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Towards a natural classification of *Astrosphaeriella*-like species; introducing *Astrosphaeriellaceae* and *Pseudoastrosphaeriellaceae* fam. nov. and *Astrosphaeriellopsis*, gen. nov.

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Abstract *Astrosphaeriella* sensu *lato* is a common genus occurring on bamboo, palms and stout grasses. Species of *Astrosphaeriella* have been collected from various countries in tropical, subtropical or temperate regions. In Asia, species have been collected in Brunei, China, Indonesia, Japan, Philippines and Vietnam. There have been several morphological studies on *Astrosphaeriella*, but molecular work and phylogenetic analyses are generally lacking. Taxa included in *Astrosphaeriella* were characterized in three main groups 1) typical *Astrosphaeriella* species (*sensu stricto*) having carbonaceous, erumpent, conical ascostromata 2) atypical *Astrosphaeriella* species (*sensu lato*) having immersed, coriaceous ascostromata with short to long papilla and 3) lophiostoma-like species having immersed ascostromata with slit-like openings. Some of the latter *Astrosphaeriella* species, having slit-like openings, have been transferred to *Fissuroma* and *Rimora* in *Aigialaceae*. In this study five type specimens of *Astrosphaeriella* were loaned from herbaria worldwide and re-examined and are re-described and illustrated. Collections of *Astrosphaeriella* were also made in Thailand and morphologically examined. Pure cultures were obtained from single spores and used in molecular studies. The asexual morph was induced on sterile bamboo pieces placed on water agar. Phylogenetic analyses of combined LSU, SSU and *TEF1* sequence data of astrosphaeriella-like species using Bayesian, Maximum parsimony (MP) and Randomized Accelerated Maximum Likelihood (RAxML) analyses were carried out. Phylogenetic analyses show that species of *Astrosphaeriella* can be distinguished in at least three families. Species of *Astrosphaeriella sensu stricto* with erumpent, carbonaceous ascostromata, form a strongly supported clade with *Pteridiospora* species and a new family, *Astrosphaeriellaceae*, is introduced to accommodate these taxa. The genera are revised

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and *Astrosphaeriella bambusae*, *A. neofusispora*, *A. neostellata*, *A. thailandica*, *A. thysanolaenae* and *Pteridiospora chiangraiensis* are introduced as new species. *Astrosphaeriella exorrhiza* is reported on a dead stem of *Thysanolaena maxima* and is the first record for Thailand. Reference specimens for *A. fusispora* and *A. tornata* are designated to stabilize the taxonomy of *Astrosphaeriella*. The coelomycetous asexual morph of *A. bambusae* is reported and forms hyaline, globose to subglobose, aseptate conidia. Species of *Astrosphaeriella sensu lato* with immersed, coriaceous ascostromata, with short to long papilla and striate ascospores, form a sister clade with *Tetraplosphaeriaceae*. The genus *Pseudoastrosphaeriella* is introduced to accommodate some of these taxa with three new species and three new combinations, viz. *P. aequatoriensis*, *P. africana*, *P. bambusae*, *P. longicolla*, *P. papillata* and *P. thailandensis*. A new family *Pseudoastrosphaeriellaceae* is introduced to accommodate this presently monotypic lineage comprising *Pseudoastrosphaeriella*. The asexual morph of *P. thailandensis* is described. *Astrosphaeriella bakeriana* forms a distinct clade basal to *Aigialaceae*. *Astrosphaeriella bakeriana* is excluded from *Astrosphaeriella* and a new genus *Astrosphaeriellopsis*, placed in Dothideomycetes genera *incertae sedis*, is introduced to accommodate this taxon. *Fissuroma aggregata* (*Aigialaceae*) is re-visited and is shown to be a cryptic species. Three new species of *Fissuroma* and a new combination are introduced based on morphology and phylogeny viz. *F. bambusae*, *F. fissuristoma*, *F. neoaggregata* and *F. thailandicum*. The asexual morph of *Fissuroma bambusae* is also reported.

Keywords Asexual morph · Bamboo · Palm · *Pseudoastrosphaeriella* · Phylogeny · *Pleosporales* · Taxonomy

Introduction

Pleosporales is the largest order of Dothideomycetes and comprises 40 families, 255 genera and more than 4700 species (Kirk et al. 2008; Zhang et al. 2009a, 2012; Liu et al. 2011, 2015; Wijayawardene et al. 2014), while Liu et al. (2015) added a new family, *Paradictyoarthriniaceae* in *Pleosporales*. Some species of *Pleosporales* are important as they cause disease on plants, animals or humans (Rinaldi et al. 1987; Schoch et al. 2006, 2009; Hipolito et al. 2009; Manamgoda et al. 2011, 2012; Zhang et al. 2009a, 2012; de Gruyter et al. 2012; Hyde et al. 2013; Seyedmousavi et al. 2013). In addition, species of *Pleosporales* are also saprotrophs which decompose organic matter (Kruys et al. 2006; Schoch et al. 2006, 2009; Kodsueb et al. 2008; Zhang et al. 2009a, 2012; Hyde et al. 2013), endophytes (Sánchez Márquez et al. 2008, 2010; Zhang et al. 2009a; Sakayaroj et al. 2010; Bhagat et al. 2012) or epiphytes on living plants (Zhang et al. 2009a, 2012; Liu et al. 2011). Since 2009, various families in *Pleosporales* have been re-visited and their taxonomy and phylogeny reassessed (Tanaka et al. 2009; Manamgoda et al. 2011, 2012; de Gruyter et al. 2012; Zhang et al. 2012; Hyde et al. 2013; Quaedvlieg et al. 2013; Woudenberg et al. 2013, 2015; Ariyawansa et al. 2014a, b, c, d, e, 2015a, b; Phookamsak et al. 2014b; Thambugala et al. 2014). However, several groups could not be resolved by phylogenetic investigations due to limited molecular data and thus their taxonomy remained ambiguous (Zhang et al. 2012; Hyde et al. 2013; Liu et al. 2011, 2014; Phookamsak et al. 2014b).

Astrosphaeriella is a well-known, almost exclusively tropical genus occurring on bamboo, palms and stout grasses, but putative species are also known from subtropical or temperate regions, such as northern Florida Japan and the southwest of England (Barr 1990; Zhou et al. 2003; Tanaka and Harada 2005; Hu 2010; Zhang et al. 2012). The genus has been reported as saprobic or parasitic from aquatic, submerged or terrestrial habitats, mostly from dead parts of monocotyledons. However, some *Astrosphaeriella* species have been found on dead parts of dicotyledons, such as *Astrosphaeriella applanata* (Fr.) Scheinpflug, *A. inaequalis* (Fabre) Scheinpflug and *A. pinicola* (Rehm) Scheinpflug (Yamamoto et al. 1954; Scheinpflug 1958; Hyde and Fröhlich 1998; Hu 2010; Tanaka and Harada 2005; Zhang et al. 2012)

Astrosphaeriella was introduced to accommodate a dothideomycete species forming carbonaceous, conical to mammiform ascostromata, with ruptured, reflexed, stellate, host remnants around the base. The asci are bitunicate, cylindrical to cylindric-clavate, pseudoparaphyses trabeculate and ascospores pale brown to brown and fusiform (Barr 1990; Sydow and Sydow 1913; Fröhlich and Hyde 2000; Tanaka and Harada 2005; Tanaka et al. 2009; Liu et al. 2011). Theissen (1917) transferred *Pemphidium erumpens* (Berk. & M.A. Curtis) Sacc. to *Astrosphaeriella erumpens* (Berk. & M.A. Curtis) Theiss. von Höhnel (1920) introduced *A. bambusella* Höhn. from Java and Saccardo (1928) combined *Amphisphaeria stellata* Pat., which was morphologically similar to *A. fusispora* Syd. & P. Syd., under *Astrosphaeriella stellata* Sacc. There have been many morphological based studies on *Astrosphaeriella* (Scheinpflug 1958; Müller and von Arx 1962; von Arx and Müller 1975; Hawksworth 1981; Hawksworth and Boise 1985; Yue and Eriksson 1986; Barr 1990; Hyde 1992, 1994a, b; Aptroot 1995a; Fröhlich and Hyde 1995; Hyde and Fröhlich 1998; San Martín and Lavín 1999; Zhou et al. 2003; Chen and Hsieh 2004). However, there has been no detailed molecular investigation of this genus (Tanaka et al. 2009; Liu et al. 2011).

Other studies include Yamamoto et al. (1954), who introduced a new taxon, *Astrosphaeriella fuscomaculans* W. Yamam. causing fuscous speckles or "Unmon-chiku" on *Phyllostachys nigra* (Lodd. ex Lindl.) Munro. The fungus infected the culms and branches of living bamboo and formed

irregularly elliptic or wholly irregular specks, which were dark brown to blackish brown with white fine stripes (Yamamoto et al. 1954). Hino and Katumoto (1956) had also reported *A. aosimensis* I. Hino & Katum. from *Livistona subglobosa* (Hassk.) Mart. These two species were reported from temperate regions of Japan. Additionally, Scheinpflug (1958) introduced three *Astrosphaeriella* species from dicotyledons from temperate regions viz. *A. applanata*, *A. inaequalis*, and *A. pinicola*. However, all species accepted by Scheinpflug (1958) and another three *Astrosphaeriella* species viz. *A. aosimensis*, *A. fuscomaculans* and *A. fusispora* were synonymized under *Microthelia* by Müller and von Arx (1962) and this was followed by von Arx and Müller (1975) and other authors (Hawksworth 1981; Hu 2010; Liu et al. 2011).

Hawksworth (1981) mentioned that *Microthelia* should clearly be regarded as an earlier synonym of the lichenicolous genus *Anisomeridium* (Hawksworth 1981; Hawksworth and Sherwood 1981; Hu 2010). Therefore, Hawksworth (1981) reinstated *Astrosphaeriella* and designated *A. stellata*, an earlier name for *A. fusispora*, as the type. Hawksworth (1981) accepted only four species in *Astrosphaeriella* viz. *A. aosimensis*, *A. stellata*, *A. trochus* (Penzig & Sacc.) D. Hawksw. and *A. venezuelensis* Barr & D. Hawsw.; whereas, *A. fusispora* was treated as a synonym of *A. stellata*. Hawksworth and Boise (1985) introduced six more species, mostly with heavily pigmented and longitudinally striate ascospores in *Astrosphaeriella*. A key to the ten species of *Astrosphaeriella* was also provided in this paper. Yue and Eriksson (1986) re-studied the genus *Rhynchostoma* and found that *R. lageniforme* Teng. which was collected from China, was similar to *Astrosphaeriella*. Therefore, Yue and Eriksson (1986) transferred *R. lageniforme* to *Astrosphaeriella* as *A. lageniformis* (Teng) J.Z. Yue & O.E. Erikss. When Barr (1990) studied the order *Melanommatales* in North America, she also introduced a new species, *Astrosphaeriella floridana* Barr from *Sabal palmetto* (Walt.) Lodd. in Florida.

Hyde (1992, 1994a, b), Fröhlich and Hyde (1995) and Hyde and Fröhlich (1998) studied various *Astrosphaeriella* species occurring on palms in tropical regions. Hyde (1992) studied fungi on submerged decaying fronds of *Nypa fruticans* Wurmb. from Brunei and introduced two new species in *Astrosphaeriella* viz. *A. nypae* K.D. Hyde and *A. striataspora* (K.D. Hyde) K.D. Hyde. Hyde (1994b) studied fungi on rachides of *Livistona* in tropical Papua New Guinea and reported *A. aosimensis* from rachides of *Livistona*. In addition, he found a freshwater *Astrosphaeriella* species, *A. aquatica* K.D. Hyde and this was reported as the first *Astrosphaeriella* species from aquatic environments (Hyde 1994a). Fröhlich and Hyde (1995) had also introduced a new species, *Astrosphaeriella fronsicola* J. Fröhl. & K.D. Hyde, associated with leaf spots of *Oraniopsis* and other palms from Queensland.

Aptroot (1995a, b) re-visited the genus *Didymosphaeria* and treated *D. fusispora* Penz. & Sacc as a synonym of *Astrosphaeriella minima* Aptroot, while *D. pustulata* I. Hino & Katum. was synonymized under *A. minoensis* (Hara) D. Hawksw. Additionally, Aptroot (1995a) introduced *Astrosphaeriella papuana* Aptroot on bamboo from Papua New Guinea, a species with striate ascospores. Furthermore, Aptroot (1995b) suggested that *Didymosphaeria japonica* I. Hino & Katum., *D. macrospora* I. Hino & Katum. and *D. tosaensis* I. Hino & Katum. should be accommodated in *Astrosphaeriella* or *Roussoella*. Their large ascospores with a distichous arrangement pointed to the genus *Astrosphaeriella*, while the compound ascostromata were similar to the genus *Roussoella*. These three species mentioned by Aptroot (1995b) were currently accommodated in the genus *Roussoellopsis* by Hino and Katumoto (1965) and the genus was placed in the family *Roussoellaceae* based on molecular data in Liu et al. (2014) and Phookamsak et al. (2014a).

A major morphological study of *Astrosphaeriella* was carried out by Hyde and Fröhlich (1998). Hyde and Fröhlich (1998) collected several *Astrosphaeriella* species from bamboo and palms in Australia, South East Asia and South America. In their study, 31 *Astrosphaeriella* species were reported, with the key to species of *Astrosphaeriella* and these accepted species have been re-described and discussed. Additionally, Hyde and Fröhlich (1998) introduced ten new species with five new combinations viz. *Astrosphaeriella aequatoriensis* K.D. Hyde & J. Fröhl., *A. angustispora* J. Fröhl. & K.D. Hyde, *A. australiensis* K.D. Hyde & J. Fröhl., *A. bakeriana* (Sacc.) K.D. Hyde & J. Fröhl., *A. lenticularis* K.D. Hyde & J. Fröhl., *A. livistonicola* K.D. Hyde & J. Fröhl., *A. lophiostomopsis* K.D. Hyde & J. Fröhl., *A. malayensis* K.D. Hyde & J. Fröhl., *A. maquilingiana* (Rehm) K.D. Hyde & J. Fröhl., *A. mauritiae* K.D. Hyde & J. Fröhl., *A. nipicola* (Cooke & Massee) K.D. Hyde & J. Fröhl., *A. papillata* K.D. Hyde & J. Fröhl., *A. samuelsii* (Boise) K.D. Hyde & J. Fröhl., *A. splendida* K.D. Hyde & J. Fröhl. and *A. uberina* (Mont.) K.D. Hyde & J. Fröhl.

Further *Astrosphaeriella* species have been introduced and contributions to the genus have also been reported. San Martín and Lavín (1999) added two new species, *Astrosphaeriella stellata* var. *palmicola* F. San Martín & P. Lavín from palms in Chiapas (currently as *A. stellata*) and *A. vaginata* F. San Martín & P. Lavín from *Bactris baculifera* Karw. ex Mart. in Tabasco, Mexico. They had also described and illustrated some *Astrosphaeriella* species found in Mexico viz. *A. stellata*, *A. tornata* (Berk. & M.A. Curtis) D. Hawksw. & Boise and *A. trochus*.

The dothideomycete species characterized by ascostromata with laterally compressed- or crest-like ostioles occurring mainly on wood and bark were previously accommodated in the genus *Lophiostoma* Ces. & De Not. (Barr 1990; Yuan and Zhao 1994; Holm and Holm 1988; Hyde et al. 2000; Tanaka

and Harada 2003; Zhang et al. 2009b). However, the crest-like ostiole, could not always distinguish *Lophiostoma* from similar *Massarina* species. Barr (1990) used cellular versus trabeculate pseudoparaphyses to separate *Lophiostoma* in *Melanommatales* from genera of *Pleosporales* and *Astrosphaeriella* was placed in the former order. Liew et al. (2000) however, has shown that this classification lacks support from molecular data.

Hyde et al. (2000) collected some *Lophiostoma* species described from palms having trabeculate pseudoparaphyses, which were not congeneric with *Lophiostoma*, but more similar to *Astrosphaeriella*. Thus, Hyde et al. (2000) expanded the already broad generic concept of *Astrosphaeriella* to accommodate lophiostoma-like species having ascostromata with slit-like ostioles (lacking crests) and trabeculate pseudoparaphyses. Five species having slit-like ostioles were included in *Astrosphaeriella* in Hyde et al. (2000) viz. *A. asiana* (K.D. Hyde) Aptroot & K.D. Hyde, *A. daemonoropis* J. Fröhl., K.D. Hyde & Aptroot, *A. fissuristoma* J. Fröhl., K.D. Hyde & Aptroot, *A. maculans* (Rehm) Aptroot et al. and *A. mangrovis* (Kohlm. & Vittal) Aptroot & K.D. Hyde. *Astrosphaeriella immersa* Taylor et al. was also introduced in Hyde et al. (2000), but the species had round ostioles.

Hsieh et al. (2000) reported *Astrosphaeriella stellata* from Taiwan, while this species was also found in Hong Kong by Tsui et al. (2001). Zhou et al. (2003) surveyed the bambusicolous fungi and reported six *Astrosphaeriella* species from Hong Kong and Yunnan, China, including *A. bakeriana*, *A. fissuristoma*, *A. maculans*, *A. splendida* and *A. stellata*.

Rogers and Barr (2003) introduced a new species, *Astrosphaeriella longispora* J.D. Rogers & M.E. Barr from Costa Rica for a species having carbonaceous, conical ascostromata, and elongate fusiform ascospores. The species had been collected from angiosperm wood.

Chen and Hsieh (2004) described seven *Astrosphaeriella* species from bamboo and grasses in Taiwan and introduced two new species, *A. macrospora* Chi Y. Chen & W.H. Hsieh and *A. pallidipolaris* Chi Y. Chen & W.H. Hsieh in their study, both species having striate ascospores. Wang et al. (2004) also introduced two new combinations in *Astrosphaeriella* viz. *A. callicarpa* (Penz. & Sacc.) Y.Z. Wang et al., and *A. polymorpha* (Rehm & Fairm.) Y.Z. Wang et al. when they revised the genus *Amphisphaeria* and transferred some *Amphisphaeria* species to *Astrosphaeriella* (Hu 2010).

Tanaka and Harada (2005) transferred *Melanopsamma aggregata* I. Hino & Katum. to *Astrosphaeriella* following the concept of Hyde et al. (2000), while Jagadeesh Ram et al. (2005) introduced *Astrosphaeriella sundarbanensis* Jagadeesh & Aptroot on dry bark of *Sonneratia apetala* Buch.-Ham. from India. Chen and Huang (2006) introduced *A. linguiformis* Chi Y. Chen & J.W. Huang on bamboo from Taiwan. Aptroot (2009) also transferred *Arthopyrenia picea* Shirley to *Astrosphaeriella picea* (Shirley) Aptroot.

It is reportedly difficult to obtain cultures from *Astrosphaeriella* species (Liu et al. 2011), thus culture characteristics and molecular data for the genus are generally lacking (Tsui et al. 2001; Tanaka and Harada 2005; Liu et al. 2011). The phylogenetic placement of *Astrosphaeriella* was first shown by Tanaka et al. (2009) who introduced a new family *Tetraplosphaeriaceae* from bambusicolous fungi in *Pleosporales* and included three strains of *Astrosphaeriella aggregata* (I. Hino & Katum.) Kaz. Tanaka & Y. Harada and *A. stellata* in their analyses. The phylogenetic results showed that *Astrosphaeriella aggregata* formed a distinct clade separate from *A. stellata* and Tanaka et al. (2009) concluded that *A. aggregata* may not congeneric with *A. stellata*. This was supported in the studies of Schoch et al. (2009) where *A. aggregata* clustered in the family *Aigialaceae*, whereas, *A. bakeriana* formed a distinct clade at the base of this family. Suetrong et al. (2009) introduced *Rimora* in the family *Aigialaceae*, based on molecular study to accommodate a mangrove species, *Rimora mangrovei* (Kohlm. & Vittal) Kohlm et al., which was previously described as *Astrosphaeriella mangrovis* (Kohlm. & Vittal) Aptroot & K.D. Hyde (≡ *Lophiostoma mangrovei* Kohlm. & Vittal) in Hyde et al. (2000). Based on phylogenetic analyses, Liu et al. (2011) introduced two new genera, *Fissuroma* and *Neoastrosphaeriella* in *Aigialaceae* to accommodate species with immersed ascostromata with slit-like ostioles, which were previously accommodated in *Astrosphaeriella*. Ren et al. (2013) introduced a new species *Astrosphaeriella thailandensis* Ren et al. on submerged wood from a freshwater stream in Thailand. Phylogenetic analyses of combined LSU and SSU sequence data showed that *A. thailandensis* formed a robust clade (97 % MP) with *A. stellata* (MFLUCC 10-0555) and separated from *A. stellata* (strain KT 998). Whereas, Pratibha and Prabhugaonkar (2015) reported the sexual morph of *Pithomyces flavus* Berk. & Broome as *Astrosphaeriella vesuvius* (berk. & broome) D. Hawksw & Boise. This species was collected from a litter of *Calamus thwaitesii* in India. Multigene phylogenetic analysis showed that *Pithomyces flavus* clusters with *Astrosphaeriella vesuvius* and was closely related to *A. bakeriana* in their analysis.

The natural placement of *Astrosphaeriella* is unresolved. Hara (1913) introduced the family *Astrocystidiaceae* Hara (as'*Asterocystidiaceae*') and accommodated *Astrosphaeriella* and *Astrocystis* in this family. Although, there is similarity in the superficial ascostromata in some species of these genera, *Astrocystis* species are unitunicate *Xylariales* in Sordariomycetes (Maharachchikumbura et al. 2015; Senanayake et al. 2015). Barr (1987, 1990) re-introduced the family *Platystomaceae* and listed *Astrosphaeriella*, *Javaria*, *Platystomum*, *Pseudotrichia*, *Thyridaria* and *Trematosphaeria* in this family. Aptroot (1995a, b) followed

Barr (1987, 1990) and placed *Astrosphaeriella* in *Platystomaceae*, whereas, Hawksworth and Ainsworth (1995) arranged *Astrosphaeriella* differently, and referred the genus to *Melanommataceae* (Hu 2010; Liu et al. 2011). Subsequently, *Astrosphaeriella* was accommodated in *Melanommataceae* (Hyde et al 2000; Lumbsch and Huhndorf 2007; Kirk et al. 2008). Tanaka et al. (2009) however, suggested that the placement of *Astrosphaeriella* in *Melanommataceae* was not suitable and rejected the monophyletic status based on their phylogenetic study (Tanaka et al. 2009; Liu et al. 2011). Lumbsch and Huhndorf (2010) treated *Astrosphaeriella* in the *Pleosporales* family *incertae sedis* and this is concurred in Liu et al. (2011).

There are presently 61 species epithets for *Astrosphaeriella* in Index Fungorum (2015), while Kirk et al. (2008) estimated that there were 51 species in the genus; 104 individual sequences are available in GenBank.

The genus *Pteridiospora* had been originally reported as saprobic on bamboo in tropical regions (Penzig and Saccardo 1897; Phookamsak et al. 2014a). Filer (1969) introduced a new species, *P. spinosispora* Filer which was isolated from mycorrhizae of sweetgum (*Liquidambar styraciflua* L.) and green ash (*Fraxinus pennsylvanica* Marsh.) in Northern Mississippi. *Pteridiospora munkii* Subhedar & V.G. Rao was found on dead leaves of *Phoenix sylvestris* (L.) Roxb. from India (Farr and Rossman 2015; Index Fungorum 2015). Guseinov (2000) had also introduced *P. chochrjakovii* on *Quercus pedunculiflora* K.Koch from Azerbaijan. This indicates that *Pteridiospora* has a wide host range and is found in tropical and temperate regions. Six epithets are listed for *Pteridiospora* in Index Fungorum (2015), with two species currently transferred to *Splanchospora* (*Pleomassariaceae*). Kirk et al. (2008) estimated there were four species in *Pteridiospora*; five sequences from putative species are available in GenBank. Phookamsak et al. (2014a) collected *P. javanica* Penz. & Sacc. from Thailand and obtained molecular data from this species. *Pteridiospora javanica* clustered with *Astrosphaeriella* sensu stricto in their analyses. Phookamsak et al. (2014a) suggested that *Pteridiospora* is related to *Astrosphaeriella* and a new family was needed to accommodate these genera. This was also suggested in Liu et al. (2011).

Astrosphaeriella-like species appear to be numerous and polyphyletic and are in need of study using molecular data. In this study, we use 20 new strains of astrosphaeriella-like taxa, three strains of *Pteridiospora* and GenBank data to establish the phylogenetic relationships of astrosphaeriella-like taxa from the basal lineages of *Pleosporales*. The new family, *Astrosphaeriellaceae* is introduced to accommodate species of *Astrosphaeriella* sensu stricto and *Pteridiospora* species with erumpent, carbonaceous ascostromata, while species of *Astrosphaeriella* sensu *lato* with coriaceous, immersed ascostromata and distinct necks are placed in a new family *Pseudoastrosphaeriellaceae*. Two new genera, *Astrosphaeriellopsis* and *Pseudoastrosphaeriella* are introduced to accommodate species excluded from *Astrosphaeriella* sensu stricto.

Material and methods

Isolation and identification

The dothideomycete species were collected from dead stems or branches of bamboo and strout grasses in Chiang Mai and Chiang Rai Provinces, Thailand. The specimens were returned to the laboratory for observation, examination and description following the methods described in Phookamsak et al. (2014a, b). Single spore isolates were obtained as described in Chomnunti et al. (2014). The ascostromata were visualized under a stereo microscope (Motic series SMZ-140) and the peridium removed from the apex towards the sides by using a razor blade. The contents inside ascostromata which comprise asci, ascospores and pseudoparaphyses were picked off with a sterile needle and soaked in sterile water in a glass container. The fungal contents were broken up further mechanically until a spore suspension was obtained. The ascospore suspensions were dropped on water agar (WA; 15 g/l sterile distilled water) and incubated at room temperature overnight. Spore germination was observed and marked under the Nikon ECLIPSE 80i compound microscope and germinating ascospores were transferred to malt extract agar (MEA; 33.6 g/l sterile distilled water, Difco malt extract) and cultivated in corn meal agar (CMA; 17 g/l sterile distilled water, Difco corn meal agar) and potato dextrose agar (PDA; 39 g/l distilled water, Difco potato dextrose) for recording growth rates and culture characters.

Isolates are deposited in Mae Fah Luang University Culture Collection (MFLUCC) in Thailand, and duplicated in BIOTEC Culture Collection (BCC), Bangkok, Thailand and Kunming Culture Collection (KUMCC). A herbarium specimen was dried by using silica gel at room temperature for 7–10 days depending on the humidity and kept in an envelope or a small box for depositing in Mae Fah Luang University (MFLU), Chiang Rai, Thailand and duplicated in Biotech Bangkok Herbarium (BBH). Facesoffungi and Index Fungorum numbers were obtained as described in Jayasiri et al. (2015) and Index Fungorum (2015).

Type specimens of *Astrosphaeriella* species were requested from FH, IFRD and S herbaria. Type species were examined and re-described as in Hyde et al. (2013) and Phookamsak et al. (2014a, b). Ascostromata were initially rehydrated in water or adding 3–5 % KOH for 5–10 min. The ascostromata

and peridium structures are illustrated from free hand sections and the squash mounts were prepared to determine the micro-morphological characters such as asci, ascospores and hamathecium.

Micro-morphological characters were obtained under the Nikon ECLIPSE 80i compound microscope and captured by using a Cannon 550D digital camera with DIC microscopy. A Sony DSC-T110 digital camera was used to capture macro-morphological characters under a Motic (series SMZ-140) stereo microscope. Squash mount preparations was obtained for determining the micro-morphology, while free hand sections of ascostromata were made for illustrating ascostromata and peridium structures. The asci were placed in Melzer's reagent for checking the reactions of the apical ring, whereas ascospores were placed in Indian ink for checking mucilaginous sheaths surrounding the ascospores. Photographic plates were edited and combined using program Adobe Photoshop version CS5 (Adobe Systems Inc., The United States) and morphological characters measured in a Tarosoft (R) Image Frame Work version 0.9.7 program. Permanent slides were prepared by adding lactoglycerol and sealing with clear nail polish (Phookamsak et al. 2014a, b).

Method to induce the asexual morph in culture

Sterile bamboo pieces (bamboo tooth picks) on water agar (WA; 15 g/l sterile distilled water) were used to encourage production of asexual morphs (Fig. 1). The bamboo can be replaced with a different substrate, e.g. grass, rice straw, depending on the host from which the taxon was isolated and availability of material;

1.) Bamboo pieces were prepared in a media bottle (250 ml.) and sterilized by autoclaving at least three times (autoclave at 121 °C with 15 psi for 15 min). While agar powder was mixed with distilled water (15 g/l) and sterilized using an autoclave.
2.) Warm water agar (WA) was poured in glass petri dishes and surface dried in laminar flow cabinet.
3.) The sterilized bamboo pieces were placed on WA (4–5 pieces) and mycelia from the growing edge of the 1-month-old cultures were cut in small pieces and placed nearby the bamboo pieces (5 pieces) on WA. This step was carried out in the laminar flow cabinet to avoid contamination (Fig. 2).
4.) The petri dish was sealed by parafilm and left at room temperature for 1 to 3 months or longer, depending on the taxon. The culture was checked to establish if the asexual morph had formed under the Motic (series SMZ-140) stereo microscope and the morphological characters were captured under Nikon ECLIPSE 80i compound microscope with a Cannon 550D digital camera.

DNA extraction, PCR amplification and sequencing

DNA extraction was performed from fresh fungal mycelia growing on MEA or PDA over 1–4 weeks at room temperature (25–30 °C). The genomic DNA were obtained by a using DNA extraction kit (A Biospin Fungus Genomic DNA Extraction Kit, BioFlux®, China) following the protocols in the manufacturer's instructions (Hangzhou, P.R. China) (Phookamsak et al. 2014a, b). The DNA products were kept at 4 °C for use in DNA amplification and duplicated at -20 °C for long term storage.

The DNA amplification was obtained by polymerase chain reaction (PCR) using the respective gene primers (ITS, LSU, SSU, *RPB2*, and *TEF1*) and amplified following the protocols described in Phookamsak et al. (2014a, b). The quality of PCR products were checked by using 1 % agarose gel electrophoresis stained with ethidium bromide and sent to sequence at Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P.R. China) (Phookamsak et al. 2014a, b).

