

A taxonomic revision and phylogenetic reconstruction of the *Jahnulales* (*Dothideomycetes*), and the new family *Manglicolaceae*

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Abstract Genera assigned to the *Jahnulales* are morphologically diverse, especially in ascospores equipped with or without appendages, sheaths or apical caps. They are predominantly freshwater fungi occurring on woody substrata, with *Manglicola guatemalensis*, *Xylomyces chlamydosporus* and *X. rhizophorae* the only species known from marine habitats. The order *Jahnulales* with 4 teleomorphic genera: *Jahnula* (15 species), *Aliquandostipite* (5), *Megalohypha* (1), *Manglicola* (2) and the anamorphic genera *Brachiosphaera* (2), *Speiropsis* (9), *Xylomyces* (8), amounting to a total of 42 species, is reviewed and nomenclatural changes are proposed. Twenty species are treated at the molecular level, with 94 sequences, 13 of which are newly generated for this review. Three species

are rejected (*Speiropsis irregularis*, *Xylomyces aquaticus*, *X. elegans*) while the phylogenetic placement of 6 *Xylomyces*, 7 *Speiropsis*, 1 *Brachiosphaera* and 1 *Manglicola* require molecular data to confirm their placement in the order. Sequences are derived from ex-holotype isolates and new collections made in Thailand. Most taxa are included in the family *Aliquandostipitaceae* and a new family *Manglicolaceae* is erected for the marine ascomycete *Manglicola guatemalensis* with its large ascomata (1,100–1,750×290–640 µm), wide ostioles and ascospores that are fusiform, unequally one-septate with the apical cell larger than the turbinate basal cell and bear apical gelatinous appendages. The genus *Jahnula* is polyphyletic grouping in three clades with *J. aquatica*, *J. granulosa*, *J. rostrata*, *J. potamophila* and *Megalohypha aqua-dulces* in the *Jahnula sensu stricto* clade. No taxonomical changes are proposed for *Jahnula* species not grouping in the *Jahnula sensu stricto* clade, until further species are isolated and sequenced.

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Introduction

The order *Jahnulales* was proposed by Pang et al. (2002) to accommodate ascomycetes with stalked/sessile and dimorphic ascomata, hyphal stalk cells that are *circa* 40 µm wide, and ascospores that are unequally 2-celled with or without various types of appendages or sheaths. Pang et al. (2002) presented molecular data for six species: *Aliquandostipite sunyatsenii* (later transferred to *Jahnula*), *A. khaoyaiensis*, *Jahnula bipolaris*, *J. australiensis*, *J. siamensiae* (a new species), and *Patescospora separans* (a new genus and species) and referred them to the family *Aliquandostipita-*

ceae. All taxa assigned to the order were freshwater species, mostly growing on submerged wood, and lacking anamorphs.

The order was based on the genus *Jahnula* with *J. aquatica* as the type species (Kirschstein 1936), however, Wegelin (1894) described *Amphisphaeria helevetica* which may be an earlier epithet and this is discussed later in the paper. Hawksworth (1984) did not refer to this earlier epithet but commented on the diagnostic structure of the ascomatal wall with the “massive pseudoparenchymatous cells up to 30 µm wide”. The concept of the genus has changed with the description of new species to include emphasis on the ascomatal wall structure, stalk, ascus morphology and the variety of caps, sheaths and appendages to the ascospores, as well as the wide hyphae now known to occur in most of the genera in the order. Campbell et al. (2007) emended the ordinal description to include wide, brown hyphae and a wider variation in ascospore characters, not known when the *Jahnulales* was proposed.

Subsequently, other genera and species were referred to the order: *Jahnula* (Hyde 1993; Pinruan et al. 2002; Raja and Shearer 2006; Raja et al. 2008; Sivichai and Boonyuen 2010), *Aliquandostipite* (Raja et al. 2005), and *Megalohypha* all known from freshwater habitats. Initially, no anamorphs were known until Campbell et al. (2007) and Prihatini et al. (2008) showed that the anamorphic fungi *Speiropsis pedatospora* and *Xylomyces chlamydo-sporus*, had a phylogenetic affinity with the *Jahnulales*. Subsequently, Sivichai et al. (2011) showed that *X. chlamydosporus* was the anamorph of *Jahnula aquatica*. No marine members of the *Jahnulales* were known until Suetrong et al. (2009, 2010) demonstrated that the marine ascomycete *Manglicola* (*M. guatemalensis*) also belonged in the order.

Molecular studies suggest that the genus *Jahnula* is polyphyletic (Campbell et al. 2007; Shearer et al. 2009; Suetrong et al. 2009) with the type species *J. aquatica* grouping with *J. granulosa*, *J. rostrata*, *J. potamophila* and *Megalohypha aqua-dulces*. Other *Jahnula* species are distally placed and may constitute more than one genus, with Shearer et al. (2009) suggesting 4 or 5 separate lineages and advocating further molecular studies to resolve their phylogenetic relationship. Currently, one family has been described, *Aliquandostipitaceae* (Inderbitzin et al. 2001) based on the genus *Aliquandostipite* (type species *A. khaoyaiensis*), to which most other genera have been referred. However, the taxonomic position of the genus *Manglicola* warrants further study as to its familial position (Suetrong et al. 2010).

The objective of this contribution is to determine the familial status of the genera assigned to the order *Jahnulales*.

Materials and methods

Sample sources

Collection, incubation and examination of freshwater and marine members of this group are well established (Vrijmoed 2000). To obtain single spore cultures ascospores were removed from ascomata with sterile forceps and placed in sterile sea water. Small drops of this spore suspension were placed on GYA (10 g glucose, 1 g yeast extract, 18 g agar, 0.5 g chloromphenicol in 1 L sea water) Petri dishes and incubated at 25°C in the dark. Germinated spores were transferred to new GYA/PDA Petri dishes and incubated at 25°C in the dark. Sporulating material was mounted in sea water for all measurements and photography. Voucher slides and type material of the new fungi have been deposited at BIOTEC Bangkok Herbarium (BBH), with cultures deposited in international culture collections and sequences in GenBank (Shearer et al. 2009; Suetrong et al. 2009, 2010). Species used in this study, their isolate numbers, sources and GenBank accession numbers are listed in Supplementary Table 1 for members of the *Jahnulales*. All isolates for the generation of new sequences were obtained from BCC, all new sequences are in bold and ex-holotype sequences carry the prefix *. Supplementary Table 2 lists representative sequences from selected orders of the Dothideomycetes with *Roccella fuciformis* and *Schismatomma decolorans* as the outgroup taxa.

DNA extraction, amplification and sequencing

Fungi were grown in potato dextrose broth with seawater, and or, freshwater at a temperature of 25°C for 14 days or until enough mycelium for DNA extraction 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 µl 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 microtube 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 14,000 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 rDNA and LSU rDNA, 5.8S rDNA and

the translation elongation factor 1-alpha (TEF-1-alpha), using the primers NS1, NS3, NS4 and NS6 for SSU rDNA (White et al. 1990), JS1, JS8, LR7 and LROR for LSU rDNA (Bunyard et al. 1994; Landvik 1996), ITS1 and ITS4 for 5.8S rDNA (White et al. 1990), and 983F, 2218R, CEFR2, CEFF2, 1577F and 1567R (Rehner 2001). DNA sequencing was performed using the primers mentioned above in an Applied Biosystem 3730XL DNA Analyzer at Macrogen, Inc in Korea. Each sequence was checked for ambiguous bases and assembled using BioEdit 6.0.7 (Hall 2004). Sequence homologies were also analysed using the BLAST search engine at the National Center 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 optimised along with other sequences obtained from the GenBank nucleotide database. The consensus sequences for each DNA region were initially aligned with ClustalW v. 1.6 (Thompson et al. 1994) and improved in 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). The tree construction procedure was performed in PAUP* 4.0b10 (Swofford 2002) on Window versions and a Power Macintosh G4 (Apple Computer, Inc., Cupertino, 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.

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 and 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 and 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 and Ronquist 2001) using a uniform [GTR+I+G] model, Iset nst=6 rates=invgamma; prset statfreqpr=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. 7.2.2 (Stamatakis 2006). The dataset was partitioned according to each gene and separated codons (two 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 1,000 pseudoreplicates 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 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.

Taxonomy

The order includes 42 species in seven genera (*Aliquandostipite*, *Brachiosphaera*, *Jahnula*, *Manglicola*, *Megalohypha*, *Speiropsis*, *Xylomyces*) and 20 species are treated in the molecular study with 94 sequences, 13 of which are newly generated for this review. Sequences are derived from ex-holotype isolates and new collections made in Thailand. Most species group in the *Aliquandostipitaceae*, while a new family is proposed for *Manglicola guatemalensis*, based on morphological and molecular evidence. Excluded taxa from the order are: *Speiropsis irregularis* (*insertae sedis*), *Xylomyces aquatica* and *X. elegans* (*Pleosporales*). Placement of *M. guatemalensis* in the *Hypsostromataceae* is rejected. Molecular data are required to confirm the position of 5 *Xylomyces*, 7 *Speiropsis*, 1 *Manglicola* and 1 *Brachiosphaera* species in

the order. Although many *Jahnula* species do not group in the *Jahnula sensu stricto* group, no taxonomical changes are proposed at this stage. This is considered premature until other *Jahnula* species are isolated and sequenced, while others await description (Abdel-Wahab, pers. com.).

Jahnulales K.L. Pang, Abdel-Wahab, El-Sharouney, E. B.G. Jones & Sivichai, Mycol. Res. 106: 1033 (2002).

emend Campbell et al. (2007)

Ascomata globose to subglobose, with a long, wide, brown, septate stalk or sessile, attached to substratum by wide, brown hyphae, immersed or superficial, ostiolate, papillate, coriaceous to sub-carbonaceous, hyaline, pale brown or black. *Peridium* thick, comprising a few layers of relatively large cells. *Hamathecium* pseudoparaphyses, hypha-like, filamentous, septate, unbranched between the asci, branching and anastomosing above the asci, persistent. *Asci* ovoid, cymbiform, saccate, clavate or cylindrical, thick-walled, fissitunicate, persistent or deliquescing, with an apical apparatus. *Ascospores* ellipsoid-fusiform, 1-septate, becoming 3–4 septate, apical cell slightly larger, hyaline or brown, slightly constricted at the septum, with or without a mucilaginous sheath, gelatinous pads, apical cellular appendages or elongate gelatinous apical appendages. Hyphae in culture wide, brown, septate, not constricted to strongly constricted at the septa. Anamorphs in the genera *Brachiosphaera*, *Speiropsis* and *Xylomyces*.

Order in the *Pleosporomycetidae*, *Dothideomycetes*.

Type species: *Jahnula aquatica* (Plöttner & Kirschst.) Kirschst., Annla. Mycol. 34: 196 (1936).

Family: *Aliquandostipitaceae* Inderb., Am. J. Bot. 88: 54 (2001).

Genera: *Aliquandostipite* (5 species), *Jahnula* (15), *Manglicola* (2), *Megalohypha* (1) (teleomorphs), *Brachiosphaera* (2), *Speiropsis* (9), *Xylomyces* (8) (anamorphs).

Aliquandostipitaceae Inderb.

Am. J. Bot. 88: 54 (2001).

Ascomata immersed-erumpent or superficial. *Hamathecium* comprising pseudoparaphyses. *Asci* fissitunicate. *Mycelium* visible on the substratum, comprising up to 50 μm , wide hyphae, which may bear ascomata. *Ascospores* 1-septate, pale brown, guttulate.

Holotype species: *A. khaoyaiensis* Inderb.

Jahnula Kirschst. Ann. Mycol. 34: 196 (1936).

= *Ascagilis* K.D. Hyde Aust. Syst. Bot. 5: 109 (1992).

Ascomata immersed, semi-immersed or erumpent, globose to subglobose, black, coriaceous, ostiolate, short-papillate, easily detached from substratum, solitary or gregarious. *Peridium* comprising 3–5 layers of relatively large, thin-walled, brown or hyaline angular cells. *Hamathecium* pseudoparaphyses wide (2–4 μm), hypha-like, filamentous, hyaline, septate, unbranched between the asci, branching and

anastomosing above the asci. *Asci* 8-spored, cylindrical or obclavate, pedicellate, thick-walled, fissitunicate, with an ocular chamber and faint ring. *Ascospores* uniseriate or 3-seriate, 1-septate, pale to dark brown, guttulate, ellipsoid-fusiform, smooth or verruculose, constricted at the septum, some with a mucilaginous appendage, pads or sheaths.

Type species: *J. aquatica* (Plöttner & Kirschst.) Kirschst.

Jahnula aquatica (Plöttner & Kirschst.) Kirschst., Ann. Mycol. 34: 196 (1936).

= *Amphisphaeria aquatica* Plöttner & Kirschst., Verh. Bot. ver. Prov. Brandenb. 48: 52 (1906).

= *Melanopsamma aquatica* (Plöttner & Kirschst.) Kirschst., Krypt.-Fl. Brandenburg 7: 226 (1911).

Ascomata arising singly or in small groups, superficial, attached to the substratum by subiculum-like hyphae, subglobose to broadly obpyriform, the base often slightly immersed, mainly 250–400(–500) μm diam, black. *Ostiole* papillate, scarcely projecting above the substrate. *Peridium* not membranous, unchanged in potassium hydroxide, variable in thickness, mainly 30–80 μm thick, often thickest in the upper parts, composed of several layers of reddish brown subglobose to polyhedral pseudoparenchymatous cells (*textura angularis*), variable in size, the outermost 20–30 μm diam with slightly verruculose walls, the inner compressed, paler and mainly 15–20 μm long and 4–8 μm wide; base of the peridium continuous, similar to the sides. *Periphyses* absent. *Hamathecium* consisting of trabeculate pseudoparaphyses, persistent, filiform, branched and anastomosing, rarely septate, 1.5–2.5 μm thick, centrum not reacting with iodine. *Asci* arising from the base of the ascomatal cavity, cylindrical, stalked, thick-walled, fissitunicate, with an internal apical cone when young, when mature with a short apical cylinder or broad rectangular indentation, possibly with some annular apical apparatus, IKI, 180–220 \times 15–18 μm , *Ascospores* with 8 uniseriate, slightly overlapping ascospores, ellipsoid to very broadly fusiform, slightly tapering towards the apices, 1-septate, slightly constricted or scarcely constricted at the septum, reddish brown, moderately thick-walled, generally with numerous small guttules, smooth-walled, without a distinct gelatinous sheath when mature, (30–)32–38(–41) \times 11–16 μm . Based on a description by Kirschstein (1936).

Note: Dr. Walter Jaklitsch has drawn our attention to a possible earlier name for *Jahnula*: *Amphisphaeria helvetica* which was collected on pine wood, Bischofszell and Heimiszwyl, Switzerland (Wegelin 1894). This provided a short description of the species with cylindrical asci and ascospore dimensions of 33–40 \times 12.5–15 μm which overlap with those of *J. aquatica*. In *A. helvetica* the apical cell is slightly larger than the basal one. Wegelin (1894) also described *Amphisphaeria dolioloides* with ascospores measuring 34–39 \times 14–15 μm but with clavate asci. Scheinpflug

(1958) referred *A. helvetica* as the basionym of *Othia helvetica*, a species in the *Botryosphaeriaceae*, which is distantly related to the *Jahnulales*. Since no type material of *A. helvetica* was available, the relationship between it and *J. aquatica* cannot be resolved. As currently circumscribed, we take *J. aquatica* as the type of the genus and order, as followed by Hawksworth (1984) and Hyde and Wong (1999).

