

# Towards a natural classification of *Annulatasceae*-like taxa: introducing *Atractosporales* ord. nov. and six new families

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**Abstract** Species with relatively small, membraneous, black ascomata, with or without long necks, unitunicate, cylindrical asci with apical rings and fusiform, hyaline ascospores with or without mucilaginous sheaths are common in freshwater habitats in tropical and temperate regions. Many of these taxa have originally been recorded as *Annulatasceae*-like taxa. Twenty genera have been included in the family *Annulatasceae*, mostly based on morphological characters, while molecular work and phylogenetic analyses are lacking for many genera. In this study, nine new *Annulatasceae*-like taxa collected from Thailand were morphologically examined. Pure cultures obtained from single ascospores were used in molecular studies. The nine new strains and several other strains of *Annulatasceae*-like Sordariomycetes species were used to establish phylogenetic and evolution relationships among the taxa, based on combined LSU, SSU, ITS and

RPB2 sequence data. Phylogenetic analyses provide evidence to introduce one new order and six new families, to accommodate taxa excluded from *Annulatasceae* sensu stricto. A new order *Atractosporales* is established based on the molecular study, including three new introduced families *Conlariaceae*, *Pseudoproboscisporaceae* and *Atractosporaceae*. *Conlariaceae* is introduced for the genus *Conlarium* which comprises two species, *Conlarium duplumascosporum* and a new Hyphomycetous asexual morph taxon *Conlarium aquaticum* which has subglobose or irregular, brown, clathrate, muriform conidia. *Pseudoproboscisporaceae* includes *Pseudoproboscispora* and *Diluvicola*, while *Atractosporaceae* includes the genera *Rubellisphaeria* and *Atractospora*. *Barbatosphaeria*, *Xylomelasma* and *Ceratostomella* form a distinct stable lineage which is introduced as a new family *Barbatosphaeriaceae* in Diaporthomycetidae families *incertae sedis*. A new family *Lentomitellaceae* is introduced in Diaporthomycetidae families *incertae sedis*, to accommodate the genus *Lentomitella*. *Woswasiaceae* is introduced to accommodate *Woswasia*, *Xylochrysis* and *Cyanoannulus* in Diaporthomycetidae families *incertae sedis*. Three new species of *Fluminicola* viz. *F. saprophytica*, *F. thailandensis* and *F. aquatica* are introduced. A new sexual morph, *Dictyosporella thailandensis*, is reported and *Dictyosporella* is excluded from *Annulatasceae* and placed in Diaporthomycetidae genera *incertae sedis*. The first sexual morph of *Sporidesmium*, *S. thailandense* is also described. The new species *Atractospora thailandensis*, *Diluvicola aquatica* and *Pseudoproboscispora thailandensis* are also introduced. *Platytrachelon* is added to *Papulosaceae* based on phylogenetic analysis and morphological characters. *Aquaticola*, *Fusoidispora* and *Pseudoannulatasceus* are excluded from *Annulatasceae* and placed in Diaporthomycetidae genera *incertae sedis*.

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*Mirannulata* is accommodated in Sordariomycetes, genera *incertae sedis*.

**Keywords** Annulatascales · Diaporthomycetidae · Phylogeny · Sexual morphs · Submerged wood

## Introduction

The majority of species from the family *Annulatasceae* and *Annulatasceae*-like taxa are recorded from freshwater habitats in tropical and temperate areas (Goh and Hyde 1996; Hyde and Goh 1998; Tsui et al. 2000; Ho et al. 2001; Cai et al. 2002a, 2003; Shearer and Raja 2010). *Annulatasceae* and *Annulatasceae*-like species appear to be predominantly saprobic on submerged wood, but individual species are also known on dead petioles of palms, submerged stems of grasses and decaying bamboo (Fröhlich and Hyde 2000; Cai et al. 2002b; Abdel-Wahab et al. 2011; Boonyuen et al. 2012).

The genus *Annulatascus* was introduced based on two tropical freshwater taxa, *A. velatispora* K.D. Hyde as the type species, and *A. bipolaris* K.D. Hyde, both of which occurred on submerged wood in northern Queensland, Australia (Hyde 1992; Dayarathne et al. 2016). The genus was originally described as representing taxa having black ascomata with long necks, unitunicate, cylindrical asci with massive, refractive, apical rings and fusiform, hyaline, aseptate ascospores with or without mucilaginous sheaths (Hyde 1992). Later, *A. bipolaris* was transferred to *Catactispora* as *C. bipolaris* (K.D. Hyde) K.D. Hyde (Hyde et al. 1999), because ascospores have a polar pad which unfurl to form long threads in water, a unique and contrasting feature from those in *Annulatascus*. However, this transfer has not yet been supported by molecular data.

A key to species and a comparative table of morphological features of *Annulatascus* was provided by Boonyuen et al. (2012) and Luo et al. (2015). Placement of species in this genus based solely on the relatively massive apical ascus ring is not very reliable and molecular data has demonstrated that *Annulatascus* is polyphyletic (Raja et al. 2003; Campbell and Shearer 2004; Abdel-Wahab et al. 2011; Luo et al. 2015). Phylogenetic analyses in Campbell and Shearer (2004) demonstrated that *A. triseptatus* S.W. Wong, K.D. Hyde & E.B.G. Jones clustered in a distinct clade which is distantly related to *A. velatispora*. Morphologically, the ascomata of *A. triseptatus* are oblate, roughened, and with short setose-like hyphae, a doughnut-shaped ascus apical ring with a subapical flange that is not visible in glycerin, unlike the globose or subglobose, glabrous ascomata and spherical ascus apical ring without a subapical flange in *A. velatispora*. A new genus

*Annulusmagnus* J. Campb. & Shearer was established for *Annulatascus triseptatus* based on morphological characters and molecular data. Luo et al. (2015) introduced *Pseudoannulatascus* Z.L. Luo, Maharachch. & K.D. Hyde for *Annulatascus biatriisporus* K.D. Hyde. *Pseudoannulatascus biatriisporus* (K.D. Hyde) Z.L. Luo, Maharachch. & K.D. Hyde differs from *Annulatascus* species in the size of asci and ascospores, the latter which have swollen ends (Hyde 1995; Boonyuen et al. 2012; Luo et al. 2015).

*Annulatascus* was first placed in *Lasiosphaeriaceae* Nannf. (Hyde 1992), then transferred to *Annulatasceae* S.W. Wong, K.D. Hyde & E.B.G. Jones with the help of ultrastructural data (sensu Barr 1990; Wong et al. 1998). One of the most distinct morphological characters of *Annulatasceae* is asci with a relatively massive, refractive, wedge-shaped, J-, apical ring (Wong et al. 1998). Ultrastructural studies on *Annulatasceae*-like taxa using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) have been published (Hyde et al. 1998, 1999; Wong et al. 1999a, b; Wong and Hyde 1999). *Diluviicola* K.D. Hyde, S.W. Wong and E.B.G. Jones is similar to *Annulatascus*, *Pseudoproboscispora* Punith. and *Rivulicola* K.D. Hyde in having a relatively massive, refractive, apical rings, however, the general structure of the apical rings is different at the TEM level (Hyde et al. 1997, 1998; Wong et al. 1999a; Wong and Hyde 1999; Boonyuen et al. 2012). Hyde et al. (1998) provided a comparative table of three *Annulatasceae*-like taxa, including *Annulatascus bipolaris*, *Diluviicola capensis* K.D. Hyde, S.W. Wong & E.B.G. Jones and *Proboscispora aquatica* S.W. Wong & K.D. Hyde, which have unfurling polar appendages at the light and ultrastructural microscope levels. The unique appendage unfurling mechanism and ontogeny were used to separate these three *Annulatasceae*-like taxa. *Fluminicola* S.W. Wong, K.D. Hyde & E.B.G. Jones was introduced to accommodate *Annulatasceae*-like taxa with ascospores having a unique appendage form which are addressed to the ascospore tip and then curled backwards to form an irregular bifurcate or cup-like structure after release in water (Wong et al. 1999b). This unfurling mechanism and ontogeny of appendages can also be used to distinguish *Fluminicola* from other similar genera. Although ultrastructural studies can separate the above genera, they all have relatively massive apical rings and are saprobic on submerged wood in freshwater. Ho and Hyde (2000) concluded that ultrastructural studies supported the description of new genera after reexamining several species in *Annulatasceae* at the ultrastructural level, combining the molecular study of Ranghoo et al. (1999).

Ranghoo et al. (1999) initially reported that *Annulatasceae* was a distinct family, using LSU rDNA molecular data. Ho and Hyde (2000) concluded that more sequences

of *Annulatasceae*-like taxa are needed in phylogenetic analyses to strengthen the data. Based on analyses of sequence data, the placements of some *Annulatasceae*-like taxa have been changed. *Ceriospora caudae-suis* Ingold was introduced by Ingold (1951) who commented that the length and coiling of the ascospore appendages made it different from all other species in the genus. Fallah and Shearer (2001) further described this species, *C. caudae-suis*, where the ellipsoidal ascospores were overlappingly uni-seriate in the cylindrical asci, which had a J-, apical ring, whereas *C. dubyi* Niessl, the type species of this genus, has fusiform ascospores arranged overlappingly bi- and triseriate in cylindrical-clavate asci, and ascus with a J + , and plug-like apical ring. A phylogenetic analyses carried out by Campbell et al. (2003) showed that *C. caudae-suis* formed a sister clade with *Pseudoproboscispora* sp. with strong support and shared similar morphological characters. Thus, Campbell et al. (2003) transferred *C. caudae-suis* to *Pseudoproboscispora caudae-suis* (Ingold) J. Campb., Shearer, J.L. Crane & Fallah. *Aquaticola ellipsoidea* W.H. Ho, K.M. Tsui, Hodgkiss & K.D. Hyde was transferred to *Atractospora ellipsoidea* (W.H. Ho, K.M. Tsui, Hodgkiss & K.D. Hyde.) Réblová & J. Fourn. based on its ascomata lying horizontally to the host surface, long-pedicellate asci and fusiform ascospores. These characters are typical of the genus *Atractospora* Réblová & J. Fourn., and *Aquaticola ellipsoidea* nested in the *Atractospora* clade with strong support (Réblová et al. 2016). Based on differences in morphology between *Ascolacicola austriaca* Réblová, Winka & Jaklitsch and *A. aquatica* Ranghoo & K.D. Hyde, the type species of this genus, the former formed a sister clade with the genus *Annulusmagnus* J. Campb. & Shearer, and a new genus *Ascitendus* J. Campb. & Shearer was established (Campbell and Shearer 2004).

It has also been demonstrated using molecular data that several *Annulatasceae*-like genera do not belong in the family *Annulatasceae*. *Brunneospora* Ranghoo & K.D. Hyde and *Fluminicola* S.W. Wong, K.D. Hyde & E.B.G. Jones were placed in *Annulatasceae* due to their cylindrical asci with a large apical ring (Wong et al. 1999b; Ranghoo et al. 2001). However, they clustered with *Papulosa* Kohlm. & Volkm.-Kohlm. (Abdel-Wahab et al. 2011) in the molecular analysis and were placed in *Papulosa* Winka & O.E. Erikss. by Maharachchikumbura et al. (2015).

A latest morphology based key of genera which belong to or are referred to the family *Annulatasceae* was provided by Maharachchikumbura et al. (2016). A comprehensive description of *Annulatasceae* was also provided, as “ascomata pale brown to black, or reddish-brown, immersed, semi-immersed, erumpent or superficial, with black or hyaline necks which rarely with setae, hyphae or

hairs; peridium coriaceous or membranous, composed of variable cells; asci cylindrical, usually with a massive, refractive, discoid or wedge-shaped, J-, apical ring; ascospores hyaline to brown, aseptate to septate, with or without appendages, sheaths and germ pores” and with a *Taeniolella*-like asexual morph and mostly saprobic on submerged wood, rarely on bamboo or other substrates in terrestrial habitats (Maharachchikumbura et al. 2016).

A *Taeniolella*-like asexual morph, was reported in *Annulatasceae* from a culture of *Chaetorostrum quincemilense* Zelski, Raja, A.N. Mill. & Shearer (Zelski et al. 2011). With use of molecular technique, asexual genera can be confidently linked to *Annulatasceae* and their sexual morphs (Shenoy et al. 2007, 2010). Ariyawansa et al. (2015) introduced *Dictyospora* Abdel-Aziz as an asexual genus in the family *Annulatasceae*, but this taxon clustered distantly with other members of *Annulatasceae*. Its morphological characters are similar to those of *Conlarium* F. Liu & L. Cai, however, the two genera are distantly separated and *Dictyospora* formed a weakly-supported clade with the sexual genus *Cyanoannulus* Raja, J. Campb. & Shearer (Ariyawansa et al. 2015).

Although most *Annulatasceae*-like taxa clustered together as a distinct clade and separated from other families in earlier studies (Réblová 2006, 2013; Réblová et al. 2014), as more and more taxa were included in phylogenetic analyses, *Annulatasceae* was revealed to be polyphyletic (Vijaykrishna et al. 2005, 2006). In a phylogenetic tree in Luo et al. (2015), five genera were placed in *Annulatasceae* sensu stricto and ten genera were placed in *Annulatasceae* sensu lato. Maharachchikumbura et al. (2016) concluded that 20 genera belong to or should be referred to the family *Annulatasceae*. *Annulatasceae* remains a heterogeneous assemblage of many genera representing different morphotypes and their phylogenetic positions are not well-studied and natural placements being doubtful due to dearth of DNA sequence data. Thus, new morphological and molecular data are required to clarify their affinities and solve the polyphyly of this family (Maharachchikumbura et al. 2016).

We are studying freshwater fungi along a north/south latitudinal gradient in the Asian region (Hyde et al. 2016). During an ongoing study on these microfungi in Thailand, we encountered several morphologically similar specimens of freshwater Sordariomycetes which are mostly saprobic on submerged bamboo, and were superficially identified as *Annulatasceae*-like species. In this paper, we use nine new strains of *Annulatasceae*-like taxa and other strains in Sordariomycetes to establish phylogenetic and evolutionary trees. One new order, six new families and nine new species are introduced to accommodate taxa excluded from *Annulatasceae* sensu stricto.

## Materials and methods

### Specimen examination

Decaying wood was collected from Prachuap Khiri Khan, a small river in Thailand in July 2015, following the procedures described in Kurniawati et al. (2010). Samples were placed in Ziploc plastic bags with sterile moist tissue paper and taken to the laboratory. The samples were processed and examined following the methods of Taylor and Hyde (2003). Specimens were examined under a Nikon SMZ-171 dissecting microscope to locate fruiting bodies. Photomicrographs were made under a Nikon ECLIPSE Ni compound microscope with a Cannon EOS 600D camera and an OLYMPUS BX53 compound microscope with a MicroPublisher 5.0 RTV camera. India ink was used for revealing gelatinous sheaths of the ascospores. The fungal structures were measured using Tarosoft (R) Image Frame Work program and images were processed with Adobe Photoshop CS5 Extended version 12.0 × 32 software (Adobe Systems, USA). Isolations were made from single ascospores or conidia as detailed in Chomnunti et al. (2014), on 2% water agar (WA) and then incubated overnight at room temperature or in an incubator (25 °C). Colonies were examined and recorded every seven days. Herbarium specimens are deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (HKAS), Kunming, China. Living cultures are deposited in the Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Institute of Botany Culture Collection (KUMCC). Facesoffungi and Index Fungorum numbers are registered as in Jayasiri et al. (2015) and Index Fungorum (2017).

### DNA extraction, PCR amplification and sequencing

All cultures were grown on PDA at 25 °C until enough mycelium was obtained and a Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, P.R. China) was used to extract total genomic DNA from the fresh mycelium in terms of instructions of manufacturer. DNA amplification was performed by polymerase chain reaction (PCR). For nucleotide sequence comparisons, fragments of four loci were analysed: LSU, SSU, ITS and RNA polymerase II subunit 2 (RPB2). Primer pairs LROR/LR5 for LSU, NS1/NS4 for SSU, ITS5/ITS4 for ITS and fRPB2-5f/fRPB2-7cR for RPB2 were utilized to amplify. The amplifications were carried out in a 25 µL reaction volume containing 9.5 µL ddH<sub>2</sub>O, 12.5 µL 2 × PCR Master Mix, 1 µL of DNA template, 1 µL of each primer (10 µM). The PCR thermal cycles for the

amplification of the gene regions were as described in Réblová et al. (2011). The PCR products were viewed on 1% agarose electrophoresis gels stained with ethidium bromide. Purification and sequencing of PCR products of four loci were carried out by Shanghai Sangon Biological Engineering Technology & Services Co. Shanghai, P.R. China. DNASTAR Lasergene SeqMan Pro v. 8.1.3 was used to obtain consensus sequences from sequences generated from forward and reverse primers.

### Phylogenetic analyses

Sequences generated in this study (Table 1) were supplemented with additional sequences obtained from GenBank, based on blast searches and the literature. Multiple sequence alignments were generated with MAFFT v. 7 (<http://mafft.cbrc.jp/alignment/server/index.html>); the alignments were visually improved with Mesquite v. 2.75 (Maddison and Maddison 2011) and MEGA v. 5.2.2 (Kumar et al. 2012). Phylogenetic analyses of the combined aligned dataset consisted of maximum likelihood (ML), Bayesian Inference (BI) and maximum parsimony (MP) analyses. Ambiguously aligned regions were excluded from all analyses and gaps were treated as “missing data” in the parsimony analyses. A ML analysis was performed using raxmlGUI v. 1.3 (Silvestro and Michalak 2011). The optimal ML tree search was conducted with 1000 separate runs, using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTR + GAMMA substitution model. Suitable models for the Bayesian analyses were first selected using models of nucleotide substitution for each gene, as determined using MrModeltest v. 2.2 (Nylander 2004), and included for each gene partition. The Bayesian analyses (MrBayes v. 3.2.1; Ronquist et al. 2012) of four simultaneous Markov Chain Monte Carlo (MCMC) chains were run from random trees for 10,000,000 generations and sampled every 1000 generations. The temperature value was lowered to 0.15, burn-in was set to 0.25, and the run was automatically stopped as soon as the average standard deviation of split frequencies reached less than 0.01. The MP analysis was performed with PAUP v.4.0b10 (Swofford 2003). Trees were inferred by using the heuristic search option with TBR branch swapping and 1000 random sequence additions. The maximum number of retained trees was limited to 5000, branches of zero length were collapsed and all multiple equally most parsimonious trees were saved. Tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), homoplasy index (HI), and log likelihood (−ln L) (HKY model) values were calculated. The robustness of the

**Table 1** Newly generated species and sequences database accession numbers used in this study

| Taxon                                     | Culture code | GenBank accession no. |          |          |          |
|---|--------------|-----------------------|----------|----------|----------|
|   |              | LSU                   | SSU      | ITS      | RPB2     |
| <i>Atractospora thailandensis</i>         | HKAS 96226   | MF374362              | MF374371 | MF374353 | MF370951 |
| <i>Conlarium aquaticum</i>                | MFLU 15-2703 | MF374363              | MF374372 | MF374354 | NS       |
| <i>Dictyosporella thailandensis</i>       | MFLU 15-2706 | MF374364              | MF374373 | MF374355 | MF370952 |
| <i>Diluvicola aquatica</i>                | MFLU 15-2701 | MF374365              | MF374373 | MF374356 | MF370953 |
| <i>Fluminicola aquatica</i>               | MFLU 15-2710 | MF374366              | MF374374 | MF374357 | NS       |
| <i>Fluminicola saprophytica</i>           | MFLU 15-2694 | MF374367              | MF374375 | MF374358 | MF370954 |
| <i>Fluminicola thailandensis</i>          | MFLU 15-2704 | MF374368              | MF374376 | MF374359 | NS       |
| <i>Pseudoproboscisporea thailandensis</i> | MFLU 15-2705 | MF374369              | MF374377 | MF374360 | NS       |
| <i>Sporidesmium thailandense</i>          | MFLU 15-2709 | MF374370              | NS       | MF374361 | MF370955 |

NS no sequence

equally most parsimonious trees was evaluated by 1000 bootstrap replications (Felsenstein 1985) resulting from a maximum parsimony analysis, each with 10 replicates of random stepwise addition of taxa. The Kishino–Hasegawa tests (Kishino and Hasegawa 1989) were performed to determine whether the trees were significantly different. The resulting trees were printed with FigTree v. 1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>) and the layout was completed with Adobe Illustrator CS v. 6.