Phylogenetic analyses

A BLAST search based on LSU sequence data was carried out to reveal the closest matches with taxa in the order *Pleosporales*. Additionally, the sequences datasets in Schoch et al. (2009), Zhang et al. (2012), Hyde et al. (2013), Wijayawardene et al. (2014) and Ariyawansa et al. (2015a) were included in this study and are listed in Table 1. The newly generated sequence data of the fungal taxa were analyzed with related taxa and additional taxa from selected families in *Pleosporales* viz. *Aigialaceae*, *Amnicolicolaceae*, *Anteagloniaceae*, *Astrosphaeriella*, *Delitschiaceae*, *Lophiotremataceae*, *Lindgomycetaceae*, *Massariaceae*, *Salsugineaceae*, *Testudinaceae* and *Tetraplosphaeriaceae* obtained from GenBank (Table 1). The LSU, SSU and *TEF1* gene datasets were aligned singly by using MAFFT: multiple sequence alignment software version 7.215 (Katoh and Standley, 2013: http://mafft.cbrc.jp/alignment/server/) and improved manually where necessary in MEGA6 version 6.0 (Tamura et al. 2013). The combined sequence alignments were obtained from MEGA6 version 6.0 (Tamura et al. 2013) and the sequence pairwise comparisons were performed in BioEdit v. 7.2 (Hall 1999). The combined sequence alignment was converted to NEXUS file for maximum parsimony analysis using ClustalX2 v. 1.83 (Thompson et al. 1997) and converted to PHYLIP file for RAxML using ALTER (alignment transformation environment: http://sing.ei.uvigo.es/ALTER/; 2015). The NEXUS file deleting the symbol was prepared for MrModeltest v. 2.2 (Nylander 2004) in PAUP v. 4.0b10 (Swofford 2002).

The Randomized Accelerated Maximum Likelihood (RAxML) was obtained using RAxML version 7.3.0 (Silvestro and Michalak 2011). One thousand non parametric bootstrap

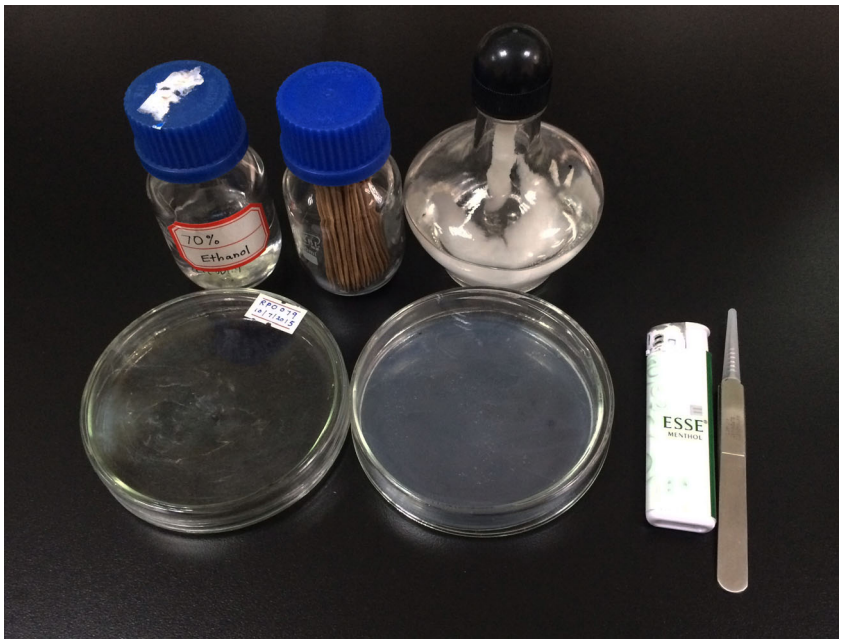


Fig. 1 General materials needed to induce the asexual morph. Alcohol burner, 70 % ethanol and lighter, extra fine forceps, sterilized bamboo pieces (bamboo tooth picks), water agar and fungal culture grown on MEA or PDA

iterations were run with generalized time reversible (GTR) and a discrete gamma distribution (Silvestro and Michalak 2011). A discrete GAMMA (Yang 1994) was complemented for each substitution model with four rate classes (Stamatakis et al. 2008). Rapid bootstrap analysis (Stamatakis et al. 2008) and searches for the best-scoring ML tree (RAxML option "-f a") were applied (Silvestro and Michalak 2011).

Maximum parsimony (MP) analysis was obtained by using PAUP v. 4.0b10 (Swofford 2002). The starting tree(s) was obtained via stepwise addition with 100 replications of sequences. One thousand bootstrap replicates with a heuristic search option and tree-bisection reconnection (TBR) of branch-swapping algorithms were obtained. Maxtrees were setup at 5000 with a zero of maximum branch length collapsed. Ambiguously aligned characters were excluded. Gaps were treated as missing data. The consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were applied for generating trees under different optimality criteria. Kishino-Hasegawa tests (KHT) (Kishino and Hasegawa 1989) were performed to determine significant parsimonious trees. Twenty-eight parsimonious trees were saved and the best parsimonious tree was selected to represent the phylogenetic relationships among the taxa (Ariyawansa et al. 2013; Phookamsak et al. 2014b).

Bayesian analysis was inferred from MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001) using a uniform [GTR+I+G] model (lset nst=6, rates = invgamma, Prset statefreqpr = dirichlet,1,1,1,1). The models of nucleotide substitution were performed by using MrModeltest 2.3 (Nylander 2004) and posterior probabilities (PP) were estimated from the Metropolis-Coupled Markov Chain Monte Carlo (MCMC) sampling approach. Four simultaneous Markov chains were run for 5000000 generations and trees sampled every 100th generations. The first 5000 trees representing the burn-in phase of convergence of the four chains were discarded. Whereas, the remaining trees were used to construct a 50 % majority rule consensus tree and calculating the Posterior Probabilities (PP). Bayesian Posterior Probabilities

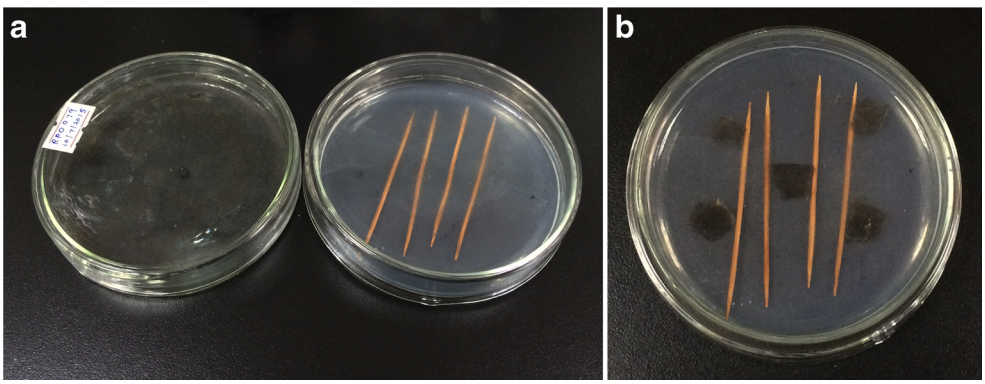


Fig. 2 Method to obtain asexual morphs in culture. **a** Representative culture on PDA after 1 month (*left*) and WA containing sterilize bamboo pieces (*right*). **b** Culture cut in small pieces and placed with bamboo pieces on WA

Table 1 Isolates used in this study and their GenBank accession numbers. The ex-type strains are in bold and the newly generated sequences are indicated in blue

Taxon	Culture/Voucher No[1]	GenBank Accession No.[2]		
		LSU	SSU	*TEF1*
Aigialus grandis	BCC 18419	GU479774	GU479738	GU479838
Aigialus grandis	BCC 20000	GU479775	GU479739	GU479839
Aigialus mangrovis	BCC 33563	GU479776	GU479741	GU479840
Aigialus mangrovis	BCC 33564	GU479777	GU479742	GU479841
Aigialus parvus	BCC 32558	GU479779	GU479743	GU479843
Aigialus parvus	BCC 18403	GU479778	GU479744	GU479842
Aigialus rhizophorae	BCC 33572	GU479780	GU479745	GU479844
Aigialus rhizophorae	BCC 33573	GU479781	GU479746	GU479845
Amniculicola immersa	**CBS 123083**	**FJ795498**	**GU456295**	**GU456273**
Amniculicola parva	**CBS 123092**	**FJ795497**	**GU296134**	**GU349065**
Anteaglonium globosum	SMH 5283	GQ221911		GQ221919
Anteaglonium parvulum	SMH 5223	GQ221909		GQ221918
Anteaglonium abbreviatum	**ANM 925a**	**GQ221877**		**GQ221924**
Ascocratera manglicola	HHUF 30032	GU479783	GU479748	GU479847
Ascocratera manglicola	BCC 09270	GU479782	GU479747	GU479846
Astrosphaeriella bambusae	MFLUCC 10-0095	JN846720	JN846741	
Astrosphaeriella bambusae	**MFLUCC 13-0230**	**KT955461**		**KT955424**
Astrosphaeriella fusispora	MFLUCC 10-0555	KT955462	KT955443	KT955425
Astrosphaeriella neofusispora	**MFLUCC 11-0161**	**KT955463**	**KT955444**	**KT955426**
Astrosphaeriella neostellata	**MFLUCC 11-0625**	KT955464		
Astrosphaeriella stellata	MAFF 239487	AB524592	AB524451	
Astrosphaeriella thailandica	**MFLUCC 11-0191**	**KT955465**	**KT955445**	**KT955427**
Astrosphaeriella thysanolaenae	**MFLUCC 11-0186**	**KT955466**	**KT955446**	**KT955428**
Astrosphaeriella tornata	MFLUCC 11-0196	KT955467	KT955447	KT955429
Astrosphaeriella vesuvius	MTCC 12224	KP814136	KP814137	
Astrosphaeriellopsis bakeriana	CBS 115556	GU301801		GU349015
Astrosphaeriellopsis bakeriana	**MFLUCC 11-0027**	**JN846730**	**JN846740**	
Clohesyomyces aquaticus	MFLUCC 11 -0092	JX276950	JX276949	
Delitschia chaetomioides	SMH3253.2	GU390656		GU327753
Delitschia chaetomioides	GKM1283	GU385172		GU327752
Delitschia didyma	UME 31411	DQ384090	NG_016519	
Delitschia winteri	CBS 225.62	DQ678077	DQ678026	DQ677922
Fissuroma aggregata	MAFF 239485	AB524590	AB524449	
Fissuroma aggregata	MAFF 239486	AB524591	AB524450	AB539105
Fissuroma bambusae	**MFLUCC 11-0160**	**KT955468**	**KT955448**	**KT955430**
Fissuroma bambusae	MFLUCC 11-0198	KT955469	KT955449	KT955431
Fissuroma maculans	**MFLUCC 10-0886**	**JN846724**	**JN846734**	
Fissuroma maculans	MFLUCC 10-0887	JN846725	JN846736	
Fissuroma neoaggregata	**MFLUCC 10-0554**	**KT955470**	**KT955450**	**KT955432**
Fissuroma neoaggregata	MFLUCC 13-0227	KT955471	KT955451	KT955433
Fissuroma thailandicum	MFLUCC 11-0189	KT955472	KT955452	KT955434

(BYPP) equal or greater than 0.95 are given below each node (Fig. 3) (Suetrong et al. 2009; Ariyawansa et al. 2014e).

The phylograms are visualized in Treeview (Page 1996) and made in Adobe Illustrator CS3 and Adobe Photoshop version CS5 (Adobe Systems Inc., The United States). The newly sequences generated in this study have been submitted in GenBank.

Results and discussion

Phylogenetic analyses

A 50 % majority rule consensus of Bayesian phylogenetic reconstruction was selected to clarify the phylogenetic relationships of taxa in the families *Astrosphaeriellaceae* and *Pseudoastrosphaeriellaceae* and the genus *Astrosphaeriellopsis*

Table 1 (continued)

Taxon	Culture/Voucher No[1]	GenBank Accession No.[2]		
		LSU	SSU	*TEF1*
Fissuroma thailandicum	**MFLUCC 11-0206**	**KT955473**	**KT955453**	**KT955435**
Flammeascoma bambusae	**MFLUCC 10-0551**	**KP744485**	**KP753952**	
Hysterium angustatum	CBS 123334	FJ161207	FJ161167	FJ161111
Hysterium angustatum	CBS 236.34	FJ161180	GU397359	FJ161096
Lepidosphaeria nicotiae	CBS 101341	DQ678067		DQ677910
Lindgomyces breviappendiculatus	**KT 1399**	**AB521749**	**AB521734**	
Lindgomyces cinctosporae	**R56-1**	**AB522431**	**AB522430**	
Lindgomyces ingoldianus	**ATCC 200398**	**AB521736**	**AB521719**	
Lophiotrema neoarundinaria	KT 2200	AB524597	AB524456	AB539110
Lophiotrema nucula	CBS 627.86	GU301837	GU296167	GU349073
Lophiotrema brunneosporum	**CBS 123095**	**GU301835**	**GU296165**	**GU349071**
Lophiotrema lignicola	**CBS 122364**	**GU301836**	**GU296166**	**GU349072**
Massaria campestris	M28	HQ599385	HQ599449	HQ599325
Massaria inquinans	M19	HQ599402	HQ599444	HQ599342
Massaria mediterranea	M45	HQ599417	HQ599452	HQ599357
Massaria platanoidea	M7	HQ599420	HQ599457	HQ599359
Massaria vomitoria	M13	HQ599437	HQ599440	HQ59375
Neoastrosphaeriella krabiensis	MFLUCC 11-0022	JN846727	JN846735	
Neoastrosphaeriella krabiensis	**MFLUCC 11-0025**	**JN846729**	**JN846739**	
Neomassariosphaeria grandispora	CBS 613.86	GU301842	GU296172	GU349036
Polyplosphaeria fusca	**MAFF 239685**	**AB524604**	**AB524463**	**AB524820**
Pseudoastrosphaeriella africana	**MFLUCC 11-0176**	**KT955474**	**KT955454**	**KT955436**
Pseudoastrosphaeriella bambusae	**MFLUCC 11-0205**	**KT955475**	**KT955455**	**KT955437**
Pseudoastrosphaeriella longicolla	**MFLUCC 11-0171**	**KT955476**	**KT955456**	**KT955438**
Pseudoastrosphaeriella thailandensis	MFLUCC 10-0553	KT955477	KT955457	KT955439
Pseudoastrosphaeriella thailandensis	**MFLUCC 11-0144**	**KT955478**	**KT955458**	**KT955440**
Pseudoastrosphaeriella thailandensis	MFLUCC 14-0038	KT955479	KT955459	KT955441
Pseudotetraploa javanica	MAFF 239498	AB524611	AB524470	AB524826
Pseudotrichia guatopoensis	SMH 4535	GU385202		GU327774
Pteridiospora chiangraiensis	**MFLUCC 11-0162**	**KT955480**	**KT955460**	**KT955442**
Pteridiospora javanica	**MFLUCC 11-0159**	KJ742940	KJ739607	KJ739605
Pteridiospora javanica	MFLUCC 11-0195	KJ742941		KJ739606
Repetophragma ontariense	**HKUCC 10830**	**DQ408575**		
Rimora mangrovei	JK 5246A	GU301868	GU296193	
Salsuginea ramicola	KT 2597.1	GU479800	GU479767	GU479861
Salsuginea ramicola	KT 2597.2	GU479801	GU479768	GU479862
Tetraplosphaeria sasicola	**MAFF 239677**	**AB524631**	**AB524490**	**AB524838**
Triplosphaeria maxima	**MAFF 239682**	**AB524637**	**AB524496**	**AB524843**
Ulospora bilgramii	CBS 110020	DQ678076	DQ678025	DQ677921
Verruculina enalia	CBS 304.66	DQ678079	DQ678028	DQ677924
Verruculina enalia	BCC 18401	GU479802	GU479770	GU479863

correlating with other selected families in the order *Pleosporales* (Fig. 3). Combined LSU, SSU and *TEF1* sequence data were analyzed using the Randomized Accelerated Maximum Likelihood (RAxML), maximum parsimony (MP) and Bayesian probability programmes.

The dataset comprises 81 taxa, with *Hysterium angustatum* Alb. & Schwein. (CBS 123334, CBS 236.34) selected as an outgroup taxon. RAxML analysis yielded a best scoring tree with a final ML optimization likelihood value of −20693.077278. The matrix had 1143 distinct alignment patterns, with 23.25 % of undetermined characters or gaps. The maximum parsimonious dataset consists of 2874 total characters, 1979 characters were constant, 715 characters were parsimony-informative and

180 available characters were parsimony-uninformative. The heuristic search resulted in a best single tree from 28 parsimonious trees with a length of 3421 steps (CI= 0.365, RI=0.705, RC=0.257, HI: 0.635). Phylogenetic trees derived from Bayesian, RAxML and MP analyses gave similar overall topologies at the family relationships and not significantly different (data not shown). The related families in *Pleosporales* showed similar results to that in the studies of Liu et al. (2011), Zhang et al. (2012), Hyde et al. (2013) and Wijayawardene et al. (2014). However, some taxa were resolved differently in the internal node relationships among the Bayesian, RAxML, and MP trees.

Strains of *Aigialaceae* formed a robust clade (Fig. 3) in all analyses with each genus having clearly resolved relationships. Six new morphologically similar strains of *Fissuroma* are included in this study. However, they cluster in three distinct species, which are introduced as *Fissuroma bambusae* Phookamsak & K.D. Hyde, *F. neoaggregata* Phookamsak & K.D. Hyde and *F. thailandicum* Phookamsak & K.D. Hyde in this paper.

A new genus, *Astrosphaeriellopsis* is introduced to accommodate a single species *A. bakeriana* which was previously included in *Astrosphaeriella*, but is not congeneric in the combined gene tree. The two strains representing *A. bakeriana* are a well resolved clade in our phylogenetic analyses which is similar to the results in Liu et al. (2011). *Astrosphaeriellopsis bakeriana* forms a single clade basal to the family *Aigialaceae* in Bayesian and MP trees, but clusters with *Astrosphaeriellaceae* in RAxML analysis. Although, the two strains do not maintain a stable position in the phylogeny, they are always distinct from other clades and we conclude that they represent a distinct genus which we introduce as *Astrosphaeriellopsis*. The genus may need to be raised to family rank with further populations of the tree.

Astrosphaeriellaceae is introduced to accommodate *Astrosphaeriella* and *Pteridiospora*. The relationships among taxa in this family have shown similar results in all analyses, with *Astrosphaeriellaceae* forming a well-resolved clade, close to *Aigialaceae* in Bayesian analysis, but are not supported in RAxML and MP. *Astrosphaeriella* sensu stricto and *Pteridiospora* forms a strongly supported clade (96 % MP, 96 % ML, 1.00 PP) in our analyses. Two *Astrosphaeriella* species (*A. tornata* and *A. vesuvius*) with reddish brown ascospores do not cluster in *Astrosphaeriella* sensu stricto. These two species form a sister clade at base of the family *Astrosphaeriellaceae* with low bootstrap value. A new genus may need to be introduced to accommodate the taxa having heavily, brown pigment, ascospores when the phylogeny is better populated.

Pseudoastrosphaeriellaceae is also introduced in this study to accommodate some species of *Astrosphaeriella* sensu *lato*. Six new strains representing four distinct species are included in the phylogenetic analyses and show similar results in Bayesian, RAxML and MP trees. *Pseudoastrosphaeriellaceae* forms a sister clade with *Tetraplosphaeriaceae*, but relationships between these families are not well-resolved, probably due to limited populations in the analyses; thus more extensive sampling is needed.

Salsugineaceae is not stable in our analyses. The family forms a sister clade with *Lindgomycetaceae* in the Bayesian analysis, but forms a single clade at base of the family *Astrosphaeriellaceae* in the RAxML analysis, while the family groups with *Anteagloniaceae* in MP analysis. In this study, *Pseudotrichia guatopoensis* (SMH 4535) clusters with the family *Amnicolicolaceae* in Bayesian and MP analyses, but the species clusters with *Testudinaceae* in RAxML analysis. This is similar to the results of Zhang et al. (2012) and Hyde et al. (2013). Other respective families form well-defined clades as basal lineages of the order *Pleosporales*.

Taxonomy

Aigialaceae Suetrong et al., in Suetrong et al., Stud. Mycol. 64: 166 (2010)

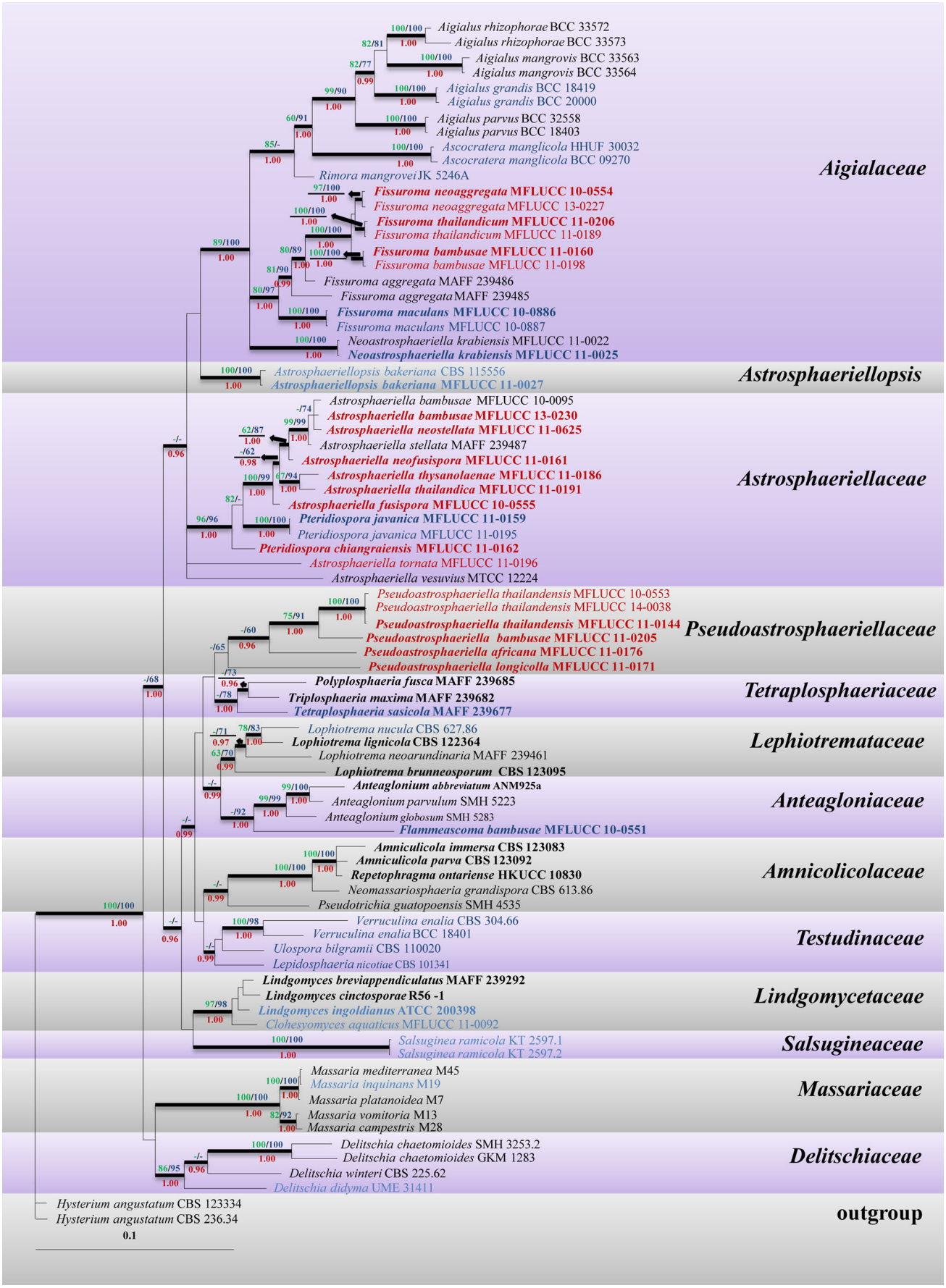
Facesoffungi number: FoF 01214

Saprobic on submerged bark or wood of mangrove trees. **Sexual morphs**: *Ascostromata* dark brown, deeply immersed, or immersed beneath the host epidermis, subglobose to conical, or hemisphaerical, glabrous, coriaceous or carbonaceous, ostiolate. *Ostioles* usually a slit-like opening. *Hamathecium* comprising trabeculate pseudoparaphyses, embedded in a hyaline gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical, short-pedicellate, apically rounded, with a non-amyloid ocular chamber. *Ascospores* overlapping uni- to bi-seriate, hyaline to brown, ellipsoidal to fusiform, septate to muriform, with a mucilaginous sheath or caps. **Asexual morph**: Undetermined (from Suetrong et al. 2009 and Hyde et al. 2013).

Type genus: *Aigialus* Kohlm. & Schatz

Notes: Suetrong et al. (2009) introduced a new family *Aigialaceae* to accommodate saprobic marine taxa, initially including three genera viz. *Aigialus*, *Ascocratera* and *Rimora* (Suetrong et al. 2009; Liu et al. 2011; Zhang et al. 2012; Hyde et al. 2013). Multigene

Fig. 3 A Bayesian 50 % majority rule consensus tree based on combined ▶ dataset of LSU, SSU and *TEF1* alignments. Bootstrap support values for maximum likelihood (ML, *blue*) and maximum parsimony (MP, *green*) equal or greater than 60 % are given above the nodes. Bayesian posterior probabilities (BYPP, *red*) equal or higher than 0.95 are given below the nodes. *Hysterium angustatum* (CBS 123334, CBS 236.34) was selected as an outgroup taxon. Ex-type strains are in *bold*. Newly generated sequences are in *red* and the type species are indicated in *blue*


Aigialus rhizophorae BCC 33572
Aigialus rhizophorae BCC 33573
Aigialus mangrovis BCC 33563
Aigialus mangrovis BCC 33564
Aigialus grandis BCC 18419
Aigialus grandis BCC 20000
Aigialus parvus BCC 32558
Aigialus parvus BCC 18403
Ascocratera manglicola HHUF 30032
Ascocratera manglicola BCC 09270
Rimora mangrovei JK 5246A
Aigialaceae
Fissuroma neoaggregata MFLUCC 10-0554
Fissuroma neoaggregata MFLUCC 13-0227
Fissuroma thailandicum MFLUCC 11-0206
Fissuroma thailandicum MFLUCC 11-0189
Fissuroma bambusae MFLUCC 11-0160
Fissuroma bambusae MFLUCC 11-0198
Fissuroma aggregata MAFF 239486
Fissuroma aggregata MAFF 239485
Fissuroma maculans MFLUCC 10-0886
Fissuroma maculans MFLUCC 10-0887
Neoastrosphaeriella krabiensis MFLUCC 11-0022
Neoastrosphaeriella krabiensis MFLUCC 11-0025
Astrosphaeriellopsis bakeriana CBS 115556
Astrosphaeriellopsis bakeriana MFLUCC 11-0027
Astrosphaeriellopsis
Astrosphaeriella bambusae MFLUCC 10-0095
Astrosphaeriella bambusae MFLUCC 13-0230
Astrosphaeriella neostellata MFLUCC 11-0625
Astrosphaeriella stellata MAFF 239487
Astrosphaeriella neofusispora MFLUCC 11-0161
Astrosphaeriella thysanolaenae MFLUCC 11-0186
Astrosphaeriella thailandica MFLUCC 11-0191
Astrosphaeriella fusispora MFLUCC 10-0555
Pteridiospora javanica MFLUCC 11-0159
Pteridiospora javanica MFLUCC 11-0195
Pteridiospora chiangraiensis MFLUCC 11-0162
Astrosphaeriella tornata MFLUCC 11-0196
Astrosphaeriella vesuvius MTCC 12224
Astrosphaeriellaceae
Pseudoastrosphaeriella thailandensis MFLUCC 10-0553
Pseudoastrosphaeriella thailandensis MFLUCC 14-0038
Pseudoastrosphaeriella thailandensis MFLUCC 11-0144
Pseudoastrosphaeriella bambusae MFLUCC 11-0205
Pseudoastrosphaeriella africana MFLUCC 11-0176
Pseudoastrosphaeriella longicolla MFLUCC 11-0171
Pseudoastrosphaeriellaceae
Polyplosphaeria fusca MAFF 239685
Triplosphaeria maxima MAFF 239682
Tetraplosphaeria sasicola MAFF 239677
Tetraplosphaeriaceae
Lophiotrema nucula CBS 627.86
Lophiotrema lignicola CBS 122364
Lophiotrema neoarundinaria MAFF 239461
Lophiotrema brunneosporum CBS 123095
Lephiotremataceae
Anteaglonium abbreviatum ANM925a
Anteaglonium parvulum SMH 5223
Anteaglonium globosum SMH 5283
Flammeascoma bambusae MFLUCC 10-0551
Anteagloniaceae
Amniculicola immersa CBS 123083
Amniculicola parva CBS 123092
Repetophragma ontariense HKUCC 10830
Neomassariosphaeria grandispora CBS 613.86
Pseudotrichia guatopoensis SMH 4535
Amnicolicolaceae
Verruculina enalia CBS 304.66
Verruculina enalia BCC 18401
Ulospora bilgramii CBS 110020
Lepidosphaeria nicotiae CBS 101341
Testudinaceae
Lindgomyces breviappendiculatus MAFF 239292
Lindgomyces cinctosporae R56 -1
Lindgomyces ingoldianus ATCC 200398
Clohesyomyces aquaticus MFLUCC 11-0092
Lindgomycetaceae
Salsuginea ramicola KT 2597.1
Salsuginea ramicola KT 2597.2
Salsugineaceae
Massaria mediterranea M45
Massaria inquinans M19
Massaria platanoidea M7
Massaria vomitoria M13
Massaria campestris M28
Massariaceae
Delitschia chaetomioides SMH 3253.2
Delitschia chaetomioides GKM 1283
Delitschia winteri CBS 225.62
Delitschia didyma UME 31411
Delitschiaceae
Hysterium angustatum CBS 123334
Hysterium angustatum CBS 236.34
outgroup
0.1

analyses indicated that *Astrosphaeriella aggregata* was not related to the *Astrosphaeriella* sensu stricto, but clustered with the family *Aigialaceae* (Schoch et al. 2009; Zhang et al. 2012). Thus, Liu et al. (2011) established a new genus *Fissuroma* in *Aigialaceae* to accommodate taxa with ascostromata having slit-like ostioles and hyaline, fusiform, didymosporous ascospores. *Fissuroma aggregata* (I. Hino & Katum.) Phookamsak et al. and *F. maculans* (Rehm) J.K. Liu et al. were transferred from *Astrosphaeriella*.

In this study, *Aigialaceae* forms a strongly supported clade (89 % MP/100 % ML/1.00 PP) in *Pleosporales* as in Schoch et al. (2009), Suetrong et al. (2009), Liu et al. (2011), Zhang et al. (2012), Hyde et al. (2013) and Wijayawardene et al. (2014). The genus *Fissuroma* is re-visited, as the phylogenetic analyses indicate that *Fissuroma aggregata* is a species complex.

Fissuroma Liu et al, in Liu et a l., Fungal Divers 51(1): 145 (2012)

Facesoffungi number: FoF 01215

Saprobic on bamboo or palms appearing as dome-shaped darkened areas on the host with slit-like ostioles. **Sexual morph**: *Ascostromata* dark brown to black, scattered to clustered, solitary to gregarious, immersed beneath host epidermis, becoming raised, hemisphaerical domes, uni-loculate, rarely bi-loculate joined at the base, glabrous, coriaceous or carbonaceous, ostiole a central, slit-like opening. *Peridium* thin- to thick-walled, of unequal thickness, poorly developed at the base, thick at sides towards the apex, composed of dark brown pseudoparenchymatous cells, with host cells, plus fungal tissue, arranged in a *textura angularis* to *textura epidermoidea*, carbonaceous at slit-like opening. *Hamathecium* composed of dense, anastomosing, trabeculate pseudoparaphyses, embedded in a hyaline gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, obclavate to cylindrical, pedicellate, apically rounded, with an ocular chamber. *Ascospores* overlapping uni-to tri-seriate, hyaline, fusiform with narrowed ends, 1-septate, slightly constricted at the central septum, surrounded by a distinct sheath. **Asexual morph**: Coelomycetous, reported as pleurophomopsis-like (Tanaka and Harada 2005; Liu et al. 2011)

Type species: *Fissuroma maculans* (Rehm) J.K. Liu, E.B.G. Jones & K.D. Hyde.

