyces aegypticus* (CY2973) and *S. clavatispora* (LP83) formed a sister clade with the above genera grouping with *Juncigena adarca* with high statistical supports (99 BSMP, 99BSML and 1.00 BYPP) confirming the polyphyletic nature of *Swampomyces* (type species *S. armeniacus*). *Swampomyces aegypticus* and *S. clavatispora* are therefore referred to a new genus (*Fulvocentrum*).

The second lineage (100 BSMP, 100 BSML and 1.00 BSPP) included *Etheirophora blepharospora*, *E. unijubata*, *S. armeniacus* and *S. triseptatus* with the genera *Etheirophora* and *Swampomyces* clearly delineated. *Falcocladium* species form a monophyletic lineage with high statistical support (100 BSMP, 100 BSML and 1.00 BSPP) and constitute the third lineage, which is sister to the second lineage comprising *Etheirophora* spp. and *Swampomyces s. s.* Three strains of *F. sphaeropeduculatum* (CBS111292-111294) showed a close relationship with *F. thailandicum* (CBS13489), while *F. turbinatum* (BCC2205) and *F. multivesiculatum* (CBS120386) grouped in a lower sub-clade. For the final lineage, *Torpedospora* is monophyletic with high statistical support (89 BSMP, 95 BSML and 1.00 BSPP). Four strains of *T. radiata* formed a monophyletic group with *Glomerulispora mangrovis* while *T. ambispinosa* was placed in a lower sub-clade.

DISCUSSION

A new lineage within the Hypocreomycetidae: The TBM clade is a major and independent lineage within the Hypocreomycetidae (the Sordariomycetes) and was proposed by Schoch *et al.* (2007). The taxonomy of the genera in the TBM clade remains inconclusive and cannot be assigned to an appropriate family, order or class. Extensive revisions of the orders assigned to the Hypocreomycetidae have taken place over the past decade with the discovery of new taxa and additional sequences (Sakayaroj *et al.*, 2005; Zhang *et al.*, 2006; Schoch *et al.*, 2007; Boonyuen *et al.*, 2011; Réblová *et al.*, 2011a). Many new lineages were revealed and at least four new lineages were present within the Hypocreomycetidae: Halosphaeriaceae (Spatafora *et al.*, 1998), Lulworthiales (Kohlmeyer *et al.*, 2000) and Koralionastetales (Campbell *et al.*, 2009). A multigene phylogeny of *Stachybotrys chartarum* revealed a new lineage of asexual fungi in the Hypocreales, but no higher taxon name was proposed (Castlebury *et al.*, 2004). A molecular study of the genera *Torpedospora*, and *Swampomyces* highlighted another marine lineage (Sakayaroj *et al.*, 2005) and Schoch *et al.* (2007) confirmed this observation with the inclusion of two other genera (*Juncigena* and *Etheirophora*). This lineage was shown to have affinity with the orders Coronophorales and Melanosporales, and referred to as the TBM clade (*Torpedospora*, *Bertia*, *Melanospora*).

Zhang *et al.* (2006) recognize the Hypocreomycetidae as a strongly supported monophyletic clade with the orders Coronophorales, Halosphaeriales (now regarded as a family in the Microascales), Hypocreales, Microascales, and Melanosporales. Hibbett *et al.* (2007) later accepted the Melanosporales as an order in the Hypocreomycetidae, with other orders as *incertae sedis*: *L*ulworthiales (Kohlmeyer *et al.*, 2000) and the TBM clade (Schoch *et al.*, 2007),

while the Halosphaeriales was referred to as a family (Halosphaeriaceae) in the Microascales. Subsequently, Boonyuen *et al.* (2011) introduced the new order Savoryellales for a group of aquatic ascomycetes that included the genera *Ascotaiwania*, *Ascothailandia* and *Savoryella* and the asexual genera *Canalisporium* and *Monotosporella*. The Savoryellales clade is distinct from the orders Microascales, Hypocreales, Coronophorales and Melanosporales (Boonyuen *et al*., 2011).