The aligned datasets from phylogenetic analyses was used in molecular dating analysis. The analysis was performed using BEAST package 1.8.0 (Drummond et al. 2012). The aligned data was partitioned for each LSU, SSU, ITS and RPB2 dataset and these were loaded to BEAUti 1.8.0. for setting up prior parameters and preparing the XML file. Unlinked substitution and clock models were selected to independently estimate for each gene partition. Taxa sets were developed for each calibration of the common ancestor nodes, associated with the most recent common ancestor, TMRCA. Since different researches have used different prior parameters and calibrations points which can affect the analysis (Gueidan et al. 2011; Prieto and Wedin 2013; Beimforde et al. 2014; Hongsanan et al. 2016; Pérez-Ortega et al. 2016; Hyde et al. 2017), thus we used the divergence time estimates from the recent paper (Hyde et al. 2017) as the reference to decide calibration points used in this study. The most recent common ancestor of Sordariomycetes and Leotiomycetes was set follow Hyde et al. (2017) using normal distribution (mean = 295, SD = 45, with 97.5% CI of 383MYA), and the Sordariomycetes crown with a normal distribution (mean = 250, SD = 45, with 97.5% of CI = 338 MYA). *Paleoophiocordyceps coccophagus* Sung et al. was used as fossil calibration of *Hypocreales*, because it is morphologically similar to *Ophiocordyceps* (Sung et al. 2008; Samarakoon et al. 2016; Hyde et al. 2017). Thus, this fossil

data was used for calibration of the *Ophiocordyceps* crown in Samarakoon et al. (2016) and Hyde et al. (2017), with an exponential distribution (offset = 100, mean = 27.5, with 97.5% CI of 200 Mya). jModeltest 2.1.1 were used to find the best model for each gene partition; GTR + GAMMA for LSU, ITS and RPB2, TrNef + I+G for SSU. We used TN93 with setting ‘‘All Equal’’ for the base frequencies to replace TrNef + I+G. The Lognormal distribution of rates with uncorrelated relaxed clockmodel (ucl) was selected for the analyses. We used the Yule process tree prior to model the speciation of nodes in the topology with a randomly generated starting tree. The number of generations was set with 100 million generations, sampling parameters every 1000 generations. After the XML file was generated from BEAUti 1.8.0, the file was loaded and run in BEAST 1.8.0. The effective sample sizes were checked by loaded.log file into Tracer v.1.6, the acceptable values are higher than 200. The first 20,000 trees representing the burn-in phase suggested by Tracer v.1.6 were discarded, thus 80,000 trees were used in LogCombiner 1.8.0. The maximum clade credibility (MCC) tree was generated by abalysing the file from LogCombiner in TreeAnnotator 1.8.0. The molecular dating tree was viewed in FigTree (Rambaut 2006).

## Results

### Phylogenetic study

Recent familial classifications of Sordariomycetes provided by Maharachchikumbura et al. (2016) and updated in Hongsanan et al. (2017) are followed here. The placement of taxa at the family level were analyzed using LSU, SSU, ITS rDNA and protein coding RPB 2 sequence data, with *Leotia lubrica* (Scop.) Pers. as the outgroup taxon (Fig. 1).

The alignment comprised 124 strains (including the out-group taxon *Leotia lubrica*) with an alignment length of 4511 characters. With parsimony analysis, 1871 characters were constant and 1857 parsimony informative. The parsimony analysis of the data matrix resulted in seven equally parsimonious trees and the first tree (TL = 15,393, CI = 0.297, RI = 0.470, HI = 0.703, RC = 0.140) is shown here (Fig. 1). The GTR + G model with gamma-distributed rate for LSU and the GTR + I+G model with inverse gamma-distributed rate for SSU, RPB2 and ITS were selected and included for each gene partition in Bayesian analyses. Both ML, MP and BI analyses generated trees with similar topologies.

The Maximum clade credibility tree of *Annulatasceae* obtained from BEAST software has a topology which differs little from the topology of the phylogenetic tree. The differences could be found in the genus *Dictyosporella* which is presented in the clade of *Atractosporales*, while the genus is close to *Distoseptisporaceae* in phylogenetic tree. *Aquaticola hyalomura* strain R038 is closely related to *Bullimyces communis* strain AF281-3 and *Ceratolenta caudata* strain CBS 125234 with high PP support in the MCC tree; however, these have low support in phylogenetic tree. The members of *Atractosporaceae*, *Barbatosphaeriaceae* and *Woswasiaceae* formed as distinct clades with high PP support in the MCC tree. Hongsanan et al. (2017) and Hyde et al. (2017) recommended the ranking for families in Sordariomycetes at ca 50–150 MYA. *Atractosporaceae* has a stem age at ca 98 MYA and *Barbatosphaeriaceae* has stem age at ca 110 MYA, and *Woswasiaceae* has stem age at ca 115 MYA which support their family status. *Atractosporales* has low PP support in the MCC tree. Hyde et al. (2017) recommend the ranking for ordinal status as 130–250 MYA. Although, the stem age of *Atractosporales* is ca 114 MYA which does not support its ordinal status, the introduction is based on morphological characters.

One new order and six new families are introduced. Nine new strains of *Annulatasceae*-like taxa were added, including eight sexual morphs and one asexual morph, which are introduced as new species. Table 2 denotes the main changes.

Species of *Sporidesmiaceae* form a weak clade (Figs. 1, 2) as in Su et al. (2016), and its sister group is not stable. It is sister to the clade comprising of *Mirannulata*, *Dictyosporella* species and *Distoseptisporaceae* in the phylogenetic tree (Fig. 1) and *Papulosaceae* in the evolution tree (Fig. 2). Therefore, we maintain *Sporidesmiaceae* in Diaportheomycetidae, families *incertae sedis* as in Maharachchikumbura et al. (2016). The sexual morph has never been linked by molecular analysis. In this study, we report the first sexual morph for *Sporidesmium* sensu stricto.

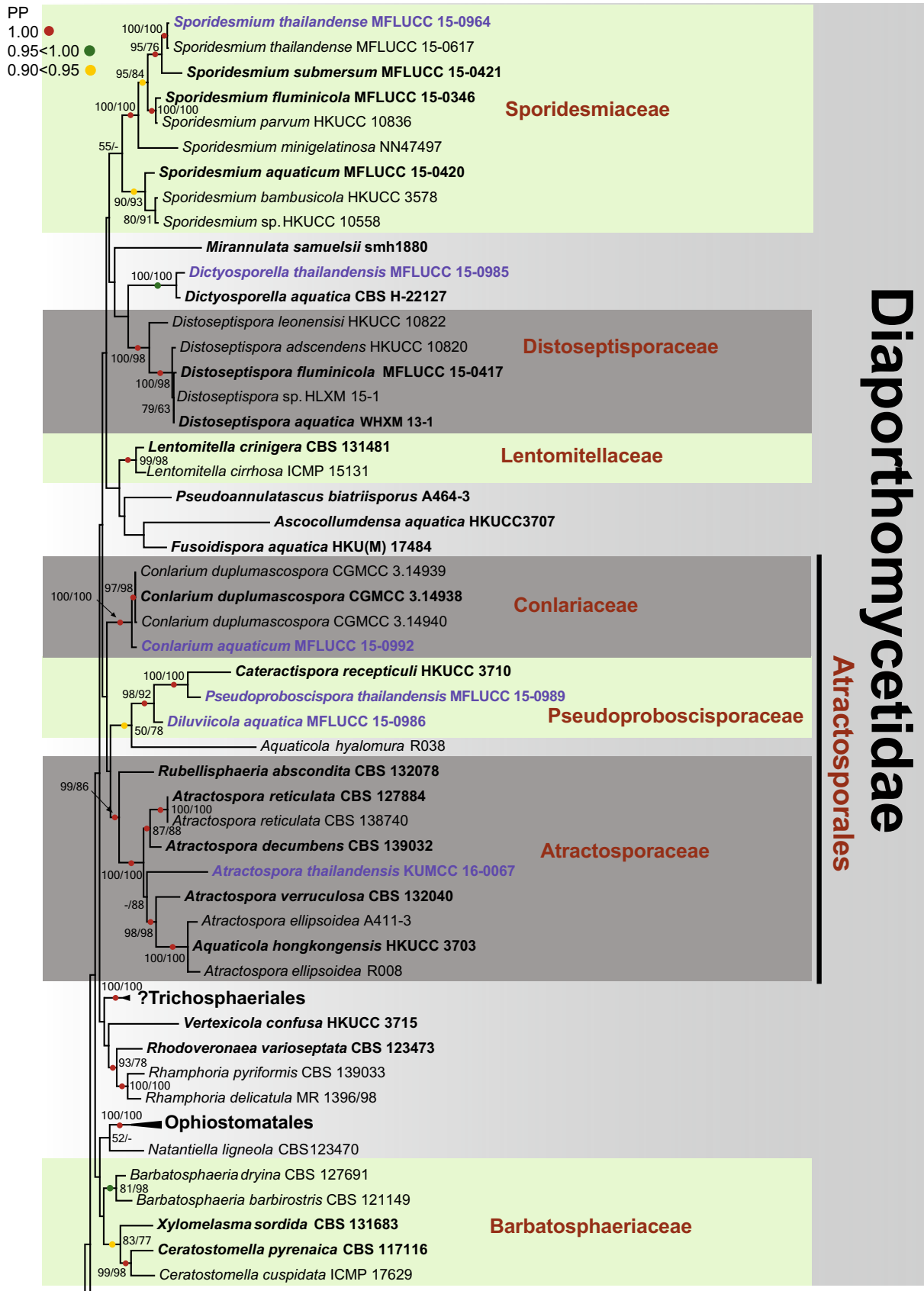
**Fig. 1** Maximum likelihood (ML) majority rule consensus tree for the combined LSU, SSU, ITS and RPB2 sequence alignment of Diaportheomycetidae and related taxa belonging to Sordariomycetes. RAxML bootstrap support values (ML), maximum parsimony bootstrap support values (MP) are given at the nodes (ML/MP) and Bayesian posterior probabilities (PP) marked with circles. The orders *Myrmecridiales*, *Phomatosporales*, *Diaporthales*, *Togniniales*, *Calosphaeriales*, *Magnaporthales*, *Sordariales*, *Boliniales*, *Chaetosphaeriales*, *Xylariales*, *Amplistromatales*, *Trichosphaeriales* and *Ophiostomatales* are compressed. Types are in bold and newly introduced sequence data are in violet. The tree is rooted to *Leotia lubrica*

*Mirannulata samuelsii* has a sister relationship to different groups in Diaportheomycetidae *Distoseptisporaceae* in the phylogenetic tree (Fig. 1) and clade comprising of *Calosphaeriales* *Togniniales* and *Diaporthales* in the evolution tree (Fig. 2). However, it was shown sister to *Lautospora* (Jones et al. 2015) which was placed in *Lautosporaceae*, Sordariomycetes, families *incertae sedis* in later study (Maharachchikumbura et al. 2016). Thus, we suggest place *Mirannulata* in Sordariomycetes, genera *incertae sedis*.

*Dictyosporella* is sister to different groups in Diaportheomycetidae: *Distoseptisporaceae* in the phylogenetic tree (Fig. 1) and the new family *Pseudoproboscisporaceae* in the evolution tree (Fig. 2). *Dictyosporella* is characterized by (1) immersed ascomata with subhyaline erumpent necks, (2) unitunicate, long cylindrical asci with sharply tapered pedicels and a small, unrefractive, wedge-shaped, J-apical ring, (3) uni-seriate, hyaline, fusiform, 3-septate ascospores with sheaths and bipolar appendages, and helicoid, brown to black, globose or subglobose conidia with holoblastic conidiogenous cells. According to phylogenetic result, a new sexual morph is introduced and *Dictyosporella* is moved from *Annulatasceae* to Diaportheomycetidae, genera *incertae sedis*.

A new family *Lentomitellaceae* is introduced in Diaportheomycetidae, families *incertae sedis*, to accommodate the genus *Lentomitella* which has dark ascomata with long or short necks, cylindrical, short-pedicellate or sessile asci with a distinct, refractive, apical ring and mostly hyaline, 1–2-seriate mostly aseptate ascospores. In this study, *Ascocollumdensa* clusters with the family *Lentomitellaceae* in the phylogenetic analysis (Fig. 1), but the species clusters at base of the evolution tree (Fig. 2). Thus, it is removed from *Lentomitellaceae*. Although the genera *Pseudoannulatascus* and *Fusoidispora* form a sister clade with *Lentomitellaceae* in the phylogenetic tree (Fig. 1), we suggest to place them in Diaportheomycetidae genera *incertae sedis*.

A new order *Atractosporales* is established based on the molecular study, for three new families *Conlariaceae*, *Pseudoproboscisporaceae* and *Atractosporaceae*, which forms a distinct group of aquatic Sordariomycetes. Dark



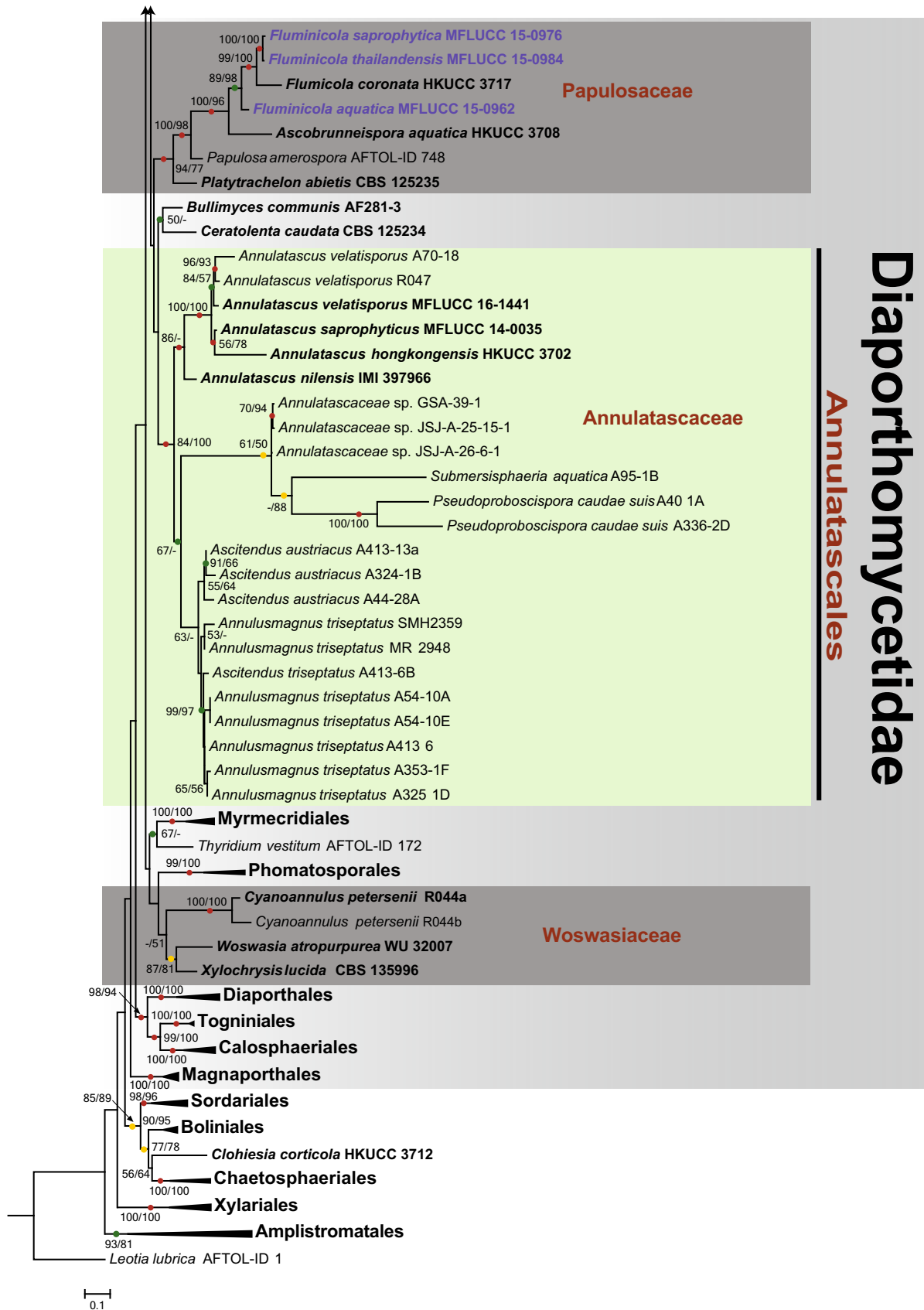


Fig. 1 continued



**Table 2** Suggested classification of *Annulatasceae*-like taxa based on the updated phylogenetic tree and divergence times

| Genera   | Position in Maharachchikumbura et al. (2016)                                      | Position in this study   | New species   |
|--|---|--|---|
| <i>Annulatascus</i>                              | <i>Annulatasceae</i> ,<br><i>Annulatascales</i>                                   | <i>Annulatasceae</i> , <i>Annulatascales</i>   |   |
| <i>Annulismagnus</i>                             | <i>Annulatasceae</i> ,<br><i>Annulatascales</i>                                   | <i>Annulatasceae</i> , <i>Annulatascales</i>   |   |
| <i>Aquaticola</i>                                | <i>Annulatasceae</i> ,<br><i>Annulatascales</i>                                   | <i>Diaporthomycetidae</i> genera <i>incertae sedis</i>   |   |
| <i>Ascitendus</i>                                | <i>Annulatasceae</i> ,<br><i>Annulatascales</i>                                   | <i>Annulatasceae</i> , <i>Annulatascales</i>   |   |
| <i>Ascobrunneispora</i> = <i>Brunneosporella</i> | <i>Papulosaceae</i> ,<br><i>Diaporthomycetidae</i> families <i>incertae sedis</i> | <i>Papulosaceae</i> , <i>Diaporthomycetidae</i> families <i>incertae sedis</i>                           |   |
| <i>Atractospora</i>                              | <i>Sordariomycetidae</i> , genera <i>incertae sedis</i>                           | <b><i>Atractosporaceae</i></b> fam. nov.,<br><b><i>Atractosporales</i></b> ord. nov.                     | <b><i>Atractospora thailandensis</i></b>  |
| <i>Barbatosphaeria</i>                           | <i>Sordariomycetidae</i> , genera <i>incertae sedis</i>                           | <b><i>Barbatosphaeriaceae</i></b> fam. nov.,<br><i>Diaporthomycetidae</i> families <i>incertae sedis</i> |   |
| <i>Ceratostomella</i>                            | <i>Sordariomycetidae</i> , genera <i>incertae sedis</i>                           | <b><i>Barbatosphaeriaceae</i></b> fam. nov.,<br><i>Diaporthomycetidae</i> families <i>incertae sedis</i> |   |
| <i>Conlarium</i>                                 | <i>Sordariomycetidae</i> , genera <i>incertae sedis</i>                           | <b><i>Conlariaceae</i></b> fam. nov.,<br><b><i>Atractosporales</i></b> ord. nov.                         | <b><i>Conlarium aquaticum</i></b>   |
| <i>Cyanoannulus</i>                              | <i>Annulatasceae</i> ,<br><i>Annulatascales</i>                                   | <b><i>Woswasiaceae</i></b> fam. nov.,<br><i>Diaporthomycetidae</i> families <i>incertae sedis</i>        |   |
| <i>Dictyosporella</i>                            | <i>Annulatasceae</i> ,<br><i>Annulatascales</i>                                   | <i>Diaporthomycetidae</i> genera <i>incertae sedis</i>   | <b><i>Dictyosporella thailandensis</i></b>  |
| <i>Diluvicola</i>                                | <i>Annulatasceae</i> ,<br><i>Annulatascales</i>                                   | <b><i>Pseudoproboscisporaceae</i></b> fam. nov.,<br><b><i>Atractosporales</i></b> ord. nov.              | <b><i>Diluvicola aquatica</i></b>   |
| <i>Fluminicola</i>                               | <i>Papulosaceae</i> ,<br><i>Diaporthomycetidae</i> families <i>incertae sedis</i> | <i>Papulosaceae</i> , <i>Diaporthomycetidae</i> families <i>incertae sedis</i>                           | <b><i>Fluminicola aquatica</i></b><br><b><i>Fluminicola saprophytica</i></b><br><b><i>Fluminicola thailandensis</i></b> |
| <i>Fusoidispora</i>                              | <i>Annulatasceae</i> ,<br><i>Annulatascales</i>                                   | <i>Diaporthomycetidae</i> genera <i>incertae sedis</i>   |   |
| <i>Lentomitella</i>                              | <i>Sordariomycetidae</i> , genera <i>incertae sedis</i>                           | <b><i>Lentomitellaceae</i></b> fam. nov.,<br><i>Diaporthomycetidae</i> families <i>incertae sedis</i>    |   |
| <i>Mirannulata</i>                               | <i>Sordariomycetidae</i> , genera <i>incertae sedis</i>                           | <i>Sordariomycetes</i> , genera <i>incertae sedis</i>  |   |
| <i>Natantiella</i>                               | <i>Sordariomycetes</i> , genera <i>incertae sedis</i>                             | <i>Sordariomycetes</i> , genera <i>incertae sedis</i>  |   |
| <i>Papulosa</i>                                  | <i>Papulosaceae</i> ,<br><i>Diaporthomycetidae</i> families <i>incertae sedis</i> | <i>Papulosaceae</i> , <i>Diaporthomycetidae</i> families <i>incertae sedis</i>                           |   |
| <i>Platytrachelon</i>                            | <i>Diaporthomycetidae</i> genera <i>incertae sedis</i>                            | <i>Papulosaceae</i> , <i>Diaporthomycetidae</i> families <i>incertae sedis</i>                           |   |
| <i>Pseudoannulatascus</i>                        | <i>Annulatasceae</i> ,<br><i>Annulatascales</i>                                   | <i>Diaporthomycetidae</i> genera <i>incertae sedis</i>   |   |
| <i>Pseudoproboscispora</i>                       | <i>Annulatasceae</i> ,<br><i>Annulatascales</i>                                   | <b><i>Pseudoproboscisporaceae</i></b> fam. nov.,<br><b><i>Atractosporales</i></b> ord. nov.              | <b><i>Pseudoproboscispora thailandensis</i></b>   |
| <i>Rhamphoria</i>                                | <i>Annulatasceae</i> ,<br><i>Annulatascales</i>                                   | <i>Annulatasceae</i> , <i>Annulatascales</i>   |   |

**Table 2** continued

| Genera                  | Position in Maharachchikumbura et al. (2016)   | Position in this study   | New species                             |
|-------------------------|--|--|---|
| <i>Rhodoveronaea</i>    | <i>Sordariomycetidae</i> , genera <i>incertae sedis</i>                              | <i>Sordariomycetidae</i> , genera <i>incertae sedis</i>  |   |
| <i>Rubellisphaeria</i>  | <i>Sordariomycetidae</i> , genera <i>incertae sedis</i>                              | <b><i>Atractosporaceae</i></b> fam. nov.,<br><b><i>Atractosporales</i></b> ord. nov.                     |   |
| <i>Sporidesmium</i>     | <i>Sporidesmiaceae</i> ,<br><i>Diaporthomycetidae</i> families <i>incertae sedis</i> | <i>Sporidesmiaceae</i> , <i>Diaporthomycetidae</i> families <i>incertae sedis</i>                        | <b><i>Sporidesmium thailandense</i></b> |
| <i>Submersisphaeria</i> | <i>Annulatascaleae</i> ,<br><i>Annulatascales</i>                                    | <i>Annulatascaleae</i> , <i>Annulatascales</i>   |   |
| <i>Vertexicola</i>      | <i>Annulatascaleae</i> ,<br><i>Annulatascales</i>                                    | <i>Annulatascaleae</i> , <i>Annulatascales</i>   |   |
| <i>Woswasia</i>         | <i>Sordariomycetidae</i> , genera <i>incertae sedis</i>                              | <b><i>Woswasiaceae</i></b> fam. nov.,<br><i>Diaporthomycetidae</i> families <i>incertae sedis</i>        |   |
| <i>Xylochrysis</i>      | Not mentioned  | <b><i>Woswasiaceae</i></b> fam. nov.,<br><i>Diaporthomycetidae</i> families <i>incertae sedis</i>        |   |
| <i>Xylomelasma</i>      | <i>Sordariomycetidae</i> , genera <i>incertae sedis</i>                              | <b><i>Barbatosphaeriaceae</i></b> fam. nov.,<br><i>Diaporthomycetidae</i> families <i>incertae sedis</i> |   |

New order, families and species are in bold

brown ascomata with dark brown, erect or lateral long or short necks, clavate but mostly cylindrical asci with a refractive, J-, apical ring, and uni-seriate to biseriate, hyaline, aseptate to multi-septate ascospores with or without sheaths or appendages are prominent characters of *Atractosporales*. *Conlariaceae* is introduced for the genus *Conlarium* comprising two species, *C. duplumascospora* and *C. aquaticum* sp. nov. *Pseudoproboscisporaceae* includes *Pseudoproboscispora* and *Diluvicola*, while *Atractosporaceae* includes the genera *Rubellisphaeria* and *Atractospora*. The ascomata of *Pseudoproboscisporaceae* and *Atractosporaceae* often lie parallel to the host surface. The ascospores of *Atractosporaceae* lack appendages, unlike in *Conlariaceae* and *Pseudoproboscisporaceae* which have appendages at one or both ends.