Notes: Liu et al. (2011) introduced *Fissuroma*, typified by *F. maculans*, to accommodate some lophiostoma-like species having hemisphaerical ascostromata with slit-like ostioles, trabeculate pseudoparaphyses and hyaline, 1-septate ascospores, with a second species, *F. aggregata* (Liu et al. 2011; Hyde et al. 2013). These species had previously been placed in *Astrosphaeriella* and Liu et al. (2011) had found that they were not congeneric with *Astrosphaeriella* sensu stricto. Based on phylogenetic analyses of combined LSU and SSU sequence data, *Fissuroma* formed a robust clade in *Aigialaceae*.

In this study, several collections similar to *Fissuroma aggregata* differed in ascospores size, and structure of mucilaginous sheath. Phylogenetic analyses of combined LSU, SSU and *TEF1* sequence data (Fig. 3) indicate that *Fissuroma aggregata* comprises at least three distinct species. *TEF1* gene pairwise comparison showed differences in 26 base positions (Fig. 9). Six strains of *Fissuroma aggregata* have been studied. Molecular analysis show them to comprise at least three species and a morphological comparison show that spore shape and sheath structure can be used to distinguish these species. *Fissuroma aggregata* is re-described in this paper and three new species with one combination are introduced.

Key to species of the genus *Fissuroma*

1. Ascostromata less than 800 μm *F. maculans*
1. Ascostromata longer than 800 μm 2
2. Ascospores with thick, distinctive sheath 3
2. Ascospores with thin sheath 4
3. Ascospores (40–)43–46(–52)×6–7(–9) μm, with spreading mucilaginous sheath surrounding the ascospores *F. thailandicum*
3. Ascospores (40–)45–47(–52)×6–8(–9) μm, with mucilaginous sheath in envelop surrounding the ascospores *F. bambusae*
4. Ascospores (43–)45–50(–55)×7–9 μm, with drawn out terminal appendages *F. fissuristoma*
4. Ascospores without appendages 5
5. Ascospores (39–)(41–)47–50(–54)×7–9 μm, ascostromata depressed conical, with short slit-like opening *F. neoaggregata*
5. Ascospores 46–61(–64)×7–9.5 μm, ascostromata hemisphaerical, with long slit-like opening *F. aggregata*

Fissuroma aggregata (I. Hino & Katum.) Phookamsak et al. in Liu et al., Fungal Divers 51(1): 145 (2011)

≡ *Melanopsamma aggregata* I. Hino & Katum., Bull. Fac. Agric. Yamaguchi Univ., 6: 53 (1955)

= *Astrosphaeriella aggregata* (I. Hino & Katum.) Kaz. Tanaka & Y. Harada, Mycoscience 46: 115 (2005)

Facesoffungi number: FoF 01216, Fig. 4

Saprobic on bamboo. **Sexual morph**: *Ascostromata* 200–300 μm high, 500–1000 μm diam., dark brown, scattered, gregarious, immersed beneath host epidermis, appearing as raised, dome-shaped regions, with, thick, rim-like ostioles on the host surface, hemisphaerical, with a flattened base, uni-loculate, glabrous, wall slightly rough, coriaceous, ostiolate. *Ostioles* central, apapillate with long carbonaceous, and thick rim-like opening. *Peridium* thick of unequal thickness, poorly developed at the base, thick at

sides towards the apex, composed of dark brown to black cells, carbonaceous at the apex, and membranacous at the base. *Hamathecium* composed of dense, 1.5–2 μm wide, filiform, anastomosing, trabeculate pseudoparaphyses, embedded in a hyaline gelatinous matrix. *Asci* 143–185×15–19.5 μm ($\bar{x}$ =164.5×17.3 μm, n=43), 8-spored, bitunicate, obclavate, with a short furcate to truncate pedicel, apically rounded, with an indistinct ocular chamber. *Ascospores* 46–61(–64)×7–9.5 μm ($\bar{x}$ =55.9×8.2 μm, n=30), overlapping bi- to tri-seriate at the base, uni-seriate

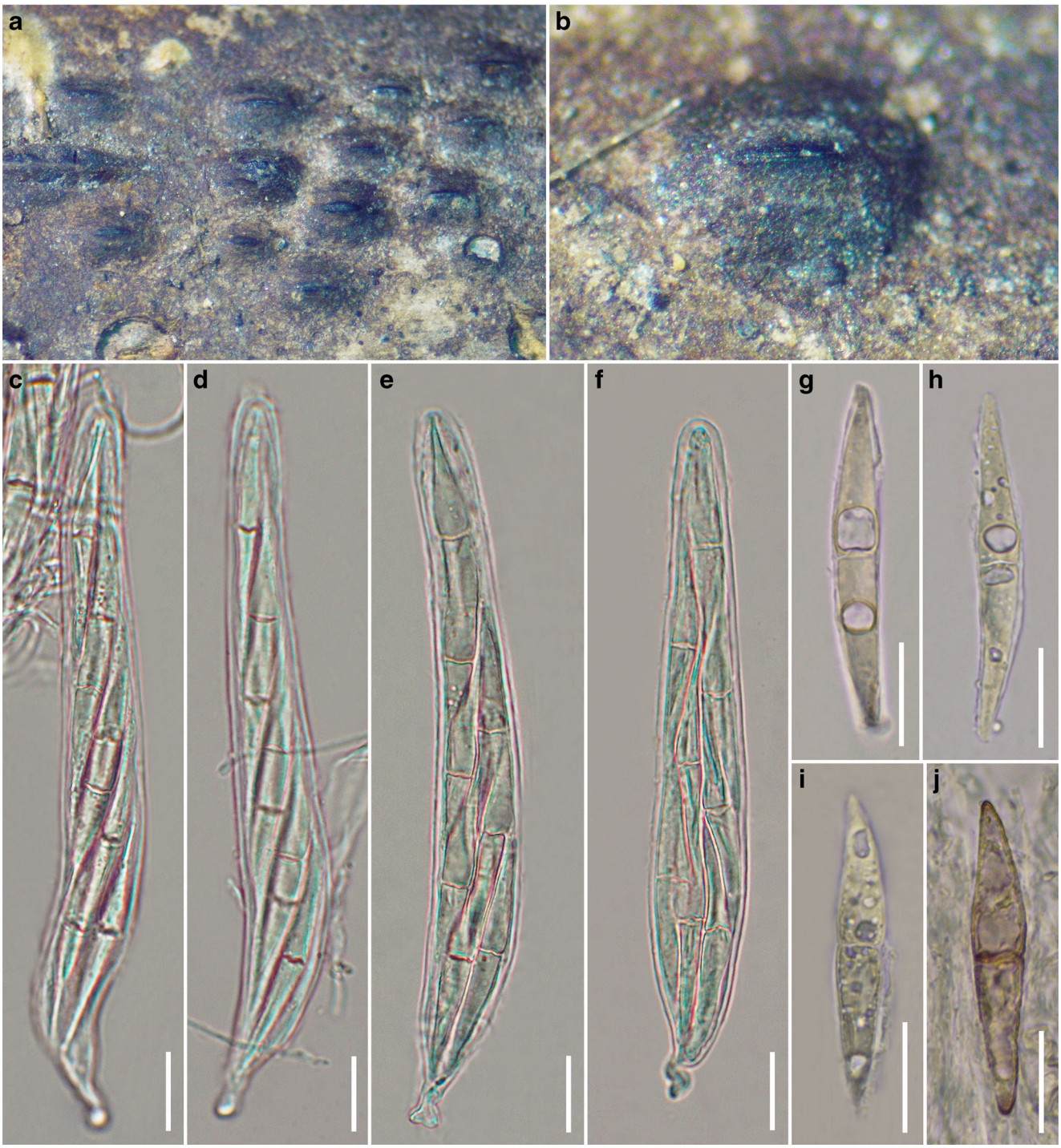
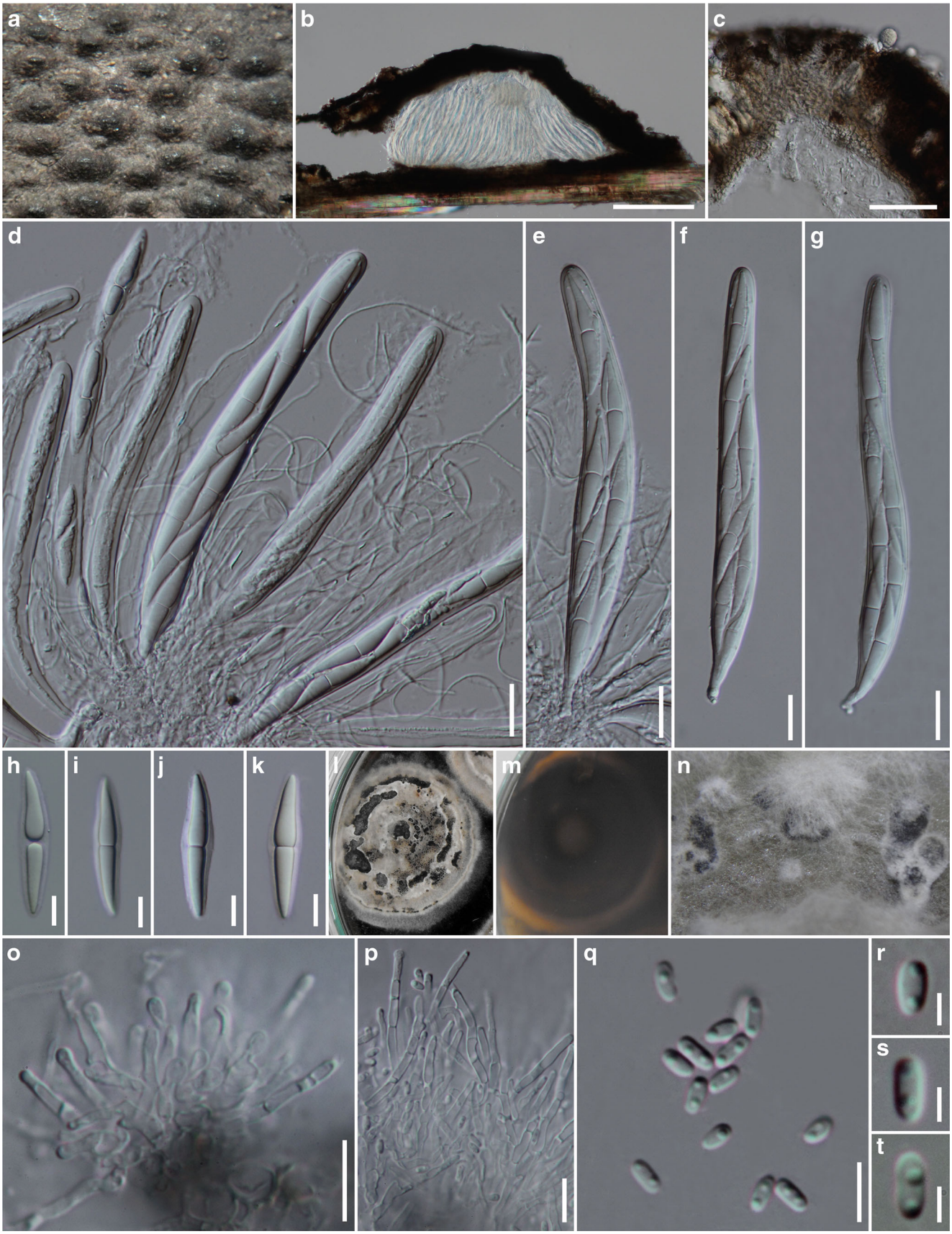


Fig. 4 *Fissuroma aggregata* (YAM 20365, holotype of *Melanopsamma aggregata*). **a**, **b** Appearance of ascostromata on host surface. **c**–**f** Asci. **g**–**i** Ascospores. **j** Old ascospore. Scale bars c–j=20 μm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◀ **Fig. 5** *Fissuroma bambusae* (MFLU11-0196, holotype). **a** Appearance of ascostromata on host surface. **b** Section through ascostroma. **c** Section through peridium. **d** Asci embedded in trabeculate pseudoparaphyses. **e–g** Asci. **h–j** Ascospores. **k** Ascospore stained with Indian ink. **l–m** Culture characters on MEA (m=from above, n=from below). **n** Conidiomata on colony. **o**, **p** Conidiophores. **q–t** Conidia. Scale bars: b=200 μm, c=50 μm, d=20 μm, d–g, h–k, o, p=10 μm, q=5 μm, r–t=2 μm

at the apex, hyaline, becoming brown at maturity, fusiform with acute ends, 1-septate, slightly constricted at the septum, asymmetric, upper cell shorter than lower cell, smooth-walled, with guttules, surrounded by a thin, distinct sheath. **Asexual morph**: Undetermined.

Material examined: JAPAN, Tokuyama, Yamaguchi, on culms of *Phyllostachys bambusoides* Siebold & Zucc. (*Poaceae*), 28 November 1954, I. Hino, YAM 20365 (**holotype** of *Melanopsamma aggregata*).

Notes: *Fissuroma aggregata* was described by Hino and Katumoto (1955) as *Melanopsamma aggregata*. Tanaka and Harada (2005) found that *Melanopsamma* was not a suitable genus for *M. aggregata* as it has bitunicate asci with broadly fusiform ascospores, while *Melanopsamma* species have unitunicate asci and ellipsoidal ascospores (Liu et al. 2011). Tanaka and Harada (2005) found that *M. aggregata* belonged in *Astrosphaeriella* following the broad sense of Hyde et al. (2000). Therefore, Tanaka and Harada (2005) transferred *M. aggregata* to *Astrosphaeriella*. Phylogenetic analyses of combined LSU and SSU sequence data showed that *Astrosphaeriella aggregata* was not related to *Astrosphaeriella* sensu stricto (*A. stellata*, KT 998) in Tanaka et al. (2009). *Astrosphaeriella aggregata* formed a single clade at the base of the family *Testudinaceae* in Tanaka et al. (2009) and was closely related to the genus *Rimora* in the family *Aigialaceae* in Schoch et al. (2009). Therefore, Liu et al. (2011) transferred *A. aggregata* to the genus *Fissuroma*.

Fissuroma bambusae Phookamsak & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF551629, Facesoffungi number: FoF 01217, Fig. 5

Etymology: The epithet "*bambusae*" refers to the host, of which the fungus was collected.

Holotype: MFLU 11-0196

Saprobic on bamboo. **Sexual morph**: *Ascostromata* 250–400 μm high, 750–1050 μm diam., dark brown, gregarious, immersed beneath host epidermis, visible as numerous, raised, dome-shaped areas on the host surface, hemisphaerical, flattened or wedge-shaped at the base, uni-loculate, glabrous with rough walls, coriaceous, ostiolate. *Ostioles* central, apapillate, with slightly long carbonaceous, and thin, slit-like opening. *Peridium* 42–77(–118) μm wide, of unequal thickness, poorly developed at the base, thick at sides towards the apex, composed of several layers, of dark brown to black, pseudoparenchymatous cells, with host cells plus fungal tissue, arranged in a *textura angularis* to *textura prismatica*. *Hamathecium* composed of dense, 0.5–1.5 μm wide, filiform, trabeculate pseudoparaphyses, anastomosing among the asci, embedded in a hyaline gelatinous matrix. *Asci* (150–)170–187(–194)×(15–)17–19(–22) μm ($\bar{x}$ =178.1×18.5 μm, n=30), 8-spored, bitunicate, cylindric-clavate or obclavate, with short furcate to truncate pedicel, apically rounded with a truncate ocular chamber. *Ascospores* (40–)45–47(–52)×6–8(–9) μm ($\bar{x}$ =46.2×7.1 μm, n=30), overlapping bi- to tri- seriate at the base, uni-seriate at the apex, hyaline, fusiform with acute ends, 1-septate, constricted at the septum, upper cell shorter and wider than lower cell, smooth-walled, surrounded by a thick, distinct sheath. **Asexual morph**: producing conidiomata on colonies growing on PDA after 3 months. *Conidiomata* large, scattered, immersed to semi-immersed, visible as black dots on PDA media agar, globose to subglobose, composed of dark brown to black, pseudoparenchymatous cells. *Conidiophores* (4.5–)(8–)10–20(–32)×1.5–3.5 μm ($\bar{x}$ =15.7×1.9 μm, n=40), arising from basal cavity, cylindrical, simple to branched, hyaline, septate, smooth-walled. *Conidiogenous cells* holoblastic, phialidic, determinate, integrated, or discrete, hyaline, lageniform or cylindrical, smooth-walled. *Conidia* (2–)3–4.5×1.5–2 μm ($\bar{x}$ =3.6×1.7 μm, n=50), hyaline, oblong, or rod-shaped with rounded ends, aseptate, with two small guttules, smooth-walled.

Culture characteristics: Colonies on MEA reaching 35–45 mm diam. after 4 weeks at 25–30 °C, colonies circular, medium dense, flattened, slightly raised near margin, dull, surface slightly rough with edge entire, fluffy to floccose, slightly radiating with concentric ring of cottonny mycelium at edge of colony; colony from above, white to white greyish with black, small droplets, laterally forming dark, dull, irregular, rough, flaky, white, sponge-like areas; from below: brown at the margin, dark brown to black at the center, slightly radiating with dark concentric ring; producing dark brown to black pigmentation in agar.

Material examined: THAILAND, Chiang Rai Province, Muang District, Khun Korn Waterfall, on dead stem of bamboo, 5 September 2010, R. Phookamsak, RP0076 (MFLU 11-0196, **holotype**), living cultures, MFLUCC 11-0160, BCC; *ibid*., on dead stem of bamboo, 17 December 2010, R. Phookamsak, RP0114 (MFLU 11-0234), living cultures, MFLUCC 11-0198, BCC.

Notes: *Fissuroma bambusae* differs from *F. thailandicum* in having a thin, slit-like ostiole, and larger ascospores ($\bar{x}$ = 46.2×7.1 μm). In *F. thailandicum* the ostiole is long, wide, shiny and rim-like, and ascospores are smaller ($\bar{x}$ =45.4×7.1 μm) (see Supplementary Table 1). In the phylogenetic

analyses (Fig. 3), *F. bambusae* forms a robust clade with *F. thailandicum* and *F. neoaggregata* (100 % MP, 100 % ML, 1.00 PP) in *Aigialaceae*, but is distinct. A comparison of the *TEF1* sequence data of *F. bambusae* and *F. thailandicum* shows a difference of 17 base positions (Fig. 9).

Tanaka and Harada (2005) reported the asexual morph of *F. aggregata* as coelomycetous and pleurophomopsis-like, with globose to subglobose conidiomata, phialidic conidiogenous cells and hyaline, globose conidia. *Fissuroma bambusae* formed the asexual morph in culture on MEA. The asexual morph is coelomycetous, but differs from *F. aggregata* in having oblong conidia (Fig. 5).

Fissuroma fissuristoma (J. Fröhl et al.) Phookamsak & K.D. Hyde, *comb. nov.*

≡ *Astrosphaeriella fissuristoma* J. Fröhl., K.D. Hyde & Aptroot, in Hyde, Aptroot, Fröhlich & Taylor, Nova Hedwigia 70(1–2): 147 (2000)

Index Fungorum number: IF551636, Facesoffungi number: FoF 01218, Fig. 6

Saprobic on *Calamus conirostris* Becc. **Sexual morph**: *Ascostromata* 300–390 μm high, 750–1030 μm diam., black, scattered, gregarious, immersed beneath host epidermis, visible as raised, glistening, dome-shaped, dark regions on the host surface, hemisphaerical, with flattened base, uni-loculate, glabrous, walls smooth, coriaceous, ostiolate. *Ostioles* central, apapillate, with long carbonaceous, and thick, slit-like opening, comprising several layers of small, black cells, of *textura globulosa*. *Peridium* 20–70(–90) μm wide, of unequal thickness, poorly developed at the base, composed of dark brown to black, pseudoparenchymatous cells, with host cells plus fungal tissue, arranged in *textura angularis* to *textura prismatica*, with palisade-like cells at the rim, carbonaceous at the apex. *Hamathecium* composed of dense, 0.8–1.8 μm wide, filiform, trabeculate pseudoparaphyses, anastomosing among the asci, embedded in a hyaline gelatinous matrix. *Asci* (124–)130–150(–166)×(16–)18–19(–26) μm ($\bar{x}$ =144.1×19.3 μm, n=30), 8-spored, bitunicate, obclavate, with subsessile to short, knob-like pedicel, apically rounded, with an ocular chamber. *Ascospores* (43–)45–50(–55)×7–9 μm ($\bar{x}$ =48.2×8.4 μm, n=30), overlapping bi- to triseriate at the base, uni-seriate at the apex, hyaline, fusiform with acute ends, 1-septate, becoming brown with 3 septa at maturity, slightly constricted at the central septum, smooth-walled, surrounded by a thin, distinct sheath, with appendages at both ends. **Asexual morph**: Undetermined.

Material examined: BRUNEI DARUSSALAM, Temburong, Batu Apoi Forest Reserve, Sungai Belalong, Kuala Belalong Field Studies Centre (KBFSC), Ashton's Trail, on dead rattan and petiole base of *Calamus conirostris* Becc., January 1994, J. Fröhlich, IFRD 294-002 (HKU(M) JF 238, **holotype**).

Notes: Hyde et al. (2000) introduced *Astrosphaeriella fissuristoma* to accommodate a lophiostoma-like species with trabeculate pseudoparaphyses and long slit-like ostioles. The species is most similar to *Astrosphaeriella mangrovei*, but differs in habitat and ascospore size (Hyde et al. 2000). Suetrong et al. (2009) found that *A. mangrovis* was not congeneric with *Astrosphaeriella*. Therefore, the genus *Rimora* was introduced to accommodate *Rimora mangrovei* in *Aigialaceae* which was previously identified as *Astrosphaeriella mangrovis* (Suetrong et al. 2009). Additionally, Liu et al. (2011) introduced a new genus *Fissuroma* to accommodate lophiostoma-like species with trabeculate pseudoparaphyses and slit-like ostioles which were described as *Astrosphaeriella* species in Hyde et al. (2000) and Tanaka and Harada (2005). Two *Fissuroma* species were phylogenetically confirmed in the family *Aigialaceae*. In this study, we examined the type of *Astrosphaeriella fissuristoma* and found that the species is morphologically similar to the species in *Fissuroma*. Therefore, we transfer *Astrosphaeriella fissuristoma* to *Fissuroma*.

Fissuroma neoaggregata Phookamsak & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF551630, Facesoffungi number: FoF 01219, Fig. 7

Etymology: The epithet "*neoaggregata*" refers to its similarity with *Fissuroma aggregata*

Holotype: MFLU 11-0146

Saprobic on bamboo. **Sexual morph**: *Ascostromata* 290–410 μm high, 870–1100 μm diam., dark brown, scattered, solitary to gregarious, immersed in host epidermis, visible as numerous, raised, dome-shaped areas on the host surface, hemisphaerical to depressed conical, with flattened base, wedge-shaped at the rim, uni-loculate, glabrous, walls slightly rough, coriaceous, ostiolate. *Ostioles* central, apapillate with short carbonaceous, and thin, slit-like opening. *Peridium* 45–70 μm wide, of unequal thickness, poorly developed at the base, composed of several layers, of small, dark brown to black, pseudoparenchymatous cells with host cells plus fungal tissue, arranged in a *textura angularis* to *textura prismatica*, with palisade-like cells at rim. *Hamathecium* composed of dense, 0.5–1 μm wide, filiform, trabeculate pseudoparaphyses, anastomosing among the asci, embedded in a hyaline gelatinous matrix. *Asci* (155–)160–190(–197)×15–17(–18) μm ($\bar{x}$ =177×16.5 μm, n=20), 8-spored, bitunicate, cylindrical to obclavate, with furcate to truncate pedicel, apically rounded, with an indistinct ocular chamber. *Ascospores* (39–)(41–)47–50(–54)×7–9 μm ($\bar{x}$ =47.7×8.7 μm, n=25), overlapping uni- to bi-seriate, hyaline, broadly fusiform, with acute ends, 1-septate, slightly constricted at the septum, smooth-walled, guttulate, surrounded by a thin sheath. **Asexual morph**: Undetermined.

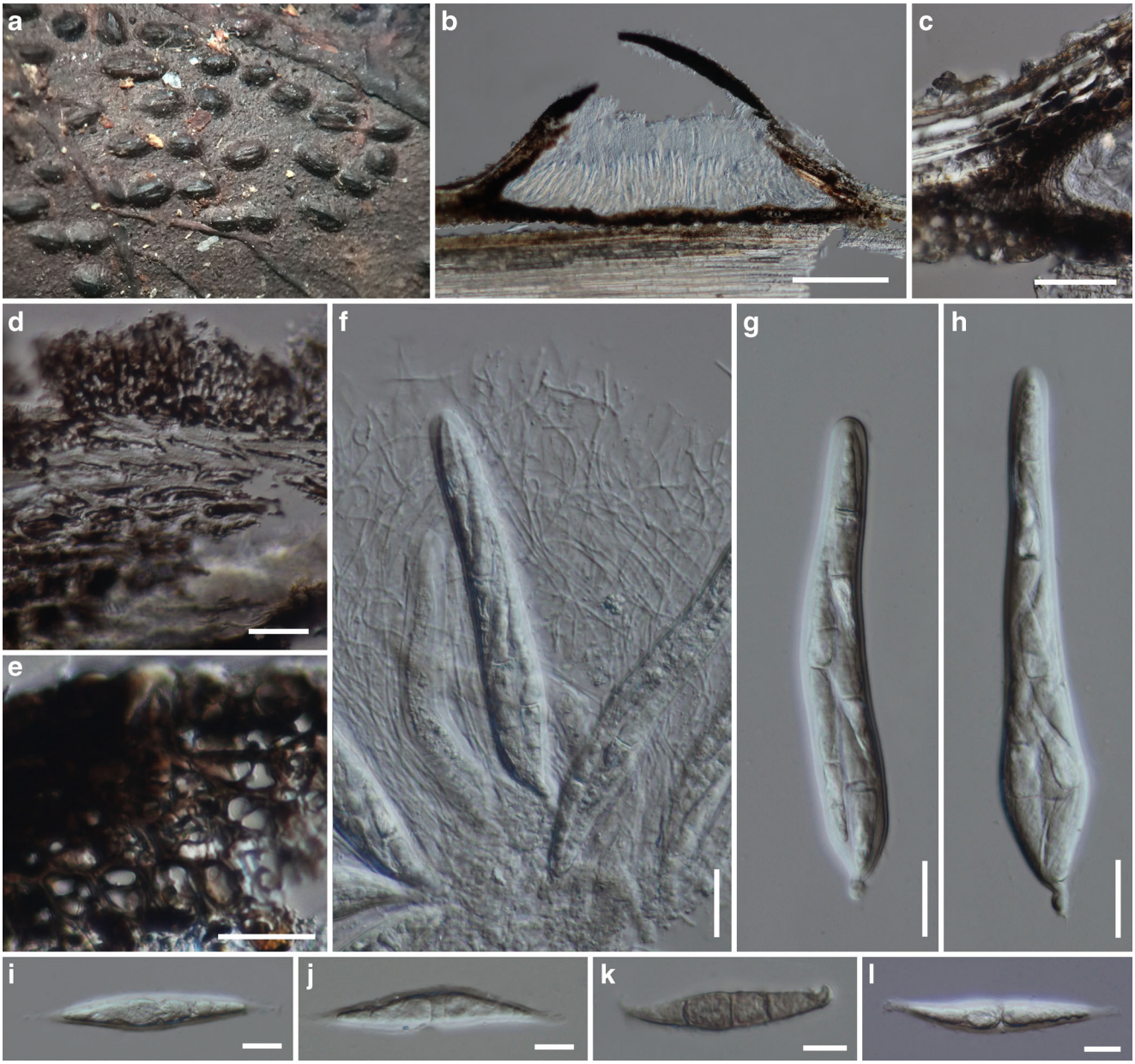


Fig. 6 *Fissuroma fissuristoma* (IFRD 294-002, holotype of *Astrosphaeriella fissuristoma*). **a** Appearance of ascostromata on host surface. **b** Section through ascostroma. **c**–**e** Section through peridium. **f** Asci embedded in trabeculate pseudoparaphyses. **g**, **h** Asci. **i**, **j** Ascospores. **k** Ascospores at maturity. **l** Ascospore stained in Indian ink. Scale bars: b=200 μm, c=50 μm, d–h=20 μm, i–l=10 μm

Culture characteristics: Colonies on MEA reaching 45–46 mm diam. after 4 weeks at 25–30 °C, colonies circular, medium dense to dense, flattened, slightly raised with low concave edge, dull, slightly rough with edge entire, velvety to woolly, smooth aspect, slightly radiating outwardly; from above, white to greyish-brown at the margin, dark grey to blacknish at the centre, with small, black droplets; from below: light brown to brown at the margin, dark grey to blackish in the middle, white brown to light brown at the center, radiating with reddish-brown concentric rings, the centre seperating from the middle part; not producing pigmentation in agar.

Material examined: THAILAND, Chiang Rai Province, Muang District, Huai Mae Sai Waterfall, on dead stem of bamboo (*Poaceae*), 10 March 2010, R. Phookamsak, RP0025 (MFLU 11-0146, **holotype**), living culture, MFLUCC10-0554, BCC; *ibid*., Khun Korn Waterfall, on dead stem of bamboo, 24 November 2012, R. Phookamsak, RP0122 (MFLU 12-2468), living culture, MFLUCC 13-0227, BCC.