Anamorph: *Xylomyces chlamydosporus* Goos, R.D. Brooks & Lamore, *Mycologia* 69: 282 (1977).

Cultures: Colonies on CMA slow growing, dark brown to black, effuse, hyphae thick-walled, septate and constricted at the septa, chlamydo-spores developing in culture, fusiform, intercalary, straight or curved, solitary or in chains, occasionally branched, with thickened septa, constricted at the septa, dark brown to blackish, end cells paler.

Sequence data: See Supplementary Table 1.

Material examined: Thailand, Trat province, Mu Ko Chang National Park, Khlong Phlu Waterfall, on submerged wood, 3 April 2006, S. Sivichai & V. Sriindrasutdhi, BIOTEC SS3895.

Habitat and host range: Submerged wood in freshwater streams.

Geographical distribution: Germany, Hong Kong, South Africa, Thailand, USA.

Phylogenetic study: Molecular data suggest that the genus is polyphyletic with *Jahnula sensu stricto* including the species *J. aquatica* (type species), *J. granulosa*, *J. potamophila*, *J. rostrata* and *Megalohypha aqua-dulces* in a clade with low bootstrap support (Campbell et al. 2007). Other *Jahnula* species group in a separate clade and may constitute other genera (Campbell et al. 2007; Suetrong et al. 2010).

Concluding remarks: Sivichai et al. (2011) demonstrated by culture techniques that the anamorph of *J. aquatica* was *Xylomyces chlamydosporus*, and frequently reported it from freshwater substrates. However, the molecular data do not support this connection (Campbell et al. 2007; current results) and reexamination of the different isolates used in the analysis is required.

Accepted species:

J. apiospora A. Carter, Raja & Shearer, *Mycoscience* 49: 326 (2008).

**J. appendiculata* Pinruan, K.D Hyde & E.B.G. Jones, *Sydowia* 54: 243 (2002).

**J. aquatica* (Plöttner & Kirschst.) Kirschst., *Ann. Mycol.* 34: 196 (1936).

**J. australiensis* K.D. Hyde, *Aust. Syst. Bot.* 6: 161 (1993).

**J. bipileata* Raja & Shearer, *Mycologia* 98: 321 (2006).

**J. bipolaris* (K.D. Hyde) K.D. Hyde, *Nova Hedw.* 68: 494 (1999).

**J. granulosa* K.D. Hyde & S.W. Wong, *Nova Hedw.* 68: 497 (1999).

J. morakotii Sivichai & Boonyuen, *Mycotaxon* 112: 476 (2010).

**J. potamophila* K.D. Hyde & S.W. Wong, *Nova Hedw.* 68: 499 (1999).

J. poonythi K.D. Hyde & S.W. Wong, *Nova Hedw.* 68: 499 (1999).

**J. rostrata* Raja & Shearer, *Mycologia* 98: 325 (2006).

**J. sangamonensis* Shearer & Raja, *Mycologia* 98: 327 (2006).

**J. seychellensis* K.D. Hyde & S.W. Wong, *Nova Hedw.* 68: 504 (1999).

**J. sunyatsenii* (Inderb.) K.L. Pang, E.B.G. Jones & Sivichai, *Amer. J. Bot.* 88: 57 (2001).

J. systyla K.D. Hyde & S.W. Wong, *Nova Hedw.* 68: 499 (1999).

15 species, * sequenced species.

Jahnula apiospora A. Carter, Raja & Shearer, *Mycoscience* 49: 326 (2008).

Ascomata scattered, superficial to partially immersed in wood, attached to the wood by broad, brown, superficial, stoloniferous hyphae (growing on the wood surface and linking up between the ascomata), membranous, globose to subglobose, 250–305×300–360 μm, black, ostiolate; ostiole circular, depressed. *Peridium* 40–45 μm wide, composed of *textura angularis* in surface view; in longitudinal section 6–10 cell layers wide, composed of an outer layer of thick-walled cells 30–33×15–19 μm, occluded by black, amorphous material along the upper two-thirds of the ascomata; inner layer of moderately thick-walled, large, brown, isodiametric to angular cells, 12–26×5–10 μm. *Hamathecium* consisting of hyaline, trabeculate, pseudoparaphyses, narrow, branched, and anastomosing above the asci, embedded in a gelatinous matrix. *Asci* 108–140×14–22 μm, basal, cylindrical to narrowly fusoid, pedicellate, fissitunicate, endoascus 18 μm wide, extending to 160–190 μm in length, with eight overlapping uniseriate to biseriate ascospores. *Ascospores* 30–40×8–12 μm, 7–9 μm wide at the septum, fusiform, slightly constricted at the septum, apiculate, unequally 1-septate; septum submedian (0.44–0.64), apical cell 18–27 μm long, basal cell shorter than apical cell, 11–16 μm long, slightly curved, hyaline when young, brown at maturity, multi-guttulate, smooth-walled, lacking a gelatinous sheath and appendages. Based on the description by Raja et al. (2008).

Anamorph: None known.

Culture: DAOM 239555 (ex-holotype).

Sequence data: None.

Habitat and host range: Decorticated wood.

Geographical distribution: Canada.

Notes: Raja et al. (2008) considered it similar to *J. aquatica*, but differs in the shape of the ascomata, and narrower ascospores in *J. apiospora*.

Jahnula appendiculata Pinruan, K.D. Hyde & E.B.G. Jones, Sydowia 54: 243 (2002).

Ascomata 305–325 µm diam, semi-immersed, becoming erumpent, globose to subglobose, hyaline to pale straw-coloured, membranous, ostiolate, short papillate, solitary, covered by short hyaline setae up to 80 µm long, with a hyaline stalk-like strand attached to the base. *Peridium* ca. 36 µm wide, comprising 4–6 rows of large angular cells with hyaline cell walls. *Pseudoparaphyses* hypha-like, septate, unbranched between asci, branching and anastomosing above. *Asci* 360–410×41–43 µm, 8-spored, cylindrical to cylindrical-clavate, fissitunicate with shallow ocular chamber and faint ring. *Ascospores* 47–55×23–27 µm, ellipsoid-fusiform, brown, 1-septate, slightly constricted at the septum, wall ornamentation minutely verrucose, surrounded by a mucilaginous sheaths and cellular appendages apically. Based on a description by Pinruan et al. (2002) and further studies.

Anamorph: None known.

Cultures: BCC11400 (ex-holotype), BCC11445, SS2448, SS2900.

Sequence data: See Supplementary Table 1.

Habitat and host range: Submerged trunk of the palm *Licuala longecalycata*.

Geographical distribution: Thailand.

Notes: This fungus differs from all other *Jahnula* species in having ascospores with a sheath and bipolar appendages. The only other species having appendages is *J. morakotii*, which has cap-like appendages and not as long as in *J. appendiculata*.

Jahnula australiensis K.D. Hyde, Aust. Syst. Bot. 6: 161 (1993).

Ascomata 180–250 µm high, 120–180 µm diam, immersed, erumpent, shedding wood particles and becoming superficial with the base remaining immersed, subglobose or obpyriform, brown above, subhyaline below, coriaceous, ostiolate, short-papillate, solitary or gregarious, associated with algae. *Peridium* comprising a few layers of relatively large, thin-walled, brown angular cells. *Hamathecium* consisting of hypha-like pseudoparaphyses up to 3.5 µm wide, filamentous, hyaline, septate, unbranched between the asci, branching and anastomosing above. *Asci* 90–140×14–18 µm, 8-spored, obclavate, pedicellate, fissitunicate, with an ocular chamber and faint ring. *Ascospores* 19–30×6–8 µm, 2-3-seriate, 1-septate, light brown, guttulate, irregularly fusiform, apical cells wider and tapering to a rounded tip, base narrower and more rounded, minutely verruculose, constricted at the septa. Based on a description by Hyde (1993) and further studies in Thailand.

Anamorph: None known.

Culture: BCC12789.

Sequence data: See Supplementary Table 1.

Habitat and host range: On submerged wood.

Geographical distribution: Australia, Thailand.

Material examined: Thailand, Khao Soi Dao Wildlife Sanctuary, 26 March 2000, S. Sivichai & N. Boonyuen, SS665.

Notes: It differs from *J. aquatica* in possessing smaller ascomata, asci and ascospores.

Jahnula bipileata Raja & Shearer, Mycologia 98: 321 (2006).

Ascomata on wood 395–400×200–205 µm, black translucent, membranous, subglobose to obpyriform, ostiolate, superficial with partially immersed base, upright to slightly horizontal, scattered, and connected to the substratum by broad, brown, septate, superficial stoloniferous hyphae. *Necks* 100–150×70–85 µm, pale brown, central; wall of the neck composed of short outwardly diverging globose to cylindrical cells. *Peridium* 20–30 µm thick, in longitudinal section composed of 3–4 cell layers; inner layer of laterally compressed, hyaline to subhyaline, isodiametric cells, outer layer of large, darkened, moderately thick-walled, globose to angular cells 30–34×20–24 µm. *Hamathecium* consisting of hyaline, septate pseudoparaphyses 2–3 µm wide, slightly constricted at the septa, filamentous, branching and anastomosing above the asci. *Asci* 170–220×10–17 µm, basal, fissitunicate, cylindrical, pedicellate, weakly developed apical chamber; with eight, overlapping uniseriate ascospores. *Ascospores* 25–30×9–10 µm, broadly ellipsoid to fusiform, dark brown, 1-septate, slightly constricted at the septum, upper cell slightly broader and more apiculate than the lower cell, rough-walled in an irregularly striate pattern, with hyaline caps at both apices up to 2×2–3 µm, lacking a sheath. Based on the description by Raja and Shearer (2006).

Anamorph: None known.

Cultures: F49-1 (ex-holotype), AF220-1.

Sequence data: See Supplementary Table 1.

Habitat and host range: Decorticated wood.

Geographical distribution: Florida, USA.

Notes: *Jahnula bipileata* is similar to *J. aquatica*, but differs in having ascomata with long cylindrical necks, and irregularly striated, rough-walled ascospores with hyaline caps.

Jahnula bipolaris (K.D. Hyde) K.D. Hyde, Nova Hedw. 68: 494 (1999).

≡ *Ascagilis bipolaris* K.D. Hyde, Aust. Syst. Bot. 5: 111 (1992).

Ascomata 260–325 µm diam, immersed, erumpent, shedding wood particles and becoming superficial with base remaining immersed, globose to subglobose, black, coriaceous, ostiolate, short-papillate, solitary or gregarious, some with algae associations. *Peridium* up to 34 µm wide, comprising 3–5 layers of relatively large thin-walled, brown angular cells. *Hamathecium* consisting hyaline, hypha-like pseudoparaphyses to 4 µm wide, filamentous, septate,

unbranched between the asci, branching and anastomosing above. *Asci* 140–200×35–40 µm, 8-spored, obclavate, pedicellate, fissitunicate, with an ocular chamber and faint ring. *Ascospores* 42–52×16–23 µm, 2–3 seriate, 1-septate, light brown, guttulate, ellipsoid-fusiform, minutely verruculose, constricted at the septum, with mucilaginous pads at each end. Based on the description by Hyde (1999) and further studies in Thailand.

Anamorph: None known.

Cultures: A421, SS44.

Sequence data: See Supplementary Table 1.

Habitat and host range: Decorticated wood.

Geographical distribution: Australia, Costa Rica, Hong Kong, Malaysia, Thailand.

Notes: *Jahnula bipolaris* was originally described as *Ascogilis bipolaris* from Australia and appears to be widely distributed in the tropics.

Jahnula granulosa K.D. Hyde & S.W. Wong, Nova Hedw. 68: 497 (1999).

Ascomata 210–280 µm diam, semi-immersed or erumpent, shedding wood particles and becoming superficial with base remaining immersed, globose to subglobose, brown to dark brown, membranous, ostiolate, short-papillate, with stalk-like strands attached to the base, with a few sparse hair-like projections, solitary. *Peridium* comprising a few layers of relatively large, thin-walled, light brown angular cells. *Hamathecium* consisting of hyaline, hypha-like pseudoparaphyses to 3 µm wide, filamentous, septate, unbranched between the asci, branching and anastomosing above. *Asci* 220–270×26–40 µm, 8-spored, obclavate, pedicellate, fissitunicate, with an ocular chamber and faint ring. *Ascospores* 26–38×15–18 µm, 2-seriate at the base of ascus, overlapping uniseriate near apex, 1-septate, dark brown, guttulate, ellipsoid-fusiform, apical cells wider and tapering to an acute apex, possibly with a germ pore, base less tapered and rounded, constricted at the septum, wall granular, surrounded by a thin mucilaginous sheath. Based on the description by Hyde and Wong (1999) and further research in Thailand.

Anamorph: None known.

Culture: SS1567.

Sequence data: See Supplementary Table 1.

Habitat and host range: On submerged wood.

Geographical distribution: South Africa.

Notes: A distinct species with dark brown ascospores, lacking appendages and a granular ornamentation of the spore wall.

Jahnula morakotii Sivichai & Boonyuen, Mycotaxon 112: 476 (2010).

Ascomata 100–180 µm diam, globose to subglobose, superficial with a septate stalk, 18–30 µm wide, or sessile. *Peridium* of large, thin-walled cells. *Hamathecium* consisting

of hyaline, septate pseudoparaphyses, hyaline, 1.5–2 µm wide, up to 150 µm in length. *Asci* 107.5–120×9–11.5 µm, 8-spored, cylindrical, pedicellate, fissitunicate, with a shallow ocular chamber and faint ring. *Ascospores* 17.5–20×5–6.5 µm, fusiform, brown, 1-septate, uniseriate or biseriate, multi-guttulate, slightly constricted at the septa, straight to curved with cellular bipolar hyaline apical appendages.

Anamorph: None known.

Culture: None.

Sequence data: None available.

Habitat and host range: Submerged wood test block of *Azadirachta indica* in freshwater stream.

Geographical distribution: Thailand.

Material examined: Thailand, Narathiwat province, Sirindhorn Peat Swamp Forest, on submerged wooden test block of *Azadirachta indica*, 10 March 2003, S. Sivichai & N. Boonyuen, BIOTEC SS2447.

Notes: *Jahnula morakotii* differs from all *Jahnula* species in having the smallest ascospores (17.5–20×5–6.5 µm) with bipolar cellular appendages and lacking a sheath. Species close in ascospore size to *J. morakotii* are *J. bipileata* (25–30×9–10 µm) and *J. australiensis* (19–30×6–8 µm); however, they lack bipolar appendages. *Jahnula appendiculata* is the only other species with bipolar appendages, but the ascospores of this species are longer and wider (45–52.5×22.5–27.5 µm) than those of *J. morakotii* (17.5–20×5–6.5 µm). In addition, ascospores of *J. appendiculata* have a thick sheath that is absent in *J. morakotii*.

During a long-term colonization study of freshwater fungi on wood submerged in the Sirindhorn peat swamp forest Narathiwat, in Southern Thailand, *J. morakotii* (Sivichai and Boonyuen 2010) was collected only once on a test block of *Azadirachta indica* and it can be considered a rare fungus and may be restricted to this unique habitat.

Phylogenetic study: None.

Jahnula poonythii K.D. Hyde & S.W. Wong, Nova Hedw. 68: 499 (1999).