The TBM as delineated by Schoch *et al.* (2007) contains three subclades of marine species: 1) *Torpedospora radiata* and *T. ambispinosa*; 2) *Swampomyces armeniacus*, *S. triseptatus* and *Juncigena adarca* and 3) *Swampomyces armeniacus*, *S. aegyptiacus, S. clavatispora, Etheirophora blepharospora* and *E. unijubata*. Recently Abdel-Wahab *et al.* (2010) demonstrated that two dematiaceous hyphomycetes grouped in the TBM clade: *Moheitospora fruticosae* in subclade 2, and *Glomerulispora mangrovis* in subclade 1, both marine species and isolated from the salt marsh plant *Suaeda fruticosa* and mangrove driftwood, respectively. *Glomerulispora mangrovis* groups with *T. radiata* with high statistical support, but whether it is its sexual state remains to be resolved (Abde-Wahab *et al*., 2010).

The data presented in this paper highlights the existence of yet another new lineage related to taxa in the TBM clade in the Hypocreomycetidae*,* the *Falcocladium* clade, a well-supported monophyletic group with no affinities to Coronophorales, Melanosporales and Savoryellales. This lineage is well supported phylogenetically and morphologically and is clearly separated from other lineages. No sexual state has been reported for *Falcocladium* species, so it is not known if they share any morphological features with taxa in these orders.

The morphology of the genus *Falcocladium* has been extensively studied and well documented (Crous *et al*., 1994, 1997, 2007; Somrithipol *et al*., 2007). There is, however, little information of the taxonomy of this genus, particularly at the molecular level. Crous *et al.* (2007) used BLASTn tool and compared DNA similarity of ITS and LSU regions of *F. thailandicum* separately with reference sequences. Their results suggested that the genus *Falcocladium* appears to belong to the Hypocreales and this genus is likely to be polyphyletic. It is worth noting that the percentages of DNA similarity were moderate (78% and 86%, respectively) with the Hypocreales. In the present study, it clearly shows that *Falcocladium* is monophyletic. *Falcocladium* species, together with the taxa in the TBM clade, form a unsupported monophyletic group with the Hypocreales, so whether these taxa truly belong to the Hypocreales remains to be seen, possibly requiring more taxa and genes for analysis.

Chaetosphaeria chaetosa: *Chaetosphaeria* is polyphyletic with most species grouping in the Chaetosphaeriales (Fig. 1), *Ch. tulsaneorum* (= *Reticulascus tulsaneorum*) forms a sister group to *Reticulascus clavatus* (Reticulascaceae, Glomerellales) while *Ch. chaetosa* groups in subclade 1 in ascomycetes *incertae sedis* (Réblová *et al*., 2011a, b, and data not shown). Two sequences of *Ch. chaetosa* formed a monophyletic group with *Juncigena adarca* and *Moheitospora fruticosa* with high bootstrap support and share many morphological features in common (Table 3), in particular, ascomata that are immersed, ostiolate, papillate with short necks, periphysate, paraphysate, asci cylindrical, persistent, and hyaline, fusiform, 1-3 septate ascospores. However, they differ in a number of respects: *Ch. chaetosa* has thick-walled asci with a thick apical thickening, paraphyses that are wide and appendaged ascospores, *J. adarca* has thin-walled asci with an apical ring, and ascospores lacking appendages.

Swampomyces aegyptiacus and *S. clavatispora* form a well-supported monophyletic group with *Ch. chaetosa*, *J. adarca*, but are all the taxa congeneric?