Notes: Liu et al. (2011) represented *F. aggregata* by a collection MFLU 11-0146; living culture, MFLUCC10-0554. However, Liu et al. (2011) did not examine the type of *F. aggregata* (≡ *Melanopsamma aggregata*), but used

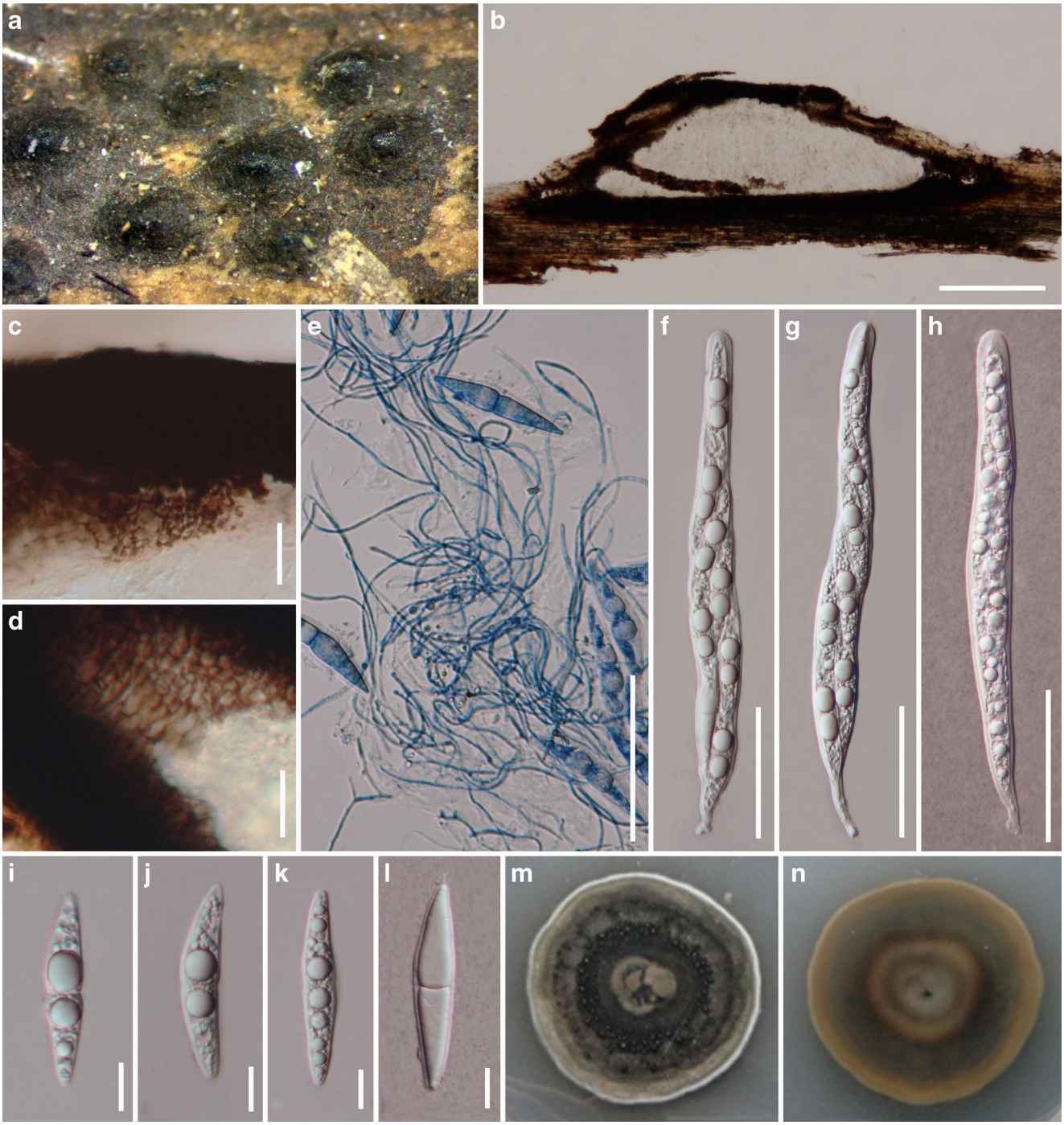


Fig. 7 *Fissuroma neoaggregata* (MFLU11-0146, holotype). **a** Appearance of ascostromata on host surface. **b** Section through ascostroma. **c**, **d** Section through peridium. **e** Pseudoparaphyses stained in cotton blue. **f**, **g** Asci. **h** Asci stained in Indian ink. **i**–**k** Ascospores. **l** Ascospore stained in Indian ink. **m**, **n** Cultures on MEA (m=from above, n=from below). Scale bars: b=200 μm, e–h=50 μm, c=20 μm, d, i–l=10 μm

morphological characters to name the species. In Liu et al. (2011), the phylogenetic analyses showed that *F. neoaggregata* clusters with *F. aggregata* (KT 984 and KT 767) in *Aigialaceae* and was separated from the type species, *F. maculans*. Thus, Liu et al. (2011) identified the *F. neoaggregata* as *F. aggregata*.

In this study, the type of *F. aggregata* (≡ *Melanopsamma aggregata*) is examined and shows that the type of *F. aggregata* differs from *F. neoaggregata* by having ascostromata with long carbonaceous, thick, opaque, rim-like openings and larger of ascospore size. Whereas, *F. neoaggregata* has ascostromata with thin, short, slightly

shiny, slit-like openings and smaller ascospores (see Supplementary Table 1). Therefore, we introduce the species with a new name, *F. neoaggregata* due to the morphological characters, phylogenetic analyses and *TEF1* gene pairwise comparison. A description and illustration are provided in this study.

Fissuroma thailandicum Phookamsak & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF551631, Facesoffungi number: FoF 01220, Fig. 8

Etymology: The epithet "*thailandicum*" refers to the country, from which the fungus was collected.

Holotype: MFLU 11-0156

Saprobic on bamboo. **Sexual morph**: *Ascostromata* 210–390 μm high, 650–1050 μm diam., dark brown, scattered, gregarious, immersed beneath host epidermis, visible as numerous, raised, dome-shaped areas on the host surface, hemisphaerical, with flattened base and wedge-shaped at the sides, uni-loculate, glabrous, coriaceous, ostiolate. *Ostioles* central, apapillate, waxy, with slit-like opening, carbonaceous at the rim. *Peridium* 15–70 μm wide, of unequal thickness, poorly developed at the apex, thick at sides towards the apex, composed of several layers, of dark brown to black, pseudoparenchymatous cells, with host cells plus fungal tissue, arranged in a *textura angularis* to *textura epidermoidea*, with palisade-like cells at the rim. *Hamathecium* composed of dense, 0.9–1.8 μm wide, filamentous, trabeculate pseudoparaphyses, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* (150–)170–190(–204)×15–18(–19.5) μm ($\bar{x}$ =176.9×17 μm, n=30), 8-spored, bitunicate, cylindrical to cylindric-clavate, with short furcate to truncate pedicel, apically rounded, with an obtuse ocular chamber. *Ascospores* (40–)43–46(–52)×6–7(–9) μm ($\bar{x}$ =45.4×7.1 μm, n=30), overlapping bi- to tri-seriate at the base, uniseriate at the apex, hyaline, fusiform with acute ends, 1-septate, slightly constricted at the septum, upper cell wider and shorter than lower cell, smooth-walled, surrounded by a thick, distinct, mucilaginous sheath. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on MEA reaching 14–17 mm diam. after 2 weeks at 25–30 °C, colonies circular, medium dense, flattened, slightly raised to umbonate, dull, smooth with edge entire, woolly, slightly radiating; from above, white to yellowish-grey at the margin, yellowish-grey to dark grey at the centre; from below: pale yellowish to yellowish-brown at the margin, yellowish-grey at the center, forming reddish-brown, concentric rings, seperating the centre from the margin; not producing pigmentation in agar.

Material examined: THAILAND, Chiang Rai Province, Mae Jun District, Huai Kang Pla Waterfall, on dead branch of bamboo, 25 October 2010, R. Phookamsak, RP0035 (MFLU 11-0156, **holotype**), living culture, MFLUCC 11-0206, BCC; *ibid*., Muang District, Khun Korn Waterfall, on dead stem of bamboo, 17 December 2010, R. Phookamsak, RP0105 (MFLU 11-0225), living culture, MFLUCC 11-0189, BCC.

Notes: *Fissuroma thailandicum* is most similar to *F. bambusae* and the differences are discussed under that species.

Astrosphaeriellaceae Phookamsak & K.D. Hyde, *fam. nov.*

Index Fungorum number: IF551632, Facesoffungi number: FoF 01221

Saprobic or *parasitic* on bamboo, palm or stout grasses. **Sexual morph**: *Ascostromata* dark opaque, solitary to gregarious, erumpent to superficial, conical or mammiform, with ruptured, reflexed, stellate, host remnants, around the base, uni-loculate, glabrous, brittle, carbonaceous, with minute apical ostiole. *Peridium* thick-walled, of unequal thickness, poorly developed at the base, composed of thick, opaque and melanized cells, with palisade-like cells at the rim. *Hamathecium* composed of dense, anastomosing, trabeculate pseudoparaphyses, embedded in a hyaline gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, pedicellate, apically rounded with an ocular chamber, or J- subapical ring. *Ascospores* overlapping uni- to bi-seriate, hyaline to pale brown, or reddish brown, subfusoid to fusiform, obclavate to ellipsoidal, or lemoniform, septate, constricted at the septum, smooth-walled, with or without appendages and mucilaginous sheath. **Asexual morph**: Coelomycetous. *Conidiomata* pycnidial, scattered, solitary, superficial, conical to hemisphaerical, or globose, uni-to bi-loculate, indistinctly ostiolate. *Pycnidial walls* thin- to thick-walled, composed of several layers of dark brown to black cells, arranged in a *textura angularis* to *textura intricata*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, phialidic, determinate, integrated, hyaline, cylindrical or cylindric-clavate or ampulliform, septate or aseptate, smooth-walled. *Conidia* hyaline, globose to subglobose, aseptate, smooth-walled.

Type genus: *Astrosphaeriella* Syd. & P. Syd.

Notes: The family *Astrosphaeriellaceae* is established to accommodate two genera viz. *Astrosphaeriella* and *Pteridiospora* in the order *Pleosporales*, Dothideomycetes. The family is characterized by a unique morphology in having large, conical, carbonaceous ascostromata, with ruptured, reflexed, stellate, host remnants, around the base, bitunicate asci with trabeculate pseudoparaphyses, fusiform or obclavate ascospores and a coelomycetous asexual morph. Phylogenetic analyses of combined LSU, SSU and *TEF1* sequence data shows that *Astrosphaeriellaceae* is most closely related to *Aigialaceae* and this concurs with the results of Schoch et al. (2009), Liu et al. (2011), Zhang et al. (2012), and Phookamsak et al. (2014a).

Key to genera of *Astrosphaeriellaceae*

1. Ascospores fusiform, yellowish brown to reddish brown, surrounded by thin distinct, mucilaginous sheath or sheath absent *Astrosphaeriella*

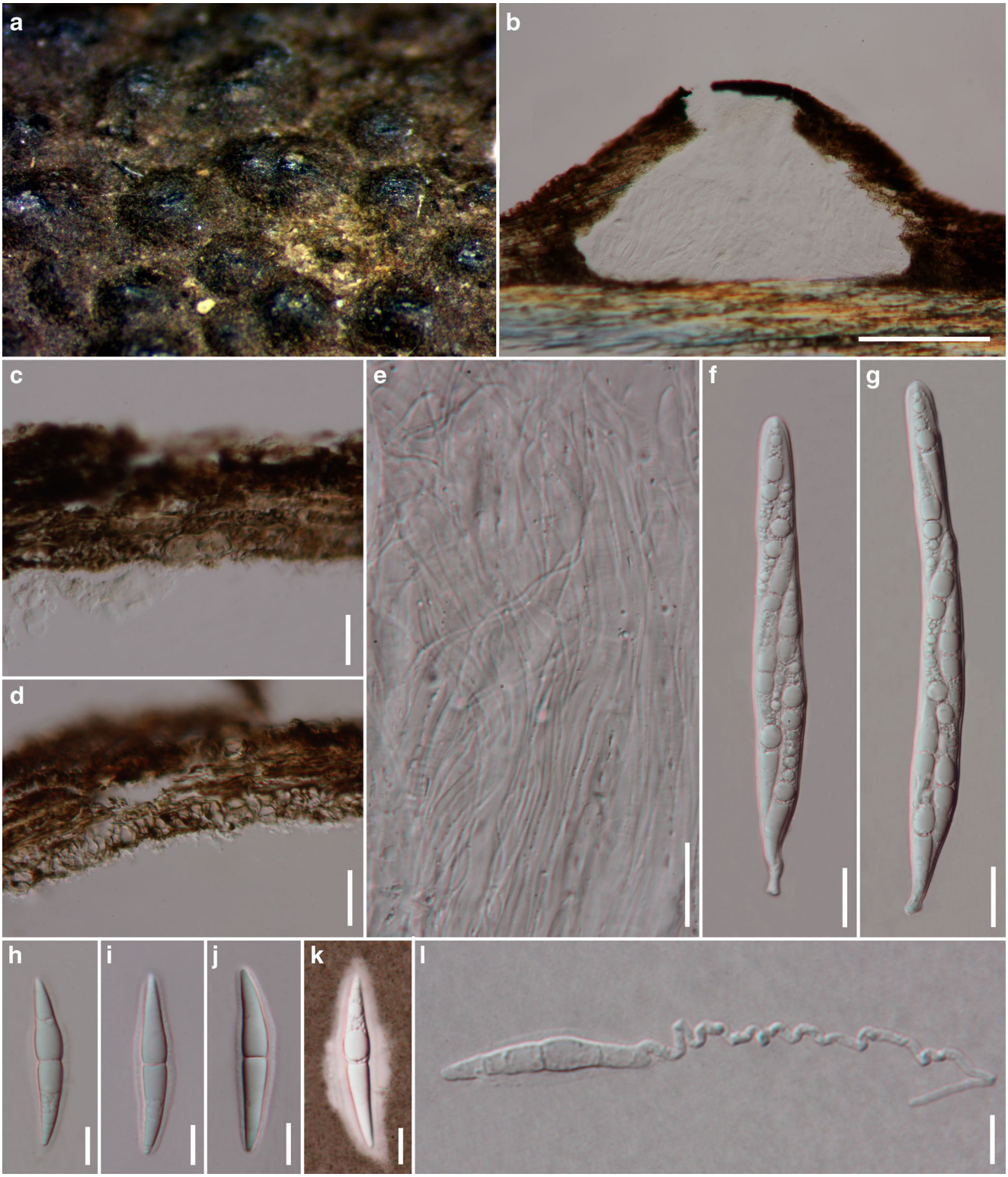


Fig. 8 *Fissuroma thailandicum* (MFLU 11-0156, holotype). **a** Appearance of ascostromata on host surface. **b** Section through ascostroma. **c**, **d** Section through peridium. **e** Pseudoparaphyses. **f**, **g** Asci. **h**–**j** Ascospores. **k** Ascospore stained in Indian ink. **l** Germination of ascospore. Scale bars: b=200 μm, e–h=50 μm, c=20 μm, d, i–l=10 μm

1. Ascospores having obclavate, hyaline to brown ascospores, surrounded by wide distinctive, wing-like, mucilaginous sheath *Pteridiospora*

Astrosphaeriella Syd. & P. Syd., Annls mycol. 11(3): 260 (1913)

Facesoffungi number: FoF 01222

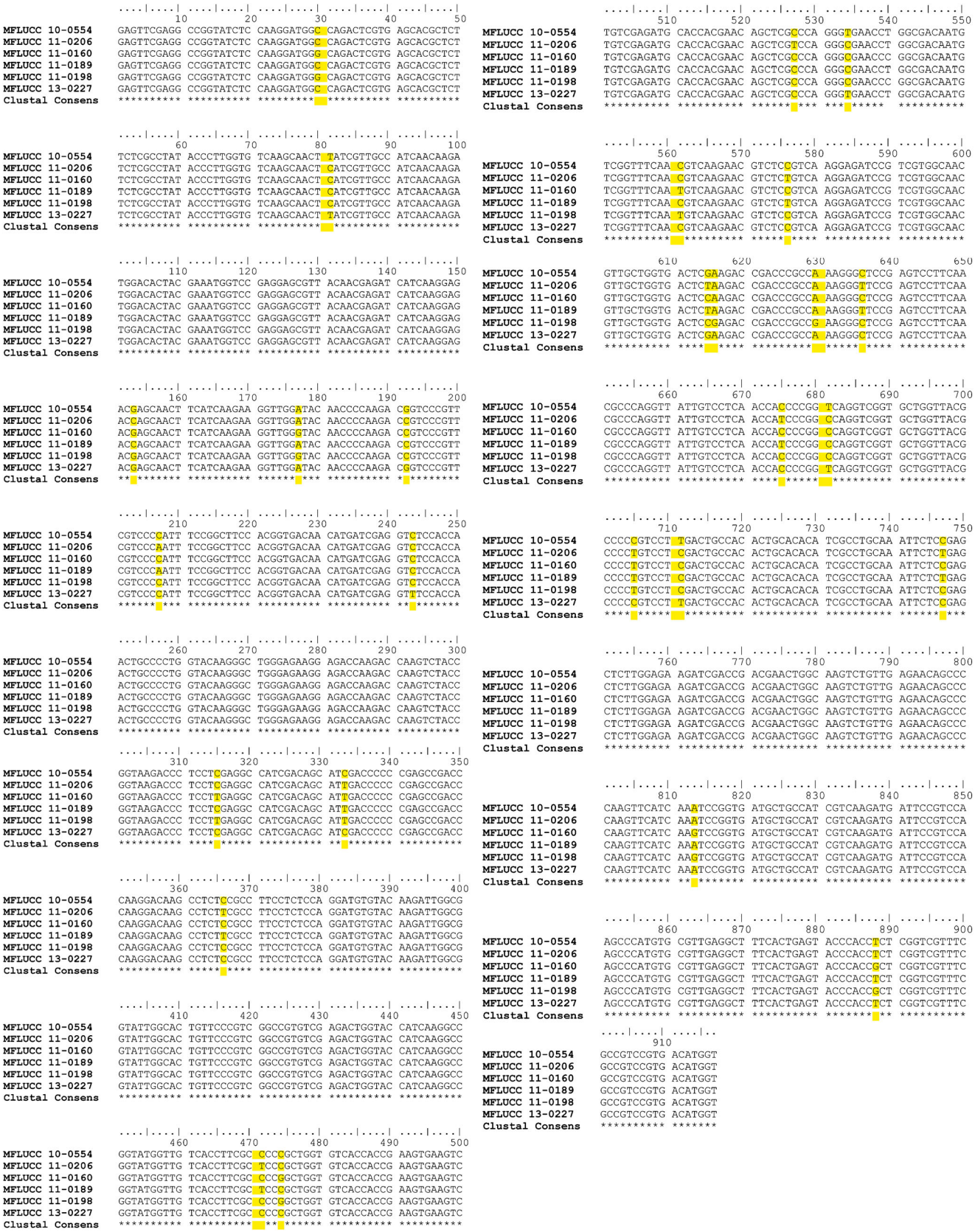


Fig. 9 *TEF1* gene comparison of *Fissuroma* species: *Fissuroma neoaggregata* (MFLUCC 10-0554, MFLUCC 13-0227), *F. thailandicum* (MFLUCC 11-0206, MFLUCC 11-0189), *F. bambusae* (MFLUCC 10-0160, MFLUCC 11-0198)

Saprobic or *parasitic* on bamboo, palm or stout grasses. **Sexual morph**: *Ascostromata* dark opaque, solitary to gregarious, erumpent to superficial, conical or mammiform, with ruptured, reflexed, stellate, host remnants, around the base, uni-loculate, glabrous, brittle, carbonaceous, with minute apical ostiolate. *Peridium* thick-walled, of unequal thickness, poorly developed at the base, thick at sides towards apex, composed of thick, opaque and melanized cells, arranged in a *textura angularis* to *textura prismatica. Hamathecium* composed of dense, anastomosing, trabeculate pseudoparaphyses, embedded in a hyaline gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, subsessile to short-pedicellate, apically rounded with narrow or obtuse ocular chamber. *Ascospores* overlapping uni- to biseriate, pale brown to brown, fusiform with rounded to acute ends, septate, constricted at the septum, smooth-walled, with or without appendages or mucilaginous sheaths. **Asexual morph**: Coelomycetous. *Conidiomata* pycnidial, scattered, solitary, immersed in host epidermis to superficial, conical or hemisphaerical to globose, indistinctly ostiolate. *Pycnidial walls* thin- to thick-walled, composed of several layers of dark brown to black cells, arranged in a *textura angularis* to *textura intricata. Conidiophores* arising from the basal cavity, reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, phialidic, integrated, hyaline, cylindrical or cylindric-clavate or ampulliform, septate or aseptate, smooth-walled. *Conidia* hyaline, globose to subglobose, or oblong, aseptate, smooth-walled.

Type species: *Astrosphaeriella fusispora* Syd. & P. Syd.

Notes: *Astrosphaeriella* is a common genus on bamboos, palms and stout grasses (Barr 1990; Zhou et al. 2003; Tanaka and Harada 2005; Hu 2010; Zhang et al. 2012). The genus was introduced by Sydow and Sydow (1913) and typified by *A. fusispora*. The genus has been morphologically well-studied (Hawksworth 1981; Hawksworth and Boise 1985; Hyde 1992, 1994a, b; Aptroot 1995a, b; Fröhlich and Hyde 1995; Hyde and Fröhlich 1998; Zhou et al. 2003; Chen and Hsieh 2004). However, phylogenetic investigation of this genus is poor (Tanaka et al. 2009; Liu et al. 2011).

Liu et al. (2011) re-circumscribed the genus *Astrosphaeriella* based on morphology and phylogeny. In their study, they introduced two new genera in *Aigialaceae* transferring species having previously been referred to members of *Astrosphaeriella*. Liu et al. (2011) mentioned that *Astrosphaeriella* is polyphyletic and formed a robust clade at the base of the family *Aigialaceae*, while *Astrosphaeriella* sensu *lato* formed a separated clade, close to *Delitschiaceae*.

In this study, *Astrosphaeriella* sensu stricto and *Pteridiospora* having conical or mammiform, carbonaceous ascostromata, form a sister clade with *Aigialaceae* (52 % ML, 0.96 PP). Several strains of *Astrosphaeriella fusispora* have been studied from bamboo and stout grasses and differ in size of ascospores, asci and ascostromata and ascospore colour and type of sheath. Phylogenetic analyses of combined LSU, SSU and *TEF1* sequence data indicate that *Astrosphaeriella fusispora* is a cryptic species. They comprise at least seven distinct species from eight strains. In a comparison of *TEF1* sequence data these *Astrosphaeriella* species are different at 53 base positions.

Astrosphaeriella has been comparable to *Acrocordiopsis*, *Caryospora*, *Caryosporella*, *Mamillisphaeria* and *Trematosphaeria* due to its carbonaceous ascostromata, and trabeculate pseudoparaphyses (Hawksworth 1981; Hyde and Fröhlich 1998; Hu 2010; Liu et al. 2011; Zhang et al. 2012). However, *Astrosphaeriella* is distinguished from other genera in its asci and ascospores shape and habitat on hosts. *Astrosphaeriella* has slightly narrow, fusiform ascospores with cylindric-clavate asci and was found in freshwater, submerged or terrestrial habitats on palms and *Poaceae*, while *Acrocordiopsis* has obovoid or ellipsoidal ascospores, with cylindrical asci and was found in marine habitat (Borse and Hyde 1989; Alias and Jones 2009; Zhang et al. 2012; Hyde et al. 2013). Recently, *Acrocordiopsis* was treated in *Salsugineaceae* by Hyde et al. (2013). *Caryospora* and *Caryosporella* differ from *Astrosphaeriella* in having broadly fusiform, or ellipsoidal ascospores, with thick walls and germ pores at the ends of ascospores and these genera have found in marine habitats (Hu 2010; Liu et al. 2011; Zhang et al. 2012). Hyde et al. (2013) placed *Caryospora* in *Zopfiaceae* and placed *Caryosporella* in *Melanommataceae* and this was supported in Wijayawardene et al. (2014). *Mamillisphaeria* differs from *Astrosphaeriella* in having sac-like asci with dimorphic ascospores and found only in freshwater habitats (Zhang et al. 2012). *Mamillisphaeria* was treated in *Melanommataceae* based on its morphology (Zhang et al. 2012; Hyde et al. 2013; Wijayawardene et al. 2014). Furthermore, *Trematosphaeria* differs from *Astrosphaeriella* in having immersed to erumpent, globose to conical ascostromata, with distinct ostioles and ellipsoidal ascospores and was reported as marine or terrestrial (Liu et al. 2011; Suetrong et al. 2011; Zhang et al. 2012; Hyde et al. 2013). Suetrong et al. (2011) introduced a new family, *Trematosphaeriaceae* to accommodate the genera *Falciformispora*, *Halomassarina* and *Trematosphaeria* with phylogenetically data.

The asexual morph of *Astrosphaeriella* has rarely been established. Tanaka and Harada (2005) described the asexual morph of *Astrosphaeriella* species and *A. aggregata* (currently name *Fissuroma aggregata*) as pleurophomopsis-like and this was reported in Kirk et al. (2008) and Liu et al. (2011). Pratibha and Prabhugaonkar (2015) have found *Pithomyces flavus* Berk. & Broome associated with *Astrosphaeriella vesuvius* (Berk. & Broome) D. Hawksw & Boise on *Calamus thwaitesii* Becc. from India. These two species were obtained from pure cultures by single spore isolation and molecular

phylogenetic analysis. Pratibha and Prabhugaonkar (2015) epitypified *Pithomyces flavus* and indicated that *P. flavus* was the asexual morph of *A. vesuvius* which was confirmed by molecular analysis. However, several pithomyces-like species have been described in Ariyawansa et al. (2015a) as *Pseudopithomyces*. In the phylogenetic analyses, *Pseudopithomyces* species cluster in *Didymosphaeriaceae*.

In this study, the sexual and asexual morphs of *Astrosphaeriella bambusae* are described and this confirms that *Astrosphaeriella* has coelomycetous asexual morph which was previously mentioned in Tanaka and Harada (2005). However, whether *Pithomyces* is the asexual morph of *Astrosphaeriella* is still questionable.

Astrosphaeriella bambusae Phookamsak & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF551633, Facesoffungi number: FoF 01223, Fig. 10

Etymology: The epithet "*bambusae*" refers to the host, on which the fungus was collected.

Holotype: MFLU 12-2471

Saprobic on bamboo, visible as numerous, black, opaque, cone-like structures on host surface with shiny apices. **Sexual morph**: *Ascostromata* 360–600 μm high, 380–660 μm diam., dark opaque, solitary to gregarious, erumpent to superficial, mammiform to conical, with ruptured, reflexed, stellate, host remnants, around the base, uni-loculate, glabrous, brittle, carbonaceous, with indistinct ostioles. *Peridium* 20–70 μm wide, of unequal thickness, poorly developed at the base, composed of thick, dark brown to black cells, arranged in a *textura angularis* to *textura prismatica*, with palisade-like cells at rim. *Hamathecium* composed of dense, 0.7–1.2 μm wide, filiform, trabeculate pseudoparaphyses, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* (125–)130–180(–194)×(9–)10–12(–15) μm ($\overline{x}$ =160×11.3 μm, n=30), 8-spored, bitunicate, fissitunicate, cylindrical, with furcate pedicel, apically rounded, with narrow or wedge-shaped ocular chamber. *Ascospores* (33–)34–39(–42)×5–6 μm ($\overline{x}$ =36.7×5.4 μm, n=30), overlapping bi-seriate, pale yellowish or pale brown, fusiform with acute ends, 1-septate, constricted at the septum, smooth-walled with a thin, distinct, mucilaginous sheath. **Asexual morph**: Coelomycetous, producing on bamboo pieces on WA cultures after 2 months. *Conidiomata* 170–330 μm high, 230–370 μm diam., pycnidial, scattered to clustered, solitary, immersed under host cortex, laterally superficial, visible as raised, black dots, covered by dark brown vegetative hyphae, hemisphaerical or dome-shaped, uni- to bi-loculate, indistinctly ostiolate. *Pycnidial walls* 20–75 μm wide, of unequal thickness, poorly developed at the base, slightly thick at the sides near the base, composed of dark brown to black pseudoparenchymatous cells, with host cells plus fungal tissue, arranged in a *textura angularis*. *Conidiophores* arising from the basal cavity, reduced to conidiogenous cells. *Conidiogenous cells* (4–)6–8(–10)×1.5–2 (–3) μm ($\overline{x}$ =6.8×1.7 μm, n=30), holoblastic, phialidic, discrete, hyaline, cylindrical to ampulliform, or lageniform, aseptate, smooth-walled. *Conidia* 1.5–3×1.6–3 μm ($\overline{x}$ =2.1×1.8 μm, n=50), hyaline, globose to subglobose, aseptate with a single guttule, smooth-walled.

Culture characteristics: Colonies on PDA reaching 45–50 mm diam. after 4 weeks at 25–30 °C, colonies circular, medium dense to dense, flattened, slightly raised, surface smooth with edge entire to fimbriate, fluffy to floccose or velvety, smooth, slightly radiating; colony from above, greenish black to greenish grey at the margin, with grey to greenish grey at the center; from below: blackish green to grey; not producing pigmentation in agar.

Material examined: THAILAND, Chiang Rai Province, Muang District, Khun Korn Waterfall, on dead stem of bamboo, 24 November 2012, R. Phookamsak, RP0129 (MFLU 12-2471, **holotype**), ex-type living culture, MFLUCC 13-0230, BCC.

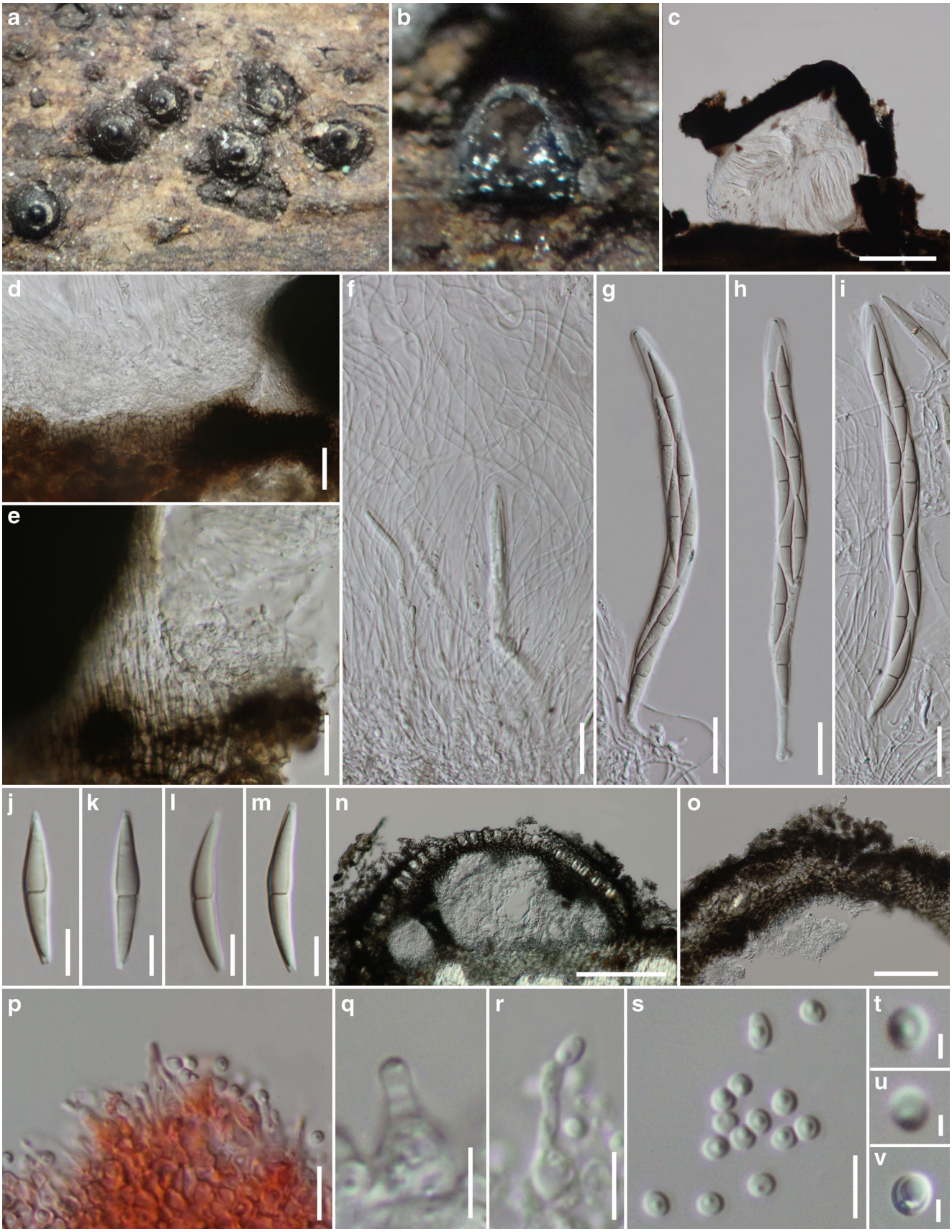
Notes: *Astrosphaeriella bambusae* differs from *A. fusispora* in having smaller ascostromata, asci and smaller, pale yellowish ascospores, surrounded by a thin, distinct, mucilaginous sheath. *Astrosphaeriella bambusae* differs from *A. thysanolaenae* in having smaller ascostromata, with a distinct, thin, mucilaginous sheath surrounding the ascospores and its occurrence on bamboo (see Supplementary Table 2).