Ascomata 350–420 µm diam, semi-immersed or erumpent, shedding wood particles and becoming superficial with the base remaining immersed, globose to subglobose, brown to dark brown, membranous, ostiolate, short-papillate, with stalk-like strands attached to the base, with sparse hair-like projections, solitary. *Peridium* comprising a few layers of relatively large thin-walled, light brown angular cells. *Hamathecium* consisting of hyaline, hypha-like pseudoparaphyses to 3 mm wide, filamentous, septate, unbranched between the asci, branching and anastomosing above. *Asci* 120–175×16–20 µm, 8-spored, cylindrical, short pedicellate, fissitunicate, with an ocular chamber and faint ring. *Ascospores* 23–28×11–13 µm, mostly uniseriate or overlapping uniseriate, 2-celled, dark brown, guttulate, ellipsoid-fusiform, both cells of approximately equal size,

tapering to rounded apices, strongly constricted at the septum, wall verruculose. Based on the description by Hyde and Wong (1999).

Anamorph: None known.

Cultures: None.

Sequence data: None.

Habitat and host range: Submerged wood in river.

Geographical distribution: Mauritius.

Jahnula potamophila K.D. Hyde & S.W. Wong, Nova Hedw. 68: 499 (1999).

Ascomata up to 450 μm high, 325–390 μm diam, erumpent, shedding wood particles and becoming superficial with base remaining immersed, subglobose, obpyriform or almost conical, hyaline and then metallic grey, coriaceous, ostiolate, short-papillate, solitary or gregarious, with algal associations. *Peridium* up to 34 μm wide, comprising 3–6 layers of relatively large thin-walled, hyaline angular cells and covered with sparse hyaline hairs. *Hamathecium* consisting of hyaline, hypha-like pseudoparaphyses to 3.5 μm wide, filamentous, septate, unbranched between the asci, branching and anastomosing above. *Asci* 200–240 \times 26–40 μm , 8-spored, obclavate, pedicellate, fissitunicate, with an ocular chamber and faint ring. *Ascospores* 46–54 \times 17–20 μm , 2-3-seriate near the base, overlapping uniseriate near the apex, 1-septate, light brown, guttulate, ellipsoid-fusiform, constricted at the septum, surrounded by a mucilaginous sheath, wavy in outline. Based on the description by Hyde and Wong (1999).

Anamorph: None known.

Culture: F111-1.

Sequence data: See Supplementary Table 1.

Habitat and host range: Submerged wood in freshwater.

Geographical distribution: Australia.

Notes: This fungus is similar to *J. bipolaris* in ascospore dimension but differs in having minute guttules, and surrounded by a narrow mucilaginous sheath.

Jahnula rostrata Raja & Shearer, Mycologia 98: 325 (2006).

Ascomata 207–264 \times 147–150 μm , hyaline and translucent when young becoming translucent brown to black at maturity, globose to subglobose, solitary, scattered, superficial on wood, connected by stoloniferous hyphae ca. 15–19 μm wide; ostiolate, papillate. *Necks* 38–40 \times 30–32 μm , composed of subglobose cells diverging from the ostiole; paraphyses not observed. *Peridium* 36–38 μm wide, in longitudinal section 3–4 cell layers wide, innermost layer of hyaline cells compressed laterally, outermost layer of large, moderately thick-walled, isodiametric cells. *Hamathecium* consisting of hyaline, septate pseudoparaphyses 3–4 μm wide, anastomosing above the asci. *Asci* 152–190 \times 32–40 μm , basal, fissitunicate, clavate, short pedicellate, with an apical chamber and eight, overlapping biseriate ascospores. *Ascospores* 32–45 \times 12–15 μm , ellipsoid or broadly

fusiform, dark brown, 1-septate, slightly constricted at the septum, upper cell of ascospores broader than lower cell, rough-walled in an irregularly striate pattern, multiguttulate, with or without a thin gelatinous sheath. Based on the description by Raja and Shearer (2006).

Anamorph: None known.

Culture: F4-3.

Sequence data: See Supplementary Table 1.

Habitat and host range: Decorticated wood.

Geographical distribution: Florida, USA.

Notes: This fungus is most similar to *J. granulosa* with its roughened ascospore cell wall (Raja and Shearer 2006).

Jahnula sangamonensis Shearer & Raja, Mycologia 98: 327 (2006).

Ascomata 468 \times 345 μm , globose to obpyriform, reddish brown to black, partially immersed in wood, ostiolate, papillate with subtending brown, septate hypha ca. 160 \times 8 μm that attach the base of the ascomata to the wood. *Necks* 116–120 \times 60–65 μm , central, with reddish brown paraphyses; wall of the neck composed of chains of elongated cells diverging from the ostiolar canal. *Peridium* 40–44 mm thick, *textura angularis* in surface view, in longitudinal section, peridial wall 4–6 cell layers wide; inner layer composed of thin-walled, narrow, flattened, elongated, subhyaline cells 11–16 \times 4–5 μm , outer layer of large, moderately thick-walled isodiametric, brown cells 14–34 \times 15–20 μm . *Hamathecium* consisting of hyaline, septate pseudoparaphyses 2–3 μm wide, anastomosing above the asci. *Asci* 164–200 \times 15–20 mm, fissitunicate, endoascus extending in water to ca. 270–500 μm in length, cylindrical, with an apical chamber, pedicellate with eight, overlapping uniseriate ascospores. *Ascospores* 20–28 \times 10–12 μm , broadly ellipsoid, 1-septate, constricted at the septum, dark brown, spore wall smooth or verruculose, multiguttulate, without a sheath. Based on the description by Raja and Shearer (2006).

Anamorph: None known.

Cultures: A402-1B (ex-holotype), A482-1B, F81-1.

Sequence data: See Supplementary Table 1.

Habitat and host range: Submerged decorticated wood.

Geographical distribution: USA.

Jahnula seychellensis K.D. Hyde & S.W. Wong, Nova Hedw. 68: 504 (1999). Figure 3 a-m.

Ascomata 160–300 μm diam, semi-immersed or erumpent with a stalk, subglobose, brown to dark brown, coriaceous, ostiolate, short-papillate, solitary or gregarious, with algal associations. *Peridium* comprising a few layers of relatively large thin-walled, brown angular cells. *Hamathecium* pseudoparaphyses to 4 μm wide, hypha-like, filamentous, hyaline, septate, unbranched between the asci, branching and anastomosing above. Two types of asci and ascospores are present in the same ascoma. *Asci* 125–150 \times

17–23 μm , 8-spored, cylindric-clavate, pedicellate, fissitunicate, with an ocular chamber and faint ring. *Ascospores* 29–36 \times 9–12.5 μm , 2-3-seriate, 1-septate, brown, guttulate, irregular fusiform, apical cells wider and tapering to a rounded tip, base narrower and more rounded, constricted at the septum, with mucilaginous pads at each end. *Asci* 112–137 \times 30–35 μm , 8-spored, obclavate, pedicellate, fissitunicate, with an apical apparatus and ring. *Ascospores* 30–40 \times 17–23 μm , 2-3-seriate, 1-septate, hyaline to pale brown, guttulate, ellipsoid, constricted at the septum, with mucilaginous pads at each end. Based on the description by Hyde and Wong (1999) and further observations in Thailand.

Anamorph: None known.

Cultures: A492, SS1536.1, SS1536.2, SS2113.2.

Sequence data: See Supplementary Table 1.

Habitat and host range: Wood submerged in freshwater.

Geographical distribution: Seychelles, Thailand.

Jahnula sunyatsenii (Inderb.) K.L. Pang, E.B.G. Jones & Sivichai, Mycol. Res. 106: 1037 (2002).

≡ *Aliquandostipite sunyatsenii* Inderb., Am. J. Bot. 88: 57 (2001).

Ascomata sessile, singly erumpent from decorticated branch immersed in small stream, rounded, 300–400 μm diam, papillate, ostiolate, light to dark brown, membranous. *Ascomal wall* one-layered, 25–40 μm thick, 2–5 cells wide, forming a *textura globulosa-angularis* in surface view. Outermost cells rounded to elongate, up to 30 μm diam, some protruding up to 13 μm above surrounding cells, inner cells elongate and laterally compressed, cell walls 1–5 μm thick, refractive. Cells at the base and towards papillum dark at times. *Ostiole* apically lined by elongate cells, ca. 10 \times 5 μm . *Hamathecium* consisting of hyaline, septate pseudoparaphyses persistent, branched, ca. 2.5 μm wide. *Asci* originating from a cushion shaped ascogenous tissue at the base of the ascomata, 128–193 \times 45–57.5 μm , when young saccate with thick-walled apex, ocular chamber and short stalk, fissitunicate, 8-spored. *Ascospores* straight or slightly curved, (39–) 46–52 \times 16–23 μm , 1-septate up to 5 μm above or 4 μm below the median septum, constricted at the septum, light brown, multi-heavily guttulate. Helmet-shaped appendages apically.

Stalked ascoma: one stalked ascoma was found, ca. 350 μm in diameter, originating from the apex of a concolorous stalk. Stalk septate at intervals of 30–40 μm , thick-walled (up to 7.5 μm), 50 μm wide and 0.5 μm long, at the base branching into 15 μm wide hyphae. *Asci* 137–143 \times 45–62 μm . *Ascospores* 50–52 \times 17–20 μm . Based on the description by Inderbitzin et al. (2001) and further observations.

Anamorph: None known.

Culture: UBC-F13876 (ex-holotype).

Sequence data: See Supplementary Table 1.

Habitat and host range: Decaying branches immersed in freshwater.

Geographical distribution: China.

Notes: Described as *Aliquandostipite* (Inderbitzin et al. 2001), it was referred to *Jahnula* based on molecular evidence (Pang et al. 2002).

Jahnula systyla K.D. Hyde & S.W. Wong, Nova Hedw. 68: 506 (1999).

Ascomata 320–340 \times 290–320 μm diam, semi-immersed and attached to a stalk, or erumpent, shedding wood particles and becoming superficial, with base remaining immersed and attached to a stalk, subglobose, light brown to dark brown, coriaceous, ostiolate, short-papillate, solitary or gregarious, with algal associations. *Peridium* comprising a few layers of relatively large, thin-walled, brown angular cells. *Pseudoparaphyses* to 3 μm wide, hypha-like, filamentous, hyaline, septate, unbranched between the asci, branching and anastomosing above. Two kinds of asci and ascospores were presented in the same ascoma. Both types of asci (6-) 8-spored (1) *Asci* 124–150 \times 22–30 μm ; ascospores 28–40 \times 8–13 μm , 2-3-seriate, 1-septate, brown to dark brown, guttulate, irregularly fusiform, apical cells wider and tapering to a rounded tip, base narrower and more rounded, minutely verruculose, constricted at the septum. (2) *Asci* 120–190 \times 34–46 μm , obclavate, pedicellate, fissitunicate, with an apical apparatus and ring. *Ascospores* 44–68 \times 13–19 μm , 2-3-seriate, 1-septate, hyaline to pale brown, guttulate, ellipsoid, slightly constricted at the septum, surrounded by a wide mucilaginous sheath. Based on the description by Hyde and Wong (1999).

Anamorph: None known.

Cultures: None.

Sequence data: None.

Habitat and host range: On submerged wood in freshwater.

Geographical distribution: Australia.

Aliquandostipite Inderb. Am. J. Bot. 88: 54 (2001).

Ascomata globose to subglobose, immersed to superficial, papillate, brown to dark brown. *Hamathecium* consisting of septate, sparsely branched pseudoparaphyses. *Asci* clavate, fissitunicate, with thickened apical region. *Ascospores* oval, 1-septate, constricted at the septum, smooth, pale brown with well developed sheath.

Type species: *A. khaoyaiensis* Inderb., Am. J. Bot. 88: 54 (2001).

Sequences derived from ex-holotype.

Accepted species:

**A. crystallinus* Raja, A. Ferrer & Shearer, Mycotaxon 91: 208 (2005).

**A. khaoyaiensis* Inderb., Am. J. Bot. 88: 54 (2001).

A. minuta Raja & Shearer, Mycoscience 48: 6 (2007).

**A. separans* (Abdel-Wahab & El-Sharouney) J. Campb., Raja, A. Ferrer, Sivichai & Shearer, Can. J. Bot. 85: 881 (2007).

**A. siamensiae* (Sivichai & E.B.G. Jones) J. Campb., Raja, A. Ferrer, Sivichai & Shearer, Can. J. Bot. 85: 879 (2007).

5 species, * sequenced species.

Aliquandostipite crystallinus Raja, A. Ferrer & Shearer, Mycotaxon 91: 208 (2005).

Ascomata globose to subglobose, 215–270×220–320 µm, hyaline becoming dark brown, papillate, immersed to erumpent, solitary or gregarious, papilla short, 30–36×60–90 µm. *Peridium* 21–25 µm wide, *textura angularis*, inner layer of hyaline thin-walled cells, outer layer of large thin-walled isodiametric cells. *Pseudoparaphyses* septate, slightly constricted at the septa, sparsely branched. *Asci* fissitunicate, clavate to cymbiform (navicular), 8-spored, 132–174×30–40 µm, with or without an apical chamber. *Ascospores* fusiform, 70–86×9–15 µm, 1-septate becoming 3-septate, hyaline, with apical appendages 2–5 µm long. Sheath swelling and becoming sigmoid or C shaped, 200–452 µm long, 4–7 µm wide. Based on the description by Raja et al. (2005).

Anamorph: None known.

Cultures: R76-1 (ex-holotype), A514-1, AF007, F83-1.

Sequence data: See Supplementary Table 1.

Habitat and host range: Decorticated wood in freshwater stream.

Geographical distribution: Costa Rica, Panama, USA.

Notes: Ascospores and hyphae contain refractive acicular crystals (Raja et al. 2005).

Aliquandostipite khaoyaiensis Inderb., Am. J. Bot. 88: 54 (2001).

Ascomata singly immersed to erumpent or superficial on old decorticated branch globose to broadly ellipsoid, 216–290 µm high, 220–344 µm wide, papillate, appearing pale brown when young or dark brown with age beneath stereomicroscope. *Peridium* membranous, one-layered, in surface view pallid brown, forming a *textura angularis-globulosa*, in transverse section cells rounded to elongate. Wall of outermost cells up to 3.5 thick and refractive, the largest cells protruding up to 8 µm. *Papilla* ca. 50 µm high, 70 µm wide. *Hamathecium* consisting of hyaline, septate pseudoparaphyses, sparsely branched, up to 3.5 µm wide. *Asci* 136–194×36–58 µm, 8-spored, clavate, fissitunicate, with thickened apical region, spores variably arranged, small peduncle observed at times. *Ascospores* oval in outline, 50–70×13–20 µm, 1-septate, constricted at the septum, 11–17 µm wide, upper cell slightly longer and narrower than lower cell, smooth, pale brown, guttulate or not, sheathed. Sheath first adpressed to the wall, gradually expanding and detaching from the polar regions towards the

septum, then balloon-like at the poles, finally surrounding the entire ascospore, ca. 150×50 µm.

Stalked ascomata: stalk to 1.6 mm long and 42 µm wide, wall up to 15 µm thick, arising singly from a superficial hypha, or singly or gregariously from substrate. Based on the description by Inderbitzin et al. (2001) and further observations in Thailand.

Anamorph: None known.

Cultures: UBC-F13875 (ex-holotype), F89-1, SS2843, SS2961, SS3028, SS3321, UAMH10371.

Sequence data: See Supplementary Table 1.

