



## Morpho-molecular characterization of microfungi associated with marine based habitats

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### Abstract

Our investigation of saprobic marine fungi in India, Thailand, Sweden and the UK yielded 57 species accommodated in 26 families. In the present study, we describe two new genera, 37 new species and 15 new host records. Novel genera, *Halocryptosphaeria* and *Halotestudina* are introduced within Diatrypaceae (Xylariales) and Testudinaceae (Pleosporales), respectively. The new species, *Amarenographium ammophilicola*, *Asterodiscus mangrovei*, *Boeremia maritima*, *Chaetopsina aurantisalinicola*, *Chloridium salinicola*, *Coniochaeta arenariae*, *C. krabiensis*, *Diaporthe krabiensis*, *D. marina*, *D. salinicola*, *Dictyosporium marinum*, *Dyfronomyces neothailandicus*, *Fusicolla gigantispora*, *Halorosellinia krabiensis*, *H. xylocarpi*, *Halotestudina muriformis*, *Hypoxylon aurantium*, *H. mangrovei*, *Lasiodiplodia krabiensis*, *Nectria marina*, *Nemania phetchaburiensis*, *N. viridis*, *Neocamarosporium artemisiae*, *N. maritimae*, *Neocosmospora rhizophorae*, *Nigrograna samueliana*, *N. rhizophorae*, *Patellaria apiculatae*, *Periconia salina*, *Peroneutypa indica*, *P. polysporae*, *Phaeoseptum carolshearerianum*, *P. manglicola*, *Rhytidhysterium bruguierae*, *Rimaconus multiguttulatus*, *Salsuginea rhizophorae* and *Xenoacremonium brunneosporum* are introduced based on multigene analyses and morphological studies. This study also provides insights into the diversity of fungi from marine based habitats and confirm that they occupy diverse marine niches. We also demonstrate how marine based substrates, including sand dunes, are fascinating substrates for discovering novel taxa. All taxa described herein are based on morphological examination of fresh specimens supported by multigene phylogenies to better integrate taxa into higher taxonomic framework and infer their phylogenetic relationships as well as establish new species.

**Key words** – 37 new taxa – Ascomycetes – Diversity – Dothideomycetes – DNA sequences – Ecology – Saline water – Sordariomycetes

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#### Hysteriales Lindau

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1. *Hysterium rhizophorae* Dayarathne & K.D. Hyde, in Hyde et al., Fungal Diversity 87: 42 (2017)
2. *Hysterium thailandicum* Jayasiri & K.D. Hyde, in Jayasiri, Hyde, Jones, Peršoh, Camporesi & Kang, Mycosphere 9(4): 817 (2018)
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#### Pleosporales Luttrell ex M.E. Barr

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4. *Aigialus grandis* Kohlm. & S. Schatz, Trans. Br. mycol. Soc. 85(4): 699 (1986) [1985]
5. *Aigialus mangrovei* Borse, Trans. Br. mycol. Soc. 88(3): 424 (1987)
6. *Aigialus parvus* S. Schatz & Kohlm., in Kohlmeyer & Schatz, Trans. Br. mycol. Soc. 85(4): 704 (1986) [1985].
7. *Ascocratera manglicola* Kohlm., Can. J. Bot. 64(12): 3036 (1986)
8. *Rimora mangrovei* (Kohlm. & Vittal) Kohlm., Volkm.-Kohlm., Suetrong, Sakay. & E.B.G. Jones, Stud. Mycol. 64: 166 (2009)

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**Dothideomycetes orders incertae sedis**

**Botryosphaeriales** C.L. Schoch et al.

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**Patellariales** D. Hawksw. & O.E. Erikss.

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**Class Sordariomycetes** O.E. Erikss. & Winka

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**Subclass Hypocreomycetidae** O.E. Erikss. & Winka

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36. *Nectria marina* Dayarathne, E.B.G. Jones & K.D. Hyde, sp. nov.

37. *Neocosmospora rhizophorae* Dayarathne, sp. nov.

**Ophiocordycipitaceae** G.H. Sung et al.

38. *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson, FEMS Microbiol. Lett. 321(2): 144 (2011)

**Subclass Sordariomycetidae** O.E. Erikss & Winka

**Boliniales** P.F. Cannon

**Boliniaceae** Rick

39. *Camarops ustulinoides* (Henn.) Nannf., Svensk bot. Tidskr. 66(4): 370 (1972)

**Chaetosphaeriales** Huhndorf et al.

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43. *Coniochaeta krabiensis* Dayarathne & E.B.G Jones, sp. nov.

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**Xylariales** Nannf.

**Diatrypaceae** Nitschke

44. *Diatrype mangrovei* Dayarathne & K.D. Hyde, Cryptog. Mycol. (2019)

45. *Halocryptosphaeria* Dayarathne, Devadatha, Sarma, V.V. & K.D. Hyde, gen nov.