Table 3. Comparison of the morphology of the genera *Swampomyces, Chaetosphaeria,* and *Juncigena*

Fungus	*Perithecia*	*Periphyses/ Paraphyses*	*Asci*	*Ascospores*	*Reference*	*Other observation*
Swampomyces clavatispora	Immersed, dark, brown, ostiolate, 160-170 × 160-190 µm Neck 50 µm long	Both present, Paraphyses numerous in a gel unbranched	80-96 × 10-13 µm, short Pedicellate. Apical thickened.	25-28 × 5-6 µm, 3 septate, clavate, hyaline	Abdel-Wahab *et al.* (2001)	Contents apricot in mass. No asexual state known. Marine
Chaetosphaeria chaetosa	170-275 × 275 µm, Immersed dark, brown, ostiolate, necks 20-70 µm	Both present, Paraphyses septate, wide	102-135 × 12-18 µm, thick-walled at the apex	25.5-36.5 × 7.5-11.5 µm, 3-septate, fusiform, hyaline with polar and equatorial appendages	Kohlmeyer (1963)	No asexual state known. Marine
Juncigena adarca	225-400 × 135-200 µm, immersed, ostiolate, papillate, necks 85-170-50-80 µm	Both present, Paraphyses thin, branched, septate	115-140 × 10-13 µm, fusiform to cylindrical, short pedunculate, apical apparatus with a ring	26.5-34.5 × 6-7 µm, 3-septate, hyaline, fusiform to elongate ellipsoidal, no appendages, smooth wall, constricted	Kohlmeyer *et al.* (1997)	Has an asexual state: *Cirrenalia adarca*. Salt marsh

Fungus	*Paraphyses*	*Ascus morphology*	*Apical apparatus*	*Ascospores*	*Asexual/Sexual stages*
S. aegypticus Pseudostroma, Wall 8-10 µm	Numerous thin-walled	Cylindrical Short Pedicel	Apically thickened	No appendages	No asexual stage
S. clavatospora Pseudostroma. Wall 14-20 µm	Numerous thin walled	Cylindrical Short Pedicel	Apically thickened	No appendages	No asexual stage
Ch. chaetosa No clypeus or pseudostroma Wall 11-30 µm	Unbranched 1.5-4.5 µm wide	Cylindrical With pedicel medium	Ascus with an apical pore	Polar and equatorial appendages	No asexual stage
J. adarca Wall 10-20 µm	Thin-walled	Fusiform to cylindrical	Apical thickening, with a ring	No appendages	Asexual *Cirrenalia adarca*

Schoch *et al.* (2007) concluded from the molecular results that *S. aegyptiacus* and *S. clavatispora* belonged to a different genus, possibly *Juncigena,* as they share a number of morphological features: immersed ascomata, periphysate, ostiolate and similar shaped and unbranched paraphyses. However, they differ in other respects (Table 3). In order to determine the taxonomy of these taxa, features that require evaluation are: morphology of the paraphyses, ascus shape and apical apparatus,

appendaged ascospores and different asexual stages or lack of one (Table 3). *Chaetosphaeria chaetosa* significantly differs from the other taxa in possessing larger ascomata, paraphyses that are up to 4.5 μm wide, a distinct apical ring to the ascus, ascospores that are wider (7.5-11.5 vs 5-7 μm) with both polar and equatorial appendages formed by fragmentation of an exosporic sheath (Jones *et al*., 1983). At the molecular level, it shows only 92.72% similarity with *Juncigena adarca*. Consequently we assign the species *S. aegyptiacus* and *S. clavatispora*, and *Ch. chaetosa* to the new genera *Fulvocentrum*, and *Marinokulati*, respectively.

Swampomyces aegyptiacus and S. clavatispora: *S. aegyptiacus* and *S. clavatispora* do not group with the type species of the genus *S. armeniacus*, but form a sister group to *J. adarca* and *Ch. chaetosa* in clade 1 (Fig. 1). The type species, *S. armeniacus*, is significantly different morphologically from *S. aegyptiacus* and *S. clavatispora*. *S. armeniacus* has large ascomata that are immersed under a large clypeus and one-septate ascospores, while *S. aegyptiacus* and *S. clavatispora* have small ascomata that are immersed under a thin stroma and 3-septate ascospores. On the other hand, *S. aegyptiacus* and *S. clavatispora* have morphological similarities with *Juncigena adarca* which include: small, single, immersed ascomata; periphysate neck; thin peridium; numerous, thin, unbranched paraphyses that are attached to the top and bottom of the ascomatal cavity; fusiform to cylindrical asci with apical apparatus (faint ring or apical thickening) and 3-septate ascospores. Based on these morphological and molecular similarities, we transferred *S. aegyptiacus* and *S. clavatispora* to a new genus *Fulvocentrum*.