Habitat and host range: On decaying branch in freshwater.

Geographical distribution: Thailand.

Aliquandostipite minuta Raja, A. Ferrer & Shearer, Mycotaxon 91: 208 (2005).

Ascomata 130–150×150–160 µm, hyaline to light brown, globose to subglobose, ostiolate, papillate, immersed to superficial and wide hyphae. *Peridium* with large cells, *textura angularis*. *Hamathecium* consisting of septate, hyaline pseudoparaphyses 2 µm wide, slightly swollen at their tips. *Asci* 740–112×36–58 µm, 8-spored, ovoid to broadly clavate, fissitunicate, with or without apical chamber. *Ascospores* 42–54×10–14 µm, hyaline becoming pale brown, 1-septate, upper cell slightly broader than the basal cell, sheath present forming a fringe at the midseptum.

Anamorph: None known.

Culture: F117-1 (ex-holotype).

Sequence data: None.

Habitat and host range: Submerged decorticated wood.

Geographical distribution: Florida, USA.

Notes: *Aliquandostipite minuta* is similar to *A. crystallinus* but differs in the dimensions of the ascomata, shape and size of the asci, ascospore sheath and the median filamentous appendages.

Aliquandostipite separans (Abdel-Wahab & El-Sharouney) J. Campb., Raja, A. Ferrer, Sivichai & Shearer, Can. J. Bot. 85: 881 (2007).

≡ *Patescospora separans* Abdel-Wahab & El-Sharouney, Mycol. Res. 106: 1033 (2002).

Ascomata 200–260×280–335 µm diam, immersed to erumpent, dark brown, coriaceous, papillate, solitary, with stalk-like thick hyphae attached to the base, with a few sparse hair-like projections. *Peridium* 25–45 µm thick, comprising 2–4 layers of cells wide, individual cells 10–16 µm wide. *Papilla* 80–150 µm high, 80–90 µm wide. *Hamathecium* consisting of hyaline, septate pseudoparaphyses, branched, up to 7 µm wide. *Asci* 44–114×24–40 µm, 8-spored, ovoid to clavate, with a rounded base, thin-walled, dissolving in water within a few seconds to 1–2 min at most, releasing spores surrounded by a thick and elaborate sheath, the asci scattered in the centrum inside locules formed by disintegration of

hamathecial tissue. *Ascospores* 30–46×9–20 µm, 1-septate, variably arranged inside the ascus, deeply constricted at the septum, upper cell slightly longer and narrower than the lower cell, smooth, hyaline, guttulate and surrounded by a multi-layered mucilaginous sheath (110–140×22–32 µm), cells of the ascospores separated later in development. Based on the description by Pang et al. (2002) and further observations.

Anamorph: None known.

Culture: CY2787 (ex-holotype).

Sequence data: See Supplementary Table 1.

Habitat and host range: Twigs in river Nile.

Geographical distribution: Egypt.

Aliquandostipite siamensiae (Sivichai & E.B.G. Jones) J. Camb., Raja, A. Ferrer, Sivichai & Shearer, Can. J. Bot. 85: 879 (2007).

≡ *Jahnula siamensiae* Sivichai & E. B. G. Jones, Mycol. Res. 106: 1037 (2002).

Ascomata 260–350×240–350 µm, globose to subglobose, gregarious, superficial with stalk (up to 300 µm long and 40–55 µm width) or sessile. Stalk septate, 25–80 µm wide. *Peridium* 3–5 µm thick, comprising a few layers of relatively large thin-walled, light brown angular cells. *Hamatecium* of pseudoparaphyses, ca. 2–3 µm thick, hypha-like, filamentous, hyaline, septate, unbranched between the asci, branching and anastomosing above. *Asci* 8-spored, fissitunicate, with an ocular chamber and a faint ring, with two spore types: (1) small ascospores formed in obclavate asci, 125–175×28–40 µm with a short peduncle; and (2) large ascospores formed in clavate asci, 137–180×33–52 µm, with a short pedicel. *Ascospores* either small, 33–45×10–13 µm, brown, or large 58–73×15–25 µm, hyaline to pale brown. Both ascospore types are biseriate, 1-septate, guttulate, ellipsoid-fusiform, smooth-walled, apical cell slightly larger, some curved, slightly constricted at the septum. Based on the description by Pang et al. (2002) and further observations in Thailand.

Anamorph: None known.

Culture: SS81.02 (ex-holotype).

Sequence data: See Supplementary Table 1.

Habitat and host range: On submerged soft wood.

Geographical distribution: Thailand.

Brachiosphaera Nawawi, in Descals, Nawawi & Webster, Trans. Br. Mycol. Soc. 67: 213 (1976).

Colonies effuse, hypha varying in width, septate, hyaline. *Conidium* round with 4–5 conidial arms, each 1–4 septate.

Type species: *B. tropicalis* Nawawi in Descals, Nawawi & Webster, Trans. Br. Mycol. Soc. 67: 213 (1976).

No ex-holotype culture.

Accepted species:

**B. tropicalis* Nawawi in Descals, Nawawi & Webster, Trans. Br. Mycol. Soc. 67: 213 (1976).

Species requiring molecular study:

B. jamaicensis (J.L. Crane & Dumont) Nawawi, in Descals, Nawawi & Webster, Trans. Br. Mycol. Soc. 67: 216 (1976).

2 species, * sequenced species.

Brachiosphaera tropicalis Nawawi in Descals, Nawawi, and Webster Trans. Br. Mycol. Soc. 67: 213 (1976).

Cultures: Colonies effuse, mycelium mostly submerged in culture media. Hypha varying in width, septate, hyaline at first, turning olivaceous brown with age. Within hyphae a few cells in a row becoming slightly widened to form ellipsoid or round-shaped conidia in a cluster. Spherical central body of conidium 44–58 µm diam, each conidial arm mostly 4–5, 82–120 µm and 1–4 septate, in nature conidia mostly with two arms. *Conidia* frequently formed chains in half-strength corn meal agar and in water agar (Chang 1994: Isolate WL0604 from Wulai, Taipei) and further observations in Thailand.

Teleomorph: None known.

Cultures: E192-1, BCC14538, BCC16746, BCC24051.

Sequence data: See Supplementary Table 1.

Material examined: Thailand, Narathiwat province, Khlong Ai-Kading Waterfall, Hala Bala Wildlife Sanctuary, in foam, 22 February 2003, S. Sivichai & N. Boonyuen, BIOTEC SS2522, and SS2523.

Habitat and host range: Foam samples and leaves in freshwater streams.

Geographical distribution: China, Dominican Republic, Hong Kong, Malaysia, Panamá, Puerto Rico, South Africa, Southern-Western France, Taiwan, Thailand, Venezuela.

Notes: Many *Brachiosphaera tropicalis* specimens (SS2522, SS2523, SS2944) were obtained from foam samples of several tropical freshwater streams in Thailand. The species is regarded as common in freshwater streams, especially in foam samples.

Morphology: *Brachiosphaera* is characterized by producing round shaped central cell from which radiate 4–5 arms, each 1–4 septate. In *Brachiosphaera tropicalis*, conidia are brown and tetra- or polyradiate, consisting of 4–8 appendages slightly constricted at the origin, much longer than 1.5 times diam of the central part; central part globose to pyramidal. Colonies are characterized by effuse, septate mycelium, mostly submerged in culture media. *Brachiosphaera tropicalis* has conidia that are very similar to those of *Actinosporella megalospora*, the anamorph of the pezizalean discomycete *Miladina lechithina* (*Pyronemataceae*), *Pezizales* which has hyaline and tetra- or polyradiate conidia. The two species are sometimes confused with each other and repeatedly misidentified.

Phylogenetic study: The placement of *Brachiosphaera* within *Jahnulales* (Campbell et al. 2007) was based on a single locus (LSU) and confirmed its unrelatedness to

Actinosporella (Descal and Webster 1978). Additionally, Prihatini et al. (2008) demonstrated that *B. tropicalis* had phylogenetic affinities to the *Jahnulales*, sharing a unique pattern of dark brown to black and thick-walled mycelia and closely related to *Speiropsis pedatospora*. More recently, Shearer et al. (2009) have shown that *B. tropicalis* has phylogenetic affinities within the *Aliquandostipitaceae*, *Jahnulales*, based on two loci (SSU and LSU rDNA).

Brachiosphaera jamaicensis (J.L. Crane & Dumont) Nawawi, in Descals, Nawawi & Webster, Trans. Br. Mycol. Soc. 67: 216 (1976).

≡ *Actinospora jamaicensis* J.L. Crane & Dumont, Can. J. Bot. 53: 843 (1975).

Cultures: Colonies effuse on half-strength cornmeal agar. *Mycelia* immersed, hyphae initially hyaline, becoming olivaceous brown when aged, septate. *Conidiophores* erect, not well differentiated from hyphae. *Conidia* produced at the apex of conidiophores, and secondary conidia usually produced sympodially by renewed branching of the conidiophore just below the initial conidium. They were also produced in succession under laboratory conditions. *Conidia* comprising a central spherical body and 6–10 bulbous, short arms 1 or 2 septate, 20–40 µm long, and 6.8–9.5 µm and 3–5 µm wide at basal and upper ends, respectively. Chang (1994) reported that *conidia* produced on natural substrata were larger than those produced in culture.

Teleomorph: None known.

Culture: None.

Sequence data: None available.

Habitat and host range: Decaying twigs, leaves, branches and foam in freshwater streams.

Geographical distribution: Jamaica, Poland, Puerto Rico, Taiwan.

Phylogenetic study: None.

Notes: Because of the lack of cultures and sequence data, the position of *B. jamaicensis* in the *Jahnulales* cannot be confirmed.

Megalohypha A. Ferrer & Shearer, in Ferrer, Sivichai & Shearer, Mycologia 99: 456 (2007).

Ascomata superficial on wood, globose to obpyriform, ostiolate, papillate, stalked or sessile, connected to the substratum by broad, brown, septate, stoloniferous hyphae. *Peridium* wall of large, hyaline, thin-walled cells. *Hamathecium* pseudoparaphysate, pseudoparaphyses septate, branched, anastomosing above the asci. *Asci* fissitunicate, broadly clavate or fusiform, short pedicellate, 8-spored. *Ascospores* pale brown to dark brown, 1-septate, symmetrical, rough-walled, longitudinally striate.

Type species: *M. aqua-dulces* A. Ferrer & Shearer, in Ferrer, Sivichai & Shearer, Mycologia 99: 458 (2007).

No ex-holotype culture.

Accepted species:

M. aqua-dulces A. Ferrer & Shearer, in Ferrer, Sivichai & Shearer Mycologia 99: 458 (2007).

Ascomata on wood, 240–300×200–250 µm, superficial, globose to obpyriform, ostiolate, papillate, hyaline, membranous, connected to substrate by a brown, septate stalk, strongly constricted at the septa, 20–25 wide and up to 450 µm long; sessile ascomata connected to the substrate by superficial, wide, brown, septate, stoloniferous hyphae. *Papillae* 55×45 µm, short, cylindrical, periphysate; wall composed of thin-walled cells. *Peridium* about 15 µm thick, in longitudinal section composed of 1–2 layers of hyaline, thin-walled cells. *Hamathecium* consisting of hyaline, septate pseudoparaphyses 3–4 µm wide, filamentous, branched and anastomosing above the asci. *Asci* 110–160×35–60 µm, fissitunicate, clavate or ellipsoid, short pedicellate, with or without an apical chamber, 8-spored. *Ascospores* 40–55×19–22 µm, irregularly arranged, ellipsoid, tapered to acute apices, brown to dark brown, 1-septate, septum appearing as a dark band, both cells of equal shape and size, rough walled with longitudinal sulcate striations, lacking appendages or a gelatinous sheath.

Anamorph: None known.

Cultures: AF005-2a, AF005-2b.

Sequence data: See Supplementary Table 1.

Habitat and host range: Decorticated wood submerged in freshwater.

Geographical distribution: Panama, Thailand.

Notes: *Megalohypha* differs from the genera assigned to the *Jahnulales* in having mycelium and ascomatal stalks that are strongly constricted at the septa, ascospores that are characterized by bipolar symmetry, acute apices, a dark band at the median septum and sulcate striations on the spore wall. Additionally, molecular data support the erection of a new genus (Campbell et al. 2007). Based on the description by Ferrer et al. (2007) and further observations in Thailand.

Manglicola Kohlm. & E. Kohlm., Mycologia 63: 840 (1971).

Ascomata solitary, obtusely clavate to fusiform, stipitate, superficial, seated in the substrate with a hypostroma, coriaceous, olive brown, with a blunt periphysate ostiole, ostiolate, epapillate. *Pseudoparaphyses* numerous, septate. *Asci* 8-spored, cylindrical, thick-walled, developing at the base of the ascoma. *Ascospores* fusiform, apiculate, 1-septate; apical cell larger, dark brown; basal cell small, light brown; deliquescent appendages apically.

Type species: *M. guatemalensis* Kohlm. & E. Kohlm., Mycologia 63: 841 (1971).

No ex-holotype culture.

Accepted species:

M. guatemalensis Kohlm. & E. Kohlm., Mycologia 63: 841 (1971).

Species requiring a molecular study:

M. samulesii Huhndorf, Mycologia 86: 266 (1994).

Manglicola guatemalensis Kohlm. & E. Kohlm., Mycologia 63: 841 (1971). Figure 4 a-t.

Ascomata 835–1,275×185–387 µm, obtusely clavate to obtusely fusiform; stipitate, ostiolate, epapillate, coriaceous, olive brown, solitary or aggregated, ascoma wall differentiated into several layers of polygonal, thick-walled cells; ascoma superficial seated on the substratum on a hypostroma, of pseudoparenchymatous cells and brown hyphae 10–20 µm in diam. *Peridium* 36–50 µm thick, composed of three to five layers of cells. *Ostirole* obtuse surrounded by hyaline, clavate hyphae, 4–6 µm diam, *periphyses* 2–3 µm in diam, simple or ramose. *Hamathecium* comprising hyaline, septate, trabeculate pseudoparaphyses 2–4 µm wide, numerous, simple, between asci, arising from the base of the centrum, and anastomosing in the upper part. *Asci* 275–326×24–28 µm (Kohlmeyer and Kohlmeyer 1971, 1979), to 440–640×30–50 µm (Suetrong et al. 2010), 8-spored, cylindrical, thick-walled, with apical apparatus and ocular chamber. *Ascospores* 80–109×18–34 µm (Kohlmeyer and Kohlmeyer 1971, 1979) to 92.5–125×22.5–40 µm (Suetrong et al. 2010), fusiform, apiculate, unequally one-septate, constricted at the septum; apical cell larger, orange brown, light brown, dark brown to chestnut brown, basal cell, turbinate, light brown; gelatinous appendages cover both apices.

Anamorph: None known.

Cultures: BCC20079, BCC21218, BCC24217, BCC24200-BCC24204, BCC24275, BCC24296-BCC24302, BCC25032-BCC25042, BCC25052-BCC25053.

Sequence data: See Supplementary Table 1.

Material examined: Thailand, Trat Province: Mu Ko Chang National Park, Ban Salak Phet, on base frond of *Nypa fruticans*, 5 October 2005, S. Suetrong, E.B.G. Jones, J. Sakayaroj, R. Choeyklin, U. Pinruan, K.L. Pang, BBH17801; Thailand, Trang Province: Ban Bang Sak, on base frond of *Nypa fruticans*, 14 November 2005, E.B.G. Jones, R. Choeyklin, A. Pinnoi, U. Pinruan; Thailand, Trat Province: Mu Ko Chang National Park, Ban Salak Phet, on base frond of *Nypa fruticans*, 14 December 2006, S. Suetrong, J. Sakayaroj, R. Choeyklin, O. Supaphon, BBH23409.