In the phylogenetic analyses (Fig. 3), *A. bambusae* clusters with *A. neostellata* and *A. stellata* (99 % MP, 74 % ML). Although, the phylogenetic relationships among these species are not well-resolved, *A. bambusae* differs from *A. neostellata* and *A. stellata* by its characters and is introduced as a new species (see Supplementary Table 2).

Astrosphaeriella exorrhiza Boise, in Hawksworth & Boise, Sydowia 38: 117 (1986) [1985]

Facesoffungi number: FoF 01224, Fig. 11

Saprobic on grasses and palms, visible as numerous, black, opaque, cone-like structures on host surface. **Sexual morph**: *Ascostromata* 630–980 μm high, 820–1200 μm diam., dark opaque, solitary to gregarious, erumpent through the host tissue, becoming superficial, conical, with host cortex persisting as ruptured, reflexed, stellate, host remnants, around the base, uni-loculate, glabrous, brittle, carbonaceous, ostiolate, with a minute papilla. *Peridium* 60–150 μm wide, of unequal thickness, poorly developed at the base, thick at sides towards the apex, composed of small, dark opaque, melanized cells, arranged in a *textura angularis* to *textura prismatica*. *Hamathecium* composed of dense, 0.8–1.3 μm wide, filiform, indistinctly septate, trabeculate pseudoparaphyses, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* 180–220(–240)×(9.5–)10–12 μm ($\overline{x}$ =213.3×11.8 μm, n=25), 8-spored, bitunicate, fissitunicate, cylindrical, with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◀ **Fig. 10** *Astrosphaeriella bambusae* (MFLU 12-2471, holotype). **a** Appearance of ascostromata on host surface. **b**, **c** Vertical sections through ascostroma. **d**, **e** Section through peridium with wedge of palisade-like cells at the rim. **f** Trabeculate pseudoparaphyses. **g**–**i** Asci. **j**–**m** Ascospores. **n** Section through conidiomata produced on bamboo pieces on WA. **o** Section through peridium. **p** Conidiogenous cells stained in Congo red. **q**, **r** Conidiogenous cells. **s**–**v** Conidia. Scale bars: c= 200 μm, *n*=100 μm, o=50 μm, d–i=20 μm, j–m=10 μm, p–s=5 μm, t–v=1 μm

subsessile to short pedicel, apically rounded, with well-developed ocular chamber. *Ascospores* (70–)77–88(–93)×7–8 μm ($\bar{x}$ =82.2×8 μm, *n*=30), overlapping bi-seriate, brown to reddish brown, narrowly fusiform with acute ends, 5-septate, broad at the septum, slightly constricted at the central septum, smooth-walled, with minute appendages at both ends. **Asexual morph**: Undetermined.

Material examined: THAILAND, Chiang Mai Province, Muang District, Doi Suthep, on dead stem of *Thysanolaena maxima* (Roxb.) Kuntze (*Poaceae*), 5 June 2011, R. Phookamsak, RP0124 (MFLU 11-0242).

Notes: *Astrosphaeriella exorrhiza* Boise was introduced by Boise in Hawksworth and Boise (1985). *Astrosphaeriella exorrhiza* is most similar to *A. venezuelensis* in having elongate-fusiform ascospores (Hawksworth and Boise 1985; Hyde and Fröhlich 1998). However, *A. exorrhiza* differs from *A. venezuelensis* in having 5 septa at maturity, with mucilage appendages, and lacks uncinate tips or a tendency to become strongly attenuated (Hawksworth and Boise 1985; Hyde and Fröhlich 1998). *Astrosphaeriella exorrhiza* was found on *Iriartea* sp., submerged dead petioles of an unidentified palm and *Miscanthus* spp. in Ecuador, Taiwan and Venezuela (Hawksworth and Boise 1985; Hyde and Fröhlich 1998; Chen and Hsieh 2004). In this study, *Astrosphaeriella exorrhiza* was found on a dead stem of *Thysanolaena maxima* in Thailand. All of these identifications will eventually need confirming with molecular data.

Astrosphaeriella fusispora Syd. & P. Syd., Annls mycol. 11(3): 260 (1913)

Facesoffungi number: FoF 01225, Fig. 12, 13

Saprobic on bamboo, visible as numerous, black, opaque, cone-like structures on host surface. **Sexual morph**: *Ascostromata* 500–680 μm high, 710–1070 μm diam. [type: 430–670 μm high, 650–810 μm diam.], dark opaque, solitary to gregarious, erumpent to superficial, conical with ruptured, reflexed, stellate, host remnants around the base, uni-loculate, glabrous, brittle, carbonaceous, ostiole central, with small, pore-like opening. *Peridium* 30–100 μm wide [type: 60–105 μm wide], of unequal thickness, poorly developed at the base, composed of several layers of thick, dark opaque and melanized cells, arranged in *textura angularis* to *textura prismatica* and with a wedge of palisade-like cells at the rim. *Hamathecium* composed of dense, 0.6–1.2 μm wide [type: 1–1.5 μm wide], filiform, trabeculate pseudoparaphyses, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* (150–)170–200×(8.5–)9–10 μm ($\bar{x}$ =180.7×9.9 μm, *n*=20) [type: (160–)170–200(–210)×10–13(–15) μm ($\bar{x}$ =184×12.7 μm, *n*=25)], 8-spored, bitunicate, cylindrical to cylindric-clavate, with furcate pedicel, apically rounded, with narrow or wedge-shaped, ocular chamber. *Ascospores* (37–)40–45)×(4–)5–6.5 μm ($\bar{x}$ =40.6×5.5 μm, *n*=25) [type: 37–40(–45)(–47)×5–7 μm ($\bar{x}$ =40.6×5.8 μm, *n*=30), overlapping uni- to bi-seriate, pale brown to brown, fusiform with acute ends, 1-septate, rarely aseptate, constricted at the central septum, upper cell larger than lower cell, smooth-walled, surrounded by a thin, distinct sheath when young, but disappearing at maturity. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on MEA reaching 20–25 mm diam. after 4 weeks at 25–30 °C, colonies circular, dense, slightly raised, to low convex, surface smooth with edge entire, velvety to woolly, slightly radiating; colony from above, dark brown at the margin, with white to white greyish at the center; from below: dark brown to black, slightly radiating; not producing pigmentation in agar.

Material examined: JAPAN, Mino Province, Kawauyemura, on dead stem of *Phyllostachis bambusoides* Siebold & Zucc. (*Poaceae*), 24 September 1912, K. Hara, S-F11298 (no. 67, **holotype**); THAILAND, Chiang Rai Province, Muang District, Huai Mae Sai Waterfall, on dead stem of bamboo, 10 March 2010, R. Phookamsak, RP0026 (MFLU 11-0147, **reference specimen designated here**), living culture, MFLUCC 10-0555, BCC.

Notes: Sydow and Sydow (1913) introduced *Astrosphaeriella fusispora* as the type species of the genus *Astrosphaeriella*. Hawksworth (1981) re-circumscribed the genus *Astrosphaeriella* and treated *A. stellata* as a new type, as it was considered as an earlier name for *A. fusispora* (*A. stellata* was published on 30th May 1913 while *A. fusispora* was published on 30th June 1913). *Astrosphaeriella fusispora* was considered as a synonym of *A. stellata* in subsequent studies (Hawksworth 1981; Hyde and Fröhlich 1998; Hyde et al. 2000; Chen and Hsieh 2004; Tanaka et al. 2009; Liu et al. 2011). However, "a nomenclatural type is that element to which the name of a taxon is permanently attached, whether as the correct name or as a synonym" (McNeill et al. 2012). *Astrosphaeriella stellata* is shown in this study to be a distinct species and thus, *A. fusispora* must be regards as the type species of the genus *Astrosphaeriella*.

Astrosphaeriella fusispora differs from *A. stellata* in having smaller ascospores, but larger asci and ascostromata (see Supplementary Table 2). *Astrosphaeriella fusispora* has ascospores with acute ends (Fig. 12, 13), while those of *A. stellata* have slightly rounded ends (Fig. 16). Liu et al. (2011) treated a

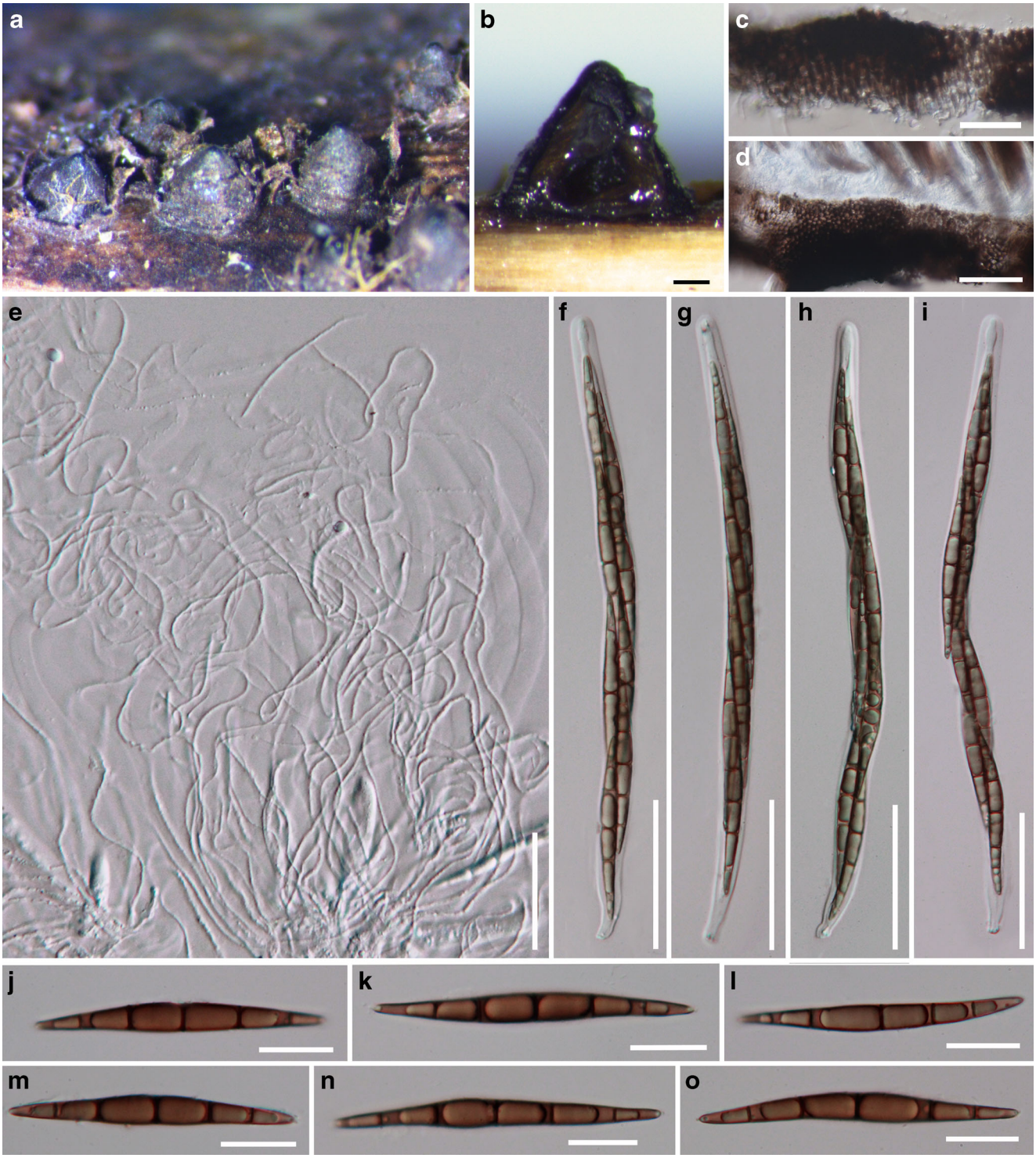


Fig. 11 *Astrosphaeriella exorrhiza* (MFLU 11-0242). **a** Appearance of ascostromata on host surface. **b** Section through ascostroma. **c**, **d** Section through peridium (c=at sides, d=at the base). **e** Trabeculate pseudoparaphyses. **f**–**i** Asci. **j**–**o** Ascospores. Scale bars: b=200 μm, d, f–i=50 μm, c, e, j–o=20 μm

collection MFLU 11-0147; living culture, MFLUCC 10-0555 to represent *A. stellata* based on its morphological characters being similar to the type of *A. stellata*. In this study, we have examined several collections with similar morphology with that of *A. fusispora* and *A. stellata*. However, the phylogenetic analyses (Fig. 3) indicate that they are different taxa. A collection MFLU 11-0147 is most similar to *A. fusispora*. Therefore, a collection MFLU 11-0147 is designated as the reference specimen of *A. fusispora*. *Astrosphaeriella fusispora* can clearly be distinguished from *A. stellata* which forms a sister clade closely related to *A. thailandica* and *A. thysanolaenae* (100 %

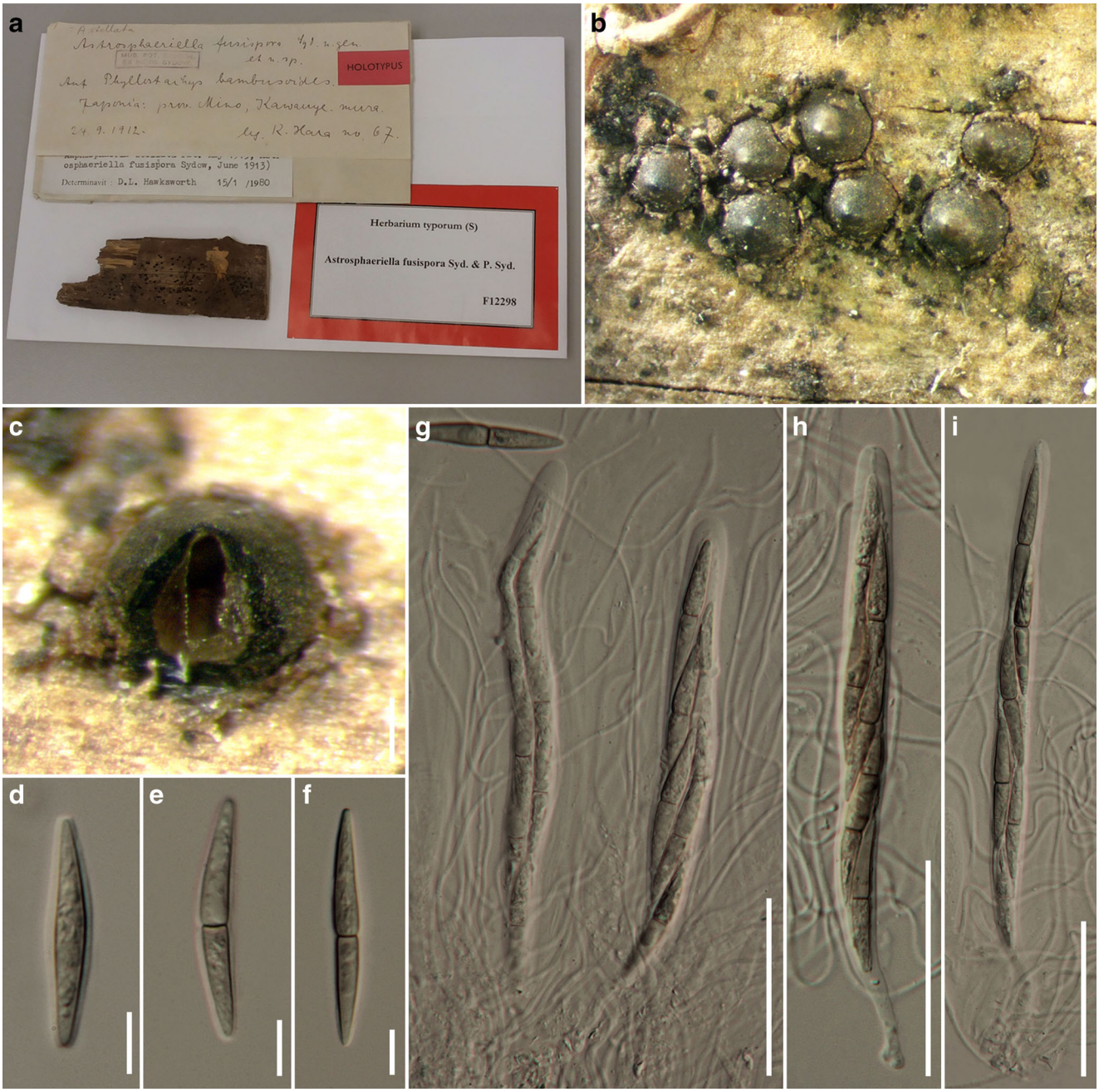


Fig. 12 *Astrosphaeriella fusispora* (S-F11298, holotype). **a** Herbarium label and specimens of *Astrosphaeriella fusispora*. **b** Appearance of ascostromata on host surface. **c** Section through ascostroma. **d–f** Ascospores. **g** Asci with trabeculate pseudoparaphyses. **h**, **i** Asci. Scale bars: c=200 *μm*, g–i=50 *μm*, d–f=10 *μm*

MP, 99 % ML, 1.00 PP). A pairwise comparison of *TEF1* sequence data shows that they are different at 14 base positions (Fig. 20).

Astrosphaeriella neofusispora Phookamsak & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF551637, Facesoffungi number: FoF 00336, Fig. 14

Etymology: The epithet "*neofusispora*" refers to its morphological characters being similar to *Astrosphaeriella fusispora*.

Holotype: MFLU 11-0197

Saprobic on bamboo, visible as numerous, black, cone-like structures on the host surface. **Sexual morph**: *Ascostromata* 520–710 *μm* high, 600–1100 *μm* diam., dark opaque, solitary to gregarious, erumpent through the host tissue, becoming superficial, conical, with ruptured, reflexed, stellate, host remnants around the base, uni-loculate, glabrous, brittle, carbonaceous, ostiolate. *Peridium* 55–110 *μm* wide, of unequal thickness, poorly developed at the base, composed of thick, opaque and melanized cells. *Hamathecium* composed of dense, 1–

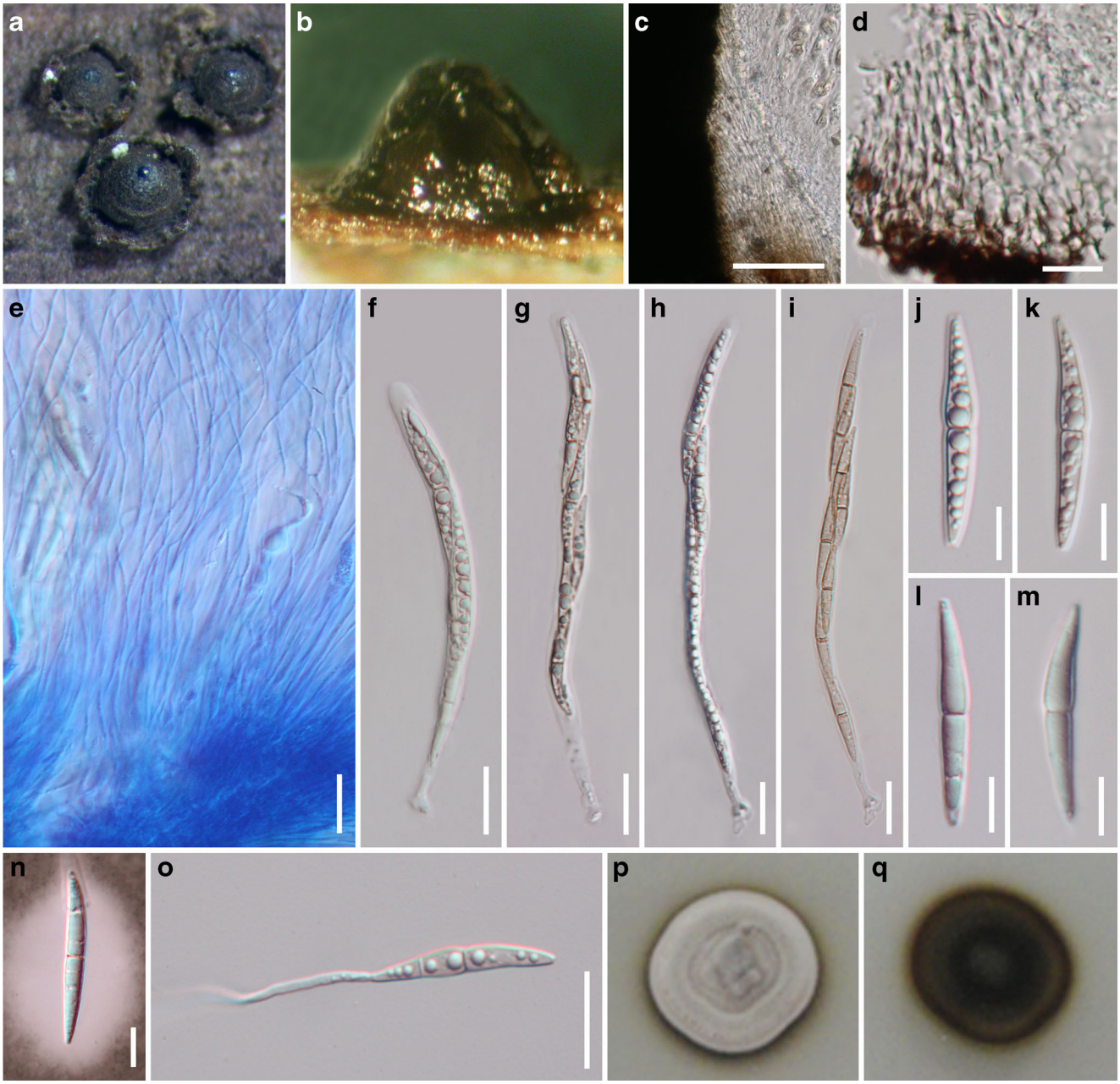


Fig. 13 *Astrosphaeriella fusispora* (MFLU 11-0147, reference specimen). **a** Appearance of ascostromata on host surface. **b** Section through ascostroma. **c**, **d** Section through peridium. **e** Trabeculate pseudoparaphyses stained in cotton blue. **f**–**i** Asci. **j**–**m** Ascospores. **n** Young ascospores strained in Indian ink. **o** Germination of ascospore. **p**, **q** Culture characters (p=from above, q=from below). Scale bars: c=50 μm, e–i, o=20 μm, d, j–n=10 μm

2 μm wide, filiform, indistinctly septate, trabeculate pseudoparaphyses, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* 150–200(–220)×12.5–14(–15) μm ($\overline{x}$ =175.5×13.8 µm, n=25), 8-spored, bitunicate, fissitunicate, cylindrical, subsessile or with short, obtuse to furcate pedicel, apically rounded, with well-developed ocular chamber. *Ascospores* (42–)45–48(–51)×6–7.5 μm ($\overline{x}$ =46.4×6.8 μm, n=30), overlapping uni- to bi-seriate, hyaline to pale yellowish when young, yellowish to olive brown, glistening when mature, fusiform with acute ends, 1(–3)-septate, constricted at the septum, upper cell larger than lower cell, smooth-walled, surrounded by a thin, distinct mucilaginous sheath. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on PDA reaching 25–30 mm diam. after 4 weeks at 25–30 °C, colonies circular, medium dense, flattened, slightly raised to umbonate, surface smooth with edge entire, fluffy to floccose or velvety,

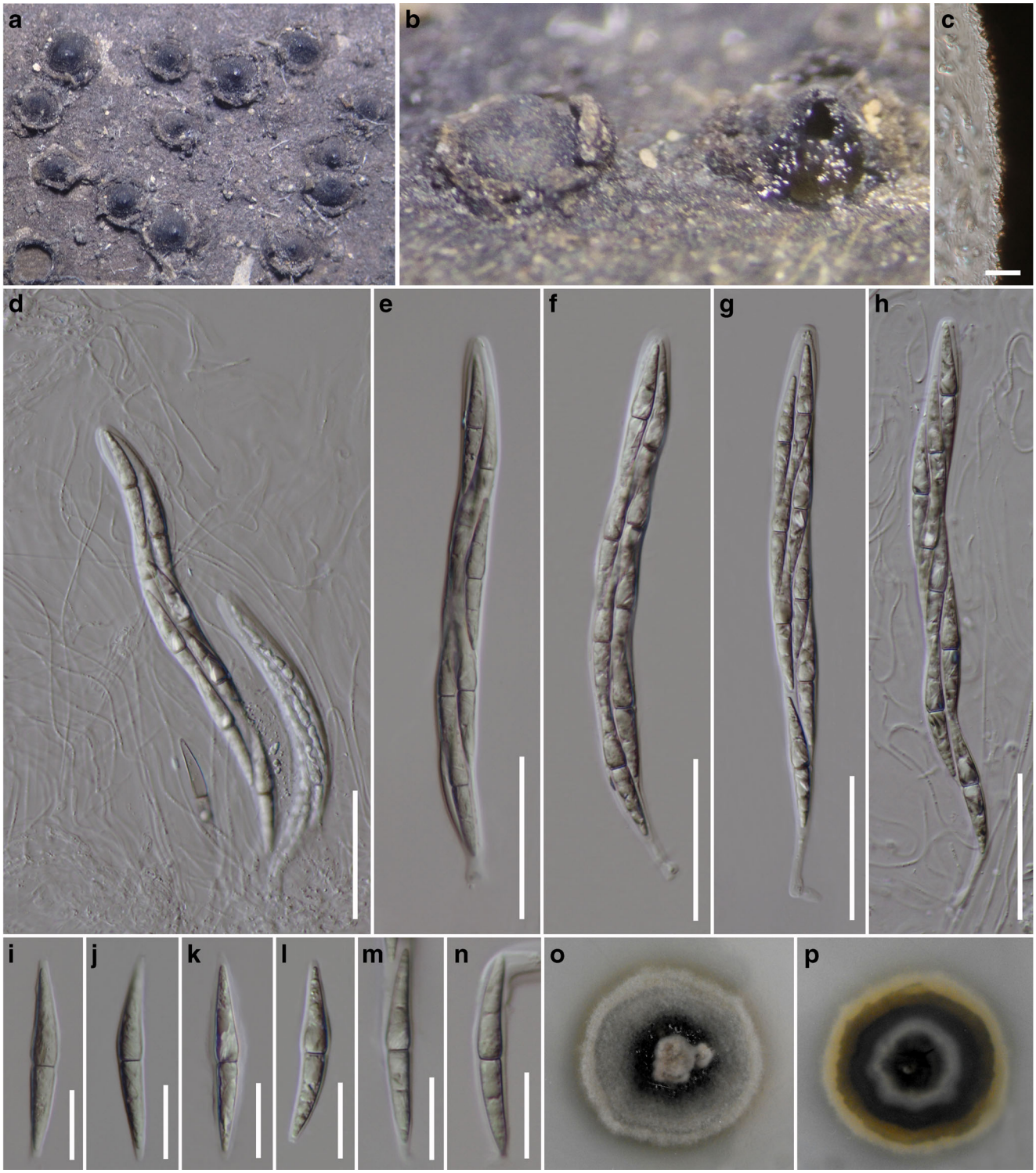


Fig. 14 *Astrosphaeriella neofusispora* (MFLU 11-0197, holotype). **a** Appearance of ascomata on host tissue. **b** Section through an ascoma. **c** Peridium structure arranged in a *textura angularis*. **d** Asci and trabeculate pseudoparaphyses. **e**–**h** Asci. **i**–**n** Ascospores. **o**, **p** Culture characters (o= from above, p=from below). Scale bars: d–h=50 μm, c, h−m=20 μm

smooth, slightly radiating with dark grey concentric ring near margin; colony from above, white at the margin, white grey to greenish-grey at the middle, brown grey to blackish at the centre with small droplets; from below: white to cream at the margin, yellowish-green to dark green in the middle, white to white grey at the center, strongly radiated with concentric sectors; producing brown pigmentation around colony.

Material examined: THAILAND, Chiang Rai Province, Muang District, Khun Korn Waterfall, on dead stem of bamboo, 5 September 2010, R. Phookamsak, RP0077 (MFLU 11-0197, **holotype**), ex-type living culture, MFLUCC 11-0161, BCC.

Notes: *Astrosphaeriella neofusispora* differs from other species in *Astrosphaeriella* sensu stricto in having larger ascospores (see Supplementary Table 2). *Astrosphaeriella neofusispora* differs from *A. fusispora* in having larger ascospores ($\bar{x}$ =46.4×6.8 μm in *A. neofusispora* versus $\bar{x}$ =40.6×5.8 μm in *A. fusispora*). *Astrosphaeriella neofusispora* is most similar to *A. bambusella* in ascospore size. However, *A. bambusella* differs from *A. neofusispora* in its larger asci and pale brown ascospores (see Supplementary Table 2).

Ariyawansa et al. (2014b) treated *Astrosphaeriella neofusispora* as a reference specimen of *A. stellata* due to its morphological similarity and few strains available. In this study, phylogenetic combined analyses of LSU, SSU and *TEF1* sequence data (Fig. 3) shows that *A. neofusispora* forms a robust clade at the base of *A. bambusae*, *A. neostellata* and *A. stellata* (62 % MP, 87 % ML, 1.00 PP). A *TEF1* gene pairwise comparison shows that *A. neofusispora* differs from other *Astrosphaeriella* sensu stricto at 13 base positions (Fig. 20).

Astrosphaeriella neostellata D.Q. Dai, Phookamsak & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF551634, Facesoffungi number: FoF 01226, Fig. 15

Etymology: The epithet "*neostellata*" refers to its morphological characters being similar to *Astrosphaeriella stellata*.