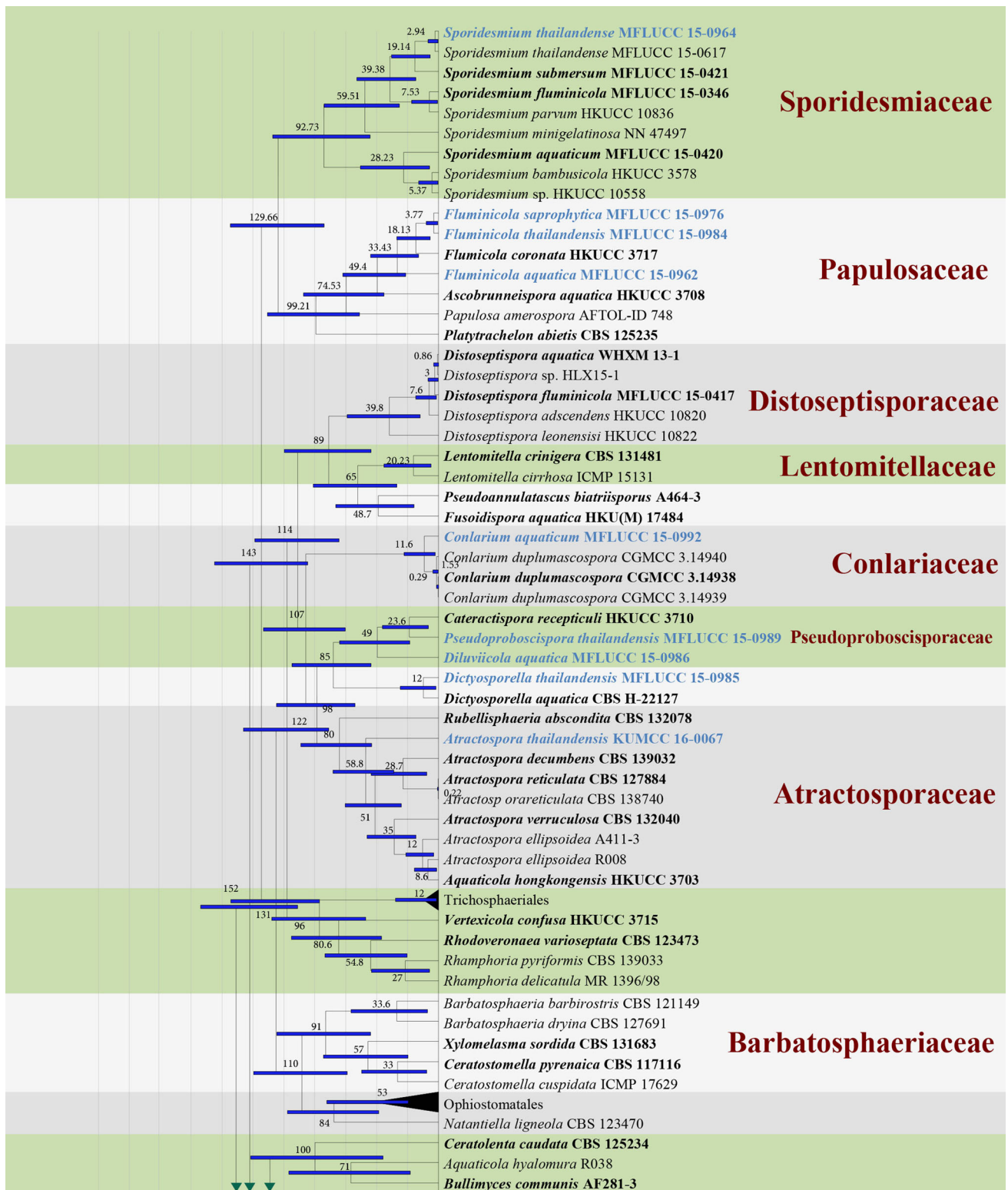
The family *Papulosaceae* forms a clade as in previous studies with each genus having clearly resolved relationships. The genus *Platytrachelon* affiliated to *Papulosaceae* in both the phylogenetic (Fig. 1) and evolution trees (Fig. 2). This is similar to the results of Réblová (2013), Réblová et al. (2014) and Jaklitsch et al. (2013). Thus, *Platytrachelon* is added to *Papulosaceae* based on the phylogenetic analysis and morphological characters. Three new species of *Fluminicola* are introduced.

*Woswasiaceae* is introduced to accommodate *Woswasia*, *Xylochrysis* and *Cyanoannulus* in *Diaporthomycetidae* families *incertae sedis*. *Woswasiaceae* is distinctive by colourful, globose to subglobose ascomata with a

cylindrical neck, ascomata nesting together in stromatic or astromatic structures. Asci are unitunicate, cylindrical to fusoid, with a J-, wedge-shaped, apical ring. Eight uniseriate, or rarely 2–4 ascospores are found in each ascus and are globose, subglobose or ellipsoidal, hyaline, unicellular or septate, verruculose or smooth-walled, thin-walled or thick-walled.

Three genera *Barbatosphaeria*, *Xylomelasma* and *Ceratostomella* form a stable clade which is introduced as a new family *Barbatosphaeriaceae* in *Diaporthomycetidae* families *incertae sedis*. *Natantiella* is close to *Ophios-tomatales*, but with weak support. This result is inconsistent with Senanayake et al. (2016). Therefore, we place *Natantiella* in *Sordariomycetes*, genera *incertae sedis* as reported in Maharachchikumbura et al. (2016).

Four strains *Vertexicola confusa*, *Rhodoveronaea varioseptata*, *Rhamphoria pyriformis* and *Rhamphoria delicatula* have only LSU sequence data and form a stable clade as in previous analyses (Réblová and Štěpánek 2009; Jaklitsch et al. 2013; Jones et al. 2015) and are sister to *Trichosphaeriales*. However, the relationships among taxa in this clade and sister clade are unstable. *Vertexicola confusa* is distant from the other three species (Réblová et al. 2016) and the whole clade separated from *Trichosphaeriales* in Senanayake et al. (2016). *Vertexicola confusa* was mentioned in Ranghoo et al. (1999) with only sequence data. Characters include black perithecia with long necks, cylindrical asci with a relative large, refractive,



**Fig. 2** The Maximum clade credibility tree of *Annulatasceae*-like taxa obtained from a Bayesian approach (BEAST). Bars correspond to the 95% highest posterior density (HPD) intervals. Divergence times estimates are presented at each node

apical ring and ascospores with appendages. It has never been validly published in any mycological journal. The asexual morph *Rhodoveronaea varioseptata*, type species

of *Rhodoveronaea*, was established by Arzanlou et al. (2007) for ramichloridium-like fungi having septate conidia with a basal and marginal conidial frill. The genus

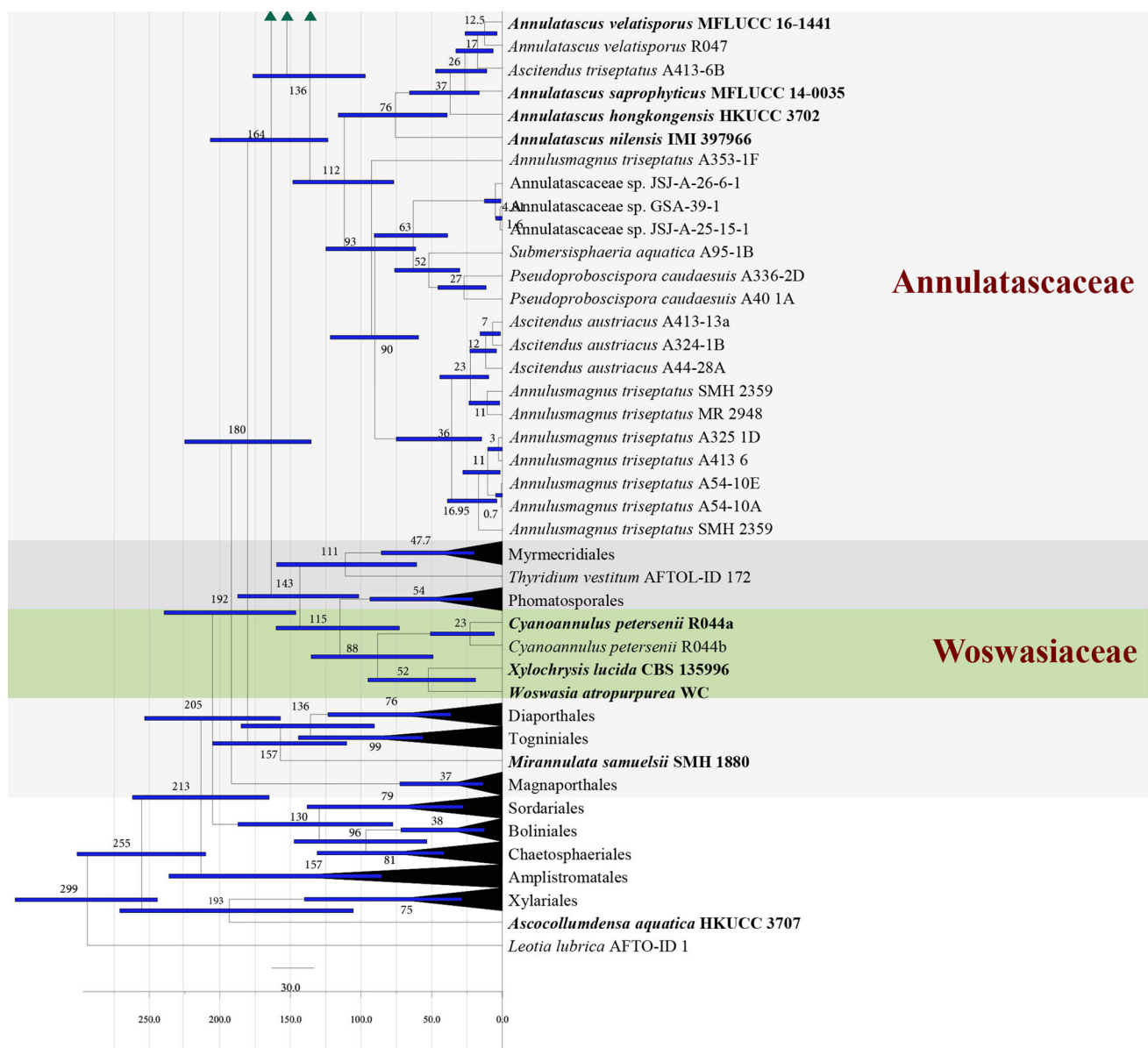


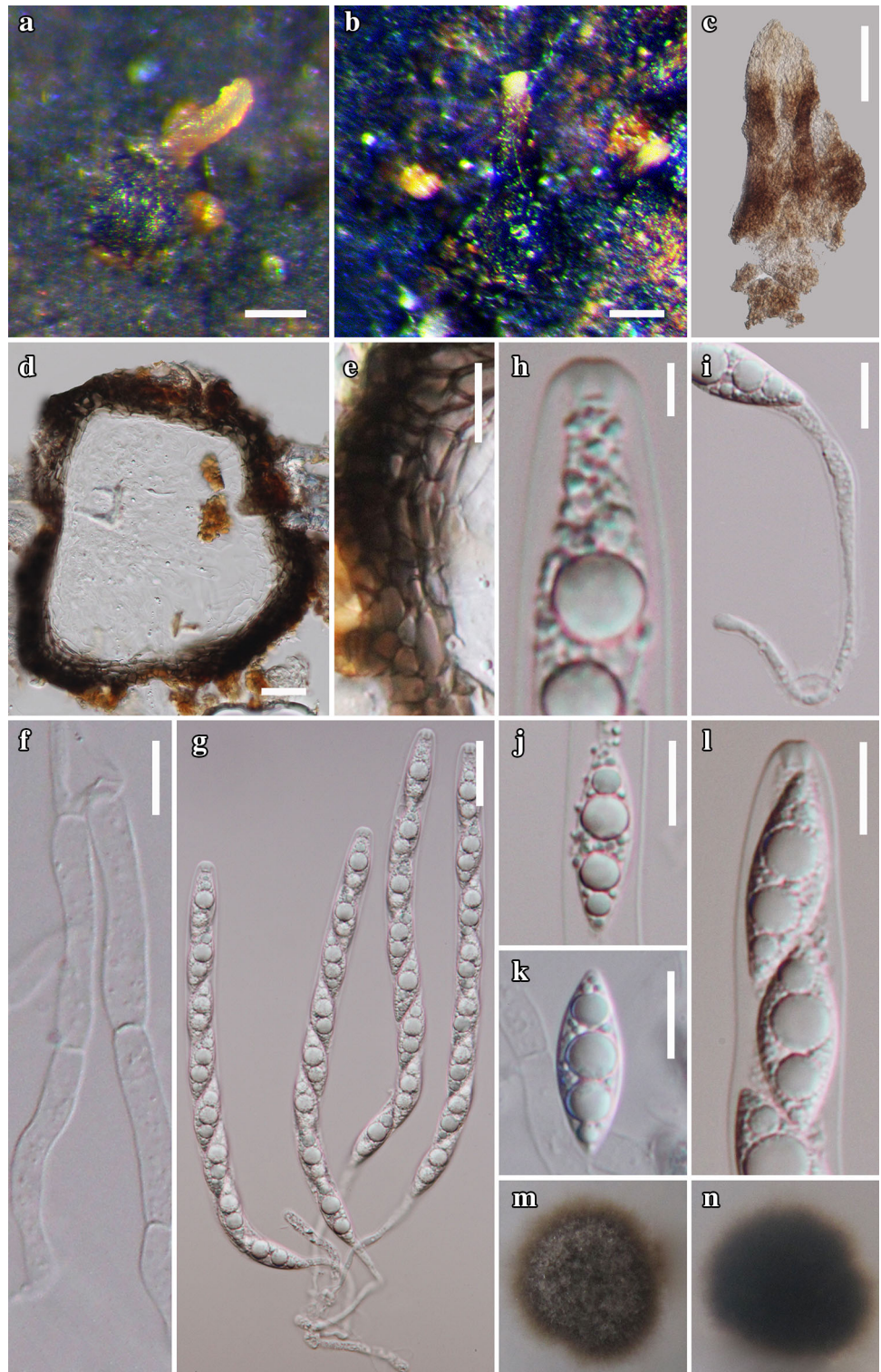
Fig. 2 continued

*Rhamphoria* is an archaic species introduced in 1876, with *R. delicatula* as the type (Niessl 1876). Fifteen records are listed under this genus in Index Fungorum (2017) and all were introduced before 1930, except *R. bevanii* in 1977 and *R. separata* in 1948. The type species needs to be recollected in order to obtain the pivotal characters of this genus. In the present, we place *Verticicola*, *Rhodoveronaea* and *Rhamphoria* in Diaporthomycetidae genera *incertae sedis* instead of *Annulatasceaceae* and Sordariomycetidae, genera *incertae sedis* as in

Maharachchikumbura et al. (2016). With exploration of further populations, the clade *Rhodoveronaea* and *Rhamphoria* may need to be raised to family rank.

Although most of the new taxa introduced in this study are single collections with a single isolate we prefer to move forward by introducing them as the taxa are relatively rare and difficult to find. The results however, advance the phylogenetic understanding of these freshwater fungi, which have been poorly understood for a long period (Vijaykrishna et al. 2006). We

**Fig. 3** *Atractospora thailandensis* (holotype) **a**, **b** Appearance of dark brown to black ascomata semi-immersed on host. **c** Ostiole with long neck. **d** Vertical section of ascoma. **e** Structure of peridium. **f** Paraphyses. **g–i** Unitunicate asci. **j** Immature ascospore in ascus. **k–l** Mature ascospores. **m** Colony on PDA (from front). **n** Colony on PDA (from reverse). Scale bars **a–c** = 100  $\mu$ m; **d, g** = 20  $\mu$ m; **e–f, i–l** = 10  $\mu$ m; **h** = 5  $\mu$ m



expect further studies to reveal a better understanding of the phylogeny and evolution of this poorly represented group of fungi (Vijaykrishna et al. 2006). Our ideas towards introducing new species follow Jeewon and Hyde (2016) and those for higher taxa of Liu et al. (2016).

### Taxonomy

*Annulatascales* D'souza, Maharachch. & K.D. Hyde, Fungal Divers. 72: 212

An order comprising a single family *Annulatascaleae* was introduced by Maharachchikumbura et al. (2015).

Species typically occur on wood in freshwater habitats and have cylindrical, thin-walled asci, with a massive, J-, refractive, apical ring and ascospores usually having appendages or sheaths (Maharachchikumbura et al. 2015). Twenty genera are included in *Annulatasceae*, but the inclusion of many are doubtful, due to lack of molecular data (Maharachchikumbura et al. 2015). *Annulatasceae* has been reported as closely related to *Cordanales* and *Papulosaceae* (Maharachchikumbura et al. 2015), *Thyridiaceae* and *Ophiostomataceae* (Maharachchikumbura et al. 2016) or *Rhamphoria pyriformis* (Pers.) Höhn., *Verticicola confusa* and *Bullimyces communis* A. Ferrer, A.N. Mill., Sarmiento & Shearer (Senanayake et al. 2016). In our phylogenetic analyses, *Annulatasceae* appears to have a distant relationship with *Bullimyces communis* and *Ceratolenta caudata* Réblová, as well as *Papulosaceae*.

In *Annulatasceae*, *Submersisphaeria aquatica* K.D. Hyde and *Pseudoproboscispora caudae-suis* (Ingold) J. Campb., Shearer, J.L. Crane & Fallah (Group 1) cluster together with *Ascitendus* J. Campb. & Shearer and *Annulismagnus* J. Campb. & Shearer (Group 2, Fig. 1), as shown in previous studies (Jaklitsch et al. 2013; Jones et al. 2015; Maharachchikumbura et al. 2015, 2016; Senanayake et al. 2015, 2016). The two groups may need to be raised to family rank when the tree is better populated.

***Atractosporales*** H. Zhang, K.D. Hyde & Maharachch., **ord. nov.**

*Index Fungorum*: IF553756; *Facesoffungi* number: FoF 03333

**Sexual morph**: *Ascomata* immersed or semi-immersed, astromatic, subglobose to conical, dark brown, with a lateral neck, often lying horizontally to the substrate surface. *Ostiole* periphysate. *Peridium* leathery to fragile, two-layered. *Paraphyses* abundant. *Asci* 8-spored, unitunicate, cylindrical, pedicellate, apically rounded, with a distinct, refractive, relatively large, wedge-shaped, J-, apical ring, *Ascospores* uni-seriate or obliquely uni-seriate, hyaline, fusiform, aseptate or transversely septate. **Asexual morph**: Hyphomycetous. *Colonies* dark brown to black, planar, smooth. *Conidiophores* micronematous or semi-macronematous, mononematous, septate, branched or unbranched, straight or flexuous, hyaline, becoming brown at maturity. *Conidiogenous cells* determinate, holoblastic, doliiform, cylindrical. *Conidia* brown, muriform, irregularly globose or subglobose. *Conidia* produced in culture of *Conlarium duplumascospora*.