TAXONOMY

Fulvocentrum E.B.G. Jones & Abdel-Wahab, **gen. nov.**

MycoBank MB 808181

Ascomata pyriform, immersed, oblique or vertical to the host surface, dark brown to black, coriaceous, ostiolate, contents apricot coloured in mass, single, developing under a thin darkened superficial pseudo stroma, covering the area where ascomata develop and composed of host cells with darkened fungal hyphae. Neck filled with periphyses. ***Peridium*** thin and consist of brown to dark brown polygonal cells. Paraphyses numerous, hyaline, in a gel, unbranched, attached to the top and bottom of the ascomatal cavity. ***Asci*** cylindrical, thin-walled, short pedicellate and apically thickened. ***Ascospores*** 3-septate, ellipsoidal, hyaline, uniseriate, constricted at the septa and smooth.

Etymology: from *fulvum* = orange in reference to the apricot color of the ascomatal centrum.

Asexual state: unknown.

Generic type: ***Fulvocentrum aegyptiaca*** (Abdel-Wahab, El-Sharouney & E.B.G. Jones) E.B.G. Jones & Abdel-Wahab

Distribution: Egypt, Saudi Arabia.

Fulvocentrum aegyptiaca (Abdel-Wahab, El-Sharouney & E.B.G. Jones) E.B.G. Jones & Abdel-Wahab, **comb. nov.**

≡ *Swampomyces aegyptiacus* Abdel-Wahab, El-Sharouney & E.B.G. Jones, *Fungal Divers*. 8: 35 (2001).

MycoBank MB 808182

Ascomata pyriform, immersed, oblique or vertical to the host surface, dark brown to black, coriaceous, ostiolate, contents apricot coloured in mass, single, developing under a thin darkened superficial pseudo stroma, covering the area where ascomata develop and composed of host cells with darkened fungal hyphae. Neck filled with periphyses. Peridium thin and consist of brown to dark brown polygonal cells. Paraphyses numerous, hyaline, in a gel, unbranched, attached to the top and bottom of the ascomatal cavity. Asci cylindrical, thin-walled, short pedicellate and apically thickened. Ascospores 3-septate, ellipsoidal, hyaline, uniseriate, constricted at the septa and smooth.

Notes: This species is very common on intertidal wood of *Avicennia marina* in Red Sea mangroves both on the Egyptian coast (Abdel-Wahab 2005) and from Arabian Gulf mangroves of the Saudi Arabia coast (Abdel-Wahab *et al*. unpublished data). So far, the species has been recorded from intertidal wood of *A. marina* and from Middle East only. The fungus neither produces ascomata nor an asexual state in culture.

Fulvocentrum clavatisporium (Abdel-Wahab, El-Sharouney & E.B.G. Jones) E.B.G. Jones & Abdel-Wahab, **comb. nov.**

≡ *Swampomyces clavatispora* Abdel-Wahab, El-Sharouney & E.B.G. Jones, *Fungal Divers*. 8: 37 (2001).

MycoBank MB 808183

Ascomata immersed, vertical, single, ostiolate, brown to dark brown, with hyaline neck, contents apricot coloured in mass, developing under a thin darkened superficial peudostroma, covering area where ascomata develop and composed of host cells with darkened fungal hyphae. Ostiolar canal filled with periphyses. ***Peridium*** comprising elongated yellow-brown to brown cells forming *textura angularis*. Paraphyses numerous, hyaline, in a gel and unbranched. ***Asci*** 8-spored, oblong, thin-walled, short pedicellate, apically thickened. ***Ascospores*** clavate, biseriate, hyaline, 3-septate, and weakly constricted at the septa.