Holotype: MFLU 15-1195

Saprobic on decaying bamboo culms. **Sexual morph**: *Ascostromata* 650–850 μm high, 700–1000 μm diam., black, scattered, erumpent to superficial, breaking the host tissue, becoming superficial, with ruptured, reflexed, stellate, host remnants around the base, immersed at the base, conical, uni-loculate, glabrous, brittle, carbonaceous, ostiole central, with pore-like opening over almost the entire length. *Peridium* 50–90 μm wide, of unequal thickness, poorly developed at the base, composed of dark brown cells. *Hamathecium* composed of dense, 1–1.5 μm wide, filiform, trabeculate pseudoparaphyses, anastomosing among the asci, embedded in a hyaline, gelatinous matrix. *Asci* 170–230×(8.5–)9.5–13.5 μm ($\bar{x}$ =194.6×11.5 μm, n=20), 8-spored, bitunicate, fissitunicate, cylindrical, with a short furcate pedicel, narrow and rounded apex, with a small ocular chamber. *Ascospores* 40–60×(5–)5.5–6.5(–8) μm ($\bar{x}$ =48.1×6.1 μm, n=20), overlapping bi- to tri-seriate, hyaline becoming pale brown, fusiform, tapering to pointed apices, 1-septate, constricted at the septum, upper cell slightly wider, smooth-walled, guttulate, surrounded by a thin sheath. **Asexual morph**: Undetermined.

Culture characteristics: Ascospores germinating on PDA within 48 h and germ tubes produced from both ends. Colonies growing slowly on PDA, reaching 10 mm diam. in 2 weeks at 28 °C, under 12 h light/12 h dark, circular, effuse, velvety to hairy, initially white, becoming brown to dark brown from above, dark brown in media from below, with light brown circular edge. Mycelium immersed in and superficial on the media, branched, septate, smooth, hyaline.

Material examined: THAILAND, Chiang Rai Province, Doi Pui, on dead culm of bamboo, 1 September 2012, D.Q. Dai, DDQ00119 (MFLU 15-1195, **holotype**); *ibid*, KUN HKAS 88704 (**isotype**), ex-type living culture, MFLUCC 11-0625, BCC.

Notes: *Astrosphaeriella neostellata* differs from *A. stellata* and *A. bambusae* in having larger ascostromata and ascospores (see Supplementary Table 2). The phylogenetic analyses (Fig. 3) show that *A. neostellata* clusters with *A. stellata* and *A. bambusae*. However, the phylogenetic relationships between *A. neostellata* and *A. bambusae* are not well-resolved in MP and Bayesian analyses, but form a well-resolved clade in maximum likelihood analysis. A comparison of ITS sequence data shows that they are different (data not shown).

Astrosphaeriella stellata (Pat.) Sacc., Syll. fung. (Abellini) 24(2): 938 (1928)

≡ *Amphisphaeria stellata* Pat., Bull. Soc. mycol. Fr. 29: 223 (1913)

Facesoffungi number: FoF 01227, Fig. 16

Saprobic on bamboo, visible as numerous, black opaque, cone-like structures on host surface. **Sexual morph**: *Ascostromata* 280–500 μm high, 420–620 μm diam., dark opaque, scattered, gregarious, erumpent to superficial, conical, with ruptured, reflexed, stellate, host remnants around the base, uni-loculate, glabrous, brittle, carbonaceous, with indistinctly ostioles. *Peridium* 20–55 μm wide, of unequal thickness, poorly developed at the base, composed of thick, opaque and melanized cells, arranged in *textura angularis*. *Hamathecium* composed of dense, 1–1.7 μm wide, filamentous, trabeculate pseudoparaphyses, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* (130–)(140–)150–180(–190)×9–11.5 μm ($\bar{x}$ =167.1×10.5 μm, n=25), 8-spored, bitunicate, fissitunicate, cylindrical, short- to long-pedicellate, with furcate pedicel, apically rounded with narrow to wedge-shaped ocular chamber. *Ascospores* (36–)38–45(–47)(–53)×5–7 μm ($\bar{x}$ =42×6.2 μm, n=30), overlapping uni- to bi-seriate, pale brown or brown, fusiform with slightly rounded ends, 1-septate, rarely aseptate, or 2-septate, slightly curved, constricted at the septum, upper cell wider than lower cell, smooth-walled, surrounded by a thin, distinct sheath, truncate at point ends. **Asexual morph**: Undetermined.

Material examined: VIETNAM, Hanoi, on Bamboo (sur Bambou), 17 April 1911, M. Duport 451, FH 00290284 (Pat 552, **holotype**).

Notes: *Astrosphaeriella stellata* was introduced by Saccardo (1928) to accommodate *Amphisphaeria stellata*

Fig. 15 *Astrosphaeriella neostellata* (MFLU 15-1195, holotype). **a** Appearance of ascostromata on host surface. **b** Vertical section through ascostroma. **c** Trabeculate pseudoparaphyses. **d**–**g** Asci. **h**–**i** Ascospores. **j** Germination of ascospores. **k**, **l** Culture characters on PDA (k=from above, l=from below). Scale bars: b=200 μm, c=50 μm, d–h, j=20 μm, h–i=10 μm

Pat. which was collected on bamboo in Vietnam. The species has commonly been found on bamboos and palms and is widely distributed in temperate and tropical regions of Australia, China, France, Hong Kong, India, Indonesia, Japan,

Taiwan, Thailand and Vietnam (Hawksworth 1981; Hyde and Fröhlich 1998; Chen and Hsieh 2004; Hu 2010; Liu et al. 2011). Hyde and Fröhlich (1998) mentioned that *Astrosphaeriella stellata* had wide ranges of ascospores size which was also shown in Liu et al. (2011). Due to the apparent difficulty in obtaining *Astrosphaeriella* cultures, the culture characteristics and molecular data for *Astrosphaeriella* is lacking (Tsui et al. 2001; Liu et al. 2011). Therefore, subsequent mycologists identified *A. stellata* based on morphology with wide ranges of ascostromata, asci and ascospore size (Hawksworth 1981; Hyde and Fröhlich 1998; Chen and Hsieh 2004; Liu et al. 2011).

In this study, eight collections of *Astrosphaeriella stellata*-like species were analyzed to resolve their phylogenetically relationships. Phylogenetic analyses of combined LSU, SSU and *TEF1* sequence data (Fig. 3), resolve these eight strains as seven distinct species. The comparison of ITS and *TEF1* gene base pairs (Fig. 20) also differentiate the taxa (data for ITS gene not shown). Based on morphological characters and phylogenetic analyses, it can be concluded that *Astrosphaeriella stellata* is distinct from *A. fusispora* and *Astrosphaeriella* sensu stricto species. These species are morphologically similar, but can be differentiated in phylogenetic relationships and some aspects such as ascospore, asci, and ascostromata size; ascospore colour and septation and culture characteristic.

Astrosphaeriella thailandica Phookamsak & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF551638, Facesoffungi number: FoF 01228, Fig. 17

Etymology: The epithet "*thailandica*" refers to the country from which the taxon was collected.

Holotype: MFLU 11-0227

Saprobic on bamboo, visible as numerous, black, opaque, cone-like structures on the host surface. **Sexual morph**: *Ascostromata* 290–530 μm high, 510–930 μm diam., dark opaque, solitary, erumpent through host surface to superficial, with ruptured, reflexed, stellate, host remnants around the base, conical, uni-loculate, glabrous, brittle, carbonaceous, ostiole central with small pore at the apex. *Peridium* 20–50(–70) μm wide, of unequal thickness, poorly developed at the base, slightly thick at sides towards the apex, composed of thick, opaque and melanized cells, arranged in a *textura angularis*. *Hamathecium* composed of dense, 1–1.8 μm wide, filiform, trabeculate pseudoparaphyses, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* (150–)170–200(–240)×(10–)11–12(–13) μm ($\bar{x}$ =185.3×11.4 μm, n=30), 8-spored, bitunicate, cylindrical to cylindric-clavate, with short, furcate pedicel, apically rounded with indistinct ocular chamber. *Ascospores* (37–)41–44(–48)×5–6(–7) μm ($\bar{x}$ =43.3×5.6 μm, n=30), overlapping bi-seriate, pale yellowish or pale brown, fusiform with acute ends, 1-septate, constricted at the septum, slightly curved, upper cell larger than lower cell, smooth-walled, surrounded by a thick, distinct sheath, with truncate or indented, point ends. **Asexual morph**: Undetermined.

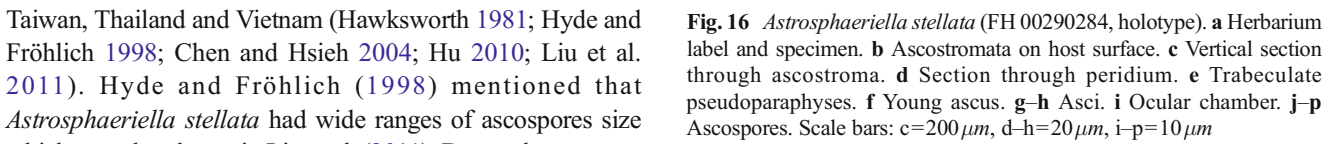


Fig. 16 *Astrosphaeriella stellata* (FH 00290284, holotype). **a** Herbarium label and specimen. **b** Ascostromata on host surface. **c** Vertical section through ascostroma. **d** Section through peridium. **e** Trabeculate pseudoparaphyses. **f** Young ascus. **g**–**h** Asci. **i** Ocular chamber. **j**–**p** Ascospores. Scale bars: c=200 μm, d–h=20 μm, i–p=10 μm

Culture characteristics: Colonies on PDA reaching 40–45 mm diam. after 4 weeks at 25–30 °C, colonies circular, medium dense to dense, flattened, slightly raised, to low convex, surface smooth with edge entire, fluffy to floccose, or velvety, colony from above part: dark brown to black at the margin, dark grey to greenish grey at the centre; from below blackish at the margin, dark grey at the center; producing brown pigmentation in agar.

Material examined: THAILAND, Chiang Mai Province, Chom Tong District, Doi Inthanon, on dead branches of bamboo, 16 November 2010, R. Phookamsak, RP0107 (MFLU 11-0227, **holotype**), ex-type living culture, MFLUCC 11-0191, BCC.

Notes: *Astrosphaeriella thailandica* is most similar to *A. thysanolaenae* but differs in having shorter, pale yellowish or pale brown ascospores and its occurrence on bamboo (versus grass) (see Supplementary Table 2). Phylogenetic results (Fig. 3) indicate that *Astrosphaeriella thailandica* is distinct from *A. thysanolaenae* which forms a distinct clade (67 % MP, 94 % ML, 1.00 PP) together with taxa in *Astrosphaeriella* sensu stricto. A pairwise comparison of *TEF1* sequence data of *A. thailandica* and *A. thysanolaenae* show they differ at 24 base pair positions (Fig. 20).

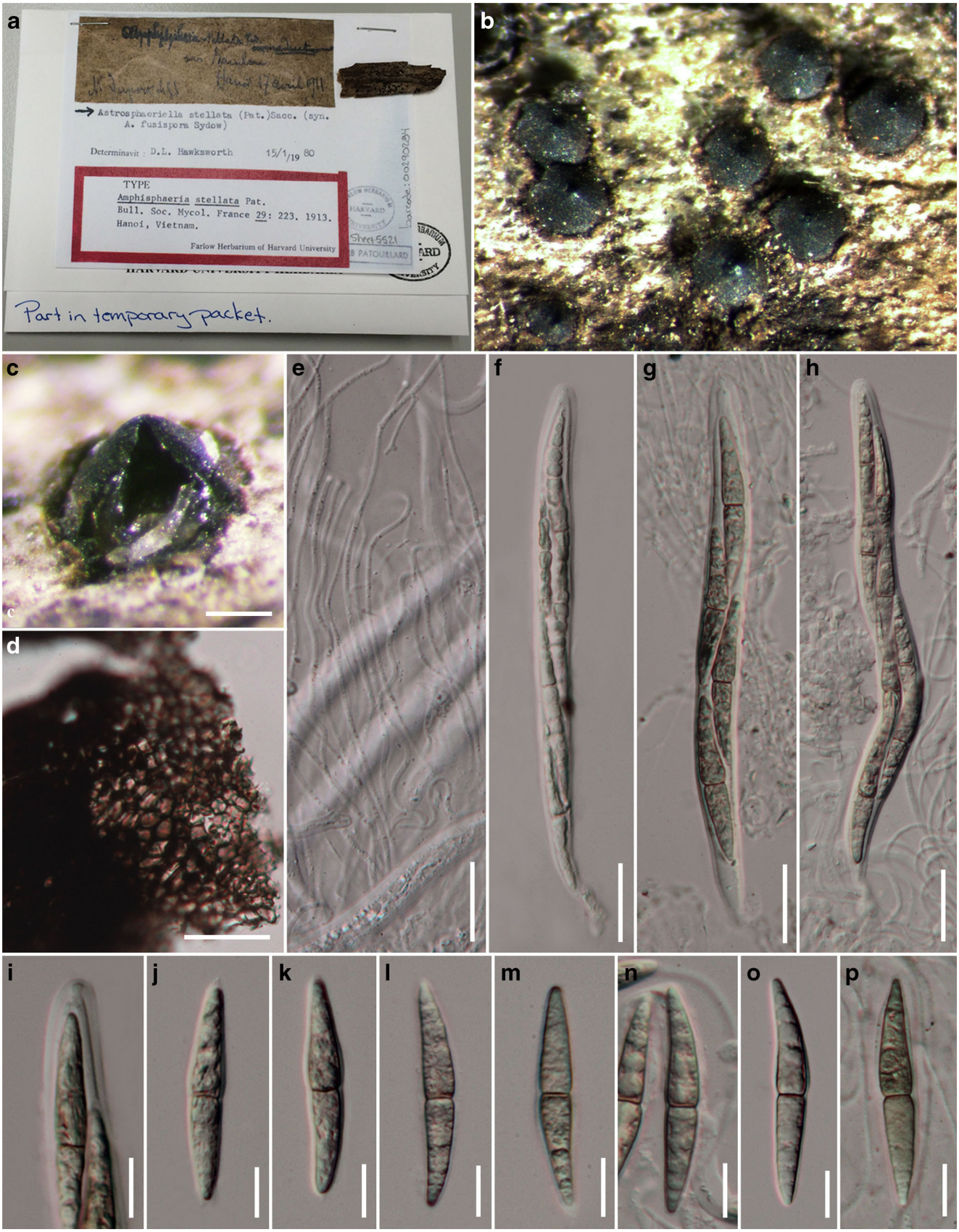
Astrosphaeriella thysanolaenae Phookamsak & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF551639 Facesoffungi number: FoF 01229, Fig. 18

Etymology: The epithet "*thysanolaenae*" refers to the host, from which the taxon was collected.

Holotype: MFLU 11-0222

Saprobic on *Thysanolaena maxima*, visible as black, opaque, cone-like structures on host surface. **Sexual morph**: *Ascostromata* 360–550 μm high, 550–780 μm diam., dark opaque, solitary, erumpent through host cortex, becoming superficial, with ruptured, reflexed, stellate, host remnants, around the base, conical, uni-loculate, glabrous, brittle, carbonaceous, ostiole central with pore-like opening. *Peridium* 30–80 μm wide, of unequal thickness, poorly developed at the base, composed of thick, opaque, melanized cells, arranged in a *textura angularis*. *Hamathecium* composed of dense, 1–1.7 μm wide, filiform, trabeculate pseudoparaphyses, anastomosing among the asci, embedded in a hyaline gelatinous matrix. *Asci* (122–)140–160(–185)×(11–)12–13(–14)(–15) μm ($\bar{x}$ =146.8×12.6 μm, n=


a
Astrosphaeriella stellata (Pat.)Sacc. (syn.
A. fusispora Sydow)
Determinavit : D.L. Hawksworth 15/1/19 80
TYPE
Amphisphaeria stellata Pat.
Bull. Soc. Mycol. France 29: 223. 1913.
Hanoi, Vietnam.
Farlow Herbarium of Harvard University
Part in temporary packet.
b
c
d
e
f
g
h
i
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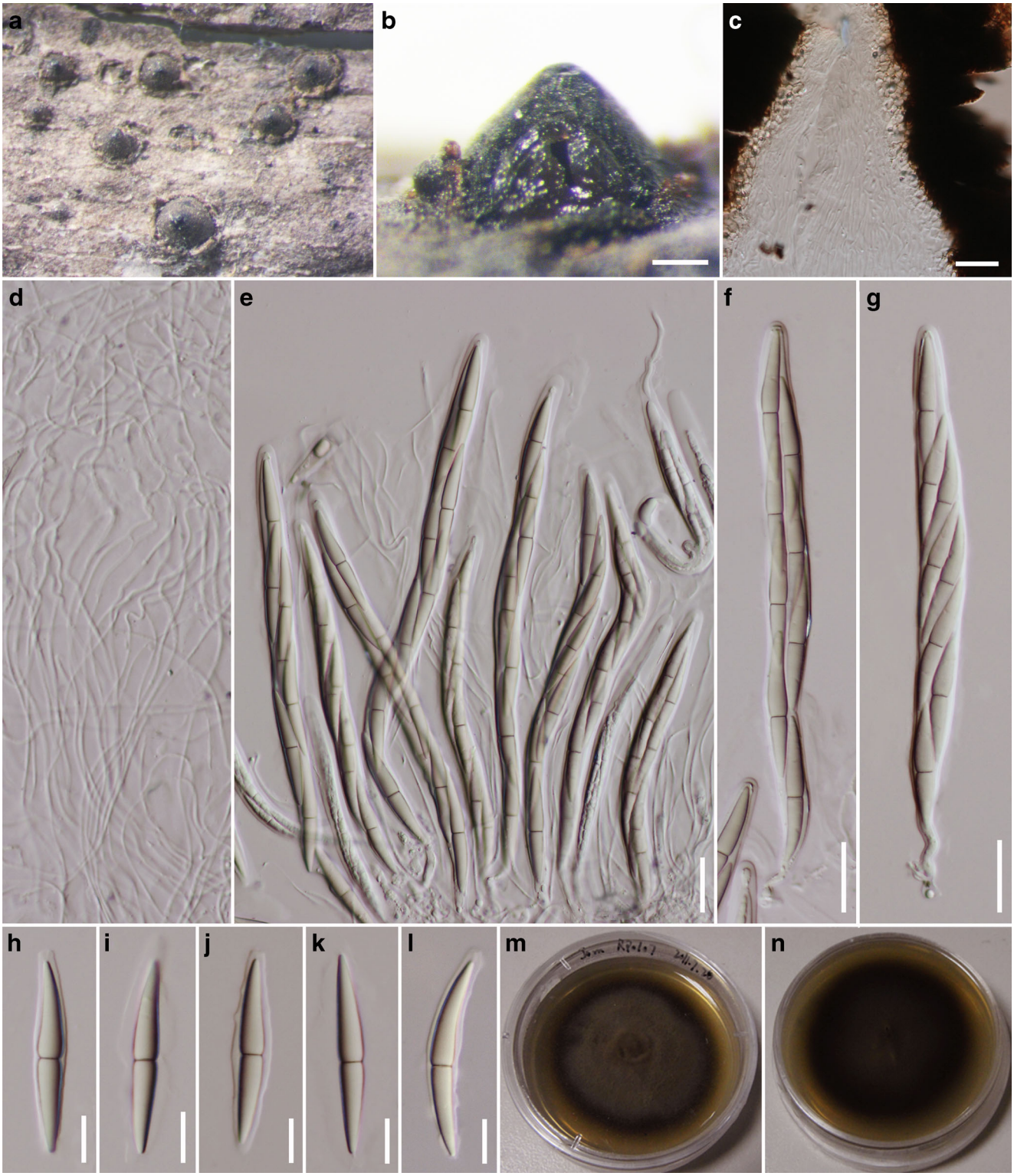

Fig. 17 *Astrosphaeriella thailandica* (MFLU 11-0227, holotype). **a** Appearance of ascostromata on host surface. **b** Section through an ascostroma. **c** Peridium structure arranged in a *textura angularis*. **d** Trabeculate pseudoparaphyses. **e** Asci with trabeculate pseudoparaphyses. **f**, **g** Asci. **h**–**l** Ascospores. **m**, **n** Culture characters (m=from above, n=from below). Scale bars: c=200 μm, c–g=20 μm, h–l=10 μm

25), 8-spored, bitunicate, cylindrical to cylindric-clavate, with a short, furcate or truncate pedicel, apically rounded with well-developed ocular chamber. *Ascospores* (29–)37–39(–44)×4–6(–7) μm ($\bar{x}$ =37.4×5.8 μm, n=30),

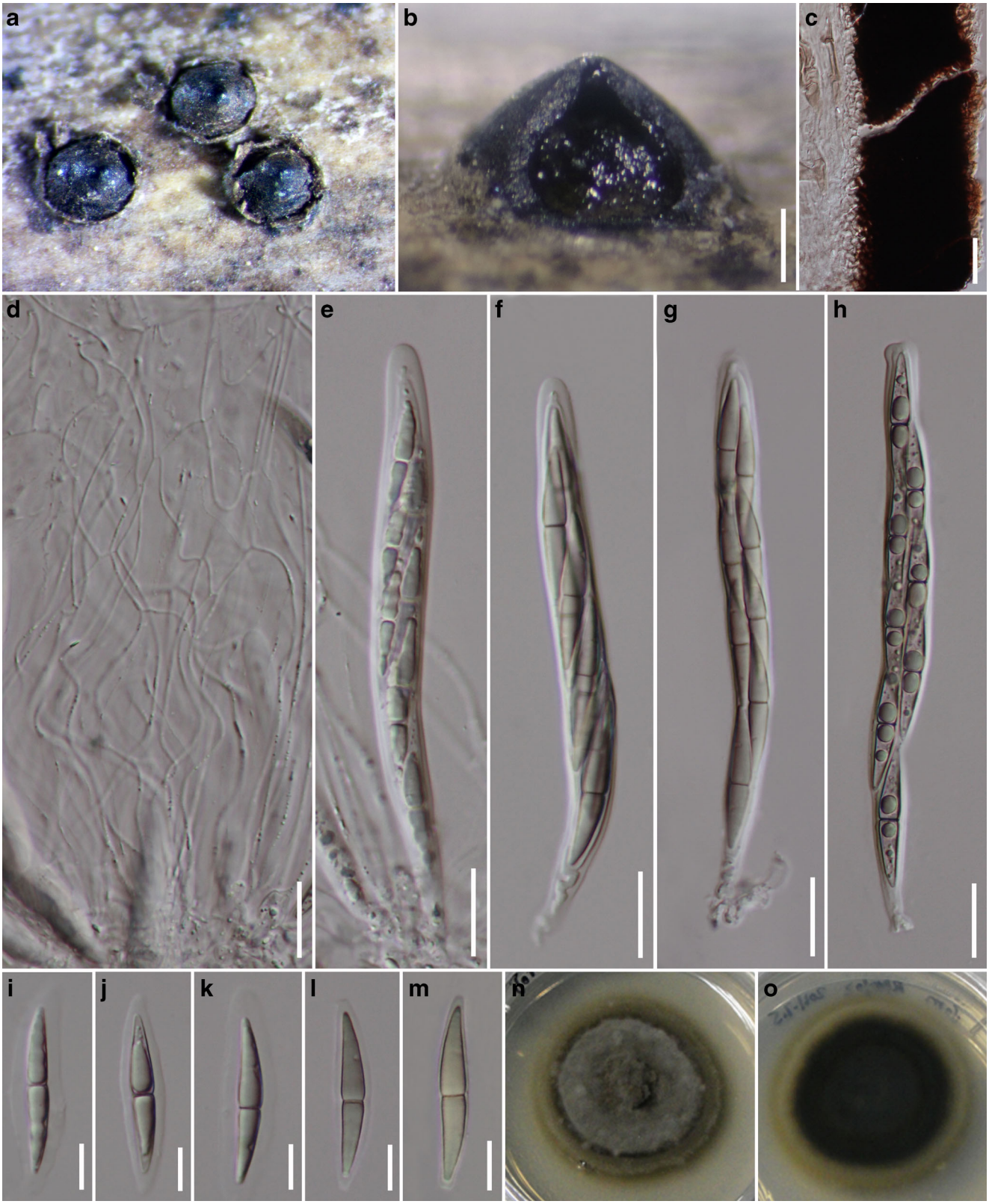

Fig. 18 *Astrosphaeriella thysanolaenae* (MFLU 11-0222, holotype). **a** Appearance of ascostromata on host surface. **b** Section through an ascostroma. **c** Peridium structure arranged in a *textura angularis*. **d** Trabeculate pseudoparaphyses. **e**–**h** Asci. **i**–**m** Ascospores. **n**, **o** Culture characters (n=from above, o=from below). Scale bars: b=200 μm, c–h=20 μm, i−m=10 μm

overlapping bi-seriate, initially hyaline, becoming yellowish brown to brown at maturity, fusiform with acute ends, slightly curved, 1-septate, constricted at the septum, upper cell larger than lower cell, smooth-walled, surrounded by a thick, distinct, mucilaginous sheath, with truncate ends. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on PDA reaching 58–65 mm diam. after 4 weeks at 25–30 °C, colonies circular, medium dense, flattened, slightly raised, surface smooth with edge entire, fluffy to floccose, slightly radiating near the margin, separated at the center by dark greenish concentric ring; colony from above: yellowish brown to greenish brown at the margin, grey at the centre; from below: pale brown to yellowish brown at the margin, dark grey to blackish at the center; not producing pigmentation in agar.

Material examined: THAILAND, Chiang Mai Province, Chom Tong District, Doi Inthanon, on dead stem of *Thysanolaena maxima*, 16 November 2010, R. Phookamsak, RP0102 (MFLU 11-0222, **holotype**), ex-type living culture, MFLUCC 11-0186, BCC.

Notes: *Astrosphaeriella thysanolaenae* is most similar to *A. thailandica* and the differences are discussed under that species.

Astrosphaeriella tornata (Berk. & M.A. Curtis) D. Hawksw. & Boise, Sydowia 38: 119 (1986) [1985]

≡ *Sphaeria tornata* Berk. & M.A. Curtis, J. Acad. nat. Sci. Philad., N.S. 2(6): 290 (1854) [1853]

= *Trematosphaeria tornata* Cooke, Grevillea 16(no. 79): 91 (1888)

Facesoffungi number: FoF 01230, Fig. 19

Saprobic on bamboo and palms, visible as numerous, black, opaque, cone-like structures on the host surface. **Sexual morph**: *Ascostromata* 430–670 μm high, 650–810 μm diam., dark opaque, gregarious, erumpent to superficial, conical with ruptured, reflexed, stellate, host remnants around the base, uni-loculate, glabrous, brittle, carbonaceous, ostiole central, with pore-like opening. *Peridium* 50–90 μm wide, of unequal thickness, poorly developed at the base, thick at the sides towards the apex, composed of thick, opaque and melanized cells. *Hamathecium* composed of dense, 1–2 μm wide, branching, rough-walled, distinctly septate, trabeculate pseudoparaphyses, anastomosing among the asci, embedded in a hyaline gelatinous matrix. *Asci* (120–)140–170(–178)(–190)×(11–)12–14(–15) μm ($\bar{x}$ =156.1×13.3 μm, n=25), 8-spored, bitunicate, cylindrical, subsessile to short pedicellate, apically rounded with an ocular chamber. *Ascospores* (47.5–)48–52(–54)×7–8(–9) μm ($\bar{x}$ =50.3×8.2 μm, n=30), overlapping uni- to bi-seriate, brown to reddish brown, fusiform with acute ends, 3-septate, slightly constricted at the central septum, widest at the middle, smooth-walled. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on PDA fast growing, reaching 70–75 mm diam. after 4 weeks at 25–30 °C, colonies circular, medium dense to dense, flat to raised, surface dull, rough, with edge erose, cotton to velvety, slightly radiating outwards; colony from above: dull green at the margin, greenish grey to white grey at the middle, dull green at the centre; from below dull green at the margin, blackish in the middle, dark green at the center; not producing pigmentation in agar.

Material examined: THAILAND, Chiang Rai Province, Muang District, Khun Korn waterfall, on dead stem of bamboo, 17 December 2010, R. Phookamsak, RP0112 (MFLU 11-0232, **reference specimen designated here**), living cultures, MFLUCC 11-0196, BCC.

Notes: Hawksworth and Boise (1985) transferred *Sphaeria tornata* to *Astrosphaeriella* because of mammiform to conical, carbonaceous ascostromata and fusiform, reddish brown ascospores (Hawksworth and Boise 1985). The type specimen of this species is in poor condition. *Astrosphaeriella tornata* is similar to *A. lenticularis*, *A. splendida*, *A. trochus* and *A. vesuvius* in having broadly fusiform, reddish brown ascospores. *Astrosphaeriella tornata* is most similar to *A. splendida*, but differs in having smaller ascospores with paler end cells. *Astrosphaeriella tornata* forms a clade at the base of the *Astrosphaeriellaceae* and is closely related to *A. vesuvius* and *Pteridiospora* (Fig. 3).