Type family: *Atractosporaceae* H. Zhang, K.D. Hyde & Maharachch.

*Notes*: Analyses of combined LSU, SSU, ITS and RPB2 sequence data in the phylogenetic tree (Fig. 1) reveals that *Atractosporaceae*, *Conlariaceae* and *Pseudoproboscisporaceae*

group together, forming a group of aquatic Sordariomycetes and this lineage is introduced here as *Atractosporales* as an order of Diaporthomycetidae. Dark brown ascomata with dark brown, erect or lateral long or short necks, clavate but mostly cylindrical asci with a refractive, J-, apical ring, uni-seriate to biseriate, hyaline, aseptate to multi-septa ascospores with or without sheaths or appendages are prominent characters of *Atractosporales*. *Conlariaceae* is introduced for the genus *Conlarium* comprising two species, *Conlarium duplumascospora* and *Conlarium aquaticum* sp. nov. *Pseudoproboscisporaceae* includes *Pseudoproboscispora* and *Diluviicola*, while *Atractosporaceae* includes the genera *Rubellisphaeria* and *Atractospora*. The ascomata of *Pseudoproboscisporaceae* and *Atractosporaceae* often lie parallel to the host surface. The ascospores of *Atractosporaceae* lack appendages, unlike in *Conlariaceae* and *Pseudoproboscisporaceae* which have appendages at one or both ends. The phylogenetic position of *Aquaticola hyalomura* is not stable in our two trees, thus it is not included in *Atractosporales*.

***Atractosporaceae*** H. Zhang, K.D. Hyde & Maharachch., **fam. nov.**

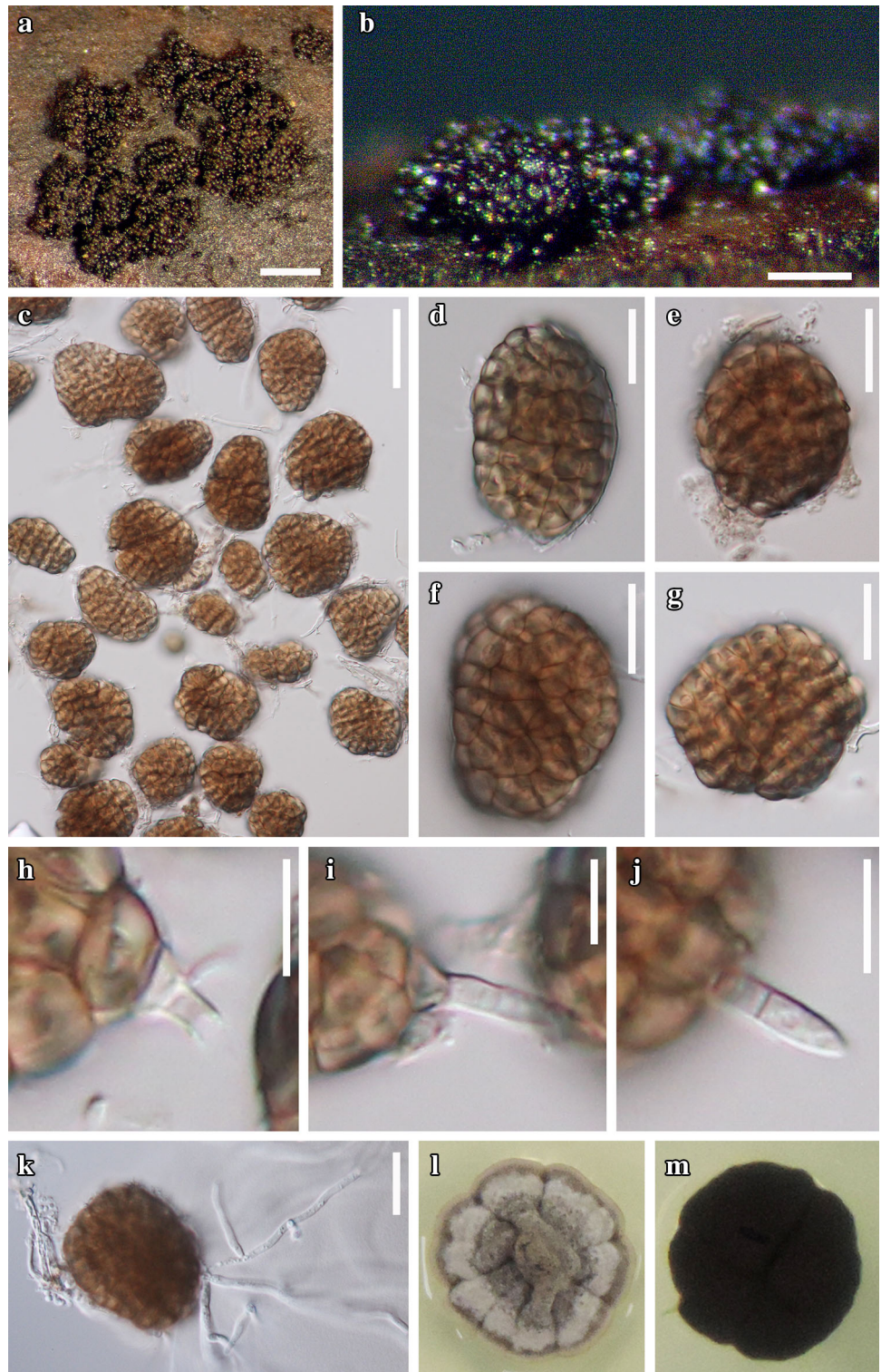
*Index Fungorum*: IF553757; *Facesoffungi* number: FoF 03334

*Saprobic* on submerged deciduous wood. **Sexual morph**: *Ascomata* immersed or semi-immersed, astromatic, subglobose to conical, dark brown, with a lateral neck, often lying horizontally on the substrate surface. *Ostiole* periphysate. *Peridium* leathery to fragile, two-layered. *Paraphyses* abundant, persistent, cylindrical, flexuous. *Asci* 8-spored, unitunicate, pedicellate, apically rounded or obtuse, with a J-, relatively small, apical ring, range from 3–4.5 µm diam., 1.5–3.5 µm high. *Ascospores* uni-seriate, hyaline, fusiform, aseptate or transversely septate. **Asexual morph**: Undetermined.

Type genus: *Atractospora* Réblová & J. Fourn., Mycol. Progr. 15(no. 21): 8 (2016)

*Notes*: This group is grossly under sampled resulting with very few taxa having molecular data. This is partly because the habitats in which they are found are poorly investigated, rather inconspicuous in size and few are known in cultures. Many of these taxa may have originally been recorded as *Annulatasceae*-like taxa. The family forms a distinct clade in the phylogenetic tree (Fig. 1), which was also shown in the study of Réblová et al. (2016). *Atractospora* and *Rubellisphaeria* are morphologically quite similar and represent a distinct group of aquatic Sordariomycetes. The genera included in this family share freshwater habitats and similar characters in having immersed to semi-immersed, usually light brown to dark brown ascomata, with a relatively thin peridium, comprising a few layers of cells of *textura angularis*. The asci

**Fig. 4** *Conlarium aquaticum* (holotype) **a–b** Colonies on submerged wood. **c–g** Conidia. **h–j** Conidia with conidiogenous cells. **k** Germinating conidium. **l** Colony on PDA (from front). **m** Colony on PDA (from reverse). Scale bars **a** = 500  $\mu\text{m}$ ; **b** = 200  $\mu\text{m}$ ; **c** = 50  $\mu\text{m}$ ; **d–g, k** = 20  $\mu\text{m}$ ; **h–j** = 10  $\mu\text{m}$



are mostly cylindrical with apical rings, while ascospores are hyaline and uni-seriate in the asci and most lack sheaths.

*Atractospora* comprises four species with the type, *Atractospora decumbens* Réblová & J. Fourn. having dark

brown ascomata, long cylindrical asci with a slender pedicel and hyaline, 3-septate, uni-seriate ascospores (Réblová et al. 2016). All species except, *A. ellipsoidea*, have fusiform, 3-septate, relatively thick-walled ascospores (Ho et al. 1999; Réblová et al. 2016). The monotypic genus

*Rubellisphaeria* has oblique, globose to subglobose ascospores with subhyaline to reddish-brown walls and ellipsoidal ascospores with a delayed formation of the middle septum (Réblová et al. 2016). *Aquaticola hongkongensis* also nests in this clade, but it has never been validly published (Ranghoo et al. 1999).

We collected a new species in this study which is typical of *Atractospora*, however the ascospores lack septa and the colonies on PDA are dark brown to grey. The phylogeny places the taxa in *Atractospora*.

***Atractospora*** Réblová & J. Fourn., Mycol. Progr. 15(no. 21): 8 (2016)

For description refer to (Réblová et al. 2016).

**Notes:** *Atractospora* was established by Réblová et al. (2016), and typified by *A. reticulata* Réblová & J. Fourn. Réblová et al. (2016) reported the genus as characterized by dark, immersed to semi-immersed ascospores lying horizontally on the host, with necks, pedicellate asci with a wedge-shaped, refractive, J-, apical ring and uni-seriate, hyaline, fusiform, aseptate or transversely septate, thick-walled ascospores with smooth or ornamented walls. However, both *Atractospora ellipsoidea* and our new species *A. thailandensis* have thin-walled ascospores. Therefore, we extend the generic description to include thin-walled ascospores.

Asexual structures were produced from vegetative mycelium of *Atractospora decumbens* and described as brown, aseptate, ellipsoidal to subglobose, thick-walled cells often forming short chains of three to five cells (Réblová et al. 2016). The authors did not regard these as asexual morph of *A. decumbens*.

***Atractospora thailandensis*** W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

**Index Fungorum:** IF553212; **Facesoffungi number:** FoF 03335; Fig. 3

**Etymology:** In reference to the host location, Thailand, where the holotype was collected.

**Holotype:** HKAS 96226

**Saprobic** on decaying wood submerged in freshwater. **Sexual morph:** *Ascomata* 150–200 µm high, 120–160 µm diam., dark brown to black, gregarious or solitary, semi-immersed, globose to subglobose, unilocular, with long, subcylindrical neck, thick-walled, ostiolate, lying horizontal to the substrate surface. *Ostiole* up to 170 µm long, subcylindrical, with long neck, straight or slight curved, filled with hyaline, septate periphysis, centrally located. *Peridium* 19–23 µm thick, comprising outer layers of brown, thick-walled, compressed cells of *textura angularis*, which are inwardly hyaline. *Paraphyses* ca 6.5 µm diam. at the base, up to 1 µm at the apex, tapering or suddenly tapering at the apex, numerous, cylindrical, unbranched, hyaline, septate, slightly constricted at the septa. *Asci* 175–

215 × 9–12 µm ( $\bar{x} = 195.7 \times 10.5 \mu\text{m}$ ,  $n = 10$ ), 8-spored, unitunicate, cylindrical with a slender tapering pedicel, up to 74 µm long, apically rounded or slightly obtuse, with a indistinct, not refractive, wedge-shaped, J-, apical ring, 2.8–3.3 µm high × 3–4 µm wide. *Ascospores* 20–26 × 7–9 µm ( $\bar{x} = 23.2 \times 8.3 \mu\text{m}$ ,  $n = 20$ ), uni-seriate, hyaline, aseptate, fusiform to long citriform, ends sharply tapered, with 2–4 prominent guttules, thin-walled. **Asexual morph:** Undetermined.

**Material examined:** THAILAND, Prachuap Khiri Khan, on submerged wood in a small river, 30 July 2015, W. Dong 56B (HKAS 96226, **holotype**); ex-type culture KUMCC 16-0067

**Culture characteristics:** On PDA, colony circular, reaching 5 mm in 30 days at 25 °C, dark brown to grey from above, black from below, surface hairy with raised elevation, dry, edge entire.

**Notes:** *Atractospora thailandensis* forms characteristic dark ascospores, lying horizontally on the host, with upright necks, cylindrical asci with a cylindrical, refractive, J-, apical ring and slender tapering pedicel and fusiform, hyaline, aseptate ascospores, with 2–4 prominent guttules. This is the only species of *Atractospora* lacking septa. *A. thailandensis* is most similar to *A. ellipsoidea*, but the former has larger ascospores (20–26 × 7–9 µm vs. 12–14 × 5–7 µm). Molecular data also separates these species (Fig. 1).

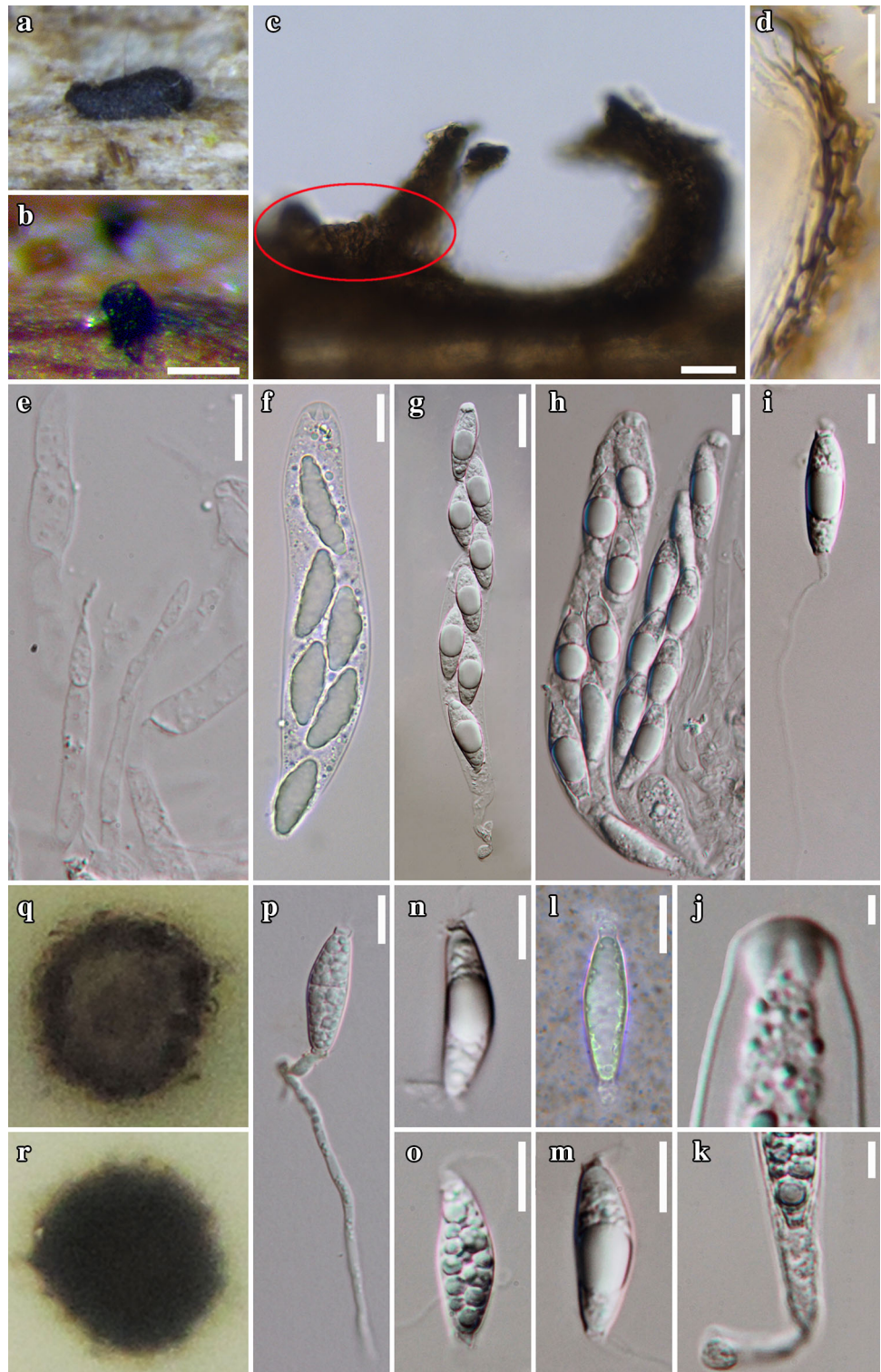
**Conlariaceae** H. Zhang, K.D. Hyde & Maharachch., *fam. nov.*

**Index Fungorum:** IF553758; **Facesoffungi number:** FoF 03336

**Saprobic** on submerged wood. **Sexual morph:** *Ascomata* perithecioid, gregarious, coriaceous, superficial or partially immersed, dark brown to black, globose to subglobose, smooth. *Ostiole* elongate, cylindrical, straight or slightly flexuous. *Peridium* composed of several layers of cells of *textura angularis*, which are usually darker in the outer layer and pale in the inner layer. *Paraphyses* cylindrical, hyaline, septate, cells sometimes slightly inflated between the septa, branched, tapering or not, not embedded in a matrix. *Asci* 8-spored, unitunicate, cylindrical, short pedicellate, apically rounded, with a distinct, refractive, massive, barrel-shaped, apical ring, 4 µm high × 5 µm wide. *Ascospores* biseriate, hyaline, fusiform, straight or slightly curved, aseptate to multi-septate, guttulate, without appendages or appendages at one or both ends. **Asexual morph:** Hyphomycetous. *Colonies* dark brown to black. *Mycelium* mostly immersed, consisting of branched, septate, thin-walled, smooth, pale brown to brown hyphae. *Conidiophores* micronematous or semi-macronematous, mononematous, septate or aseptate, unbranched or irregularly branched, straight or flexuous, hyaline, becoming



**Fig. 5** *Diluvicola aquatica* (holotype) **a–b** Ascomata semi-immersed or superficial on host. **c** Vertical section of ascoma. **d** Structure of peridium. **e** Paraphyses. **f–h, j–k** Unitunicate asci. **i** Ascospore with long appendages. **l** Ascospore mounted in India ink. **m–o** Ascospores mounted in water. **p** Germinating ascospore. **q** Colony on PDA (from front). **r** Colony on PDA (from reverse). *Scale bars* **b** = 100  $\mu$ m; **c, g** = 20  $\mu$ m; **d–f, h–i, l–p** = 10  $\mu$ m; **j** = 2  $\mu$ m; **k** = 5  $\mu$ m

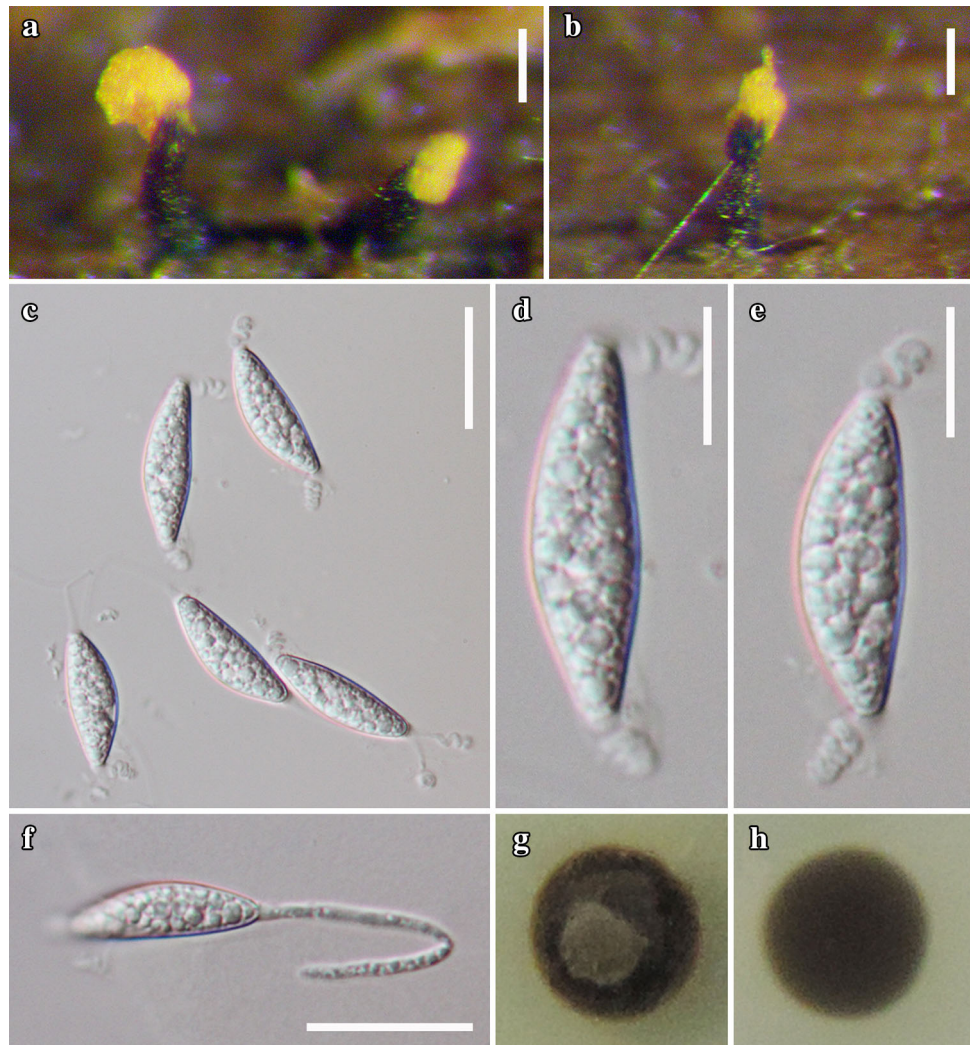


brown when old. *Conidiogenous cells* holoblastic, determinate, doliiform, cylindrical. *Conidia* brown, muriform, irregularly globose or subglobose, septate, constricted at the septa (asexual morph description from Liu et al. 2012).