Asexual state: unknown.

Distribution: Egypt, Saudi Arabia.

Notes: This species is very common on intertidal wood of *Avicennia marina* in Egyptian mangroves along the Red Sea coast (Abdel-Wahab 2005). Recently, the species has been commonly recorded from Saudi Arabian mangroves from Red Sea and Arabian Gulf (Abdel-Wahab *et al*. unpublished data)

Marinokulati E.B.G. Jones & K.L. Pang, **gen. nov.**

MycoBank MB 808184

Ascomata subglobose to pyriform, subcoriaceous, wall thick, necks long (385 µm), ostiolate, and periphysate. Paraphyses wide 1.5 to 4.5 µm, septate, apically free. ***Asci*** 8-spored, unitunicate, cylindrical, attenuate at the base, thick-walled at the apex with a perforated apical apparatus. ***Ascospores*** biseriate, fusiform to elongate ellipsoidal, 3-septate, constricted at the septa, hyaline with polar and equatorial appendages. Asexual state not reported.

Etymology: in reference to the marine origin of the fungus in Bahasa.

Asexual state: unknown.

Generic type: *Marinokulati chaetosa* (Kohlm.) E.B.G. Jones & K.L. Pang.

Marinokulati chaetosa (Kohlm.) E.B.G. Jones & K.L. Pang, **comb. nov.** **Fig. 2**

≡ *Chaetosphaeria chaetosa* Kohlm., Nova Hedw. 6: 307 (1963).

MycoBank MB 808186

Ascomata subglobose to pyriform, immersed to superficial, subcoriaceous, wall thick, ostiolate, papillate, necks long (385 µm), and periphysate dark brown or black. Paraphyses wide 1.5 to 4.5 µm, septate, apically free. ***Asci*** 8-spored, unitunicate, cylindrical to clavate, attenuate at the base, thick-walled at the apex with a perforated apical apparatus, persistent. ***Ascospores*** biseriate, fusiform to elongate ellipsoidal, 3-septate, constricted at the septa, hyaline with polar and equatorial appendages. Appendages formed by fragmentation of an exosporic sheath, polar appendage caducous. Asexual state not reported.

Asexual state: unknown.

Material examined: Turkey, Isme, decayed driftwood; UK, Cornwall, decayed driftwood, 2012.

Substrate: rotten driftwood.

Distribution: Bulgaria, Denmark, Germany, Italy, Turkey, Spain, UK, USA.

Notes: The species is widely distributed in temperate climates on intertidal and drifting wood. It differs from *Chaetosphaeria sensu stricto* (type species *Ch. innumera*) in ascospores with polar and equatorial appendages formed by fragmentation of an exosporic sheath (Jones *et al*., 1983), it lacks asexual phialidic stages, and its marine habitat. Previously, *Ch. chaetosa* has not been examined at the molecular level (Réblová 2000; Réblová & Winka 2000; Jones *et al*., 2009) and this study resolves its taxonomic position.

The higher order assignment of this unique group of species poses many problems as clearly they show no affinities with the Coronophorales and Melanosporales and thus the redundancy of the term TBM clade. We therefore propose the introduction of the following families: Clade 1: Juncigenaceae, Clade 2: Etheirophoraceae, Clade 3: Falcocladiaceae and Clade 4: Torpedosporaceae.

Ecologically, the taxa discussed here come from diverse habitats and share few common morphological characteristics: *Falcocladium* are asexual species found in terrestrial tropical habitats on leaf litter; *Etheirophora*, *Fulvocentrum*, *Marinokulati* and *Swampomyces* are marine species on driftwood, and *Juncigena adarca*, and its asexual state, is known only from the senescent leaves of the salt marsh reed *Juncus roemerianus*. The connection of the *Falcocladium* to the other marine fungal genera is not known. Their natural habitats are also considerably different. However, there is molecular evidence to support that *Falcocladium* has an affinity with marine fungi in one way or the other. The link of these fungi is yet to be discovered.