Habitat and host range: Superficial on frond bases of *Nypa fruticans* and *Rhizophora mangle*.

Geographical distribution: Brunei, Guatemala, Thailand.

Phylogenetic study: Suetrong et al. (2009, 2010) confirmed the placement of *M. guatemalensis* in the *Jahnuales*.

Notes: The taxonomic position of *M. samulesii* remains unresolved as morphologically it differs from *M. guatemalensis*, a rare tropical ascomycete currently referred to *Hypsostromataceae*, a family in the *Pleosporales* (See Fig. 1).

Speiropsis Tubaki, J. Hattori. Bot. Lab. 20: 171 (1958).

Conidiophores macronematous, mononematous, with short, pale branches at the apex; stipe straight or curved, mid to dark brown, smooth septate. *Conidiogenous cells* discrete or integrated formed at the ends of the branches, polyblastic, determinate, clavate, cylindrical or ellipsoidal, usually with 2 or 3 terminal protuberances or denticles on which branched chains of conidia are formed. *Conidia* cylindrical or cuneiform, hyaline or brown, smooth, aseptate.

Cultures: Colonies effuse, brown or blackish brown, hairy or velvety. Mycelium partly superficial, partly immersed. Stroma absent. Setae and hyphopodia absent.

Type species: *Speiropsis pedatospora* Tubaki, J. Hattori, Bot. Lab. 20: 171 (1958).

No ex-holotype cultures.

Accepted species:

**S. pedatospora* Tubaki, J. Hattori, Bot. Lab. 20: 171 (1958).

Species requiring a molecular study:

S. aquatica Aramb., Cabello & Mengasc, Boln. Soc. Argent. Bot. 25: 222 (1987).

S. belauensis Matsch., Matsush, Mycol. Mem 4: 15 (1985).

S. hyalospora Subram. & Ledha, Can. J. Bot. 42: 1062 (1964).

S. ixorae Subram. & Sudha., Kavka 14: 37 (1987).

S. rogergoosensis T.S.K. Prasad & Bhat, Mycotaxon 82: 127 (2002).

S. scopofromis Kuthub. & Nawawi, Trans. Br. Mycol. Soc. 89: 584 (1987)

S. simplex Marsush., in Kabayasi et al., Bull. Natn. Sci. Mus., Tokyo 14: 475 (1971).

Eight species,*sequenced species.

Rejected species:

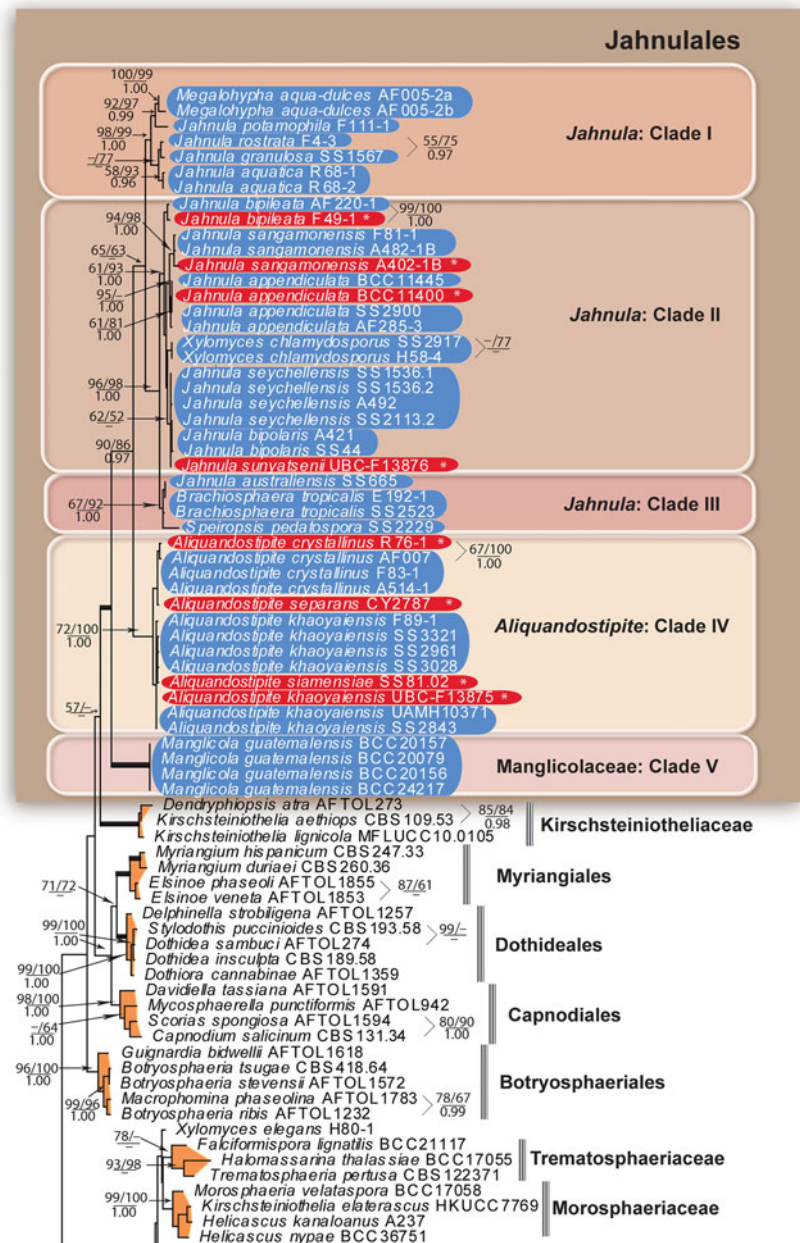
S. irregularis R.H. Petersen, Mycologia 55: 26 (1963).

Current name: *Arbusculina irregularis* (R.H. Petersen) Marvanová & Descals, Trans. Br. Mycol. Soc. 89: 499 (1987).

Speiropsis pedatospora Tubaki, J. Hattori, Bot. Lab. 20: 171 (1958). Figure 5 a-g.

Colonies hyaline, olivaceous to brown, *Conidiophores* 60–130 µm high, 3.5–5 µm wide, brown, apical region subhyaline to pale brown. *Conidia* branched with 2–6 arms, arms 65–85 µm long, each with 3–5 septa. Colonies on malt agar, olivaceous to fuscous, aerial

Fig. 1 One of two MPTs inferred from combined SSU and LSU rDNA sequences of *Jahnulales* and other taxa of the Dothideomycetes, generated with maximum parsimony (BSMP, left) and likelihood (BSML, right) bootstrap values 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. Taxa in red are generated from ex-holotype cultures, those in blue are from verified cultures



hyphae sparse, septate, 1.5–2.5 μm wide. *Conidiophores* hyaline 50–65 μm long rarely 100 μm . *Conidia* 3–5 arms, 80–90 μm long, 4.5–6.5 μm wide, pale coloured. Based on Tubaki (1958) and further observations in Thailand.

Teleomorph: None known.

Culture: SS2229.

Sequence data: See Supplementary Table 1.

Habitat and host range: Dead leaves.

Geographical distribution: Japan, Thailand.

Notes: This is the only species currently included in the *Jahnulales*, as it is the only species with available molecular data. The remaining species listed above require study at the molecular level. This genus may be polyphy-

letic as indicated by the transfer of *S. irregularis* to *Asbusculina* (Marvanová and Descals 1987). Therefore the only species accepted in the *Jahnulales* is *S. pedatospora*.

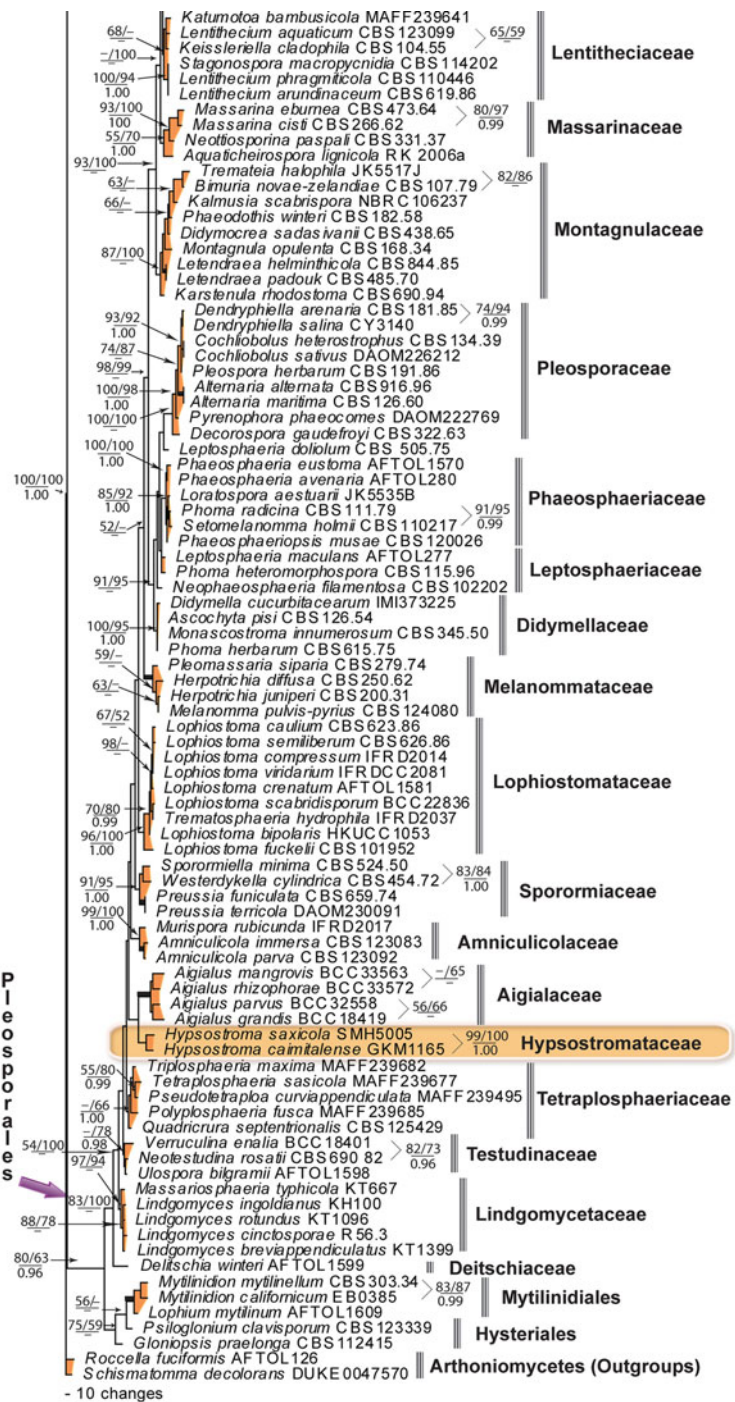
Xylomyces Goos, R.D. Brooks & Lamore, Mycologia 69: 282 (1977).

Colonies on natural substrate thin, effused. *Mycelium* immersed and superficial; hyphae branched, septate, hyaline to fuscous. *Conidiophores* and *conidia* lacking. *Chlamydo-spores* intercalary, multi-septate, fusiform, fuscous.

Known teleomorphs: in *Jahnulales* *Pleosporales*.

Type species: *Xylomyces chlamydosporus* Goos, Brooks & Lamore, Mycologia 69: 282 (1977).

Fig. 1 (continued)



No ex-holotype cultures.

Accepted species:

**X. chlamydosporus* Goos, R.D. Brooks & Lamore, Mycologia 69: 282 (1977).

Species requiring molecular study:

X. foliicola W.B. Kendr. & R.F. Castañeda, Univ. Waterloo, Biol. Ser. 33: 54 (1990).

X. giganteus Goh, W.H. Ho, K.D. Hyde & K.M. Tsui, Mycol. Res. 101: 1324 (1997).

X. punctatus Goh, W.H. Ho, K.D. Hyde & K.M. Tsui, Mycol. Res. 101: 1328 (1997).

X. pusillus Goh, W.H. Ho, K.D. Hyde & K.M. Tsui, Mycol. Res. 101: 1328 (1997).

X. rhizophorae Kohlm. & Volkm. -Kohlm., Fungal Divers. 1: 160 (1998).

five species, * sequenced species.

Rejected species:

X. aquaticus (Dudka) K.D. Hyde & Goh, Mycol. Res. 103: 1573 (1999).

≡ *Camposporium aquaticum* Dudka, Ukr. Bot. Zh. 23: 91 (1966).

≡ *Vargamyces aquaticus* (Dudka) Tóth, Acta Bota. Acade. Sci. Hung. 25: 403 (1979).

≡ *Sporidesmium ontariense* Matsush., Matsush. Mycol. Mem. 3: 16 (1983).

X. elegans Goh, W.H. Ho, K.D. Hyde & K.M. Tsui, Mycol. Res. 101: 1324 (1997).

Both species are rejected as molecular data places them in the Pleosporales (Prihatini et al. 2008)

Xylomyces chlamydosporus Goos, R.D. Brooks & Lamore, Mycologia 69: 282 (1977). Figure 6 a-b.

Colonies on natural substrates effused, thin, reddish brown. *Mycelium* immersed and superficial, composed of branched, septate, dark, anastomosing hyphae, (5–) 7–15 (–25) μm diam. *Conidiophores* and *conidia* lacking. *Chlamydo-spores* abundant, intercalary, solitary or in chains of 2 to 5, occasionally branched, with 5 to 9 septa, constricted at the septa, fusiform, brown to blackish, 100–450 \times 35–42 μm . Based on Goos et al. (1977) and observations in Thailand.

Teleomorph: in the *Jahnulales*.

Cultures: SS2917, H58-4.

Sequence data: See Supplementary Table 1.

Habitat and host range: Submerged wood in freshwater streams.

Geographical distribution: Thailand, USA.

Notes: Sivichai et al. (2011), based on a culture study, demonstrated that *Xylomyces chlamydosporus* is the anamorph of *Jahnula aquatica*. However, this is not supported by sequence data in our study (Fig. 1).

Xylomyces foliicola W.B. Kendr. & R.F. Castañeda, Univ. Waterloo, Biol. Ser. 33: 54 (1990).

Colonies spreading, hypophyllous, brown to dark brown. *Mycelium* mostly superficial, but some immersed in the substrate, composed of septate, branched, anastomosing, smooth-walled, brown hyphae, 2–3 μm wide. *Conidiophores* and conidiogenous cells absent. *Chlamydo-spores* present, resembling phragmoconidia, intercalary, fusiform, brown, sometimes pale brown at the ends, in chains or solitary, usually unbranched, smooth-walled, 4–11-septate, mostly 11-septate, 126–152 \times 7–9 μm . Based on a description by Castañeda and Kendrick (1990).

Teleomorph: None known.

Cultures: None.

Sequence data: None.

Habitat and host range: Decaying leaves.

Geographical distribution: Cuba.

Xylomyces giganteus Goh, W.H. Ho, K.D. Hyde & K.M. Tsui, Mycol. Res. 101: 1324 (1997).