Type genus: *Conlarium* F. Liu & L. Cai

*Notes:* This group was introduced to accommodate a single genus *Conlarium* with both sexual and asexual morphs. Species of *Conlarium* formed a sister clade to members of *Atractosporaceae* and *Pseudoproboosciporaceae* in our phylogenetic analyses. *Conlariaceae* is

**Fig. 6** *Pseudoproboscispora thailandensis* (holotype) **a**, **b** Appearance of necks of ascomata on host. **c–e** Ascospores with unfurling appendages. **f** Germinating ascospore. **g** Colony on PDA (from front). **h** Colony on PDA (from reverse). Scale bars **a–b** = 100  $\mu\text{m}$ ; **c, f** = 20  $\mu\text{m}$ ; **d–e** = 10  $\mu\text{m}$



characterized by superficial, dark, globose to subglobose ascomata with cylindrical, mostly straight necks, cylindrical asci with a distinct, refractive, massive, barrel-shaped, apical ring and short pedicel and biseriate, fusiform, aseptate to multi-septate, guttulate ascospores with or without appendages. The distinct morphological characters and phylogeny warrant a new family *Conlariaceae*. The prime features distinguishing *Conlariaceae* from *Atractosporaceae* are superficial, ascomata with mostly straight necks, biseriate ascospores with globose or papillary appendages at one or both ends. In *Atractosporaceae*, ascomata are immersed to semi-immersed, with a lateral neck, often lying horizontally on the substrate surface. The ascospores are mostly uni-seriate in the asci and most lack sheaths.

**Conlarium** F. Liu & L. Cai, *Mycologia* 104(5): 1180 (2012)

For description refer to Liu et al. (2012).

**Conlarium aquaticum** W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

*Index Fungorum*: IF553759; *Facesoffungi* number: FoF 03337; Fig. 4

*Etymology*: In reference to the aquatic habitat.

*Holotype*: MFLU 15-2703

*Saprobic* on decaying, submerged wood in freshwater.

**Sexual morph**: Undetermined. **Asexual morph**: Colonies sporodochial, broadly punctiform, gregarious or scattered, raised, dark brown to black, velvety. *Mycelium* mostly immersed on natural substratum, consisting of branched, septate, thin-walled, smooth, pale brown to brown hyphae. *Conidiophores* absent or reduced to conidiogenous cells. *Conidiogenous cells* up to 18  $\mu\text{m}$  long, monoblastic, holoblastic, integrated, determinate, cylindrical, hyaline to pale brown, smooth. *Conidia* 45–70  $\times$  20–57  $\mu\text{m}$  ( $\bar{x}$  = 54.9  $\times$  39.3  $\mu\text{m}$ ,  $n$  = 30), acrogenous, solitary, dry, subglobose, ellipsoidal, oblong or irregular, brown, clathrate, muriform, 6–12-transversely septate, 4–10-

longitudinal septate, slightly constricted at septa, smooth, thin-walled. *Conidial secession* schizolytic.

**Material examined:** THAILAND, Prachuap Khiri Khan, on submerged wood in a small river, 30 July 2015, W. Dong 136A (MFLU 15-2703, **holotype**); ex-type culture MFLUCC 15-0992.

**Culture characteristics:** On PDA, colony circular, reaching 10 mm in 30 days at 25 °C, grey to dark brown from above, black from below, umbonate, rough, wrinkled, dry, edge undulate.

**Notes:** The monotypic genus *Conlarium* was established to accommodate the holomorphic species *C. duplumascospora* (Liu et al. 2012). Phylogenetic analyses showed that our isolate separate with *C. duplumascospora* with high support (100% ML, 100% MP, 100% PP) (Fig. 1). Morphologically, *C. aquaticum* is similar to the asexual morph of *Conlarium* in muriform conidia. However, they can be distinguished by the number of septa and conidial size (2–4 transversely septa, 1–3 longitudinal septa, 15.5–35 × 11–26.5 µm in *C. duplumascospora* vs. 6–12 transverse septa, 4–10 longitudinal septa, 45–70 × 20–57 µm in *C. aquaticum*).

**Pseudoproboscisporaceae** H. Zhang, K.D. Hyde & Maharachch., **fam. nov.**

**Index Fungorum:** IF553760; **Facesoffungi number:** FoF 03338

**Saprobic** on decaying bamboo submerged in freshwater.

**Sexual morph:** *Ascomata* scattered or solitary, immersed with neck erumpent through host surface, dark brown to black, ostiolate. *Ostirole* central, erect, dark brown, apical with mass of releasing spores yellowish. *Peridium* comprising several layers of flattened cells. *Paraphyses* wide, hypha-like, septate, tapering. *Asci* 8-spored, cylindrical, unitunicate, pedicellate, with a relatively large, J-, apical ring. *Ascospores* uniseriate or overlappingly uniseriate, ellipsoidal or fusiform, aseptate or 1–3-septate, hyaline, with bipolar filamentous appendages. Appendages initially coiled or proboscis-like then unfurling to form long threads on release. **Asexual morph:** Undetermined.

Type genus: *Pseudoproboscispora* Punith.

**Notes:** This family includes the genera *Cateractispora*, *Pseudoproboscispora* and *Diluvicola* based on molecular study. The prominent morphological characters are appendages which are at first coiled and then unfurl in water to form long threads. The ascomata are light brown to dark brown, with central or lateral dark brown necks. *Asci* are clavate to cylindrical, with a refractive, wedge-shaped, J-, apical ring. *Ascospores* are uni-seriate to biseriate, fusiform, hyaline, 0–1- or 3-septa, with appendages.

*Cateractispora* Ranghoo, K.D. Hyde & E.C.Y. Liew has never been validly published. We observed a photo-plate of this species in the PhD thesis of Ranghoo (1998) without

any description. The plate shows that ascospores appendages of *C. recepticuli* are at first coiled and proboscisporalike, and then unfurl in water to form long polar, thread-like appendages, as in *Pseudoproboscispora aquatica* (S.W. Wong & K.D. Hyde) Punith. (= *Proboscispora aquatica* S.W. Wong & K.D. Hyde), the type species of *Pseudoproboscispora*. It has also been observed by Cai et al. (2006), in which they named the same plate as *P. aquatica*. According to morphological characters, *Cateractispora* is likely to be an invalidly published synonym of *Pseudoproboscispora*. However, *Pseudoproboscispora caudae-suis* (Ingold) J. Campb., Shearer, J.L. Crane & Fallah clusters in *Annulismagnaceae* distant from *C. recepticuli*. *Pseudoproboscispora* should be compared with *Diluvicola* as both have unfurling appendages. These taxa can be separated as the appendages are cap-like in *Diluvicola* and stretch to form filaments and are proboscis-like in *Pseudoproboscispora* and unwind.

*Aquaticola hyalomura* W.H. Ho, K.M. Tsui, Hodgkiss & K.D. Hyde also clusters as a sister group to *Pseudoproboscisporaceae* in the phylogenetic tree (Fig. 1). However, it clustered in a different clade in the MCC tree (Fig. 2). Réblová et al. (2016) showed that *Aquaticola* is polyphyletic. This is confirmed in our study as *A. hongkongensis* and *A. hyalomura* (type species) clustered in two distinct clades. Due to this unstable phylogenetic relationship, *Aquaticola hyalomura* is not placed in *Pseudoproboscisporaceae*.

Our new collection MFLU 15-2705 has immersed, dark brown ascomata with central, erect, dark brown necks and hyaline ascospores with proboscis-like appendages, which unfurl to form thread-like appendages. The species is typical of *Pseudoproboscispora*. Another new collection MFLU 15-2701 is typical of *Diluvicola*.

**Diluvicola** K.D. Hyde, S.W. Wong & E.B.G. Jones, *Fungal Diversity Res. Ser. 1*: 141 (1998)

For description refer to Hyde et al. (1998).

**Diluvicola aquatica** W. Dong, H. Zhang & K.D. Hyde, **sp. nov.**

**Index Fungorum:** IF553762; **Facesoffungi number:** FoF 03340; Fig. 5

**Etymology:** In reference to aquatic habitat.

**Holotype:** MFLU 15-2701

**Saprobic** on decaying bamboo submerged in freshwater.

**Sexual morph:** *Ascomata* 70–100 µm high, 115–145 µm diam., scattered or solitary, semi-immersed or superficial, subglobose or ellipsoidal, dark brown, ostiolate. *Ostirole* 35–45 µm long, 18–22 µm wide, dark brown, growing laterally to the host surface, curving upwards. *Peridium* 15–20 µm thick, two-layered, outer layer comprising 4–5 layers of dark brown, large, compressed cells of *textura*

*angularis*, inner layer comprising 3–4 layers of hyaline, compressed, elongate cells. *Paraphyses* numerous, cylindrical, unbranched, hyaline, septate, constricted at the septa, tapering towards the apex, ca 6.2 µm diam. at the base, up to 1.5 µm at the apex, not embedded in a gelatinous matrix. *Asci* 120–180 × 14–20 µm ( $\bar{x}$  = 143.1 × 16.3 µm, n = 10), 8-spored, unitunicate, clavate to cylindrical, pedicellate, with a distinct, refractive, wedge-shaped, J-, apical ring, 3.2–4 µm high × 4.2–4.5 µm wide. *Ascospores* 23–28 × 7–10 µm ( $\bar{x}$  = 25.3 × 8.3 µm, n = 20), obliquely uni-seriate or partially biseriate or sometimes triseriate, fusiform, straight or slightly curved, smooth or rugose, aseptate when young, 0–1-septate when mature, slightly constricted at the septa, hyaline, thin-walled, guttulate, with bipolar filamentous appendages. Appendages initially coiled or cap-like then unfurl to form long threads after releasing, up to 98 µm long. **Asexual morph:** Undetermined.

*Material examined:* THAILAND, Prachuap Khiri Khan, on submerged bamboo in a small river, 30 July 2015, W. Dong 55A (MFLU 15-2701, **holotype**); ex-type culture MFLUCC 15-0986.

*Culture characteristics:* On PDA, colony circular, reaching 10 mm in 40 days at 25 °C, dark brown to black from above, black from below, raised, rough, dry, edge entire or undulate.

*Notes:* *Diluviicola capensis*, the type species of *Diluviicola*, is characterized by semi-immersed or superficial, dark brown ascomata with short, dark brown necks growing laterally to the host surface and curving upwards, clavate to cylindrical asci with a distinct, refractive, wedge-shaped, J-, apical ring and obliquely uni-seriate or partially biseriate or sometimes triseriate, fusiform, 0–1-septate, hyaline, guttulate ascospores with cap-like appendages, which form long threads after releasing (Hyde et al. 1998). *Diluviicola aquatica* resembles *D. capensis* in having black ascomata with short necks growing laterally to the ascomata, fusiform, hyaline ascospores with cap-like appendages and the same unfurling mechanism (Hyde et al. 1998). However, *D. aquatica* was considered to differ from *D. capensis* as the latter has obpyriform ascomata with hyaline necks and uni-seriate ascospores as compared with subglobose or ellipsoidal ascomata with dark brown necks and mostly biseriate ascospores in *D. aquatica*.

*Pseudoproboscispora* Punith., Kew Bull. 54(1): 234 (1999)  
For description refer to Wong and Hyde (1999).

*Pseudoproboscispora thailandensis* W. Dong, H. Zhang & K.D. Hyde, **sp. nov.**

*Index Fungorum:* IF553761; *Facesoffungi* number: FoF 03339; Fig. 6

*Etymology:* In reference to the freshwater habitat.

*Holotype:* MFLU 15-2705

*Saprobic* on decaying bamboo submerged in freshwater.

**Sexual morph:** *Ascomata* scattered or solitary, immersed with neck erumpent through host surface, dark brown to black, ostiolate. *Ostiole* 200–300 µm long, 30–70 µm wide, central, erect, dark brown, apical with mass of releasing spores appear yellowish. *Peridium* comprising several layers of compressed brown-walled cells. *Paraphyses* wide, hypha-like, septate, tapering distally. *Asci* deliquesced. *Ascospores* 23–27 × 7–9 µm ( $\bar{x}$  = 24.5 × 8.2 µm, n = 15), fusiform, less straight or mostly curved, smooth, aseptate, hyaline, minutely guttulate, with bipolar filamentous appendages. Appendages initially coiled or proboscis-like then unfurl to form long threads after releasing. **Asexual morph:** Undetermined.

*Material examined:* THAILAND, Prachuap Khiri Khan, on submerged bamboo in a small river, 30 July 2015, W. Dong 41C (MFLU 15-2705, **holotype**); ex-type culture MFLUCC 15-0989.

*Culture characteristics:* On PDA, colony circular, reaching 5 mm in 30 days at 25 °C, dark brown to black from above, dark brown from below, raised, rough, dry, edge entire.

*Notes:* *Pseudoproboscispora thailandensis* has the typical appendages and unfurling mechanism found in *Pseudoproboscispora*. *P. aquatica*, the type species, has a hyaline neck, while *P. thailandensis* has a dark brown neck. In addition, it differs in having aseptate rather than three-septate ascospores. The asci were not seen in this collection because they had deliquesced. Like many aquatic ascomycetes, the asci can only be seen at an early stage.

**Diaporthomycetidae** families *incertae sedis*

**Barbatosphaeriaceae** H. Zhang, K.D. Hyde & Maharachch., **fam. nov.**

*Index Fungorum:* IF553763; *Facesoffungi* number: FoF 03341

*Saprobic* on decaying wood or other plant materials. **Sexual morph:** *Ascomata* astromatic, leathery to fragile, dark brown to black, solitary or usually aggregated in circular to oval nests or in short rows, globose to subglobose, slightly roughened, with long necks, both venter and neck are sparsely covered with a pubescence that disappears with age, necks remaining covered with septate hairs, ostiolate. *Ostiole* cylindrical, long, straight to slightly flexuous, periphysate, when in circular groups decumbent to perpendicular, covering, piercing the periderm in a group but never united in a disk. *Peridium* two layered, outer layer composed of brown cells of *textura prismatica*, with wide lumina tending to be longer toward the interior and becoming smaller outwards, inner layer composed of elongate, hyaline cells of *textura prismatica*. *Paraphyses* abundant, persistent, cylindrical,

unbranched, hyaline, septate, constricted at the septa, tapering to the apex, longer than the asci. *Asci* unitunicate, clavate, tapering toward the stipe, with a shallow, refractive, J-, apical ring, floating freely in centrum at maturity. *Ascospores* 1- or 2-seriate, oblong to ellipsoidal, septate, non- or slightly constricted at the median septum, hyaline, smooth-walled. **Asexual morph:** Hyphomycetous. Ramichloridium- and Sporothrix-like. *Conidiophores* of the *sporothrix*-type micronematous to semi-micronematous, unbranched or branched, cylindrical to flask- or irregular-shaped, hyaline, denticulate. *Conidiogenous cells* polyblastic, integrated, and terminal with several denticles producing conidia holoblastically. *Conidia* ellipsoidal to suballantoid, curved, unicellular, hyaline. *Conidiophores* of the *Ramichloridium*-type macronematous, simple, erect, septate, brown, paler and thin-walled toward the apex. *Conidiogenous cells* polyblastic, integrated, terminal, cylindrical, tapering with rachis, proliferating sympodially. *Conidia* ellipsoidal, straight or curved, unicellular, hyaline (Réblová 2007). Conidia produced in culture of *Barbatosphaeria barbirostris*.

Type genus: *Barbatosphaeria* Réblová

*Notes:* This family presently comprises three genera, *Barbatosphaeria*, *Ceratostomella* and *Xylomelasma*, which was also apparent in Réblová et al. (2016). The genera are morphologically quite similar and represent a distinct group. They share similar characters in having dark, long-necked, astromatic ascomata, mostly surrounded by sparse mycelium or pubescence, clavate or cylindrical-clavate asci, arising from ascogenous hyphae and mostly ellipsoidal ascospores. In our study, *Barbatosphaeriaceae* forms a sister clade to *Natantiella ligneola* and the order *Ophiostomatales* Nannf. However, it formed a sister clade comprising of *Ophiostomatales*, *Phomatosporales* and *Amplistromatales*. Therefore, *Barbatosphaeriaceae* can not be phylogenetically assigned to any orders and we place it in Diaporthomycetidae families *incertae sedis*.

*Barbatosphaeria* is phenotypically similar to *Lentomitella* in ascospore morphology and the long-necked, dark ascomata surrounded by sparse mycelium or pubescence. However, *Barbatosphaeria* can be distinguished as ascomata form circular formations with the pubescence disappearing with age; clavate asci arising from croziers on proliferating ascogenous hyphae without a high-level branching system and with a shallow apical ring and basal pedicel and smooth ascospores (Réblová 2007). Both genera are clearly separated in our phylogenetic tree (Fig. 1) and other studies (Réblová 2007).

*Ceratostomella* sensu lato comprises 110 species in Index Fungorum (2017), with the type, *Ceratostomella vestita* Sacc. *Lentomitella* was introduced by Höhnelt (1905) based on *C. vestita* to accommodate taxa distinct from *Ceratostomella* in having ornamented (longitudinally striate) ascospore walls. *Ceratostomella* was discussed and

redescribed by Réblová (2006), who emended the generic concept based on lectotype species *C. rostrata* (Tode: Fr.) Sacc. *Ceratostomella* can be characterized by dark, astromatic, elongate ascomata surrounded by sparse mycelium; clavate to cylindrical-clavate asci arising from branched, discrete ascogenous hyphae, with short pedicels and a shallow, indistinct, J- apical ring and pale brown aseptate, ascospores ranging from suballantoid to irregularly ellipsoid, to globose, to reniform., sometimes with terminal pores (from emended generic concept in Réblová 2006).

*Xylomelasma* is similar to *Ceratostomella* in having pigmented ascospores before discharge, similar ascogenous hyphae and asci floating within the centrum at maturity, but differs in having cylindrical, septate paraphyses, generally larger cylindrical asci with a distinct, refractive, apical ring and ellipsoidal to oblong, obliquely uni-seriate ascospores (Réblová 2006).

The genus *Natantiella* was shown to cluster with *Barbatosphaeriaceae* in Senanayake et al. (2016). In our study, however, it is close to *Ophiostomatales*, but with weak support. This is similar to Jaklitsch et al. (2013) and Réblová et al. (2014). Therefore, we place *Natantiella* in Sordariomycetes, genera *incertae sedis* as in Maharachchikumbura et al. (2016).

***Lentomitellaceae*** H. Zhang, K.D. Hyde & Maharachch., *fam. nov.*

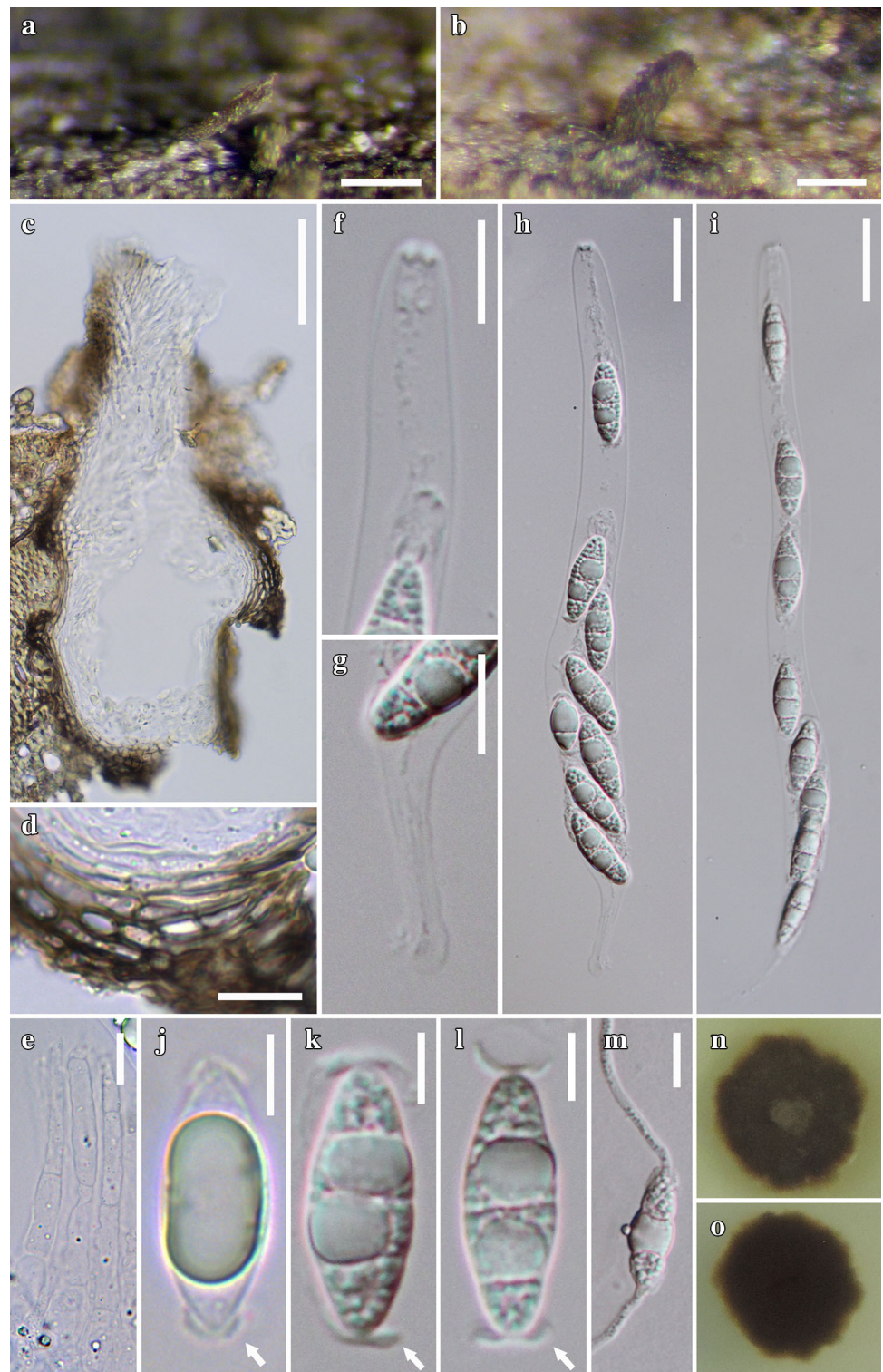
*Index Fungorum:* IF553764; *Facesoffungi number:* FoF 03342

*Saprobic* on branch or decorticated wood. **Sexual morph:** *Ascomata* solitary or gregarious, immersed to superficial, globose, subglobose or conical, glabrous or slightly roughened, dark brown. *Necks* dark brown, central, elongate, periphysate. *Peridium* coriaceous, two-layered, outer layer composed of brown, thick-walled cells of *textura prismatica* to *textura angularis*, externally with a row of heavily melanized cells, inner layer composed of subhyaline to hyaline, thinner-walled, elongated and compressed cells. *Paraphyses* numerous, cylindrical, tapering. *Asci* 8-spored, cylindrical-clavate, sessile, with a refractive, J-, apical ring. *Ascospores* obliquely 1–2-seriate, hyaline, ellipsoidal, aseptate to several septate. **Asexual morph:** Hyphomycetous. *Conidiophores* macronematous, mononematous, hyaline, short. *Conidiogenous cells* hyaline, cylindrical, tapering toward the apex, terminal or intercalary, bearing several hyaline denticles. *Conidia* ellipsoidal to globose, apiculate at the base, aseptate, (Conidia produced in culture of *Lentomitella crinigera*, description from Réblová 2006).