This study has revealed an additional new lineage within the Hypocreomycetidae. In the meantime, some genera within this sub-class have been re-defined and four new families (Juncigenaceae, Etheirophoraceae, Falcocladiaceae and Torpedosporaceae) have been proposed. Using multi-gene phylogeny can significantly improve and clarify the taxonomic status of asexual fungi which were previously unassigned into a certain group.

Juncigenaceae E.B.G. Jones, Abdel-Wahab & K.L. Pang, **fam. nov.**

MycoBank MB 808177

Saprobic fungi growing on lignicolous substrates in marine habitats. ***Ascomata*** perithicioid, immersed, ostiolate, papillate, coriaceous, brown to dark-

brown, contents apricot coloured in mass. Paraphyses numerous, narrow, unbranched, persistent, connected to the top and bottom of the ascomatal cavity. ***Asci*** 8-spored, unitunicate, thin-walled, persistent, cylindrical to fusiform, with apical apparatus, short pedicellate. ***Ascospores*** uni- to biseriate, ellipsoidal, clavate to fusiform, 3-septate, hyaline, constricted at the septa, with or without equatorial and polar appendages. Asexual stage: helicoid conidia when present.

Habitat: intertidal wood, mangrove and herbaceous wood and roots, bark and leaves, marine.

Family type: *Juncigena* Kohlm., Volkm.-Kohlm. & O.E. Erikss.

Included genera: *Juncigena* Kohlm., Volkm.-Kohlm. & O.E. Erikss., Bot. Mar. 40: 291 (1997). (1 species) – MycoBank MB 27750; *Fulvocentrum* E.B.G. Jones & Abdel-Wahab, this study. (2 species) – MycoBank MB 808181; *Marinokulati* E.B.G. Jones & K.L. Pang, this study. (1 species) – MycoBank MB 808184; *Moheitospora* Abdel-Wahab, Abdel-Aziz & Nagah., Mycol. Prog. 9: 551 (2010). (2 species) – MycoBank MB 543122.

Etheirophoraceae Rungjindamai, Somrithipol & Suetrong, **fam. nov.**

MycoBank MB 808178

Ascomata immersed, ostiolate, periphysate, papillate, clypeate, coriaceous, light coloured, paraphysate. Peridium of *textura angularis*. **Asci** 8-spored, cylindrical to oblong, pedicellate, none amyloid, thin-walled, unitunicate, persistent. **Ascospores** biseriate, 1-septate, ellipsoidal, with a filamentous appendage at one or both ends. **Appendages** bristle-like origin not determined. No asexual state known.

Habitat: intertidal wood, bark. Bark of *Rhizophora mangle* seedlings.

Family type: *Etheirophora* Kohlm. & Volkm.-Kohlm.

Distribution: Bahamas, Bermuda, Colombia, Fiji, Mexico, Peru, US (Hawaii).

Notes: Kohlmeyer & Volkmann-Kohlmeyer (1989) introduced the genus to accommodate three marine species from tropical locations, including a species previously referred to as *Keissleriella blepharospora*. Initially Kohlmeyer & Kohlmeyer (1965) considered the asci of *K. blepharospora* to be bitunicate but not fissitunicate and the species lacks ascospore appendages. The genus was assigned to the Sphaeriales by Kohlmeyer *et al*. (1989) and to the Halosphaeriales by Hawksworth *et al*. (1995) and Kirk *et al*. (2001). However, molecular data clearly shows it does not belong in the Halosphaeriaceae, but in a new family and order *incerate sedis*. Further taxon sampling is required to determine its ordinal status within the Hypocreomycetidae.

Included genera: *Etheirophora* Kohlm. & Volkm.-Kohlm., Mycol. Res. 92: 414 (1989). (3 species) – MycoBank MB 25298; *Swampomyces* Kohlm. & Volkm.-Kohlm., Botanica Marina 30: 198 (1987). (2 species) – MycoBank MB 6004.