Colonies on natural substrate effuse, thin, brown. *Mycelium* mostly immersed and partly superficial, composed of branched, septate, 4–15 μm wide, dematiaceous, anastomosing hyphae. *Stromata* lacking. Setae and hyphopodia absent. *Conidiophores* and *conidia* not developed. *Chlamydo-spores* narrowly fusiform or long-fusiform, intercalary, straight or curved, solitary or in chains, occasionally branched, (140–) 191–575 \times 25–50 μm , with 6–26 septa, yellowish brown to mid brown, uniform in colour or end cell paler, thick-walled with scarce irregular longitudinal striations. Based on the description by Goh et al. (1997).

Teleomorph: None known.

Cultures: SAPR15 (HKU(M) 2167), SAPR40 (HKU(M) 2211), ENG6A (HKU(M) 3228).

Sequence data: None.

Habitat and host range: Submerged wood in freshwater streams.

Geographical distribution: Australia.

Xylomyces punctatus Goh, W.H. Ho, K.D. Hyde & K.M. Tsui, Mycol. Res. 101: 1328 (1997).

Colonies on natural substrate scattered, effuse, dark brown. *Mycelium* mostly immersed or partly superficial, composed of pale olivaceous brown, branched, septate, 2–3 μm wide, smooth or sometimes punctuate hyphae. *Stromata* lacking. Setae and hyphopodia absent. *Conidiophores* and *conidia* not developed. *Chlamydo-spores* intercalary or terminal, solitary, fusiform or slightly clavate, straight or slightly curved, 44–74 \times 10–16 μm , with 3–7 septa, constricted at the septa, guttulate, pale reddish brown, end cells paler, with distinctive blackish punctuate wall ornamentation. Based on the description by Goh et al. (1997).

Teleomorph: None known.

Cultures: None.

Sequence data: None.

Habitat and host range: Submerged wood in freshwater streams.

Geographical distribution: Hong Kong.

Xylomyces pusillus Goh, W.H. Ho, K.D. Hyde & K.M. Tsui, Mycol. Res. 101: 1328 (1997).

Colonies on natural substrate scattered, effuse, dark brown. *Mycelium* mostly immersed and partly superficial, composed of subhyaline to pale olivaceous brown, branched, septate, 1.5–3 μm wide, smooth hyphae. *Stromata* lacking. Setae and hyphopodia absent. *Conidiophores* and *conidia* not developed. *Chlamydo-spores* intercalary or terminal, solitary, fusiform or slightly clavate, usually slightly curved, 42–56 \times 7–11 μm , with 3–7 septa, not constricted or sometimes slightly at the septa, guttulate, smooth, pale olivaceous brown, uniform in colour or

sometimes central cells slightly darker. Based on the description by Goh et al. (1997).

Teleomorph: None known.

Cultures: None.

Sequence data: None.

Habitat and host range: Submerged wood in freshwater streams.

Geographical distribution: Hong Kong.

Xylomyces rhizophorae Kohlm. & Volkm.-Kohlm, Fungal Divers. 1: 160 (1998).

Hyphae 1.5–4 µm diam, mostly superficial, septate, branched, light to dark brown. *Conidiophores* and *conidia* absent. *Chlamydospores* mostly terminally, rarely intercalary, single or in chains, rarely branching, filamentous, straight or curved, mostly widest at the tips, tapering towards the base, 95–370(–500) × (8–) 9–16 µm, 5.5–8 µm wide at the base, with 11–43(–64) transverse septa, rarely with longitudinal or oblique septa, constricted at some of the septa, smooth, dark brown, more or less uniform in colour throughout.

Teleomorph: None known.

Cultures: None.

Sequence data: None.

Habitat and host range: Washed up prop root of a mangrove tree.

Geographical distribution: Martinique.

Notes: *Xylomyces*, typified by *X. chlamydosporus*, is characterized by lacking conidiophores and conidiogenous cells but producing large, dark, multi-septate, fusiform chlamydospores (Goos et al. 1977). Castañeda and Kendrick (1990) described a second species of *Xylomyces*, *X. foliicola* from Cuba on decaying leaves of *Quercus oleoides* var. *sagraeana*. However, *Xylomyces foliicola* was concluded to be atypical of the genus in having a terrestrial occurrence and the propagules of this species might be genuine ‘conidia’, which are borne singly or in short unbranched chains (Goh et al. 1997). In the same study, Goh and co-workers described four more *Xylomyces* species (*X. elegans*, *X. giganteus*, *X. punctatus* and *X. pusillus*) from submerged wood in freshwater habitats. *Xylomyces rhizophorae*, discovered on dead submerged roots and branches of *Rhizophora mangle*, differs from other *Xylomyces* species in having narrower chlamydospores with consistently more septa and in its marine occurrence (Kohlmeyer and Volkmann-Kohlmeyer 1998). Hyde and Goh (1999) added the last species *Xylomyces aquaticus*, a taxon transferred from *Vargamyces aquaticus*. This species was originally described as *Camposporium aquaticum* by Dudka (1966) and later transferred to *Vargamyces aquaticus* (Tóth 1979).

Phylogenetic study: Nuclear ribosomal RNA genes of only three *Xylomyces* species have been sequenced (*X. aquaticus*, *X. chlamydosporus* and *X. elegans*). Both

combined analysis of SSU and LSU rDNA genes (Campbell et al. 2007; Shearer et al. 2009) and ITS regions (Prihatini et al. 2008) put *X. chlamydosporus* in the *Jahnulales*. However, *X. aquaticus* and *X. elegans* were inferred to be related to the *Pleosporales* (Prihatini et al. 2008) and this placement of *X. elegans* was confirmed by Shearer et al. (2009) using sequence of the SSU rRNA gene.

Concluding remarks: Since both *X. aquaticus* and *X. elegans* did not group with the type species of the genus (*X. chlamydosporus*), the taxonomic placement of the two species needs to be reexamined. *Xylomyces aquaticus* is unusual in the genus in producing true conidia (Gönczöl et al. 1990), which clearly does not conform to the generic description of the genus. On the other hand, *X. elegans* is unique in the genus in having broadly fusiform chlamydospores. Whether this character is important in the delineation between *X. chlamydosporus* and *X. elegans* will require addition *Xylomyces* species in the phylogenetic analysis.

Results

Molecular phylogenies of combined SSU and LSU rDNA sequence data

The BLAST search based on SSU and LSU rDNA revealed the closest match with taxa in the subclass *Pleosporomycetidae*, order *Jahnulales* (genera *Aliquandostipite*, *Brachiosphaera*, *Jahnula*, *Manglicola*, *Megalohypha*, *Speiroopsis* and *Xylomyces*). Sequences were aligned and analyzed separately by maximum parsimony, maximum likelihood and Bayesian inference, and the resulting trees compared. The phylogenetic analyses of combined SSU and LSU rDNA sequence data were performed, along with various orders of the Dothideomycetes (*Botryosphaerales*, *Capnodiales*, *Dothideales*, *Hysteriales*, *Myriangiales*, *Mytilinidiales* and *Pleosporales*) from sequences retrieved from GenBank (Supplementary Table 2). Major insertions were present in the genes of twelve species (*Botryosphaeria stevensii*, *Dendryphiopsis atra*, *Guignardia bidwellii*, *Halomassarina thalassia*, *Herpotrichia juniperi*, *Kirschsteiniothelia aethiops*, *Neottiosporina paspali*, *Phaeosphaeria avenaria*, *Phaeosphaeria eustoma*, *Quadricrura septentrionalis* and *Roccella fuciformis*, SSU; *Capnodium salicinum*, LSU) inclusion or exclusion of these insertions did not affect the overall tree topology.

All major taxonomic clades presented in the multigene phylogeny of Campbell et al. (2007), Schoch et al. (2006, 2009), Shearer et al. (2009) and Suetrong et al. (2009, 2010) yielded the same topology. The final alignment of combined SSU and LSU rDNA sequences for 164 taxa was

2639 characters long, with *Roccella fuciformis* and *Schizatomma decolorans* as the outgroup. The alignment is available in TreeBASE No. 11942 (<http://www.treebase.org/treebase-web/home.html>).

The unweighted parsimony dataset consisted of 2,639 total characters, 1,573 (59.6%) were constant characters, 740 (28.0%) parsimony informative characters and 326 (12.4%) parsimony uninformative characters. Heuristic searches were 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 maximum parsimony resulted in a single MPT in a length of 4,189 steps (CI=0.372, RI=0.819, RC=0.304, HI=0.628). One hundred successive searches using a rapid hill-climbing algorithm from distinct randomised starting trees in RAxML yielded a best scoring likely tree (data not shown) with a log likelihood -26515.129553. 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 parsimony and maximum likelihood (Campbell et al., 2007; Schoch et al. 2006, 2009; Shearer et al. 2009; Suetrong et al. 2009, 2010). However, the internal node relationships of some taxa were resolved differently between the maximum parsimony, maximum likelihood and Bayesian trees (Fig. 1).

Five clades are identified in Fig. 1, clade I comprising a group of species regarded as *Jahnula sensu stricto* by Campbell et al. (2007) and Shearer et al. (2009): *J. aquatica* (type species), *J. granulosa*, *J. rostrata*, with *Megalohypha aqua-dulces* and *J. potamophila* as a sister group. This clade has high support, while the remaining *Jahnula* species group in clades II and III. Clade II comprises seven *Jahnula* species, while *J. australiensis*, *Brachiosphaera tropicalis* and *Speiropsis pedatospora* group in clade III. Species grouping in Clade II (*Jahnula sensu lato*) may comprise

Fig. 2 One of 82 MPTs inferred from combined LSU+5.8S rRNA+TEF-1-alpha sequences of *Jahnulales* generated with maximum parsimony. Maximum parsimony (BSMP, left) and likelihood (BSML, right) bootstrap values 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

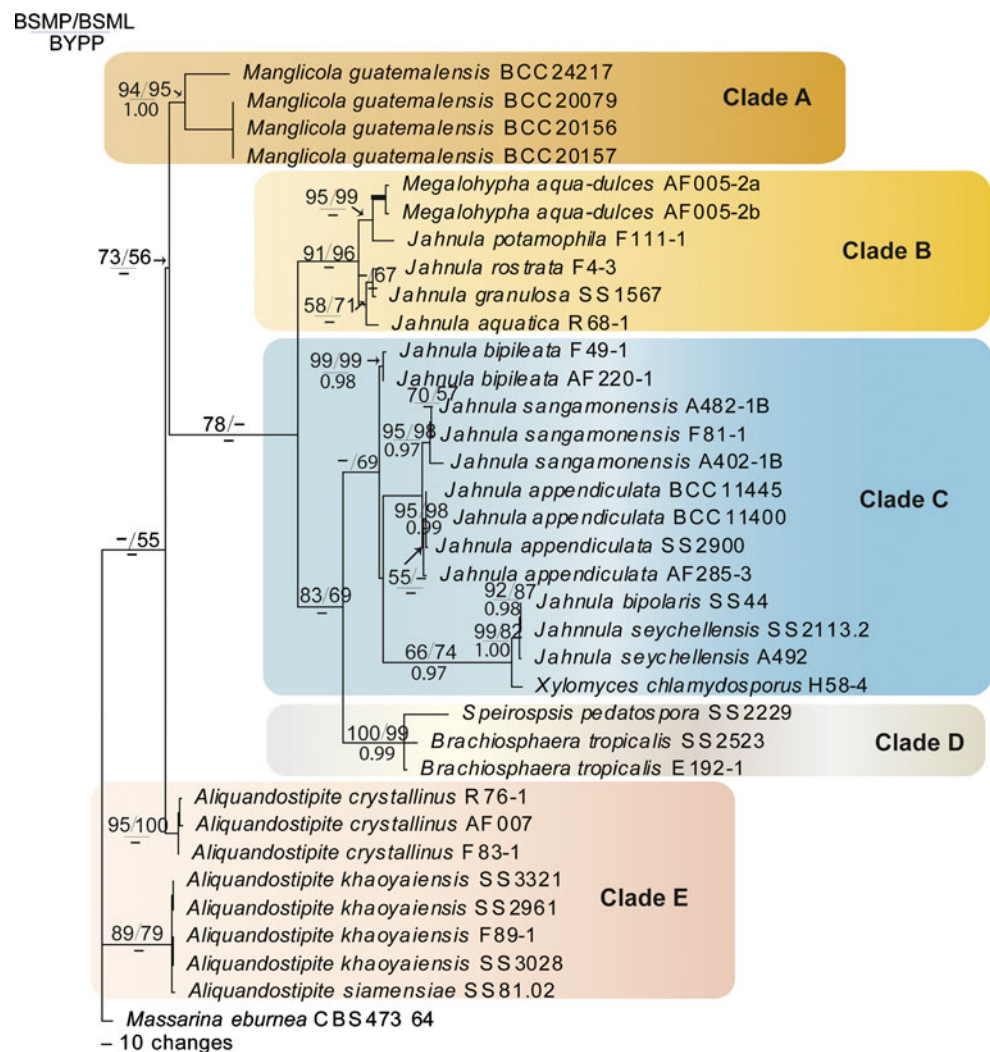
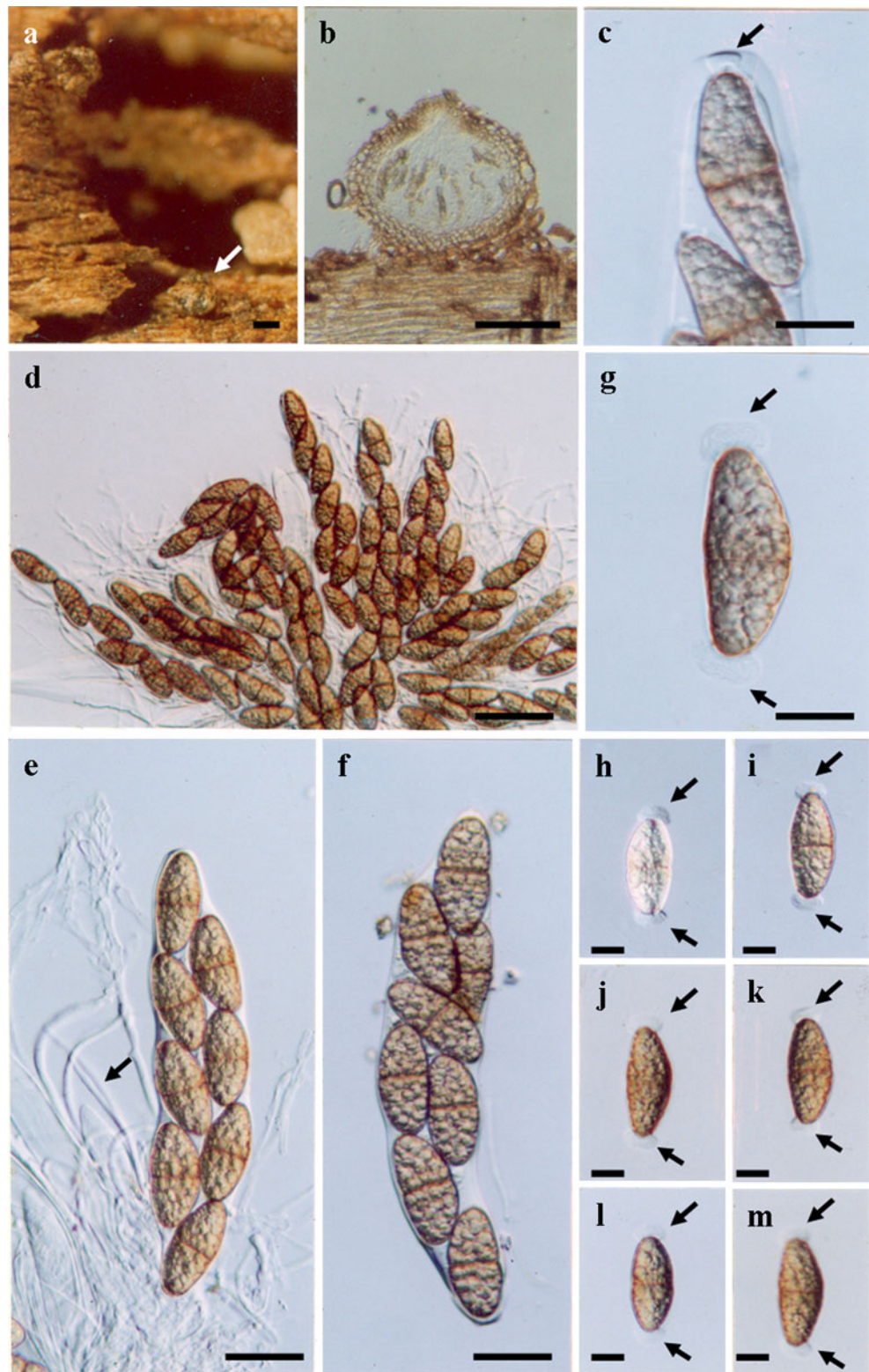
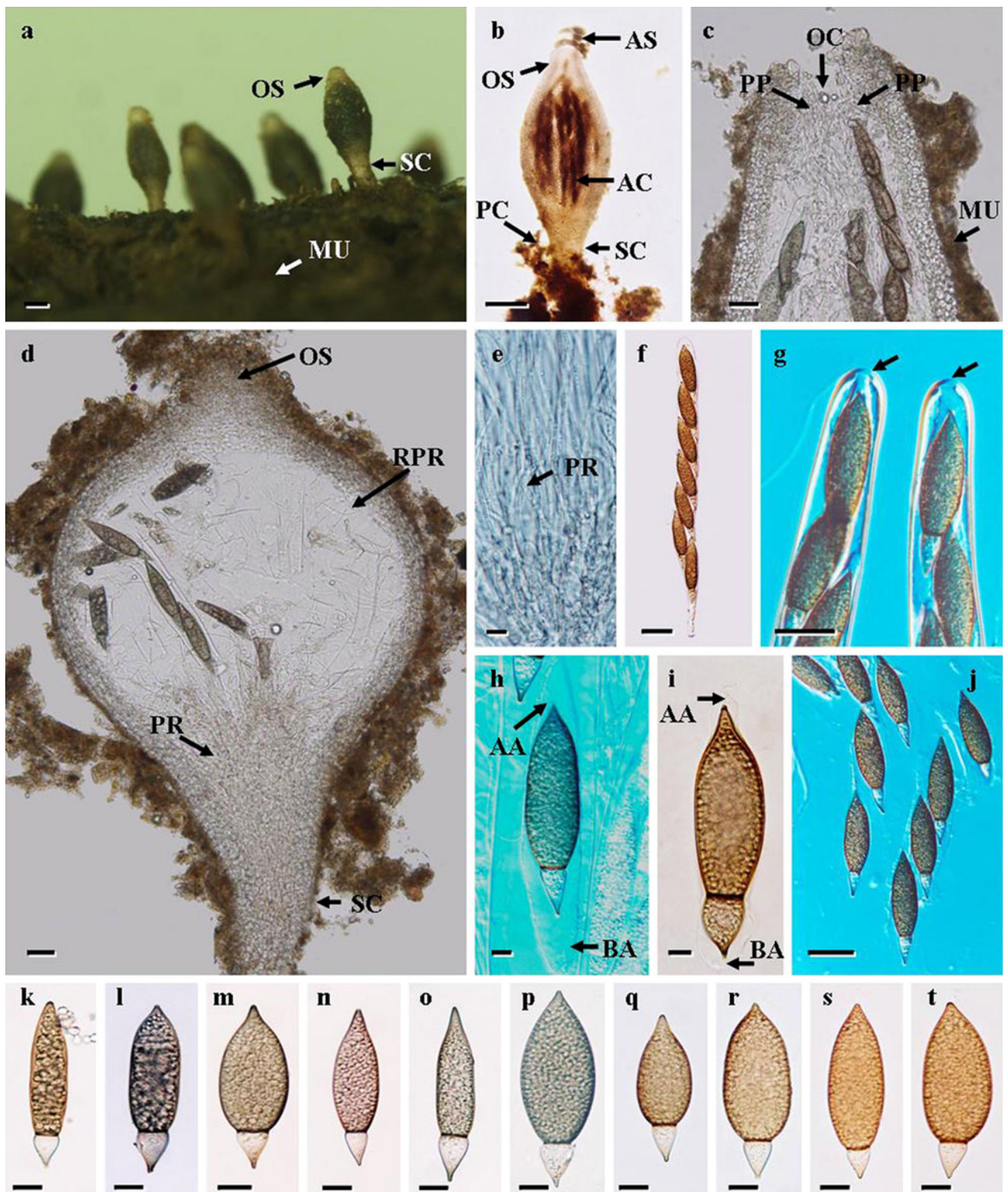