Pteridiospora Penz. & Sacc., Malpighia 11(9-10): 399 (1897)

Facesoffungi number: FoF 01231

Saprobic on bamboo, visible as cone-like structures on the host surface. **Sexual morph**: *Ascostromata* dark opaque, scattered, solitary or forming a groups (3–4 ascostromata) on darkened areas on the host surface, erumpent through host tissue, becoming superficial, mammiform to conical, with ruptured, reflexed, stellate, host remnants around the base, uni-loculate, glabrous, brittle, carbonaceous, ostiolate. *Ostioles* central with pore-like opening or minute papilla. *Peridium* thin-walled, of unequal thickness, poorly developed at the base, composed of opaque dark cells. *Hamathecium* composed of dense, filiform, anastomosing, trabeculate pseudoparaphyses, embedded in hyaline gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, broadly cylindrical to cylindric-clavate, short pedicellate, apically rounded with ocular chamber or faint apical ring. *Ascospores* overlapping uni- to bi-seriate, hyaline to brown, obclavate to ellipsoidal, or subfusoid, with rounded to acute ends, narrow towards apex, septate, constricted at the septum, asymmetric, smooth-walled, surrounded by irregular, distinct, mucilaginous sheath, expanded and widest below the basal cell. **Asexual morph**: Coelomycetous. *Conidiomata* pycnidial, scattered, gregarious, forming conidiomata, radiating outwards on the colonies, semi-immersed to superficial, globose to subglobose, uni-loculate, glabrous, covered by thick, vegetative hyphae, indistinctly ostiolate. *Pycnidial walls* composed of two cell layers of equal thickness,

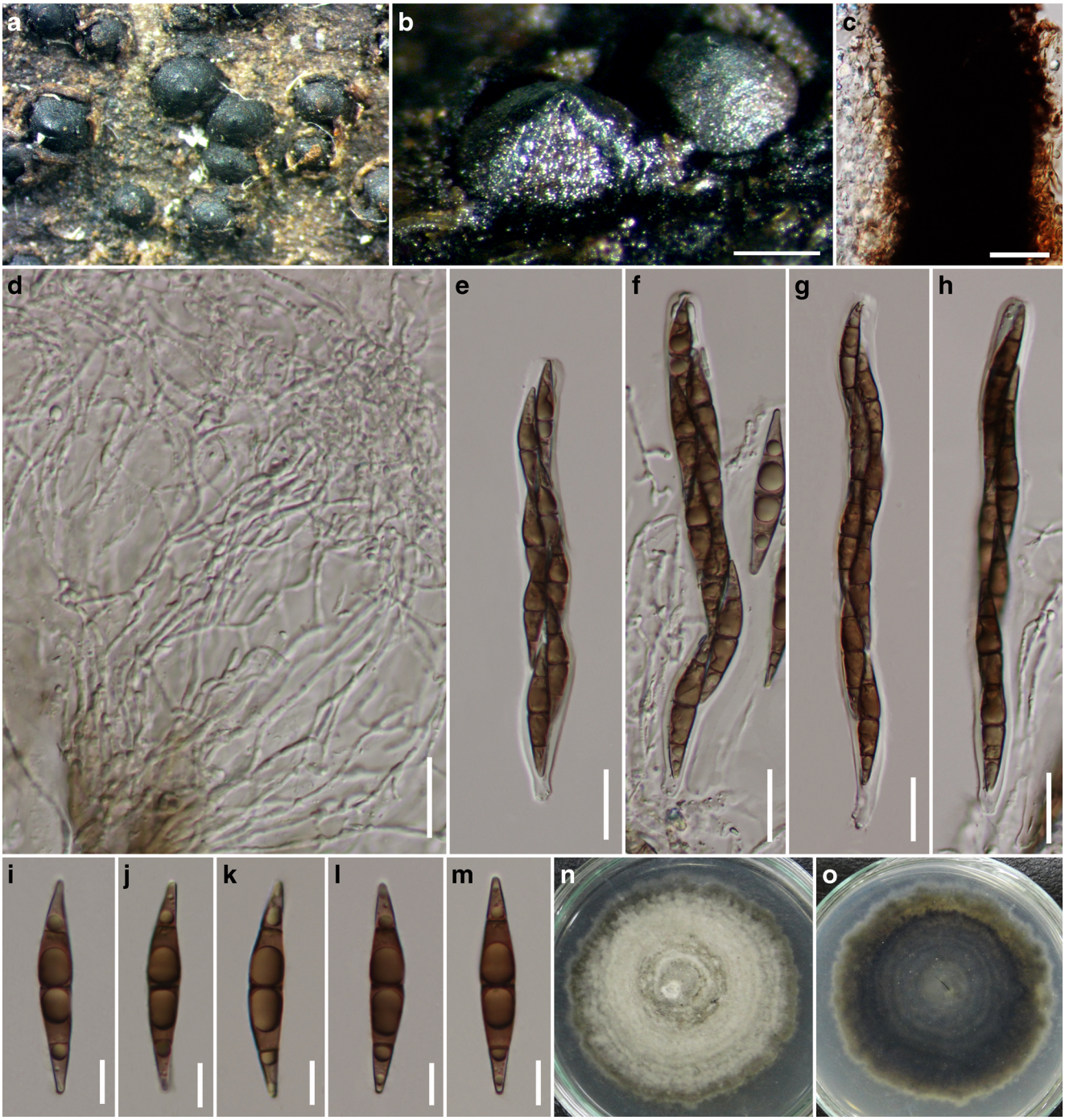

Fig. 19 *Astrosphaeriella tornata* (MFLU11-0232, reference specimen). **a** Ascostromata visible as cone-like structures on the host surface. **b** Section through ascostroma. **c** Peridium structure arranged in a *textura angularis* to *textura intricata*. **d** Pseudoparaphyses. **e**–**h** Asci. **i**–**m** Ascospores. **n**, **o** Culture characters on PDA (n=from above, o=from below). Scale bars: b=500 μm, c–h=20 μm, i–m=20 μm

outer layer comprising brown to dark brown cells, of *textura intricata*, inner layers comprising dark brown to black cells, of *textura angularis*. *Conidiophores* reduced to conidiogenous cells, arising from the basal cavity. *Conidiogenous cells* enteroblastic, phialidic, determinate, integrated or discrete, hyaline, oblong to cylindrical or ampulliform, aseptate, unbranched, smooth-walled. *Conidia* hyaline, globose to subglobose, forming chains, aseptate, smooth-walled.

Type species: *Pteridiospora javanica* Penz. & Sacc.

Notes: *Pteridiospora* was introduced by Penzig and Saccardo (1897) with *P. javanica* as the type species. The genus was introduced to accommodate fungi having mammiform, carbonaceous ascostromata with obclavate to

Fig. 20 *TEF1* gene comparison of *Astrosphaeriella* species: *Astrosphaeriella bambusae* (MFLUCC 13-0230), *Astrosphaeriella fusispora* (MFLUCC 10-0555), *Astrosphaeriella neofusispora* (MFLUCC 11-0161), *Astrosphaeriella thailandica* (MFLUCC 11-0191), *Astrosphaeriella thysanolaenae* (MFLUCC 11-0186)

ellipsoidal, or subfusoid ascospores with a thick, distinct mucilaginous sheath (Penzig and Saccardo 1897; Phookamsak et al. 2014a). The genus *Pteridiospora* has been poorly studied (Phookamsak et al. 2014a). The type species, *P. javanica* was collected on dead culms of *Bambusae* from Tjibodas, Java, Indonesia. Phookamsak et al. (2014a) collected *P. javanica* on bamboo in Thailand and designated their collection as an epitype. Phookamsak et al. (2014a) illustrated the species and reported the asexual morph of *Pteridiospora* which was produced in culture as coelomycetous, having hyaline, globose, aseptate conidia. The phylogenetic analyses showed *Pteridiospora* to cluster with *Astrosphaeriella* (Phookamsak et al. 2014a).

Pteridiospora is similar to *Astrosphaeriella* species, in having superficial, mammiform to conical, carbonaceous ascostromata and trabeculate pseudoparaphyses (Hyde and Fröhlich 1998; Tanaka et al. 2009; Liu et al. 2011; Phookamsak et al. 2014a). *Pteridiospora* differs from *Astrosphaeriella* in its asymmetrical ascospores, with a large, distinctive, irregular mucilaginous sheath (Phookamsak et al. 2014a). Phylogenetic analyses (Fig. 3) show that *Pteridiospora* forms a sister clade, distinct from *Astrosphaeriella* sensu stricto.

Pteridiospora was previously accommodated in the *Pleosporaceae* (Müller and von Arx 1962; Filer 1969; von Arx and Müller 1975). Lumbsch and Huhndorf (2010) accommodated the genus in the Dothideomycetes genera *incertae sedis*. Phookamsak et al. (2014a) showed that *Pteridiospora* clusters with *Astrosphaeriella* and in this paper a new family is designated to accommodate these genera.

Pteridiospora chiangraiensis Phookamsak & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF551640, Facesoffungi number: FoF 01232, Fig. 21

Etymology: The epithet "*chiangraiensis*" refers to the Province in Thailand, of which the fungus was found.

Holotype: MFLU 11-0198

Saprobic on bamboo, visible as mammiform to conical structures, on the host surface. **Sexual morph**: *Ascostromata* 300–450 μm high, 420–680 μm diam., opaque dark, solitary, erumpent through the host tissue, becoming superficial, conical to mammiform, flattened at the base, with ruptured, reflexed, stellate, host remnants around the base, uni-loculate, glabrous, brittle, slightly glistening, carbonaceous, ostiolate. *Ostioles* central, with pore-like opening. *Peridium* 30–50 μm wide, of unequal thickness, poorly developed at the base, composed of opaque dark cells. *Hamathecium* composed of dense, 1–2 μm wide, filiform, trabeculate pseudoparaphyses, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* (145–)160–180(–210)×(13–)15–17(–19) μm ($\bar{x}$ =181.6×15.9 μm, n=25), 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, short, with obtuse pedicel, apically rounded with well-developed ocular chamber. *Ascospores* (33–)35–38(–40)×6–8 μm ($\bar{x}$ =36.1×7.4 μm, n= 30), overlapping uni-to bi-seriate, initially hyaline, becoming brown when mature, subfusoid to ellipsoidal, or obclavate, with rounded to acute ends, narrow towards the apex, 1-septate, constricted at the septum, upper cell longer than lower cell, surrounded by an irregular, distinct mucilaginous sheath, with large, wing-like appendage extending from the lower cell. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on SDA fast growing, reaching 16–18 mm diam. after 7 days at 25–30 °C, colonies irregular, dense, raised to umbonate, surface dull and rough, with undulate edge, floccose to velvety, wrinkled, slightly radiating, with black concentric ring; colony from above: white to cream at the margin, dark brown to grey at the centre; from below: white grey and black sectors; not producing pigmentation in agar.

Material examined: THAILAND, Chiang Rai Province, Muang District, Khun Korn Waterfall, on dead stem of bamboo, 5 September 2010, R. Phookamsak, RP0078 (MFLU 11-0198, **holotype**), ex-type living cultures, MFLUCC 11-0162, BCC.

Notes: *Pteridiospora chiangraiensis* is similar to *P. javanica* in having asymmetric, subfusoid to ellipsoidal or obclavate ascospores, with an irregular, distinct mucilaginous sheath. However, *P. chiangraiensis* differs from *P. javanica* due to its smaller ascospores, which are brown at maturity, while *P. javanica* has larger, hyaline ascospores. In the phylogenetic analyses (Fig. 3), *P. chiangraiensis* forms a sister clade basal to *P. javanica*.

Pseudoastrosphaeriellaceae Phookamsak & K.D. Hyde, *fam. nov.*

Index Fungorum number: IF551650, Facesoffungi number: FoF 01233

Saprobic on bamboo and palms. **Sexual morph**: *Ascostromata* scattered to clustered, solitary to gregarious, immersed beneath host epidermis, erumpent through host surface by papilla, slightly conical to lenticular or hemisphaerical, uni- to bi-loculate, glabrous, coriaceous, ostiolate, papillate, or with short to long neck. *Peridium* composed of small, dark brown to black, pseudoparenchymatous cells, comprising host cells plus fungal tissue, internally arranged in a *textura angularis* to *textura prismatica*, poorly developed at the base. *Hamathecium* trabeculate pseudoparaphyses. *Asci* 8-spored, bitunicate, clavate to cylindric-clavate, pedicellate, apically rounded, with ocular chamber. *Ascospores* brown to reddish brown, fusiform to clavate, septate, constricted at the septum, rough-walled, with or without striations, or with longitudinal ridges towards the ends, some with a mucilaginous sheath surrounding the ascospores. **Asexual morph**: Coelomycetous.

Type genus: *Pseudoastrosphaeriella* Phookamsak, Z.L. Luo & K.D. Hyde

Notes: The family *Pseudoastrosphaeriellaceae* is introduced to accommodate a monotypic genus, *Pseudoastrosphaeriella* in the order *Pleosporales*, Dothideomycetes. The genus is not congeneric with *Astrosphaeriella* and characterized by hemispherical to lenticular ascostromata, immersed beneath host epidermis, erumpent through host surface by a papilla, with short to long necks, trabeculate pseudoparaphyses, brown to reddish brown, fusiform to obclavate ascospores, some with striations or longitudinal ridges and forming coelomycetous asexual morphs. Species of *Pseudoastrosphaeriella* were previously accommodated in the genus *Astrosphaeriella*. Hawksworth and Boise (1985) were the first who accommodated the species having coriaceous ascomata with papilla or necks and striate ascospores in *Astrosphaeriella*. Subsequently,

the species having similar morphological characters were added to this genus (Hyde 1992; Aptroot 1995a; Hyde and Fröhlich 1998; Chen and Hsieh 2004; Hu 2010). Liu et al. (2011) studied a putative strain represented by *A. africana* and mentioned that *A. africana* might need to be excluded from the genus *Astrosphaeriella* based on its phylogeny. *Pseudoastrosphaeriellaceae* forms a sister clade with *Tetraplosphaeriaceae*, but the relationships between these two families are not significantly resolved in the phylogenetic analyses.

Pseudoastrosphaeriella Phookamsak, Z.L. Luo & K.D. Hyde, *gen. nov.*

Index Fungorum number: IF551641, Facesoffungi number: FoF 01234

Etymology: Pseudo- (Gr., false), the generic epithet "*Pseudoastrosphaeriella*" refers to the similarity to *Astrosphaeriella*.

Saprobic on bamboo and palms, visible as dark, raised structures on host surface, often with necks. **Sexual morph**: *Ascostromata* dark brown, scattered, rarely clustered, solitary to gregarious, immersed beneath host epidermis, erumpent through host surface by papilla, slightly conical to lenticular or hemisphaerical, with flattened base, uni-loculate, rarely bi-loculate, glabrous, coriaceous, ostiolate. *Ostioles* central, cylindrical, straight to obique, internally periphysate, brittle, carbonaceous, papillate or with short to long neck. *Peridium* thick-walled, of unequal thickness, composed of small, dark brown to black, pseudoparenchymatous cells, outer layers comprising host cells and fungal tissue, internally arranged in a *textura angularis* to *textura prismatica*, poorly developed at the base. *Hamathecium* composed of dense, filiform, anastomosing, trabeculate pseudoparaphyses, embedded in a hyaline gelatinous matrix. *Asci* 8-spored, bitunicate, clavate to cylindric-clavate, short to long pedicellate, apically rounded, with ocular chamber. *Ascospores* overlapping uni- to tri-seriate, brown to reddish brown, fusiform to clavate, septate, constricted at the septum, rough-walled, with or without striations, or with longitudinal ridges towards the ends, some with a mucilaginous sheath surrounding the ascospores. **Asexual morph**: Coelomycetous. *Conidiomata* pycnidial, scattered, solitary, immersed, laterally superficial, conical or hemisphaerical to globose, ostiolate. *Pycnidial walls* thick-walled, of unequal thickness, composed of dark brown to black, pseudoparenchymatous cells, arranged in a *textura angularis* to *textura intricata. Conidiophores* arising from the basal cavity, cylindrical, unbranched or branched, reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, phialidic, discrete, hyaline, cylindrical or cylindric-clavate or ampulliform, septate, smooth-walled. *Conidia* hyaline, globose to subglobose, or oblong, aseptate, smooth-walled.

Notes: *Pseudoastrosphaeriella* is introduced to accommodate astrosphaeriella-like species and are placed in the family *Pseudoastrosphaeriellaceae*. The main characters that distinguish *Astrosphaeriella* and *Pseudoastrosphaeriella* are the ascostromata. In *Astrosphaeriella*, the ascostromata are usually erumpent through the host epidermis, raised conical, and have a star-like or rounded flange and small papilla, and the peridium comprises an opaque, black, amorphous, brittle layer. In *Pseudoastrosphaeriella*, the ascostromata remain immersed beneath the host epidermis, are hemisphaerical or dome-shaped with short to long necks and the peridium comprises small, dark brown to black, pseudoparenchymatous cells, comprising host cells and fungal tissue at the outside, internally arranged in a *textura angularis* to *textura prismatica*.

Based on phylogenetic evidence and morphology, we accept *Pseudoastrosphaeriella africana*, *P. bambusae*, *P. longicolla* and *P. thailandensis* in *Pseudoastrosphaeriella. Pseudoastrosphaeriella aequatoriensis* and *P. papillata* are also accommodated in the genus based on them being morphologically similar. *Astrosphaeriella macrospora*, *A. papuana* and *A. striataspora* may also need transferring to *Pseudoastrosphaeriella*, but should be recollected.

Type species: *Pseudoastrosphaeriella thailandensis* Phookamsak & K.D. Hyde

Key to species of the genus *Pseudoastrosphaeriella*

1. Ascostromata with long neck 2
1. Ascostromata with short neck 3
2. Ascospores with striations or longitudinal ridges, appendages absent *P. longicolla*
2. Ascospores rough-walled, or minutely striate, with small appendages at both ends *P. papillata*
3. Ascospores (2–)5-septate, surrounded by evanescent mucilaginous sheath *P. aequatoriensis*
3. Ascospores with 1(–3)-septate, mucilaginous sheath present or absent 4
4. Ascospores with minute striation *P. bambusae*
4. Ascospores with wide, longitudinal ridges 6
5. Ascospores (39–)40–48(–50)×5–7 μm, fusiform with acute ends, lacking mucilaginous sheaths *P. africana*
5. Ascospores 35–45(–49)×5–6(–7) μm, fusiform with knob-like at point ends, with mucilaginous sheath surrounding ascospores *P. thailandensis*

Pseudoastrosphaeriella aequatoriensis (K.D. Hyde & J. Fröhl.) Phookamsak & K.D. Hyde, *com. nov.*

≡ *Astrosphaeriella aequatoriensis* K.D. Hyde & J. Fröhl., Sydowia 50(1): 86 (1998)

Index Fungorum number: IF551642

Description details see Hyde and Fröhlich (1998)

Notes: Hyde and Fröhlich (1998) introduced *Astrosphaeriella aequatoriensis* to represent species having lenticular to conical ascostromata with necks and brown, fusiform ascospores with longitudinal ridges. *Astrosphaeriella*

aequatoriensis is more similar to the species in *Pseudoastrosphaeriella* in having coriaceous ascostromata with short to long papilla, and striate ascospores. Therefore, we transfer *A. aequatoriensis* to *Pseudoastrosphaeriella*.

Pseudoastrosphaeriella aequatoriensis differs from other species in having ascospores with 2(–5) septa, wide longitudinal ridges and a thin, evanescent mucilaginous sheath. *Pseudoastrosphaeriella aequatoriensis* has been reported only on *Phytelephas* and other palms from Ecuador (Hyde and Fröhlich 1998).

Pseudoastrosphaeriella africana (D. Hawksw.) Phookamsak & K.D. Hyde, *com. nov.*

≡ *Astrosphaeriella africana* D. Hawksw., in Hawksworth & Boise, Sydowia 38: 116 (1986) [1985]

Index Fungorum number: IF551643, Facesoffungi number: FoF 01235, Fig. 22

Saprobic on submerged bamboo, visible as raised, brown to dark brown, hemispherical regions with short papilla on the host surface. **Sexual morph**: *Ascostromata* 200–330 μm high, 520–750 μm diam., dark brown, scattered, solitary, immersed beneath host epidermis, erumpent through host surface by papilla, hemisphaerical to lenticular, with flattened base, wedge-shaped at the rim, uni-loculate, glabrous, coriaceous, ostiole central, with short papilla, internally periphysate, brittle, carbonaceous. *Peridium* 25–110 μm wide, of unequal thickness, thicker at the sides, especially towards the apex, composed of dark brown to black, pseudoparenchymatous cells, comprising host cells plus fungal tissue at outside, arranged vertically in a *textura angularis* to *textura prismatica*, poorly developed at the base, (20–30 μm wide). *Hamathecium* composed of dense, 0.8–1.8 μm wide, filiform, trabeculate pseudoparaphyses, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* (104–)110–130(–135)×(12–)13–15(–17) μm ($\bar{x}$ =122.4×14.4 μm, n=30), 8-spored, bitunicate, clavate to cylindric-clavate, with slightly long furcate or knob-like pedicel, apically rounded, with an indistinct ocular chamber. *Ascospores* (39–)40–48(–50)×5–7 μm ($\bar{x}$ = 44.8×6.1 μm, n=30), overlapping uni-seriate at the base, bi- to tri-seriate at the apex, initially hyaline to pale brown, becoming brown to dark brown at maturity, fusiform, with acute ends, 1(–3)-septate, constricted at the septum, swollen immediately above central septum, lower cell longer than upper cell, rough-walled with distinct longitudinal ridges towards the ends. **Asexual morph**: Undetermined.

Culture characteristic: Colonies on PDA reaching 19–20 mm diam. after 4 weeks at 25–30 °C, colonies irregular, medium dense, raised, umbonate, surface dull and smooth, slightly rough with well-defined, undulate edge, floccose to velvety, slightly radiating; colonies from above: blackish at the margin, dull green at the middle, and white grey at the centre; from below: dark brown to black; producing orange brown pigmentation in agar.

Material examined: THAILAND, Chiang Rai Province, Mae Jun District, Huai Kang Pla Waterfall, on dead stems of bamboo, 25 October 2010, R. Phookamsak, RP0092 (MFLU 11-0212, **reference specimen designated here**), living culture, MFLUCC 11-0176, BCC.

Notes: *Pseudoastrosphaeriella africana* was introduced as *Astrosphaeriella africana* in Hawksworth and Boise (1985). The species is characterized by coriaceous, hemisphaerical ascostromata, with short necks and fusiform, reddish brown ascospores, with longitudinal ridges (Hawksworth and Boise 1985; Hyde and Fröhlich 1998; Chen and Hsieh 2004; Liu et al. 2011). Hawksworth and Boise (1985) and Liu et al. (2011) mentioned that *A. striaspora* was similar in its striate, but shorter ascospores. Chen and Hsieh (2004) introduced *A. macrospora* and *A. pallidipolaris* also with striate ascospores. *Astrosphaeriella macrospora* differs from *A. africana* in having significantly larger ascospores with five septa, whereas in *A. pallidipolaris* ascospores are 5(–6)-septate with paler end cells (Chen and Hsieh 2004; Liu et al. 2011).

Pseudoastrosphaeriella africana is most similar to *P. bambusae*, *P. thailandensis* and *Astrosphaeriella macrospora* in having reddish brown to dark brown ascospores with 1(–3)-septate, with longitudinal ridges and enlarged above the central septum. However, *P. africana* differs from *P. thailandensis* and *P. bambusae* and the differences are discussed under those species (see Supplementary Table 3).

Liu et al. (2011) showed that *Astrosphaeriella* was polyphyletic which *A. africana* (with striate ascospores) forming a distinct clade from *Astrosphaeriella* sensu stricto at the base of the order *Pleosporales*. Therefore, Liu et al. (2011) concluded that *A. africana* and *A. bakeriana* may need to be excluded from *Astrosphaeriella* and a new genus should be designated to accommodate these taxa when further supporting data are available. Liu et al. (2011) used strain MFLUCC 10-0553 to represent *A. africana*. However, this collection has much smaller ascospores when compared with the original description (Hawksworth and Boise 1985). Therefore, in the present study we designate collection MFLU 11-0212 (culture = MFLUCC 11-0176) as a reference specimen to represent *P. africana* (≡ *A. africana*) as the morphology is typical. The reference strains of *P. africana* forms a distinct clade (60 % ML, 0.96 PP), closely related to *P. bambusae* in the phylogenetic analyses (Fig. 3).

Pseudoastrosphaeriella africana was first reported on *Poaceae* (possibly *Phragmites australis* (Cav.) Trin. ex Steud.) from Tanzania, Africa (Hawksworth and Boise

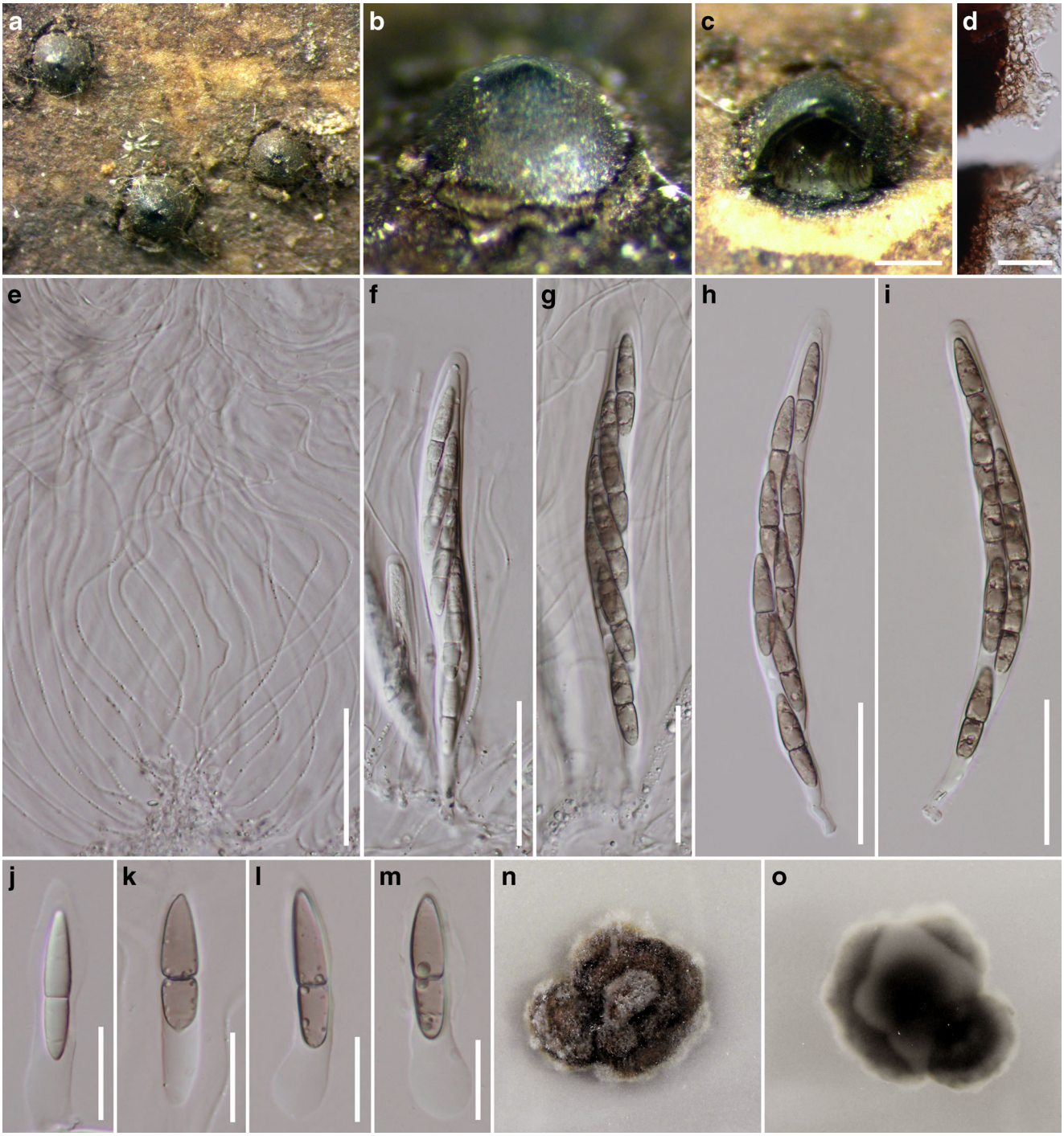

Fig. 21 *Pteridiospora chiangraiensis* (MFLU 11-0198, holotype). **a**, **b** Ascostromata visible as cone-like structures on the host surface. **c** Section through ascostroma. **d** Peridium structure arranged in *textura angularis*. **e** Pseudoparaphyses. **f** Young ascus. **g**–**i** Asci. **j** Young ascospore. **k**–**m** Mature ascospores. **n**, **o** Cultures on SDA (n=from above, o=from below). Scale bars: c=200 μm, d–h=50 μm, d, i–l=20 μm

1985; Liu et al. 2011). Later, Hyde and Fröhlich (1998) reported the species on various hosts: *Arenga*, bamboo, *Calamus*, *Daemonorops*, *Phragmites* and palms from tropical parts of Australia, Brunei, Malaysia, Philippines and Tanzania, while Chen and Hsieh (2004) found the species on bamboo in Taiwan. Liu

Fig. 22 *Pseudoastrosphaeriella africana* (MFLU 11-0212, reference specimen). **a** Ascostromata immersed in host. **b** Section through ascostroma. **c** Section through upper part of peridium **d** Section through side part of peridium. **e** Asci embedded in trabeculate pseudoparaphyses stained in Indian ink. **f** Young ascus. **g**–**i** Asci. **j** Young ascospore. **k**–**o** Ascospores. Scale bars: b=200 μm, c–e=50 μm, f–i=20 μm, j–o=10 μm


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et al. (2011) also reported *P. africana* on bamboo in Thailand. These reports need to be verified against the reference specimens.

Pseudoastrosphaeriella bambusae Phookamsak & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF551644, Facesoffungi number: FoF 01236, Fig. 23

Etymology: The epithet "*bambusae*" refers to the host, of which the species was collected

Holotype: MFLU 11-0155

Saprobic on submerged bamboo, visible as numerous, raised, black, knob-like structures with short papilla on the host surface. **Sexual morph**: *Ascostromata* 180–330 μm high, 400–890 μm diam., dark brown to black, scattered, rarely clustered, solitary to gregarious, immersed in host cortex, becoming raised, erumpent through host tissue by papilla, hemisphaerical to lenticular, or depressed conical, with flattened base, uni-loculate, rarely bi-loculate, glabrous, coriaceous, ostiolate, with short papilla. *Papilla* 170–250 μm high, 120–180 μm wide, black, central, cylindrical, straight, internally periphysate, brittle, carbonaceous, arranged vertically in a *textura prismatica. Peridium* 15–65 μm wide, of unequal thickness, thick at the sides, especially towards the apex, composed of dark brown to black, pseudoparenchymatous cells, comprising host cells plus fungal tissue at outside, internally arranged in a *textura angularis*, poorly developed at the base (15–40 μm wide), with palisade-like cells at the rim. *Hamathecium* composed of dense, 1–1.8 μm wide, filiform, trabeculate pseudoparaphyses, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* (102–)105–130(–136)×(14.5–)15–18(–19.5) μm ($\bar{x}$ = 119.7×16.1 μm, n=30), 8-spored, bitunicate, cylindric-clavate to clavate, with furcate or foot-like pedicel, apically rounded, with well-developed ocular chamber. *Ascospores* (37–)(39–)41–44(–48)×5–7 μm ($\bar{x}$ =42.5×6.5 μm, n=30), overlapping uni-seriate at the base, bi- to tri-seriate at the apex, slightly reddish brown, fusiform, with acute or rounded ends, 1(–3)-septate, slightly constricted at the central septum, swollen above central septum, rough-walled with minute striations, occasionally upper cell shorter than lower cell, surrounded by mucilaginous sheath. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on MEA slow growing, reaching 8–10 mm diam. after 3 weeks at 25–30 °C, colonies circular, medium dense, flat, slightly raised to umbonate, surface dull and smooth with edge entire, velvety, slightly radiating with black concentic ring separated the margin from the centre; colony from above: dull, dark green; from below: black; not producing pigmentation in agar.

Fig. 23 *Pseudoastrosphaeriella bambusae* (MFLU 11-0155, holotype). **a**, **b** Appearance of ascostromata on host surface. **c** Section through ascostroma. **d**, **e** Section through peridium. **f** Papilla with internal periphysate. **g** Trabeculate pseudoparaphyses. **h** Asci embedded in trabeculate pseudoparaphyses. **i**–**k** Asci. **l**–**o** Ascospores. **p** Ascospore stained in Indian ink. **q**, **r** Culture characters (q=from above, r=from below). Scale bars: c=200 μm, d–k=20 μm, l–p=10 μm

Material Examined: THAILAND, Chiang Rai Province, Mae Jun District, Huai Kang Pla Waterfall, on dead branches of submerged bamboo, 25 October 2010, R. Phookamsak, RP0034 (MFLU 11-0155, **holotype**), ex-type living culture, MFLUCC 11-0205, BCC.