Type genus: *Lentomitella* Höhnelt.

*Notes:* *Lentomitellaceae* is introduced to accommodate the presently monotypic genus *Lentomitella* in Diaportheomycetidae, families *incertae sedis*. The genus is

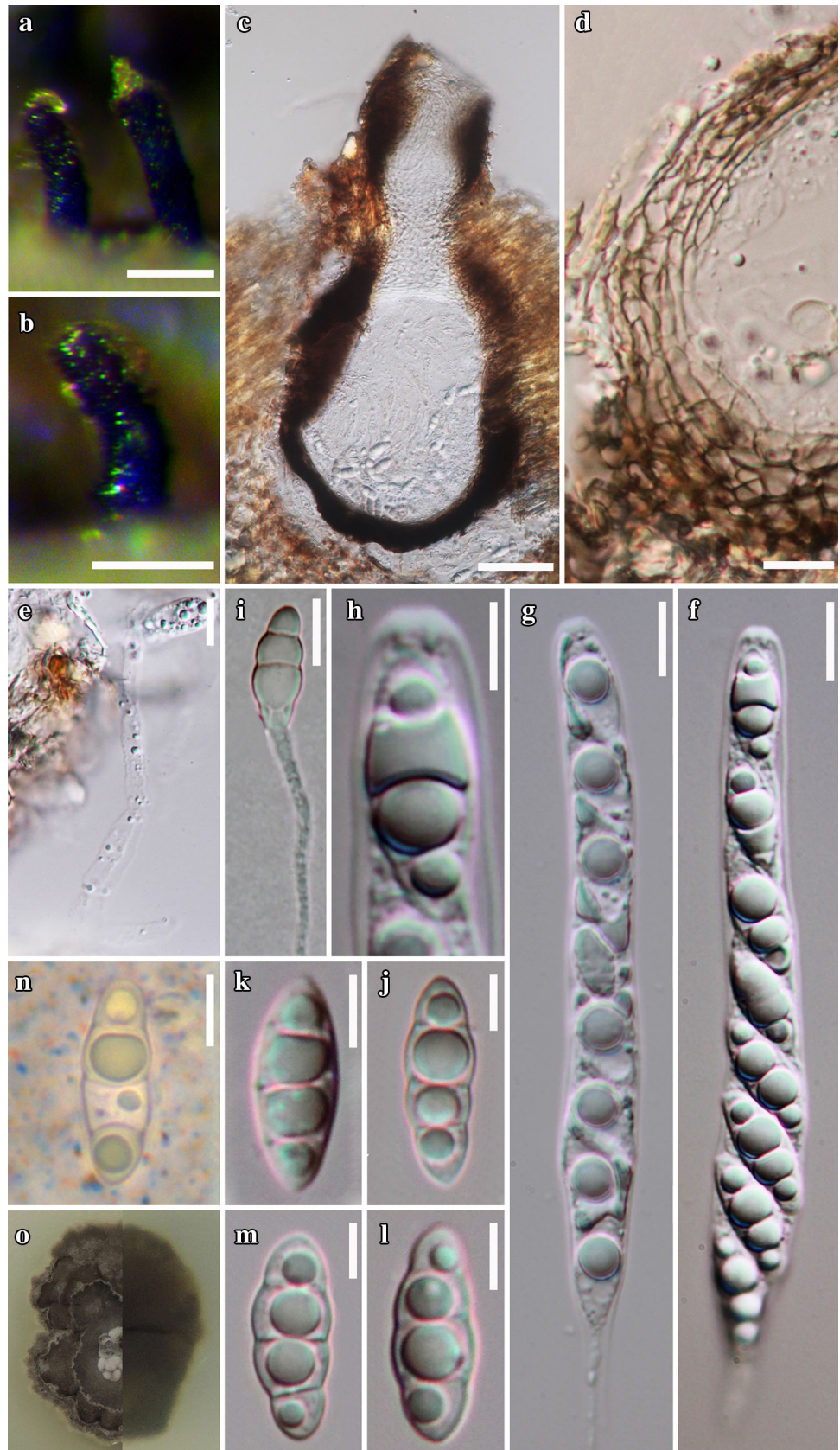
**Fig. 7** *Fluminicola aquatica* (holotype) **a, b** Appearance of necks on host. **c** Vertical section of ascoma. **d** Structure of peridium. **e** Paraphyses. **f–i** Unitunicate asci. **j** Young ascospore with bipolar appendages (arrowed). **k–l** Mature ascospore with bipolar appendages (arrowed). **m** Germinating ascospore. **n** Colony on PDA (from front). **o** Colony on PDA (from reverse). Scale bars **a** = 200  $\mu\text{m}$ ; **b** = 100  $\mu\text{m}$ ; **c** = 50  $\mu\text{m}$ ; **d–g, m** = 10  $\mu\text{m}$ ; **h–i** = 20  $\mu\text{m}$ ; **j–l** = 5  $\mu\text{m}$



characterized by dark ascomata, with long or short necks, cylindrical, short-pedicellate or sessile asci, with a distinct, refractive, apical ring and mostly hyaline, 1–2-seriate mostly aseptate ascospores. *Lentomitella* was segregated from *Ceratostomella* by Höhnelt (1905), based on the type

species *C. vestita* Sacc. *Lentomitella* accommodates taxa that have dark brown ascomata with long necks, cylindrical-clavate, sessile asci with a distinct, J-, apical ring, relatively wide, distinctly tapering paraphyses, hyaline, ellipsoidal, aseptate or several-septate ascospores, often

**Fig. 8** *Fluminicola saprophytica* (**holotype**) **a**, **b** Appearance of necks on host. **c** Vertical section of ascoma. **d** Structure of peridium. **e** Paraphyses. **f–h** Unitunicate asci. **i** Germinating ascospore. **j–m** Ascospores mounted in water. **n** Ascospores mounted in India ink. **o** Colony on PDA (from *front* and *reverse*). Scale bars **a** = 100  $\mu$ m; **b** = 200  $\mu$ m; **c** = 50  $\mu$ m; **d–g**, **i** = 10  $\mu$ m; **h**, **j–n** = 5  $\mu$ m



with longitudinal ridges and a hyphomycetous asexual morph (Réblová 2006). Sequence data and morphological characters also warrant the distinction between *Lentomitella* and *Ceratostomella* (Réblová 2006). *Lentomitella* comprises seven species in Index Fungorum (2017), with the type *L. vestita* (Sacc.) Höhn, with the current name *L. cirrhosa* (Réblová 2006). All species, except *L. pallibrunnea* (pale brown ascospores) and *L. tropica* (pale brown ascospores), have hyaline ascospores. A mucilaginous sheath was observed in some spores of *L. unipretoriae* (Marincowitz et al. 2008).

The clade comprising species of *Pseudoannulatasascus*, *Fusoidispora* and *Ascocollumdensa* forms a sister clade with *Lentomitellaceae* in phylogenetic tree with weak support (Fig. 1). The monotypic genus *Pseudoannulatasascus* was segregated from *Annulatasascus* to accommodate *Annulatasascus biatriisporus* K.D. Hyde based on molecular study and morphological differences that are large asci, and larger, long-fusiform ascospores with swollen ends (Hyde 1995; Luo et al. 2015). The ascomata are immersed, ellipsoidal with long, black and cylindrical necks, asci unitunicate, cylindrical, short-pedicellate with a large, refractive, spherical, apical ring; and ascospores overlapping uni-seriate, aseptate, hyaline, long fusiform with a thin mucilaginous sheath (Luo et al. 2015). *Fusoidispora* is characterized by dark brown ascomata lying horizontal to the host surface with very short necks, long cylindrical, pedicellate asci with a refractive, discoid, J-, apical ring and hyaline, fusoid to sickle-shaped, guttulate, aseptate to rarely 5-septate ascospores with globose mucilaginous pads at the ends (Vijaykrishna et al. 2005). *Pseudoannulatasascus* and *Fusoidispora* were accepted in *Annulatasaceae* in the study of Maharachchikumbura et al. (2016). But they are distant from *Annulatasaceae* in our phylogenetic tree. Therefore, we suggest placement of *Pseudoannulatasascus* and *Fusoidispora* in Diaporthomycetidae genera *incertae sedis* rather than *Annulatasaceae* until more evidence is available. The monotypic genus *Ascocollumdensa* was introduced by Ranghoo et al. (1999) with *A. aquatica* Ranghoo, K.D. Hyde & E.C.Y. Liew as the type species. It has never been validly published. It was briefly described as having “black perithecia with long necks, cylindrical asci with a relative large refractive apical ring and ascospores with appendages”. It needs recollecting, examining and sequencing because it has been placed in Xylariomycetidae, Diaporthomycetidae and Lulworthomycetidae in different papers. We do not include *Ascocollumdensa* in *Lentomitellaceae* until more evidence is available.

***Papulosaceae*** Winka & O.E. Erikss., Mycoscience 41 (2): 102 (2000)

*Notes:* The family *Papulosaceae* was introduced by Winka and Eriksson (2000) to accommodate the monotypic

marine genus *Papulosa*. Maharachchikumbura et al. (2015) placed two freshwater genera, *Brunneosporella* (as *Ascobrunneispora aquatica* Ranghoo, K.D. Hyde & E.C.Y. Liew) and *Fluminicola* in this family. It is characterized by immersed or semi-immersed, black to dark brown, globose to ellipsoidal ascomata, with long periphysate necks, cylindrical asci with a short pedicel and a refractive, discoid, J-, apical ring, and uniseriate to biseriate, hyaline or brown, ellipsoidal or fusiform, unicellular to 3-septate ascospores, with or without cup-like, bipolar appendages (Kohlmeyer and Volkmann-Kohlmeyer 1993; Maharachchikumbura et al. 2016). Maharachchikumbura et al. (2016) placed the genus in Diaporthomycetidae families *incertae sedis* and provided a discussion concerning the family. Su et al. (2016) showed that *Papulosaceae* formed a sister clade to *Sporidesmiaceae* and *Trichosphaeriaceae*. In our study, it clusters with *Annulatascales* (Fig. 1) and *Sporidesmiaceae* (Fig. 2).

The monotypic genus *Platytrachelon*, typified by *P. abietis* (Réblová) Réblová also nests in *Papulosaceae* with strong support in our study, as shown in previous studies (Jaklitsch et al. 2013; Réblová 2013; Réblová et al. 2014). The genus was placed in Diaporthomycetidae genera *incertae sedis* in Maharachchikumbura et al. (2016) due to its simple and inconspicuous morphology. *Platytrachelon abietis* is characterized by immersed, dark brown to black, globose to subglobose ascomata with long, central necks, cylindrical-clavate asci with a long pedicel and a refringent wedge-shaped apical ring, and uniseriate, hyaline, fusiform, 3(–5)-septate ascospores (Réblová 2013). The genus produces a hyphomycetous asexual morph which has ellipsoid, pale brown, 1-septate conidia. *Platytrachelon abietis* fits the characters of *Papulosaceae* well except for the freshwater habitat. We therefore place the genus in *Papulosaceae*.

***Fluminicola*** S.W. Wong, K.D. Hyde & E.B.G. Jones, Fungal Diversity Res. Ser. 2: 190 (1999)

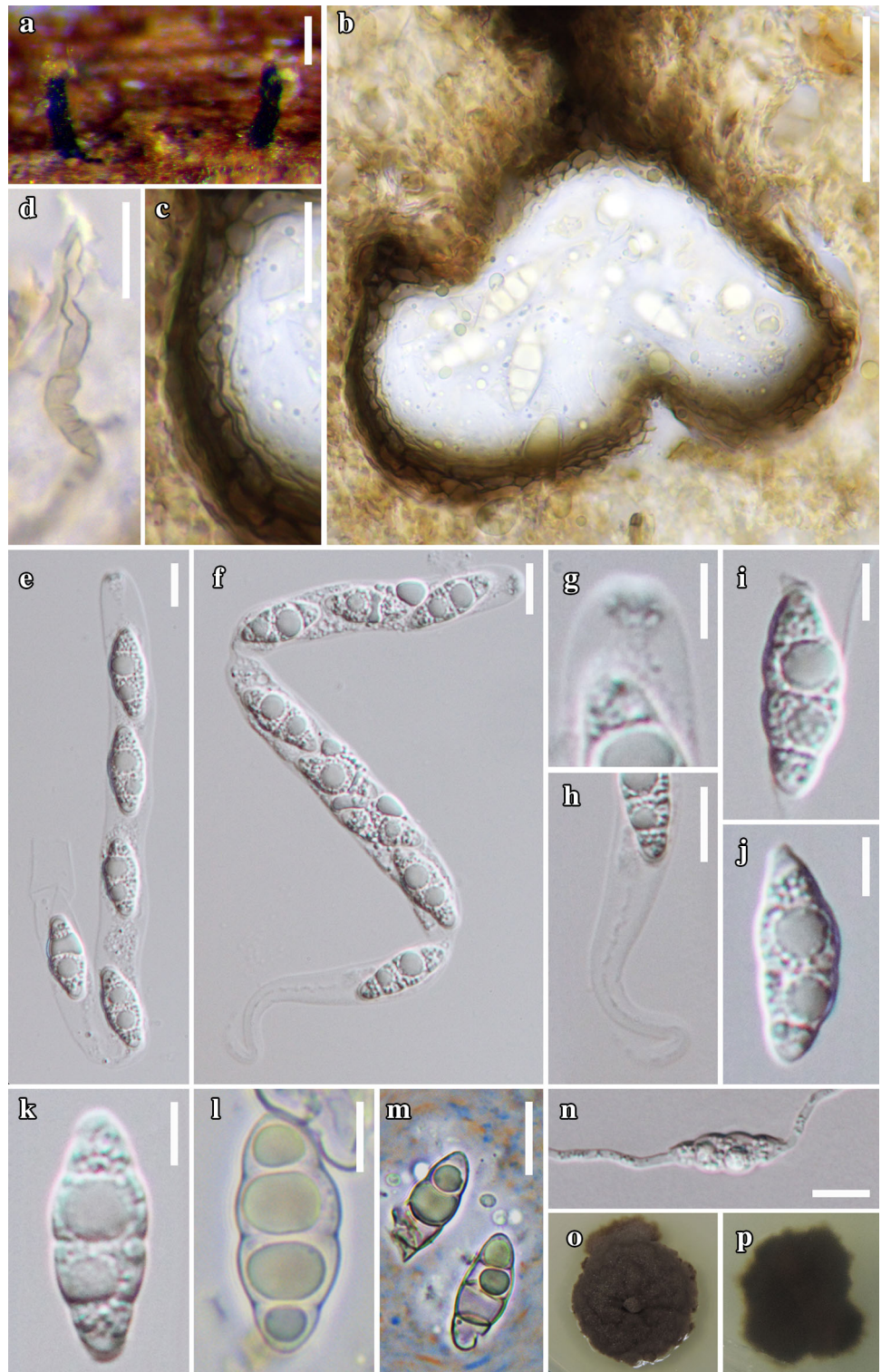
*Notes:* The genus *Fluminicola* was established for the freshwater species *F. bipolaris* (Wong et al. 1999b). It is characterized by cylindrical asci with a relatively massive, refractive apical ring and fusiform, hyaline ascospores with irregular bifurcate or cup-like bipolar appendages (Wong et al. 1999b). *Fluminicola* was placed in *Annulatasaceae* by Wong et al. (1999b), and transferred to *Papulosaceae* by Réblová (2013) based on phylogenetic analyses. This was also shown by Maharachchikumbura et al. (2015) and our study (Fig. 1). The asexual morph of *Fluminicola* remains to be discovered.

***Fluminicola aquatica*** W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

*Index Fungorum:* IF553765; *Facesoffungi* number: FoF 03344; Fig. 7



**Fig. 9** *Fluminicola thailandensis* (holotype)  
**a** appearance of necks on host.  
**b** Vertical section of ascoma.  
**c** Structure of peridium.  
**d** Paraphyses. **e–h** Unitunicate asci.  
**i–k** Ascospores mounted in water.  
**l** Ascospore mounted in Melzer's Reagent.  
**m** Ascospores mounted in India ink clearly showing mucilaginous sheath.  
**n** Germinating ascospore.  
**o** Colony on PDA (from front).  
**p** Colony on PDA (from reverse). Scale bars  
**a** = 100  $\mu$ m; **b** = 30  $\mu$ m; **c–f**,  
**h, k, n** = 10  $\mu$ m; **g, i–j, l–**  
**m** = 5  $\mu$ m



**Etymology:** In reference to the freshwater habitat.  
**Holotype:** MFLU 15-2710  
**Saprobic** on decaying wood submerged in freshwater.  
**Sexual morph:** *Ascomata* 140–180  $\mu$ m high, 100–140  $\mu$ m in diam., scattered or solitary, immersed with neck

erumpent through host surface, uniloculate, ellipsoidal, glabrous, brown, coriaceous. *Ostiole* 155–175  $\mu$ m long, 55–65  $\mu$ m wide, cylindrical, central, brown, periphysate. *Peridium* 15–25  $\mu$ m wide, two-layered, outer layer comprising 5–6 layers of dark brown, thick-walled, large cells

of *textura angularis*, inner layer comprising 4–5 layers of pale brown to hyaline, thin-walled, compressed cells of *textura angularis*. *Paraphyses* ca 6.6  $\mu\text{m}$  diam. at the base, up to 1.9  $\mu\text{m}$  at the apex, tapering, cylindrical, unbranched, hyaline, septate, constricted at the septa. *Asci* 180–200  $\times$  9–14  $\mu\text{m}$  ( $\bar{x}$  = 185.7  $\times$  10.2  $\mu\text{m}$ ,  $n$  = 10), 8-spored, unitunicate, cylindrical, subcylindrical or lanceolate, with a short, narrow pedicel, ca 20  $\mu\text{m}$  long, with an indistinct, refractive, relatively small, discoid, J-, apical ring, 1.9–2.1  $\mu\text{m}$  high  $\times$  2.7–3  $\mu\text{m}$  wide. *Ascospores* 19–22  $\times$  6–8  $\mu\text{m}$  ( $\bar{x}$  = 20.4  $\times$  6.7  $\mu\text{m}$ ,  $n$  = 20), obliquely uni-seriate, hyaline, fusiform, straight or slightly curved, with a single prominent guttule, aseptate when young, becoming 1–2 guttulate and 3-septate when mature, slightly constricted at the septa, smooth and thin-walled, with bifurcate bipolar appendages. **Asexual morph:** Undetermined.

**Material examined:** THAILAND, Prachuap Khiri Khan, on submerged wood in a small river, 30 July 2015, W. Dong 42B (MFLU 15-2710, **holotype**); ex-type culture MFLUCC 15-0962.

**Culture characteristics:** On PDA, colony circular, 10 mm in 15 days at 25 °C, dark brown to black from above, black from below, umbonate, rough, dry, edge entire.

**Notes:** Morphologically, our collection should be placed in *Fluminicola* as it has characteristic features of cylindrical asci with an apical ring and fusiform, hyaline ascospores with bifurcate bipolar appendages. *Fluminicola aquatica* is similar to *F. bipolaris*. It differs from *F. bipolaris* in the colour of the ascomata (brown vs. black) and longer asci (180–200  $\times$  9–14  $\mu\text{m}$  vs. 107–192  $\times$  9–12  $\mu\text{m}$ ). Additionally, the arrangement of ascospores in the asci can distinguish the taxa. *Fluminicola bipolaris* has a relatively massive, refractive, apical ring (3–6  $\mu\text{m}$  high  $\times$  1.5–3  $\mu\text{m}$  wide), while *F. aquatica* has a relatively small, apical ring (1.9–2.1  $\mu\text{m}$  high  $\times$  2.7–3  $\mu\text{m}$  wide). *Fluminicola coronata* Ranghoo, K.D. Hyde & E.C.Y. Liew has never been validly published. It was described as having “black perithecia with long necks, cylindrical asci with a relative large refractive apical ring and ascospores with appendages” (Ranghoo et al. 1999). Molecular data confirmed that *F. aquatica* can be distinguished from *F. coronata* and the other two taxa within this genus defined in this study.