Fig. 3 *Jahmula seychellensis*
a–b Ascomata with short stalk (arrowed); **b** Section of ascomata; **c** Ascus apical pore (arrowed); **d** Asci and pseudoparaphyses (arrowed); **e–f** Asci; **g–m** Ascospore with bipolar pads (arrowed). Scale bars: a, b=100 μ m; c, g=10 μ m; d=40 μ m; e–f=20 μ m; h–m=10 μ m



more than one genus, for example, *J. bipolaris*, *J. seychellensis* and *J. sunyatsensii* all have ascospores with apical gelatinous pads and could be referred to the genus *Ascagilis* (Hyde 1992). However, until a wider molecular study is

undertaken with 4–6 loci, we believe it to be premature, and regard all species in Clades I–III as belonging in *Jahmula*. This is a conclusion also drawn by Shearer et al. (2009) with fewer species sampled.



Four *Aliquandostipite* species form a well supported monophyletic group and this is in agreement with Shearer et al. (2009), although in their study *A. separans* is on a long branch. Strains of *A. khaoyaiensis* and *A. siamensiae* group

together, with ascospore measurements in the same range. They differ in that the sheath in *A. khaoyaiensis* balloons out while in *A. siamensiae* it is narrow and ascospores in the latter are dimorphic: small and brown ($33\text{--}45 \times 10\text{--}13 \mu\text{m}$)

◀ **Fig. 4** *Manglicola guatemalensis* on the surface of *Nypa fruticans*, partially immersed in mud; **b** Ascoma seated on the substrata a hypostroma, composed of pseudoparenchymatous cells (PC) and dark ascospores (AS) in the mature asci (AC), visible through thin wall; spores exuded at ostiole (OS); **c, d** Longitudinal section of ascoma. **c**. Ascoma covered with mud (arrowed MU). Periphyses (PP) are simple; reticulate (arrowed RPR) and anastomosing; pseudoparaphyses in the upper part of the centrum arising from the venter wall; ostiolar canal (OC), trabeculate pseudoparaphyses (PR) arise between the asci from the base of the centrum; **d** Longitudinal section of ascoma with stalk, asci and pseudoparaphyses; **e** Narrow pseudoparaphyses; **f** Cylindrical ascus; **g** Ascus tip with ocular chamber (arrows); **h, i** Ascospore in ascus with apical and basal appendages (arrow). Ascospore in ascus (h) with apical (AA) and basal (BA) appendages; **j–t** Bicelled ascospores. Scale bars a, b=250 μ m; d=100 μ m; c, f, g, j=50 μ m; k–t=20 μ m; e, h, i=10 μ m

or large (58–73 \times 15–25 μ m) and hyaline to pale brown (Pang et al. 2002). Further studies are required to determine whether they may be conspecific. All *Aliquandostipite* species have well developed ascospore sheaths, with the exception of *A. siamensiae*. Clade V is a monophyletic group comprising four strains of *Manglicola guatemalensis*, a marine mangrove taxon reported from Brunei, Guatemala, and Thailand. This clade is distinct from taxa assigned to *Aliquandostipitaceae*, and a new family is proposed.

Molecular phylogenies of combined LSU and 5.8S rDNA and TEF-1-alpha sequence data

The BLAST search based on 5.8S and LSU rDNA revealed the closest match with taxa in the subclass *Pleosporomycetidae*, order *Jahnulales* (*Aliquandostipite sunyatsenii*, *Brachiosphaera tropicalis*, *Jahnula granulosa*, *Speiroopsis pedatospora* and *Xylomyces chlamydosporus*) with 84–98% similarity.

The combined LSU and 5.8S rDNA and TEF-1-alpha data set consisted of 35 taxa, with *Massarina eburnea* as the outgroup. The phylogenetic analyses of combined LSU and 5.8S rDNA and TEF-1-alpha sequence data were performed, along with various taxa of the *Jahnulales* from GenBank. Sequences were aligned and analyzed separately by maximum parsimony, maximum likelihood and Bayesian analysis. The maximum parsimony dataset consisted of 2,218 total characters, 1,649 (74.3%) characters were constant, 283 (12.7%) characters are parsimony informative and 286 (13.0%) characters were parsimony uninformative. Heuristic searches were run for 100 replicates of random stepwise addition of sequence that treated gaps as missing data. The maximum parsimony resulted in eighty-two MPTs in a length of 871 steps (CI=0.785, RI=0.833, RC=0.654, HI=0.215). 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. One hundred successive searches using a rapid

hill-climbing algorithm from distinct randomised starting trees in RAxML yielded a best scoring likely tree (data not shown) with a log likelihood -7413.460154. 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 parsimony and maximum likelihood (Campbell et al. 2007; Prihatini et al. 2008; Shearer et al. 2009; Suetrong et al. 2009, 2010). The alignment is available in TreeBASE No 11942 (<http://www.treebase.org/treebase-web/home.html>). A single MPT is shown as a phylogram, representing the best topology with the best K-H-likelihood scores (Fig. 2).

The dataset of combined LSU and 5.8S rDNA and TEF-1-alpha also comprises five clades but the topology of the tree differs from that in Fig. 1. Clades A, B, D and E correspond to clades V, I, III and IV respectively in Fig. 2, while the resolution of subclades in Clade C differs in their support at the nodes. In this tree, *Aliquandostipite* species are not monophyletic while *Manglicola guatemalensis* groups with moderate support with *Jahnula*, *Megalohypha*, *Brachiosphaera* and *Speiroopsis* species (73BSMP and 56BSML) Figs. 3, 4, 5 and 6.

Taxonomical changes

1. *Manglicolaceae* Suetrong & E.B.G. Jones, **fam. nov.** Mycobank 563225

Etymology: Named after the type genus.

Family in the *Jahnulales*, *Dothideomycetes*, *Ascomycota*. *Ascomata* obtusely clavate to fusiform, stipitate, solitary or aggregated, superficial, ostiolate, epapillate, brown, coriaceous and large (1,100–1,750 \times 290–640 μ m). *Ostiole* wide ca. 100–200 μ m at the apex. *Hamathecium* comprising trabeculate, hyaline pseudoparaphyses simple, numerous, and septate. *Asci* cylindrical, bitunicate, thick-walled, with an ocular chamber. *Ascospores* uniseriate, pale brown to brown, fusiform, apiculate, unequally 1-septate, constricted at the septum, apical cell larger, basal cell turbinate, with gelatinous appendages at both ends.

Type genus: Manglicola Kohlm. & E. Kohlm., Mycologia 63: 840 (1971).

Notes: The *Manglicolaceae* shares a number of features with the *Aliquandostipitaceae*: ascomata with short stalks, cylindrical asci, appendaged ascospores, wide hyphae (ca. 40 μ m wide), and all are saprobic species in aquatic habitats. However, they differ in that *Manglicolaceae* ascomata are large, with a wide ostiole, have large unequally 1-septate ascospores that are pale brown with a turbinate basal cell, few asci per ascoma, and they differ by their marine mangrove habitat.

Huhndorf (1994) referred *Manglicola* to the *Hypsostromataceae*, a family with no previously known relationship

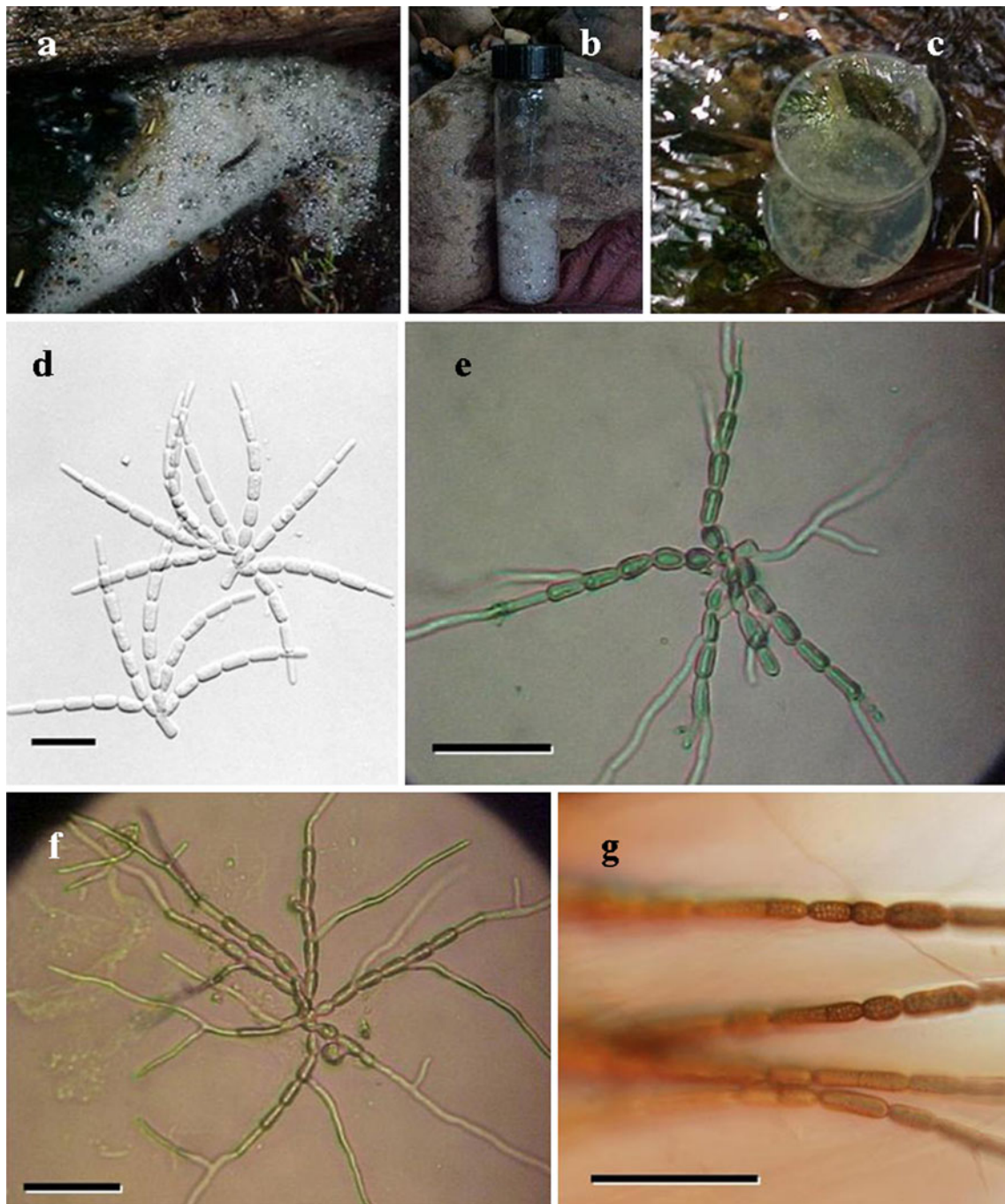


Fig. 5 a–g *Speiropsis pedatospora*; a–c Foam sample collected at Khao-Yai National Park, Thailand; d Conidia; e–f Geminating conidium; g Hyphae and thick-walled mycelium in culture on Corn Meal Agar; Scale bars d–g=30 μ m