Notes: *Pseudoastrosphaeriella bambusae* is most similar to *P. africana* and *P. thailandensis*. However, *P. bambusae* differs from *P. africana and P. thailandensis* in having reddish brown ascospores, with inconspicuous striations, while *P. africana* and *P. thailandicum* having slightly dark brown ascospores, with wide, distinct longitudinal ridges (see Supplementary Table 3). *Pseudoastrosphaeriella bambusae* forms a robust clade (75 % MP, 91%ML, 1.00 PP), closely related to *P. thailandensis* in the phylogenetic analyses (Fig. 3).

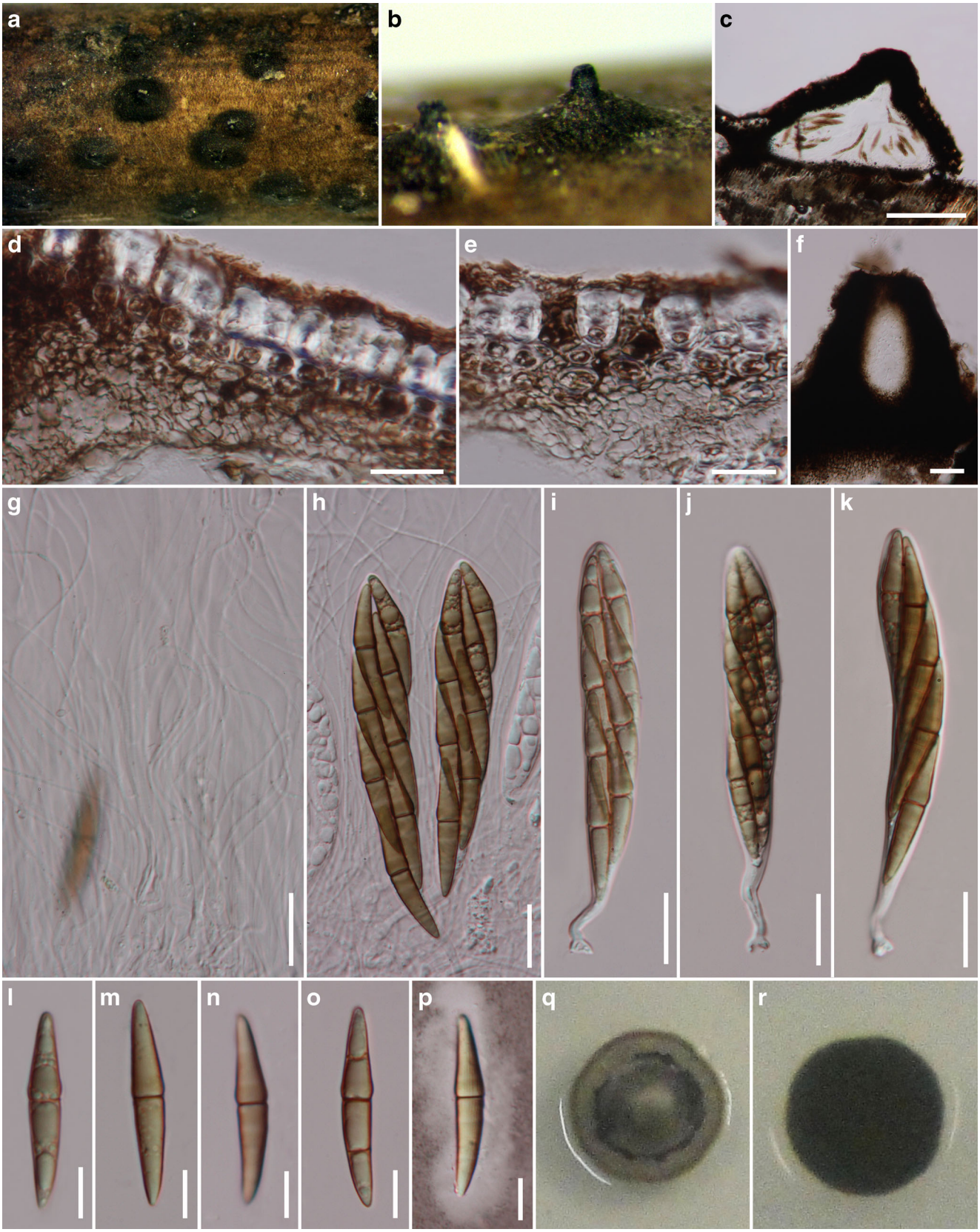
Pseudoastrosphaeriella longicolla Phookamsak & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF551645, Facesoffungi number: FoF 01237, Fig. 24

Etymology: The epithet "*longicolla*" refers to ascostromata with long neck.

Holotype: MFLU 11-0207

Saprobic on bamboo, visible as numerous, black domes, with spike-like structures on host surface, covered by dense, brown vegetative hyphae. **Sexual morph**: *Ascomata* 350–570 μm high (excluding neck), 900–1200 μm diam., scattered, gregarious, immersed in host epidermis, becoming raised, erumpent through host tissue by papilla, depressed conical or lageniform, flattened at the base, uni-loculate, glabrous, coriaceous, ostiolate. *Necks* 1.4–2.5 mm high, 110–175 μm diam., black, central, cylindrical, oblique, internally periphysate, brittle, carbonaceous. *Peridium* 10–60 μm wide, of unequal thickness, thick at the sides towards the apex, composed of small, dark brown to black, pseudoparenchymatous cells, outer layers comprising host cells plus fungal tissue, arranged vertically in a *textura angularis*, poorly developed at the base, with palisade-like cells at rim. *Hamathecium* composed of dense, filiform, 0.7–2 μm wide, trabeculate pseudoparaphyses, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* (140–)160–180(–200)(–240)×(10–)12–15(–17) μm ($\bar{x}$ =179.3×13.7 μm, n=30), 8-spored, bitunicate, cylindric-clavate, with a furcate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pedicel, apically rounded with well-developed ocular chamber. *Ascospores* (48–)50–60(–63.5)×5.5–7(–7.5) μm ($\bar{x}$ =55×6.3 μm, n=30), overlapping uni- to bi-seriate, pale yellowish when young, becoming reddish brown to dark brown at maturity, fusiform, with acute ends, 1-septate, constricted at the septum, slightly swollen above central septum, rough-wall, with longitudinal ridges. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on MEA fast growing, reaching 50–55 mm diam. after 4 weeks at 25–30 °C, colony circular, dense, raised, umbonate to convex, surface smooth and dull, with edge entire, floccose to fluffy, smooth to slighly curved; colony from above: dark brown to black; from below: black; producing brown pigmentation in agar.

Material examined: THAILAND, Chiang Mai Province, Chom Tong District, Doi Inthanon, on dead branch of submerged bamboo, 16 November 2010, R. Phookamsak, RP0087 (MFLU 11-0207, **holotype**), ex-type living culture, MFLUCC 11-0171, BCC.

Notes: *Pseudoastrosphaeriella longicolla* is similar *P. papillata* by having coriaceous ascostromata with long necks and was found on submerged bamboo. However, *P. longicolla* differs from *P. papillata* due to its ascospores. *Pseudoastrosphaeriella longicolla* has larger ascospores, with reddish brown, narrowly fusiform and distinct longitudinal ridges. Whereas, *P. papillata* has smaller, broadly fusiform to clavate, rough-walled ascospores, with small appendages at the both ends. Hu (2010) described a species *Astrosphaeriella francensis* in her study, but it was not validly published. *Pseudoastrosphaeriella longicolla* is also similar to this species in having coriaceous ascostromata, with long necks and narrow fusiform, striate ascospores. However, *Pseudoastrosphaeriella* differs from *Astrosphaeriella francensis* due to its ascospore septation which are often only 1-septate in *P. longicolla*. Whereas, *A. francensis* having ascospores with 1–3(–5) septa (see Supplementary Table 3).

Based on phylogenetic analyses (Fig. 3), *Pseudoastrosphaeriella longicolla* forms a distinct clade at the base of *Pseudoastrosphaeriellaceae*, close to *P. africana*.

Pseudoastrosphaeriella papillata (K.D. Hyde & J. Fröhl.) Phookamsak & K.D. Hyde, *com. nov.*

≡ *Astrosphaeriella papillata* K.D. Hyde & J. Fröhl., Sydowia 50(1): 109 (1998)

Index Fungorum number: IF551646, Facesoffungi number: FoF 01238, Fig. 25

Saprobic on submerged bamboo, visible as numerous, raised, black, dome-shaped, with spike-like structures in dark area on host surface. **Sexual morph**: *Ascostromata* 300–430 μm high (excluding neck), 550–820 μm diam., black, scattered, solitary to gregarious, immersed in host cortex, becoming raised, erumpent through host tissue by papilla, hemisphaerical or depressed conical, flattened at the base, uni-loculate, glabrous, coriaceous, ostiolate. *Neck* 490–800 μm high, 120–150 μm diam., central, cylindrical, oblique to curved, single, rarely furcate at the apex, brittle, carbonaceous. *Peridium* 10–130 μm wide, of unequal thickness, thick at the sides towards the apex, composed of small, hyaline to dark brown or black, pseudoparenchymatous cells, outer layers comprising host cells plus fungal tissue, arranged vertically in a *textura angularis* to *textura prismatica*, poorly developed at the base, with palisade-like cells at the rim. *Hamathecium* composed of dense, 0.7–1.5 μm wide, filiform, trabeculate pseudoparaphyses, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* (130–)135–165(–172)×(15–)17–20(–22) μm ($\bar{x}$ =152.6×18.2 μm, n=25), 8-spored, bitunicate, fissitunicate, cylindric-clavate, with slightly furcate, rounded pedicel, apically rounded, with indistinct ocular chamber. *Ascospores* (29.5–)30–40(–45)×6–8(–9.5) μm ($\bar{x}$ =38.4×8 μm, n=30) overlapping uni- to bi-seriate, yellowish brown to grey brown, broadly fusiform to clavate, with drawn out, narrow, often curved, rounded ends, initially 1-septate, rarely 2–3-septate, constricted at the central septum, slightly swollen above the central septum, walls rough, with small, pad-like, mucilaginous appendages at both ends. **Asexual morph**: Undetermined.

Material examined: BRUNEI, Temburong, Batu Apio Forest Reserve, Sungai Belalong, Kuala k. Field Studies Centre, Sungai Esu, on dead submerged bamboo, 1 February 1994, K.D. Hyde, (IFRD 8704, **holotype**).

Notes: *Pseudoastrosphaeriella papillata* was described by Hyde and Fröhlich (1998) from dead, submerged bamboo from a small stream in Brunei. *Pseudoastrosphaeriella papillata* may be a common species on submerged bamboo and wood in tropical regions (Luo et al. 2004; Pinruan et al. 2007) with 19.5 % percentage occurrence on submerged bamboo in the Liput River in Philippines (Cai et al. 2003). The collections however, need verifying against the reference specimen of *P. papillata*.

In this study, the type specimen of *Astrosphaeriella papillata* is examined and found that *A. papillata* has morphological characters related to *Pseudoastrosphaeriella*. Thus, we transfer *Astrosphaeriella papillata* to *Pseudoastrosphaeriella*.

Fig. 24 *Pseudoastrosphaeriella longicolla* (MFLU11-0207, holotype). **a** Appearance of ascostromata and necks on host surface. **b**, **c** Section through ascostroma. **d** Section through peridium. **e** Asci embedded in pseudoparaphyses. **f** Young ascus. **g**–**j** Asci. **k**–**p** Ascospores. Scale bars: c=200 μm, d–j=50 μm, k–p=20 μm


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Pseudoastrosphaeriella papillata is similar to *P. longicolla* and the differences are discussed under that species (see Supplementary Table 3).

Pseudoastrosphaeriella thailandensis Phookamsak, Z.L. Luo & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF551647, Facesoffungi number: FoF 01239, Fig. 26

Etymology: The epithet "*thailandensis*" refers to the country from which the fungus was collected.

Holotype: MFLU 11-0145

Saprobic on bamboo, visible as raised, brown to dark brown, hemisphaerical regions, with short necks on host surface. **Sexual morph**: *Ascostromata* 170–340 μm high, 700–940 μm diam., dark brown, scattered, rarely clustered, gregarious, immersed in host cortex, becoming raised, erumpent through host surface by short papilla, depressed conical or hemisphaerical, with flattened base, uni-loculate, rarely bi-loculate, glabrous, coriaceous, ostiolate. *Neck* 165–225 μm high, 110–130 μm diam., central, cylindrical, straight, internally periphysate, brittle, carbonaceous. *Peridium* 55–65 μm wide, of unequal thickness, thick at the sides, especially towards the apex, composed of small, dark brown to black, pseudoparenchymatous cells, outer layers comprising host cells plus fungal tissue, internally arranged vertically in a *textura angularis* to *textura prismatica*, poorly developed at the base, with palisade-like cells at the rim. *Hamathecium* composed of dense, 1–1.5 μm wide, filiform, anastomosing, trabeculate pseudoparaphyses, embedded in a hyaline gelatinous matrix. *Asci* (102–)110–120(–125)×(12–)13–15(–17.5) μm ($\bar{x}$ =116.4×14.2 μm, n=20), 8-spored, bitunicate, cylindric-clavate, with furcate or foot-like pedicel, apically rounded, with well-developed ocular chamber. *Ascospores* 35–45(–49)×5–6(–7) μm ($\bar{x}$ =41.1×6.2 μm, n=25), overlapping uniseriate at the base, bi- to tri-seriate at the apex, brown to reddish brown, fusiform, acute or occasionally swollen at ends, 1(–3)-septate, constricted at the central septum, wall rough with striations, surrounded by a mucilaginous sheath. **Asexual morph**: produced on bamboo pieces on WA after 8 weeks. *Conidiomata* 150–340 μm high, 120–470 μm diam., pycnidial, scattered, solitary, immersed, laterally superficial, visible as black dots covered by brown, sparse hyphae, conical or dome-shaped to globose, standing on tufts of hyphae, indistinctly ostiolate. *Pycnidial walls* 25–75 μm wide, of unequal thickness, slightly thick at sides, composed of two cell layers of dark brown to black, pseudoparenchymatous cells, inner layers comprising dark brown to black cells, arranged in a *textura angularis*, outer layers comprising several layers, of *textura intricata*.

Fig. 25 *Pseudoastrosphaeriella papillata* (IFRD 8704, holotype of *Astrosphaeriella papillata*). **a** Herbarium label and specimens. **b** Appearance of ascostromata on host surface. **c** Section of ascostroma. **d** Section of peridium. **e** Pseudoparaphyses. **f–i** Asci. **j–m** Ascospores. Scale bars: c=200 μm, d–e=50 μm, f–h=20 μm, j–m=10 μm

Conidiophores arising from the basal cavity, cylindrical, unbranched or branched, reduced to conidiogenous cells. *Conidiogenous cells* (3–)4–7(–9)(–11)×1.5–2 (–3) μm ($\bar{x}$ =6.7×2 μm, n=30), holoblastic, phialidic, integrated to discrete, hyaline, cylindrical or cylindric-clavate or ampulliform, 1–2-septate, smooth-walled. *Conidia* 2–3×1.5–2 μm ($\bar{x}$ =2.7×2 μm, n=50), hyaline, globose to subglobose, or oblong, aseptate, with a single guttule, smooth-walled.

Culture characteristics: Colonies on MEA slow growing, reaching 25–28 mm diam. after 4 weeks at 25–30 °C, irregular, dense, raised, umbonate to convex, surface dull and rough, edge undulate, with entire margin, floccose to fluffy, wrinkled, slightly radiating, margin separating from the center by black concentric rings; colonies from above dark grey to black; from below black; producing brown pigmentation in agar.

Material examined: THAILAND, Chiang Rai Province, Muang District, Huai Mae Sai Waterfall, on dead stem of bamboo, 10 March 2010, R. Phookamsak, RP0024 (MFLU 11-0145, **holotype**), living cultures, MFLUCC 10-0553, BCC; *ibid*., Khun Korn Waterfall, on dead branchs of bamboo, 10 September 2010, R. Phookamsak, RP0060 (MFLU 11-0180, **paratype**), living cultures, MFLUCC 11-0144, BCC; N 20°3′39″, E 99°52′29″, 700 m, saprobic on decaying stem of bamboo submerged in stream, November 2013, Z.L. Luo, Zl-12 (MFLU 15–0083, KUN HKAS 86458) living cultures, MFLUCC 14-0038.

Notes: *Pseudoastrosphaeriella thailandensis* is most similar to *P. africana* and *P. bambusae*. It differs in the size and shape of the ascospores and being lighter at the ends. Phylogenetically *P. thailandensis* forms a well-resolved clade (100 % MP, 100 % ML, 1.00 PP), in the family *Pseudoastrosphaeriellaceae* (Fig. 3).

Pseudoastrosphaeriella thailandensis produced a coelomycetous asexual morph on bamboo pieces on WA. The micro-conidia are characterized by globose to subglobose, or obovoid, hyaline and aseptate conidia, which is similar to *Astrosphaeriellaceae*. However, they differ as conidiomata in *Pseudoastrosphaeriella* are initially immersed, and then erumpent through host surface and eventually become superficial. Whereas, in *Astrosphaeriella* conidiomata are superficial on the host surface.


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· IFRD 8704 holotype
· Astrosphaeriella papillata
· Submerged bamboo
· Brunei. Temburong Batu Apio Forest Reserve, Sungai Belalong. Kuala Field Studies Centre, Sungai Esu.
· 1994-2-1
业科学研究院资源昆虫研究所
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Other genus excluded from *Astrosphaeriellaceae*

Astrosphaeriellopsis Phookamsak, J.K. Liu & K.D. Hyde, *gen. nov.*

Index Fungorum number: IF551648, Facesoffungi number: FoF 01240

Etymology: -opsis (Gr., resembling), the generic epithet "*Astrosphaeriellopsis*" refers to the resemblance to *Astrosphaeriella*.

Saprobic on palms, visible as numerous, flattened, black spots on host surface. **Sexual morph**: *Ascostromata* scattered or gregarious, rarely clustered at the base, immersed beneath host epidermis, becoming raised, hemisphaerical, flattened at the base, uniloculate, glabrous, black, carbonaceous, ostiolate. *Ostioles* central, with minute papilla. *Peridium* thick-walled, of unequal thickness, poorly developed at the base, composed of dark brown to black cells. *Hamathecium* composed of dense, filiform, anastomosing, trabeculate pseudoparaphyses, embedded in a hyaline gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, cylindric-clavate, short- to long-pedicellate, apically rounded, with ocular chamber. *Ascospores* overlapping uni- to tri-seriate, hyaline, becoming brown at maturity, fusiform, septate, constricted at the septum, smooth-walled, with an inconspicuous striations and mucilaginous sheath. **Asexual morph**: Undetermined.

Notes: *Astrosphaeriellopsis bakeriana*, the type species of the genus *Astrosphaeriellopsis* was described by Saccardo (1918) as *Winterina bakeriana* Sacc. which was collected on *Livistona chinensis* in Singapore. Hyde and Fröhlich (1998) examined the type specimen of *Winterina bakeriana* and compared its morphological characters with their collections from Hong Kong and Papua New Guinea. Therefore, Hyde and Fröhlich (1998) introduced a new combination *Astrosphaeriella bakeriana* (Sacc.) K.D. Hyde & J. Fröhl. following morphological examination. Additionally, they also synonymized *A. aosimensis* under the name *A. bakeriana*. Liu et al. (2011) collected *A. bakeriana* from *Borassus* in Thailand and obtained molecular data from this strain. Based on their phylogenetic analyses, *A. bakeriana* formed a distinct clade separating from *Astrosphaeriella* sensu stricto and close to the family *Delitschiaceae*.

In this study, *Astrosphaeriella bakeriana* forms a single clade at the base of the family *Aigialaceae*, separating from *Astrosphaeriellaceae* which is concurs with Liu et al. (2011). Therefore, we exclude *A. bakeriana* from the genus *Astrosphaeriella* and designate a new genus, *Astrosphaeriellopsis* to accommodate this taxon. Based on phylogenetic analyses, *Astrosphaeriellopsis bakeriana* forms a clade which is not closely related with any of the basal Dothideomycete families comprising astrosphaeriella-like taxa. Therefore, we place this genus in the *Pleosporales* genera *incertae sedis* until more extensive taxa populations are included in the phylogenies.

Fig. 26 *Pseudoastrosphaeriella thailandensis* (MFLU11-0145 and MFLU11-0180) **a**, **b** Appearance of ascostromata on host surface (MFLU11-0145). **c** Vertical section through ascostroma (MFLU11-0180). **d** Section of peridium (MFLU11-0180). **e** Germinating ascospore (MFLU11-0145). **f** Pseudoparaphyses (MFLU11-0145). **g**–**i** Asci (MFLU11-0145). **j**–**m** Ascospores (MFLU11-0145). **n** Ascospore stained in Indian ink to show sheath (MFLU11-0145). Asexual morph observed from MFLU11-0145: **o** Conidiomata on bamboo pieces on WA. **p** Section of conidioma. **q** pycnidial walls. **r**–**t** Conidiogenous cells. **u**–**af** Conidia. Scale bars: c=200 μm, p=100 μm, d−n, q=20 μm, r, u=10 μm, s−t, v−af=2 μm

Type species: *Astrosphaeriellopsis bakeriana* (Sacc.) J.K. Liu, Phookamsak & K.D. Hyde

Astrosphaeriellopsis bakeriana (Sacc.) J.K. Liu, Phookamsak & K.D. Hyde, *comb. nov.*

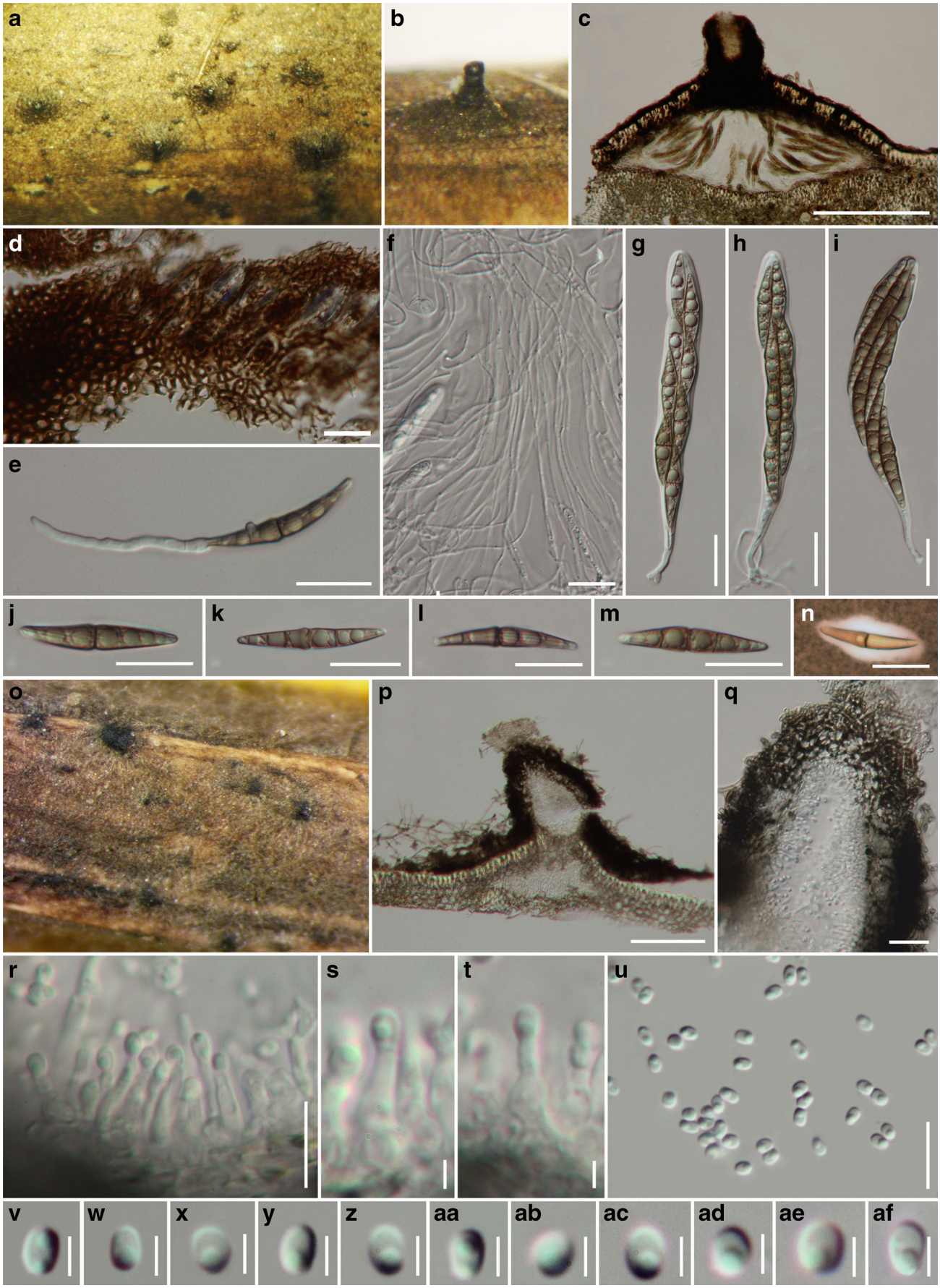
≡ *Winterina bakeriana* Sacc., Bull. Orto Bot. Univ. Napoli 6: 45 (1918)

= *Astrosphaeriella bakeriana* (Sacc.) K.D. Hyde & J. Fröhl., Sydowia 50(1): 93 (1998)

Index Fungorum number: IF551649, Facesoffungi number: FoF 01241, Fig. 27

Saprobic on palms. **Sexual morph**: *Ascostromata* 130–250 μm high, 450–750 μm diam., black, scattered, rarely clustered at the base, gregarious, immersed beneath host epidermis, hemisphaerical, flattened at the base, uniloculate, glabrous, carbonaceous, ostiolate. *Ostioles* central, with minute papilla. *Peridium* 30–60 μm wide, of unequal thickness, poorly developed at the base, thick at the rim towards the apex, composed of dark brown to black cells, arranged in a *textura angularis*. *Hamathecium* composed of dense, 1–1.5 μm wide, filiform, persistent, septate, trabeculate pseudoparaphyses, anastomosing among asci, embedded in a hyaline gelatinous matrix. *Asci* 95−155×10.5−17 μm ($\bar{x}$ =120×13 μm, n=20), 8-spored, bitunicate, fissitunicate, cylindric-clavate, long pedicellate, apically rounded, with an ocular chamber. *Ascospores* 32−40×5−6.5 μm ($\bar{x}$ =36×5.5 μm, n=30), overlapping bi- to tri-seriate, hyaline, becoming brown at maturity, fusiform to clavate, with acute or slightly rounded ends, 1-septate, upper cell slightly shorter and wider than lower cell, constricted at the central septum, rough-walled, with inconspicuous striations and mucilaginous sheath. **Asexual morph**: Undetermined.

Material examined: THAILAND, Krabi Province, Nuea Khlong District, on petiole of *Borassus* sp., 26 September 2010, J.K. Liu, JKA0053 (MFLU 11-1149, **holotype**), ex-type living culture, MFLUCC 11-0027, BCC.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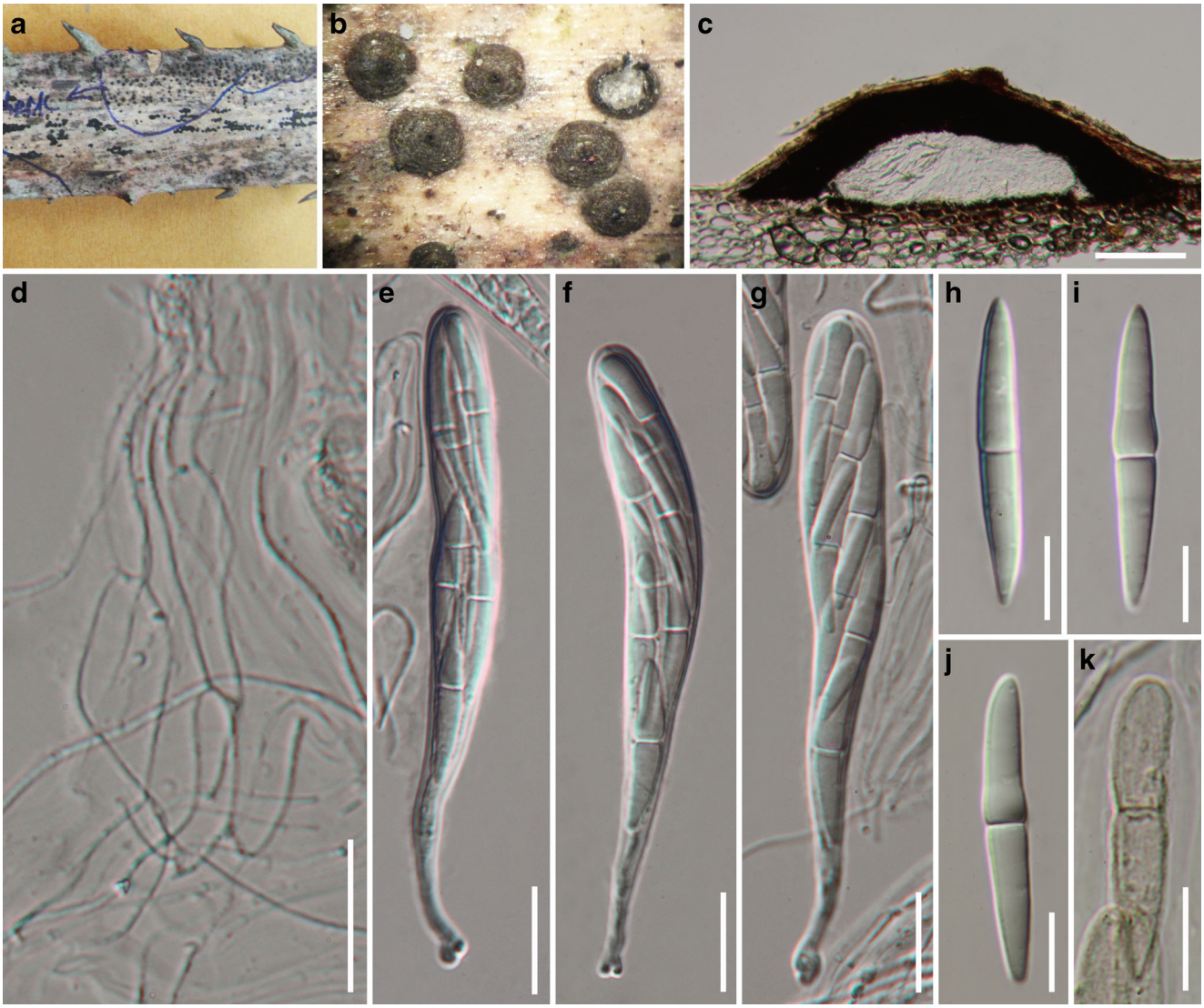

Fig. 27 *Astrosphaeriellopsis bakeriana* (MFLU 11-1149, holotype). **a**, **b** Appearance of ascostromata on host surface. **c** Section through ascostroma. **d** Pseudoparaphyses. **e**–**g** Asci. **h**–**j** Ascospores. **k** Old ascospore. Scale bars: c=100 μm, d–g=20 μm, h–k=10 μm

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