***Fluminicola saprophytica*** W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

**Index Fungorum:** IF553766; **Facesoffungi number:** FoF 03345; Fig. 8

**Etymology:** In reference to saprobic life mode of the fungus.

**Holotype:** MFLU 15-2694

**Saprobic** on decaying bamboo submerged in freshwater.

**Sexual morph:** *Ascomata* 200–230  $\mu\text{m}$  high, 145–175  $\mu\text{m}$  diam., scattered or solitary, immersed with neck erumpent through host surface, globose, subglobose or ellipsoidal, black. *Ostiole* central, brown, with straight necks. *Peridium* 12–23  $\mu\text{m}$  thick, two-layered, outer wall comprising 3–4 layers of brown, thick-walled, large cells of *textura angularis* to *textura prismatica*, inner wall comprising 2–3 layers of pale brown to hyaline, thin-walled, compressed cells of *textura angularis*. *Paraphyses* ca 6.5  $\mu\text{m}$  diam. at the base, up to 2.5  $\mu\text{m}$  at the apex, tapering, sparse, unbranched, hyaline, septate, constricted at the septa, often with minute guttules. *Asci* 120–140  $\times$  8–12  $\mu\text{m}$  ( $\bar{x}$  = 116.2  $\times$  9.4  $\mu\text{m}$ ,  $n$  = 10), 8-spored, unitunicate, cylindrical or subcylindrical, pedicellate, tapering to a point, apically rounded or slightly obtuse, with an indistinct, refractive, small, discoid, J-, apical ring, 2.4–2.7  $\mu\text{m}$  high  $\times$  2.4–2.9  $\mu\text{m}$  wide. *Ascospores* 15–19  $\times$  6–7  $\mu\text{m}$  ( $\bar{x}$  = 17.0  $\times$  6.5  $\mu\text{m}$ ,  $n$  = 10), obliquely uni-seriate and partially overlapping, 3-septate, fusiform, straight or slightly curved, constricted at the septa, with 4 prominent guttules, hyaline, smooth and thick-walled, with an irregular sheath. **Asexual morph:** Undetermined.

**Material examined:** THAILAND, Prachuap Khiri Khan, on submerged bamboo in a small river, 30 July 2015, W. Dong 04A (MFLU 15-2694, **holotype**); ex-type culture MFLUCC 15-0976.

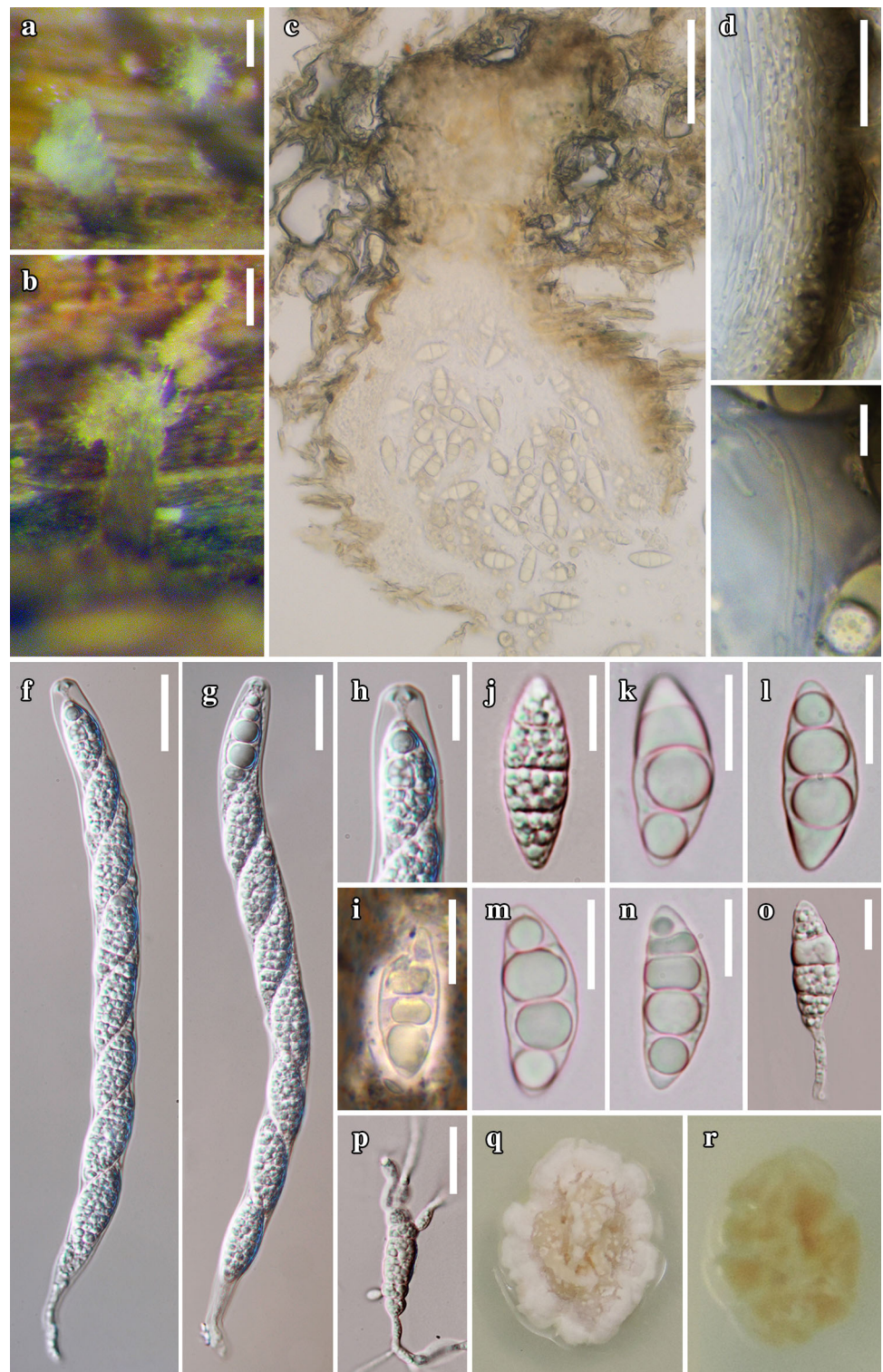
**Culture characteristics:** On PDA, colony irregular, reaching 15 mm in 40 days at 25 °C, dark brown to grey from above, black from below, umbonate, veined or rough, dry, edge curled.

**Notes:** The ascospores of *Fluminicola saprophytica* are of similar length and shape as those of the type species, *F. bipolaris*, but lack bifurcate, bipolar appendages in contrast to the latter species. It also differs from *F. bipolaris* in its smaller apical rings, 2.4–2.7  $\mu\text{m}$  high  $\times$  2.4–2.9  $\mu\text{m}$  wide vs. 3–6  $\mu\text{m}$  high  $\times$  1.5–3  $\mu\text{m}$  wide. *Fluminicola saprophytica* is similar to *F. aquatica* in having immersed ascomata with neck erumpent through host surface, and 3-septate, fusiform, guttulate, hyaline ascospores. However, *F. saprophytica* has smaller ascospores with an irregular sheath and without bifurcate bipolar appendages contrasting with those of *F. aquatica*. *Fluminicola saprophytica* clustered together with *F. aquatica* in our phylogenetic tree (Fig. 1).

***Fluminicola thailandensis*** W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

**Index Fungorum:** IF553767; **Facesoffungi number:** FoF 03346; Fig. 9

**Fig. 10** *Sporidesmium thailandense* (holotype) **a**, **b** Appearance of ascomata on host. **c** Vertical section of ascoma. **d** Peridium. **e** Paraphyses. **f–g** Unitunicate asci. **h** Ascus with a wedge-shaped apical ring. **i–n** Ascospores. **i** is mounted in India ink showing the sheath. **o** Ascospore with germ tube on substratum. **p** Germinating ascospore. **q** Colony on PDA (from front). **r** Colony on PDA (from reverse). Scale bars **a**, **b** = 100  $\mu$ m; **c** = 50  $\mu$ m; **d**, **f**, **g**, **p** = 20  $\mu$ m; **e** = 5  $\mu$ m; **h–o** = 10  $\mu$ m



**Etymology:** In reference to Thailand, where the holotype was collected.

**Holotype:** MFLU 15-2704

**Saprobic** on decaying bamboo submerged in freshwater.

**Sexual morph:** Ascomata 60–100  $\mu$ m high, 130–180  $\mu$ m

diam., scattered or solitary, deeply immersed with neck erumpent through host surface, ellipsoidal with the base tortuous toward the interior, black. *Ostiole* central or lateral, brown, with straight upright, black necks, ca 230  $\mu$ m long. *Peridium* 14–18  $\mu$ m thick, two-layered, outer layer

comprising 3–4 layers of dark brown, thick-walled, large cells of *textura angularis*, inner layer comprising 2–3 layers of pale brown, thin-walled, compressed cells of *textura angularis*. *Paraphyses* ca 2.7  $\mu\text{m}$  diam. at the base, up to 0.5  $\mu\text{m}$  at the apex, tapering, sparse, unbranched, thick-walled, brown, septate, not constricted at the septa. *Asci* 186–193  $\times$  8–12  $\mu\text{m}$  ( $\bar{x}$  = 189.5  $\times$  10.2  $\mu\text{m}$ ,  $n$  = 10), 8-spored, unitunicate, cylindrical, pedicellate, up to 52  $\mu\text{m}$  long, tapering to a point, apically rounded or slightly obtuse, with an indistinct, refractive, small, discoid, J-, apical ring, 1.5–3  $\mu\text{m}$  high  $\times$  2.7–3.9  $\mu\text{m}$  wide. *Ascospores* 14–20  $\times$  7–9  $\mu\text{m}$  ( $\bar{x}$  = 19.2  $\times$  7.6  $\mu\text{m}$ ,  $n$  = 20), uni-seriate or overlapping uni-seriate, 3-septate, fusiform, constricted at the septa, with 2 prominent guttules, hyaline, smooth and thick-walled, with a thin mucilaginous sheath. **Asexual morph:** Undetermined.

*Material examined:* THAILAND, Prachuap Khiri Khan, on submerged wood in a small river, 30 July 2015, W. Dong 22A (MFLU 15-2704, **holotype**); ex-type culture MFLUCC 15-0984.

*Culture characteristics:* On PDA, colony irregular, reaching 15 mm in 25 days at 25 °C, dark brown to black from above, black from below, raised, rough, dry, edge undulate.

*Notes:* *Fluminicola thailandensis* is most similar to *F. saprophytica* in having fusiform, 3-septate, guttulate ascospores. However, it can be distinguished from *F. saprophytica* by its longer asci (186–193  $\times$  8–12  $\mu\text{m}$  vs. 120–140  $\times$  8–12  $\mu\text{m}$ ) and wider ascospores (7–9  $\mu\text{m}$  vs. 6–7  $\mu\text{m}$ ). In our phylogenetic tree, two taxa formed a sister clade with strong support (100% ML, 100% MP) (Fig. 1).

**Sporidesmiaceae** Fr. [as ‘Sporidemiacei’], Summa veg. Scand., Sectio Post. (Stockholm): 504 (1849)

*Saprobic* on woody plants in terrestrial and aquatic habitats. **Sexual morph:** *Ascomata* scattered or solitary, immersed, uniloculate, subglobose or ellipsoidal, coriaceous. *Ostiole* cylindrical, periphysate. *Peridium* three-layered. *Paraphyses* numerous, tapering, hypha-like. *Asci* 8-spored, unitunicate, long cylindrical, with a tapering pedicel, with a distinct, J-, apical ring. *Ascospores* obliquely uni-seriate, fusiform, hyaline. **Asexual morph:** Hyphomycetous. *Colonies* hairy or velvety, effuse, brown, grey or black. *Conidiophores* macronematous, monone-matous, solitary or sometimes aggregated, unbranched, brown. *Conidiogenous cells* monoblastic, holoblastic, terminal, cylindrical, brown, proliferating once percurrently at apex. *Conidia* acrogenous, solitary, brown, obclavate.

Type genus: *Sporidesmium* Link

*Notes:* The family *Sporidesmiaceae* was introduced by Fries (1849), but the taxon has not been widely used in modern polymorphic classification. The family is typified by *Sporidesmium* with *S. ehrenbergii* as the lectotype

species (see below) and Shenoy et al. (2006) showed the genus to be polyphyletic. Su et al. (2016) treated *Ellisembia* as a synonym of *Sporidesmium* based on molecular data and placed it in the monotypic *Sporidesmiaceae* and this is followed here. In our study, *Sporidesmiaceae* shows a sister relationship to *Distoseptisporaceae* and *Lentomitellaceae* in the phylogenetic tree (Fig. 1), while it clusters with *Papulosaceae* in the evolution tree (Fig. 2). We report the first molecular-based sexual morph for *Sporidesmiaceae* in this study.

**Sporidesmium** Link, Mag. Gesell. naturf. Freunde, Berlin 3(1–2): 41 (1809)

Synonymy:

=*Podoconis* Boedijn, Bull. Jard. bot. Buitenz, 3 Sér. 13(1): 133 (1933)

=*Ellisembia* Subram., Proc. Indian natn Sci. Acad., Part B. Biol. Sci. 58(4): 183 (1992)

*Facesoffungi* number: FoF 01750

*Saprobic* on woody plants in terrestrial and aquatic habitats. **Sexual morph:** *Ascomata* scattered or solitary, immersed with neck erumpent through host surface, uniloculate, subglobose or ellipsoidal, glabrous, brown, coriaceous. *Ostiole* cylindrical, periphysate. *Peridium* three-layered, outer layer comprising several layers of dark brown to black, oblong and rounded cells, middle layer comprising 3–4 layers of pale brown to hyaline, compressed cells of *textura prismatica*, inner layer comprising 4–5 layers of hyaline, large cells of *textura angularis* or irregular cells. *Paraphyses* numerous, tapering, hypha-like, hyaline, septate, unbranched, persistent, embedded in a gelatinous matrix. *Asci* 8-spored, unitunicate, long cylindrical, with a tapering pedicel, apically rounded, with a distinct, relatively small, refractive, wedge-shaped, J-, apical ring. *Ascospores* obliquely uni-seriate, fusiform, hyaline, 3-septate. **Asexual morph:** Hyphomycetous. *Colonies* hairy or velvety, effuse, brown, grey or black. *Mycelium* superficial or immersed, composed of branched, septate, thin-walled, smooth, hyaline to pale brown hyphae. *Conidiophores* macronematous, monone-matous, solitary, or sometimes aggregated in groups, cylindrical, straight or curved, erect or somewhat repent, unbranched, brown, septate, smooth-walled. *Conidiogenous cells* monoblastic, integrated, terminal, cylindrical, brown, proliferating percurrently at apex. *Conidia* acrogenous, solitary, pale brown to brown, obclavate, or cylindrical with tapering ends, with hyaline apex and truncate base, euseptate or distoseptate, length determinate, truncate at the base, some with basal scar, smooth-walled (description of asexual morph from Su et al. 2016).

Lectotype: *Sporidesmium ehrenbergii* M.B. Ellis

*Notes:* The generic concept of *Sporidesmium* has been defined and emended several times in its history (Link

1809; Ellis 1958, 1971; Wu and Zhuang 2005; Shenoy et al. 2006; Su et al. 2016). Su et al. (2016) provided a detailed discussion on this genus and provided a description based on the lectotype *S. ehrenbergii* M.B. Ellis. The type species of this genus needs to be epitypified (sensu Ariyawansa et al. 2014), in order to fix the generic concept (Su et al. 2016). The sexual morph of *Sporidesmium* has never been confirmed. In this study, we collected a sexual morph taxon which clustered with *Sporidesmium* species with high support (Fig. 1). This is the first sexual morph of *Sporidesmium* confirmed by molecular study.

***Sporidesmium thailandense*** W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

*Index Fungorum*: IF553768; *Facesoffungi* number: FoF 03347; Fig. 10

*Holotype*: MFLU 15-2709

*Etymology*: In reference to Thailand, where the holotype was collected.

*Saprobic* on decaying wood submerged in freshwater.

**Sexual morph**: *Ascomata* 150–190 µm high, 190–230 µm in diam., scattered or solitary, immersed with neck erumpent through host surface, uniloculate, subglobose or ellipsoidal, glabrous, brown, coriaceous. *Ostiole* 290–320 µm long, 80–90 µm wide, cylindrical, central or lateral, brown at the base, becoming hyaline towards the apex, periphysate. *Peridium* 25–35 µm thick, three-layered, outer layer comprising several layers of dark brown to black, oblong and rounded cells, middle layer comprising 3–4 layers of pale brown to hyaline, compressed cells of *textura prismatica*, inner layer comprising 4–5 layers of hyaline, large cells of *textura angularis* or irregular cells. *Paraphyses* ca 3.5 µm diam. at the base, up to 1 µm at the apex, tapering, numerous, hypha-like, hyaline, septate, unbranched, persistent, embedded in a gelatinous matrix. *Asci* 160–220 × 11–14 µm ( $\bar{x}$  = 189.1 × 12.5 µm, n = 10), 8-spored, unitunicate, long cylindrical, with a tapering pedicel up to 22 µm long, apically rounded, with a distinct, relatively small, refractive, wedge-shaped, J-, apical ring, 2–2.2 µm high × 4.5–4.8 µm wide. *Ascospores* 23–28 × 8–10 µm ( $\bar{x}$  = 25.2 × 8.9 µm, n = 20), not overlapping, obliquely uni-seriate, fusiform, straight or slightly curved, hyaline, 3-septate, slightly constricted at the septa, thin-walled, guttulate when young, with 2–5 prominent guttules when mature, with a thin sheath. **Asexual morph**: Undetermined.

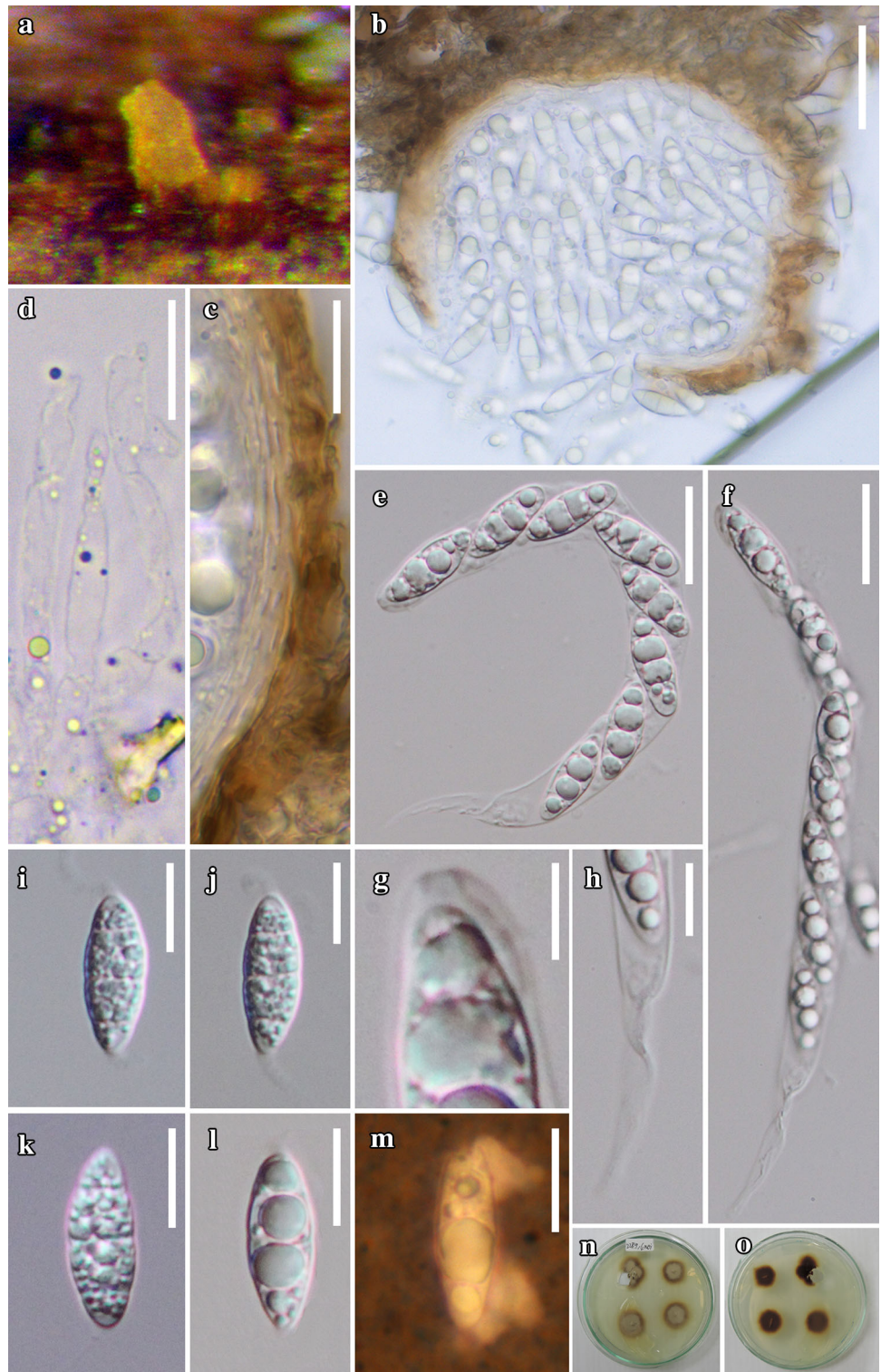
*Material examined*: THAILAND, Prachuap Khiri Khan, on submerged wood in a small river, 30 July 2015, W. Dong 11A (MFLU 15-2709, **holotype**); ex-type culture MFLUCC 15-0964.