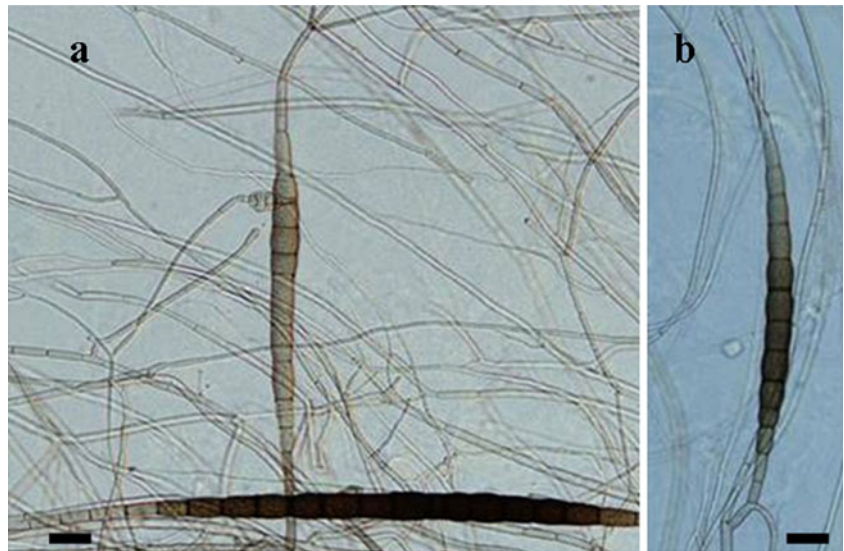
to any group in the *Dothideomycetes*, but “probably with affinities to the *Melanommatales*” (Mugambi and Huhndorf 2009). Characteristics that unite *Manglicola* and the *Hypsostromataceae* include superficial, large, elongate ascomata (stalked) with a soft texture, trabeculate pseudo-paraphyses, stipitate asci attached in a basal arrangement in the centrum and fusiform, septate ascospores (Huhndorf 1994). Our dataset places *Hypsostroma saxicola* and *H.*

caimitalense (*Hypsostromataceae*) in the *Pleosporales* with high bootstrap support (Fig. 1).

2. Genera in the *Aliquandostipitaceae*

Genera that are well circumscribed include *Aliquandostipite*, *Megalohypha*, and the anamorphs *Speiropsis*, *Brachiosphaera* and *Xylomyces*. *Jahnula potamophila*

Fig. 6 *Xylomyces chlamydo-sporus*. **a, b** Chlamydo-spores of *Xylomyces chlamydo-sporus* produced on CMA. Scale bars a–b=10 μ m



might be better placed in *Megalohypha*, and the position of *J. australiensis* requires further consideration based on the molecular evidence presented in this study.

Taxa rejected from inclusion in the family are:

Speiropsis irregularis R.H. Petersen, *Mycologia* 55: 26 (1963).

Current name:

Arbusculina irregularis (R.H. Petersen) Marvanová & Descals, *Trans. Br. Mycol. Soc.* 89: 499 (1987).

Xylomyces aquaticus (Dudka) K.D. Hyde & Goh, *Mycol. Res.* 103: 1573 (1999).

X. elegans Goh, W.H. Ho, K.D. Hyde & K.M. Tsui, *Mycol. Res.* 101: 1324 (1997).

Both species are referred to the *Pleosporales* based on molecular evidence.

Conclusions

The fact that taxa in the *Jahnulales* share many morphological features, such as wide brown hyphae, ascomata with stalks, bicelled ascospores with or without polar appendages/apical caps/pads, makes it difficult to delineate genera using morphology alone. *Manglicola* with its large ascomata and wide ostioles, apiculate appendaged ascospores with polar appendages is clearly defined, as are most *Aliquandostipite* taxa with their clavate/ovoid asci, fusiform, smooth-walled ascospores with a large gelatinous sheath that balloons out in water. However, *A. siamensiae* has a narrow sheath.

Within the *Jahnula* clades, there is considerable variation in the morphology of the different taxa. Species in *Jahnula sensu stricto* share the following features: cylindrical to

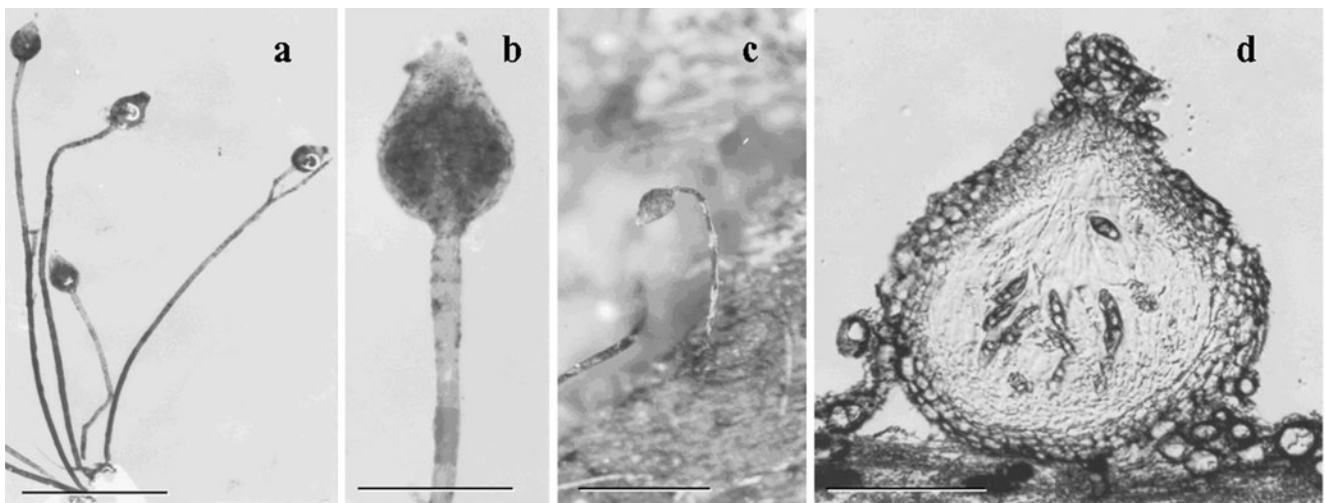


Fig. 7 *Jahnula* sp. **a, b** Ascomata with long stalk; **c** Long stalk on timber species of *Buchanania latifolia*; **d** Section of ascoma with short stalk. Scale bars a, c=200 μ m, b=100 μ m, d=150 μ m

clavate asci, brown to dark brown ascospores, 1-septate ascospores lacking a sheath or polar caps or appendages; that distinguish them from other *Jahnula*-like species.

A notable feature of the *Jahnulales* is the procession of stalked ascospores, with the stalks varying in length from 18–30×450–500 µm, to 1.6 mm. However, this is not a constant feature, as illustrated in Fig. 7 of an undescribed *Jahnula* species which has both sessile and stalked ascospores. Indeed it is interesting to speculate as to the function of these long stalks. Does it raise the ascospores from the substratum (Fig. 7c) above the water level for the discharge of ascospores, or to keep it clear from the debris on the surface of the wood?

Jahnula seychellensis and *J. systyla* possess dimorphic asci and ascospores, with one type larger than the other (*J. seychellensis*-Asci 1: 125–150×17–23 µm, Asci 2: 112–137×30–35 µm, Ascospores 1: 29–36×9–12.5 µm, Ascospores 2: 30–40×17–23 µm; *J. systyla*-Asci 1: 124–150×22–30 µm, Asci 2: 120–190×34–46 µm, Ascospores 1: 28–40×8–13 µm, Ascospores 2: 44–68×13–19 µm). Isolates were made of both types of ascospores in *J. seychellensis* and sequenced to determine if they were the same species. Sequence data confirmed that they are identical as shown in Fig. 1, sequence SS1536.1 has large spores, while SS1536.2 has smaller spores.

The *Jahnulales* are a unique group of ascomycetes with their stalked ascospores, variously appendaged ascospores, and wide vegetative hyphae, predominantly aquatic, tropical and growing on wood or palm fronds. However, little is known of their role in nature other than they are saprobes of submerged wood and palm fronds in aquatic habitats. Simonis et al. (2008) indicated that *Jahnula bipileata* is able to cause soft rot of wood and thus they may play a role in the decomposition of organic matter in freshwater and marine habitats. In a study of long term submergence of 9 timber test blocks in a stream and a peat swamp in Thailand, *Jahnula appendiculata* and *Xylomyces chlamydosporus* were recovered on eight of the timbers at the peat swamp site after a three year exposure period. A *Jahnula* sp. was only recovered on one test block. No *Jahnula* species was recovered on the test blocks in a fast flowing river at the Hala Bala Wildlife Sanctuary. The marine species *Manglicola guatemalensis* was known only from two previous collections (Hyde 1988; Kohlmeyer and Kohlmeyer 1971) until further collections were made on the brackish water palm *Nypa fruticans* (Suetrong et al. 2010). Ascospores are often covered in mud on the bases of fronds of the palm.

Key to the genera in the *Jahnulales*

1. Conidial genera.....2
 1. Ascospores with broad gelatinous sheaths.....4
 2. Ascospores with thin gelatinous sheath.....4
 3. Ascospores with arcicular crystals and broad, fusiform gelatinous sheath.....4
 4. Ascospores lacking arcicular crystals.....4
 5. Ascospores sheath small, constricted at the septum, with numerous median filamentous appendages.....4
 6. Ascospores sheath long and sigmoid, not constricted at the septum, with two short tapering appendages apically.....4
 7. Ascospores 30–40×9–20 µm.....4
 8. Ascospores 50–70×13–20 µm.....4
1. Ascospores with appendages, caps or gelatinous apical pads.....5
 1. Ascospore wall verruculose.....3
 2. Ascospore wall smooth.....4
 3. Ascospore 20–28×10–12 µm, wall minutely verruculose.....3
 4. Ascospores 23–28×11–13 µm, wall verruculose.....3
 5. Ascospores narrow, 6–8 µm.....4
 6. Ascospores wider than 8 µm.....5
 7. Ascospores 30–41×11–16 µm, cells equal in length.....5
 8. Ascospores 30–40×8–12 µm, apical cell larger.....5
2. Conidiophores and conidia lacking, chlamydosporous, intercalary, multiseptate.....*Xylomyces*

2. Conidiophores and conidia present.....3
3. Conidia tetra- or polyradiate with 4–8 appendages (arms).....3
3. Conidia catenate, branched.....*Speiropsis*
4. Ascospores larger than 1,150 µm, wide ostioles (100–200 µm), marine.....*Manglicola*
4. Ascospores smaller than 1,000 µm, ostioles *circa* smaller than 100 µm wide, not marine.....5
5. Ascospores with a thin or broad gelatinous sheath.....*Aliquandostipite*
5. Ascospores with apical caps, appendages, or lacking such structures.....6
6. Ascospores with longitudinal sulcate striations, lacking appendages or a gelatinous sheath.....*Megalohypha*
6. Ascospores lacking longitudinal sulcate striations.....*Jahnula*

Key to *Aliquandostipite* species

1. Ascospores with broad gelatinous sheaths.....2
1. Ascospores with thin gelatinous sheath.....*A. siamensis*
2. Ascospores with arcicular crystals and broad, fusiform gelatinous sheath.....3
2. Ascospores lacking arcicular crystals.....4
3. Ascospores sheath small, constricted at the septum, with numerous median filamentous appendages.....*A. minuta*
3. Ascospores sheath long and sigmoid, not constricted at the septum, with two short tapering appendages apically.....*A. crystallinus*
4. Ascospores 30–40×9–20 µm.....*A. separans*
4. Ascospores 50–70×13–20 µm.....*A. khaoyaiensis*

Key to *Jahnula* species

1. Ascospores with a sheath and polar appendages.....*J. appendiculata*
1. Ascospores lacking pads, appendages or a sheath.....2
 1. Ascospores with appendages, caps or gelatinous apical pads.....5
 2. Ascospore wall verruculose.....3
 3. Ascospore wall smooth.....4
 4. Ascospore 20–28×10–12 µm, wall minutely verruculose.....*J. sangamonensis*
 5. Ascospores 23–28×11–13 µm, wall verruculose.....*J. poonythii*
 6. Ascospores narrow, 6–8 µm.....*J. australiensis*
 7. Ascospores wider than 8 µm.....5
 8. Ascospores 30–41×11–16 µm, cells equal in length.....*J. aquatica*
 9. Ascospores 30–40×8–12 µm, apical cell larger.....*J. apiospora*
2. Ascospores with gelatinous apical pads.....7

6. Ascospores with a sheath.....10
 6. Ascospores 18–20×5–6.5 μm, with polar appendage
*J. morakotii*
 7. Ascomata dimorphic, ascospores 39–49×17–
 23 μm.....*J. seychellensis*
 7. Ascomata not dimorphic.....8
 8. Ascospores shorter than 30 μm.....*J. bipileata*
 8. Ascospores longer than 30 μm.....9
 9. Ascospores 42–52×16–33 μm.....*J. bipolaris*
 9. Ascospores 39–52×16–23 μm, helement
 shaped.....*J. sunyatsenii*
 10. Ascospores with thin sheaths.....11
 10. Ascospores with wide sheaths.....12
 11. Ascospores 26–37×15–18 μm, ascomata with
 short papilla.....*J. granulosa*
 11. Ascospores 32–45×12–15 μm, ascomata with a
 neck.....*J. rostrata*
 12. Ascospores 46–54×17–20 μm, sheath margin
 wavy.....*J. potamophila*
 12. Ascospores 44–68×13–19 μm, sheath margin not
 wavy.....*J. systyla*

Key to *Xylomyces* species

1. Width of chlamydo-spores at least 25 μm.....2
 1. Width of chlamydo-spores less than 20 μm.....5
 2. Chlamydo-spores with fewer than 15 septa, 110–
 455×25–45 μm.....*X. chlamydo-sporus*
 2. Chlamydo-spores with more than 15 septa.....3
 3. Chlamydo-spores with 6–26 septa, 140–575×25–
 50 μm.....*X. giganteus*
 3. Chlamydo-spores with 11–43 (68) septa, 95–370
 (–500) × (8–) 9–16 μm.....*X. rhizophorae*
 4. Chlamydo-spores with distinct punctuate wall orna-
 mentations, 44–74×10–16 μm.....*X. punctatus*
 4. Chlamydo-spores lack distinct punctuate wall orna-
 mentations.....5
 5. Chlamydo-spores 3–7 septate, 42–56×7–11 μm...
*X. pusillus*
 5. Chlamydo-spores 4–11 septate predominantly 11,
 126–152×7–9 μm.....*X. foliicola*

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