Falcocladiaceae Somrithipol, E.B.G. Jones & K.L. Pang, **asex. fam. nov.**

MycoBank MB 808179

Saprobic on leaf litter and leaves. ***Conidiomata*** hyaline, sporodochial or synnematal, or penicillate, arising directly from the mycelium or from a stroma or from microsclerotia, thick-walled, non-septate stipe extensions that terminate in thin-walled sphaeropendunculate vesicles. ***Conidiophore*** branches hyaline, non- to multi-septate, up to three series of branches per conidioma (primary, secondary tertiary), smooth, subcylindical. ***Conidiogenous cells*** phialidic, in whorls of

2-6 ampulliform with elongated necks and minute collarettes. ***Conidia*** hyaline, smooth, 0-1 septate, falcate, with short apical and basal appendages. The four species are distinguished by the morphology of the terminal vesicle, conidial measurements and septation. No sexual state known.

Family type: *Falcocladium* S.F. Silveira, Alfenas, Crous & M.J. Wingf., Mycotaxon 50: 447 (1994) (Four species).

Distribution: Brazil, Thailand.

Notes: *Falcocladium* was erected by Crous *et al*. (1994) with *F. multivesiculatum* as the type species. Subsequently, three other species were described: *F. sphaeropedunculatum* (Crous *et al.*, 1997), *F. thailandicum* (Crous *et al*., 2007) and *F. turbinatum* (Somrithipol *et al*., 2007). This genus occurs on a wide range of substrata including *Eucalyptus grandis*, *E. camaldulensis* leaves, and leaf litter collected from tropical forests. Further taxon sampling is required to determine its ordinal status within the Hypocreomycetidae and establish the sexual stage.

Torpedosporaceae E.B.G. Jones & K.L. Pang, **fam. nov.**

MycoBank MB 808180

Saprobic on lignicolous substrates and leaves. *Ascomata* perithecioid, immersed or superficial, subglobose, ostiolate, papillate, subcarbonaceous to coriaceous, brown. ***Paraphyses*** narrow, irregular, persistent or early deliquescing. ***Asci*** 8-spores, unitunicate, thin-walled, clavate to ellipsoidal, lacking an apical apparatus, short pedicellate. ***Ascospores*** cylindrical to ellipsoidal, 3-septate, hyaline, with several radiating appendages at one or both ends. No known asexual stage.

Habitat: intertidal wood, mangrove wood and roots, bark and leaves, marine.

Family type: *Torpedospora* Meyers

Distribution: widely distributed in temperate and tropical locations, Bahamas, France. Denmark, India, Italy, Ivory coast, Japan, Liberia, Libya, Malaysia, Mexico, New Zealand, Norway, Samoa, Seychelles, Sierra Leone, UK, USA. The distribution of *T. ambispinosa* is less well known.

Notes: Jones (1995) queried the assignment of *Torpedospora ambispinosa* to *Torpedospora* as they differ morphologically in ascomatal features and in the ontogeny of ascospore appendages. In *T. radiata,* the ascospores possess 3-5 radiating subterminal appendages at one pole, that appear to be fibrillar (Jones & Moss 1978), whereas *T. ambispinosa* has subterminal appendages at both poles, that are rigid, straight or curved. Ascospores of *T. ambispinosa* in mass are bright orange, but are hyaline in *T. radiata*. At the molecular level they group in the same clade with good branch support, which raises the question as to whether they are congeneric (Sakayaroj *et al*., 2005; Schoch *et al*., 2007). Schoch *et al*. (2007) suggested that the monophyly of the genus should be further tested with a more complete data set. The marine hyphomycete *Glomerulispora mangrovis*, with irregularly helicoid conidia forming muriform-like spores, groups with *T. radiata* with variable support (Abdel-Wahab *et al*., 2010; Fig. 1 this paper).

Included genera: *Glomerulispora* Abdel-Wahab & Nagah., Mycol. Prog. 9: 552 (2010). (2 species) – MycoBank MB 543125; *Torpedospora* Meyers, Mycologia 49: 496 (1957). (2 species) – MycoBank MB 5501.

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