*Culture characteristics*: On PDA, colony irregular, reaching 10 mm in 40 days at 25 °C, white to yellow from above, yellow from below, umbonate, rough or wrinkled, dry, edge undulate.

*Notes*: The sexual morphs of *Sporidesmium* have been associated with the dothideomycetous genera, *Akaropeltella*, *Eupelte* and *Placosoma*, as well as the sordariomycetous genera *Miyoshiella* and *Umbrinosphaeria* (Casagrande 1969; Sivanesan 1984; Hyde et al. 2011; Wijayawardene et al. 2012). *Miyoshiella fusispora* Kawamura (type), *M. larvata* Réblová and *M. triseptata* (Shoemaker & White) Réblová were considered to be associated with *Sporidesmium* in Réblová (1999). They all have cylindrical asci with an apical ring and fusiform, 3-septate ascospores (Shoemaker 1985; Réblová 1999), which are similar to the new collection. However, *Miyoshiella* can be separated by the characters of ascomata (superficial, black, pyriform ascomata often associated with asexual morph in *Miyoshiella* vs. immersed, brown, subglobose or ellipsoidal ascomata with neck erumpent through host surface and without asexual morph surrounded in the new collection) and peridium (two-layered peridium with outer layer comprising dark cells of *textura angularis*, inner layer comprising hyaline, compressed cells in *Miyoshiella* vs. three-layered peridium with outer layer comprising dark, oblong and rounded cells, middle layer comprising brown, compressed cells of *textura prismatica*, inner layer comprising hyaline, large cells of *textura angularis* or irregular cells in the new collection) (Shoemaker 1985; Réblová 1999). *Umbrinosphaeria caesariata* (Clinton & Peck) Réblová resembles the new collection in having cylindrical, pedicellate asci and fusiform, septate, guttulate ascospores, but it differs by its larger ascomata often sparsely covered with conidiophores and sterile, and 7(–12)-septate, 2–3-seriate ascospores (middle cells becoming brown when old) (Réblová 1999). None of these taxa can be linked by molecular data and as this character form is common amongst freshwater fungi, we treat the new collection as a distinct species until the above taxa are sequenced. Therefore, this sexual morph is introduced as a new species in *Sporidesmium* and it is the first sexual morph species proven by molecular studies.

*Sporidesmium thailandense* is similar to *Fluminicola saprophytica* in having immersed ascomata with necks erumpent through host surface, cylindrical asci and obliquely uni-seriate, fusiform, 3-septate, guttulate ascospores. However, they can be distinguished by the characters of neck (black in *F. saprophytica* vs. brown at the base, becoming hyaline towards the apex in *S. thailandense*), size and shape of apical ring (2.4–2.7 µm high × 2.4–2.9 µm wide, indistinct, discoid apical ring in *F. saprophytica* vs. 2–2.2 µm high × 4.5–4.8 µm wide, distinct, wedge-shaped apical ring in *S. thailandense*) and spore size (15–19 × 6–7 µm in *F. saprophytica* vs. 23–28 × 8–10 µm in *S. thailandense*). Additionally, ascospores lack of sheaths in *F. saprophytica*.

**Fig. 11** *Dictyosporella thailandensis* (holotype)  
**a** Appearance of necks on host.  
**b** Vertical section of ascoma.  
**c** Structure of peridium.  
**d** Paraphyses. **e–h** Unitunicate asci.  
**i–l** Ascospores mounted in water. **m** Ascospore mounted in India ink showing the appendages. **n** Colony on PDA (from front). **o** Colony on PDA (from reverse). Scale bars **b** = 30  $\mu$ m; **c, d, h–m** = 10  $\mu$ m; **e, f** = 20  $\mu$ m; **g** = 5  $\mu$ m



**Woswasiaceae** H. Zhang, K.D. Hyde & Maharachch., *fam. nov.*

*Index Fungorum*: IF553769; *Facesoffungi* number: FoF 03348

*Saprobic*, hyperparasitic or hypersaprotrophic on stromata of *Diaporthe*. **Sexual morph**: *Ascomata* aggregated

or in groups, stromatic, immersed to erumpent, globose, subglobose or ellipsoid, coloured, seated in a stroma or basal stroma. *Ostiole* centrally located, cylindrical, upright or slightly horizontal to the substrate surface, pale brown, dark brown to black. *Paraphyses* abundant, persistent, hyaline, septate. *Asci* 8-spored, unitunicate, cylindrical to

fusoid, with a distinctive, J-, apical ring. *Ascospores* uniseriate or overlapping uni-seriate, globose, subglobose, ellipsoidal or fusiform, hyaline, unicellular or septate, thin-walled or thick-walled, verruculose or smooth-walled, with or without gelatinous sheath. **Asexual morph:** Produced in culture with or without sporodochial conidiomata. *Conidiophores* aggregated, hyaline to subhyaline, with a clavate or penicillate head or conidiogenous cells developing directly on hyphae. *Conidiogenous cells* monoblastic or polyblastic, terminal, sympodially proliferating, cylindrical, hyaline. *Conidia* ellipsoid to obovoid, tapering towards the base, hyaline, aseptate, smooth-walled (sexual morph and asexual morph description from Jaklitsch et al. 2013 and Réblová et al. 2014).

**Type genus:** *Woswasia* Jaklitsch, Réblová & Voglmayr

**Notes:** *Woswasiaceae* is distinctive among lignicolous Diaporthomycetidae in forming coloured, globose to subglobose ascomata with a cylindrical neck and ascomata nested together in stromatic or nonstromatic tissues. Asci are unitunicate, cylindrical to fusoid, with a J-, apical ring. Ascospores are globose, subglobose or ellipsoidal, hyaline, unicellular or septate, verruculose or smooth-walled and thin-walled or thick-walled (Jaklitsch et al. 2013 and Réblová et al. 2014). Three genera, *Woswasia*, *Xylochrysis* and *Cyanoannulus* are included according to our phylogenetic analysis. A close relationship of *Woswasia* and *Xylochrysis* is supported in both the phylogenetic (Fig. 1) and evolution trees (Fig. 2) as well as in recent studies (Jaklitsch et al. 2013; Réblová et al. 2014; Senanayake et al. 2016). *Xylochrysis* is phenotypically more similar to *Woswasia* as it often has aggregated astromatic ascomata or with bases slightly immersed and seated on a small, basal stroma. The venter is surrounded by a golden yellow layer of cells. Asci are cylindrical, with a J-, wedge-shaped, apical ring (Réblová et al. 2014). The asexual morphs produced from culture have similar holoblastic conidiogenesis and branched conidiophores with clavate heads (Réblová et al. 2014). Réblová et al. (2014) compared the two genera and mentioned the main differences as phenotypical characters of ascomata (ascomata with stromata with colour changing with the addition of water and 3% KOH in *Woswasia*, whereas astromatic or base seated on an inconspicuous stroma which colour not changing in *Xylochrysis*) and morphology of ascospores (globose to subglobose, verruculose and thick-walled in *Woswasia*, whereas ellipsoidal, smooth and thin-walled in *Xylochrysis*). In addition, *Woswasia* is apparently hyperparasitic or hypersaprotrophic on stromata of *Diaporthe oncostoma* (Duby) Fuckel (Jaklitsch et al. 2013), while *Xylochrysis* is saprobic and terrestrial (Réblová et al. 2014).

*Cyanoannulus*, monotypic with *C. petersenii* Raja. is stable and close to *Woswasia* and *Xylochrysis*, but has weak support (Fig. 1, Réblová et al. 2014, Senanayake

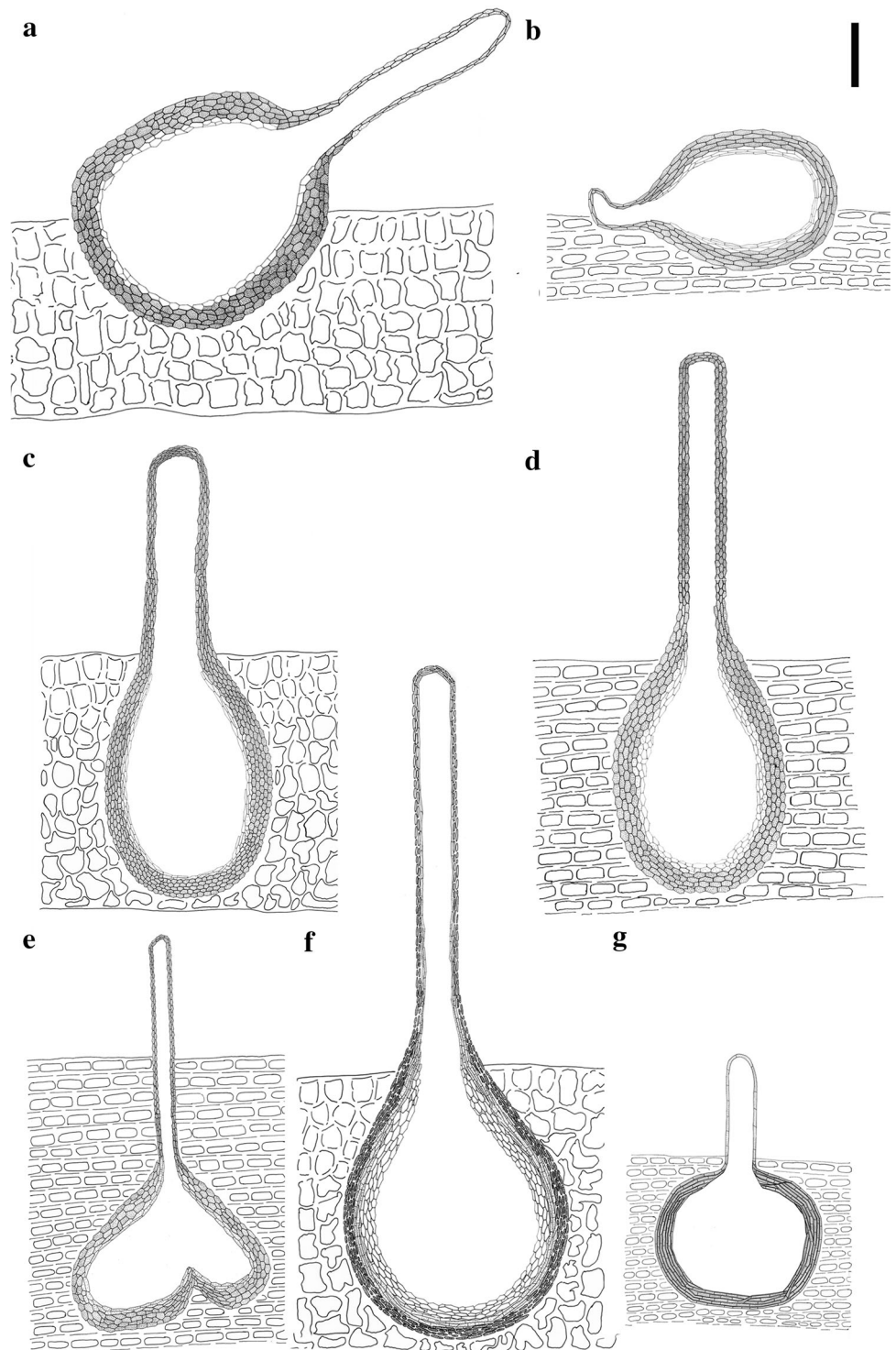
et al. 2016). It was saprobic on submerged wood in freshwater streams and previously placed in *Annulatascaceae* based on LSU sequence data (Raja et al. 2003). *Cyanoannulus* is characterized by immersed pale reddish-brown, globose ascomata, slightly horizontal to the substrate surface, central, long, cylindrical necks becoming tapering and lighter towards the apex, fusoid asci with a large, bipartite, J-, but cotton blue positive, apical ring and thick-walled ascospores with a narrow channel at the apices and with a mucilaginous sheath (Raja et al. 2003). The characters, such as coloured ascomata, morphology of asci fits *Woswasiaceae* well. Therefore, no other placement is presently more suitable than *Woswasiaceae* for *Cyanoannulus*. *Woswasia* resembles *Cyanoannulus* in having pale brown to dark ascomata with long necks, a two layered peridium, J-, but cotton blue positive asci and hyaline ascospores. *Woswasia* is easily distinguished from *Cyanoannulus* by its characteristic colour changing stromata that is dark pink, purple or brown on surface when dry, purple or nearly violet when rehydrated, turning immediately green to black after the addition of 3% KOH; the stromata surface comprises a layer of intricate, hyaline, pink to brownish hyphae; ascomata separate towards the margins, are then superficial, the venter entirely enclosed in a dense pink hyphal subiculum with a glabrous neck (Jaklitsch et al. 2013). *Cyanoannulus* has no stromata. Because *Woswasiaceae* has been shown sister to *Phomatosporales* (Fig. 1), *Magnaporthales* (Senanayake et al. 2016) or *Ceratolenta caudata* (Jaklitsch et al. 2013; Réblová et al. 2014), we place the genus in Diaportheomycetidae, families *incertae sedis* until more taxa are discovered.

#### Diaportheomycetidae genera *incertae sedis*

*Aquaticola* W.H. Ho, K.M. Tsui, Hodgkiss & K.D. Hyde, Fungal Diversity Res. Ser. 3: 88 (1999)

**Notes:** The genus *Aquaticola* is polyphyletic (Réblová et al. 2016) and therefore its characters must be taken from the type species, *Aquaticola hyalomura* W.H. Ho, K.M. Tsui, Hodgkiss & K.D. Hyde (Ho et al. 1999) whose placement is not stable. It clusters close to the family *Pseudoproboscisporaceae* in our phylogenetic tree (Fig. 1), while it is close to *Bullimyces communis* and *Ceratolenta caudata* in the evolution tree (Fig. 2). This species which occurs on submerged wood in freshwater, has white to pale brown ascomata, small, broadly oblong asci with a relatively wide, ring-like, apical ring and tapering base, and small, overlapping uni-seriate to biserial ascospores with distinct guttules. *Aquaticola ellipsoidea* W.H. Ho, K.M. Tsui, Hodgkiss & K.D. Hyde is somewhat similar, but has dark brown to black ascomata, cylindrical asci and 1-celled, inequilaterally fusoid to

**Fig. 12** Peridium of new species. **a** *Atractospora thailandensis* (HKAS 96226, **holotypus**). **b** *Diluvicola aquatica* (MFLU 15-2701, **holotypus**). **c** *Fluminicola aquatica* (MFLU 15-2710, **holotypus**). **d** *Fluminicola saprophytica* (MFLU 15-2694, **holotypus**). **e** *Fluminicola thailandensis* (MFLU 15-2704, **holotypus**). **f** *Sporidesmium thailandense* (MFLU 15-2709, **holotypus**). **g** *Dictyosporella thailandensis* (MFLU 15-2706, **holotypus**). Scale bars 50  $\mu\text{m}$



rhomboid ascospores (Ho et al. 1999). In the phylogenetic tree (Fig. 1), *A. ellipsoidea* clusters with *Atractospora* species and was synonymized as *Atractospora ellipsoidea* by Réblová et al. (2016). *Aquaticola hongkongensis* Ranghoo, K.D. Hyde & E.C.Y. Liew which has never been validly published and groups with *Atractospora* species.

The type species of *Aquaticola* needs to be recollected and epitypified (sensu Ariyawansa et al. 2014) to establish if *Atractospora* is a synonym, however, they appear to be morphologically distinct. We tentatively place *Aquaticola* in Diaporthomycetidae genera *incertae sedis* instead of in *Annulatascaceae* (Maharachchikumbura et al. 2016).



**Dictyospora** Abdel-Aziz, in Ariyawansa et al., Fungal Diversity 75: 143 (2015)

*Saprobic* on grass stem or decaying bamboo submerged in freshwater. **Sexual morph:** *Ascomata* scattered or solitary, immersed with neck erumpent through host surface, uniloculate, globose, subglobose or ellipsoidal, glabrous, brown, coriaceous. *Ostiole* long, cylindrical, periphysate. *Peridium* comprising of several layers of brown, thick-walled, compressed cells of *textura porrecta*. *Paraphyses* numerous, cylindrical, hyaline, septate, unbranched, persistent. *Asci* 8-spored, unitunicate, long cylindrical, with a tapering pedicel, apically rounded, with an indistinct, J-, apical ring. *Ascospores* uni-seriate or overlapping uni-seriate, fusiform, hyaline, 3-septate, with filamentous bipolar appendages. **Asexual morph:** *Mycelium* superficial and immersed in the substrate, composed of branched, septate, smooth, yellow–brown to brown hyphae. *Conidiophores* reduced. *Conidiogenous cells* holoblastic. *Conidia* terminal and lateral, unicellular, determinate, helicoid when young and quickly becoming a mass of cells, brown to black. Conidial cells are generally similar in shape, colour and size that are globose, subglobose, rounded or with truncate base, brown to dark-brown (from Ariyawansa et al. 2015).

**Notes:** The genus *Dictyospora* was introduced for an asexual morph species *D. aquatica*, which is characterized by helicoid, brown to black, globose or subglobose conidia (Ariyawansa et al. 2015). *Dictyospora* was placed in the family *Annulatasceae* (Ariyawansa et al. 2015; Maharachchikumbura et al. 2016). In our phylogeny (Fig. 1), *Dictyospora* grouped in *Distoseptisporaceae*, while in the evolution tree *Dictyospora* clustered with *Pseudoproboscisporaceae*. Given this, we suggest that *Dictyospora* is temporarily assigned to *Diaorthomycetidae*, genera, *incertae sedis*.

Phylogenetic analyses showed that our collection grouped together with *Dictyospora aquatica* with high support, but is a different species (Fig. 1). Thus, a new species, *Dictyospora thailandensis* is introduced in *Dictyospora*. This is the first account of a sexual morph for this genus.

**Dictyospora thailandensis** W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

*Index fungorum:* IF553770; *Facesoffungi* number: FoF 03349; Fig. 11

**Etymology:** In reference to Thailand, where the holotype was collected.

**Holotype:** MFLU 15-2706

*Saprobic* on decaying bamboo submerged in freshwater. **Sexual morph:** *Ascomata* 50–100  $\mu\text{m}$  high, 40–90  $\mu\text{m}$  in diam., scattered or solitary, immersed with neck erumpent

through host surface, uniloculate, globose, subglobose or ellipsoidal, glabrous, brown, coriaceous. *Necks* 70–100  $\mu\text{m}$  long, 40–60  $\mu\text{m}$  wide, cylindrical, central or slightly lateral, hyaline to pale yellow, thin-walled, periphysate. *Peridium* 14–19  $\mu\text{m}$  thick, comprising of several layers of brown, thick-walled, compressed cells of *textura porrecta*. *Paraphyses* ca 3.5  $\mu\text{m}$  diam. at the base, up to 1  $\mu\text{m}$  at the apex, tapering, numerous, cylindrical, hyaline, septate, unbranched, persistent. *Asci* 130–155  $\times$  9–12  $\mu\text{m}$  ( $\bar{x}$  = 138.6  $\times$  10.5  $\mu\text{m}$ , n = 10), 8-spored, unitunicate, long cylindrical, pedicellate, up to 35  $\mu\text{m}$  long, suddenly tapered at one-third, apically rounded, with an indistinct, small, refractive, wedge-shaped, J-, apical ring, 1.3–1.6  $\mu\text{m}$  high  $\times$  2.1–2.3  $\mu\text{m}$  wide. *Ascospores* 18–22  $\times$  6–8  $\mu\text{m}$  ( $\bar{x}$  = 20  $\times$  7.1  $\mu\text{m}$ , n = 20), uni-seriate or overlapping uni-seriate, fusiform, straight, hyaline, 3-septate, slightly constricted at the septa, thin-walled, with large prominent guttules when old, with filamentous bipolar appendages. **Asexual morph:** Undetermined.

**Material examined:** THAILAND, Prachuap Khiri Khan, on submerged bamboo in a small river, 30 July 2015, W. Dong 22B (MFLU 15-2706, **holotype**); ex-type culture MFLUCC 15-0985.

**Culture characteristics:** On PDA, colony circular, researching 30 mm in 35 days at 25 °C, brown to grey from above, dark brown from below, flat, surface rough, dry, edge entire.

**Notes:** *Dictyospora thailandensis* is characterized by immersed ascomata with subhyaline, erumpent necks, long, unitunicate cylindrical asci with tapering pedicels, and small, refractive, wedge-shaped, J-, apical rings, and uni-seriate, hyaline, fusiform, 3-septate ascospores with bipolar appendages. Its ascospores are quite similar to the phylogenetically unrelated taxa, *Fluminicola aquatica* and *Sporidesmium thailandense*. However, in *Fluminicola aquatica*, ascomata have central, relatively long, dark brown necks and ascospores have bifurcate bipolar appendages, while in *Dictyospora thailandensis*, ascomata have lateral, short, hyaline to pale yellow necks and ascospores have filamentous bipolar appendages. *Sporidesmium thailandense* differs in its necks (brown at the base, becoming hyaline towards the apex) and larger ascospores (23–28  $\times$  8–10  $\mu\text{m}$  vs. 18–22  $\times$  6–8  $\mu\text{m}$ ), as well as ascospores that lack appendages. Line drawing of the peridia of *D. thailandensis* as well as other *Annulatasceae*-like new species are shown in Fig. 12.

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