46. *Halocryptosphaeria bathurstensis* (Dayarathne & K.D. Hyde) Dayarathne, V.V. Sarma, Devadatha & K.D. Hyde, comb. nov.

47. *Halocryptovalsa avicenniae* (Abdel-Wahab, Bahkali & E.B.G. Jones) Dayarathne & K.D. Hyde, Cryptog. Mycol. (2019)

48. *Halodiatrype salinicola* Dayarathne & K.D. Hyde, Mycosphere 7(5): 617 (2016)

49. *Peroneutypa scoparia* (Schwein.) Carmarán & A.I. Romero, in Carmarán, Romero & Giussani, Fungal Diversity 23: 84 (2006)

50. *Peroneutypa indica* Devadatha V.V. Sarma & E.B.G Jones, sp. nov.

51. *Peroneutypa polysporae* Devadatha, V.V. Sarma & E.B.G Jones, sp. nov.

**Hypoxylaceae** DC.

52. *Hypoxylon aurantium* Dayarathne, E.B.G. Jones & K.D. Hyde, sp. nov.

53. *Hypoxylon mangrovei* Dayarathne, E.B.G. Jones & K.D. Hyde, sp. nov.

**Xylariaceae** Tul. & C. Tul. (= Clypeosphaeriaceae G. Winter)

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55. *Halorosellinia krabiensis* Dayarathne, & K.D. Hyde, sp. nov.

56. *Halorosellinia xylocarpi* Dayarathne & K.D. Hyde, sp. nov.

57. *Nemania maritima* Y.M. Ju & J.D. Rogers, Nova Hedwigia 74(1-2): 102 (2002)

58. *Nemania viridis* Dayarathne, E.B.G. Jones & K.D. Hyde, sp. nov.

59. *Nemania phetchaburiensis* Dayarathne, E.B.G. Jones & K.D. Hyde, sp. nov.

**Introduction**

Mangrove forests are considered “biodiversity hotspots” for fungi (Shearer et al. 2007). The first fungus from a marine mangrove habitat was described in the middle of the nineteenth century by Durieu & Montagne (1846-1850). However, no further reports of mangrove fungi were published until 1955 when Cribb and Cribb collected fungi on mangrove bark, roots and wood from

Australia (Cribb & Cribb 1955). Extensive investigations on mangrove fungi have been carried out in the Caribbean and Pacific Islands (Kohlmeyer 1984, Kohlmeyer & Kohlmeyer 1993, Jones & Abdel-Wahab 2005) and in South East Asia (Sarma & Hyde 2001). When compared to other parts of the world, currently most mangrove associated fungi are described from South East Asia (Schmit & Shearer 2003). Marine fungi in Asia have been relatively well investigated in Brunei, India, Malaysia, Taiwan, Seychelles & Thailand (Hyde and Jones 1988, Hyde 1993a, 1988, Maria & Sridhar 2003, Prasannarai & Sridhar 2003, Sakayaroj et al. 2004, Alias et al. 2010, Pang et al. 2016).

Jones et al. (2019) listed accepted names and classification of marine fungi, which included 1257 marine species that belonged in 539 genera, majority of which are Ascomycota. To date 943 marine ascomycetes species are known and distributed over 353 genera (Abdel-Wahab et al. 2010, Jones et al. 2009, Pang et al. 2010, Abdel-Wahab & Nagahama 2011, Jones et al. 2015, Dayarathne et al. 2016, [www.marinefungi.org](http://www.marinefungi.org)). The marine Ascomycota are found in eight classes, Dothideomycetes, Eurotiomycetes, Laboulbeniomycetes, Lecanoromycetes, Leotiomyces, Lichinomycetes, Arthoniomycetes and Sordariomycetes, of which the Dothideomycetes and the Sordariomycetes are the two largest groups (Jones & Pang 2012, [www.marinefungi.org](http://www.marinefungi.org)). This study will focus on the latter two subclasses. They are well-adapted for growth in marine habitats, mostly by bearing spores with appendages which facilitate buoyancy in water and their entrapment and adherence to substrates (Jones 1995). Sordariomycetes are characterized predominantly by perithecial ascomata and inoperculate unitunicate asci, often deliquescing at maturity (Jones et al. 2015, Maharachchikumbura et al. 2015, 2016). Marine Sordariomycetes have been classified into several orders based on their morphology and phylogeny including Coronophorales, Chaetosphaeriales, Diaporthales, Hypocreales, Koralionastetales, Lulworthiales, Magnaporthales, Microascales, Ophiostomatales, Phyllachorales, Savoryellales, Sordariales, Tirisporellales, Torpedosporales, and Xylariales (Jones et al. 2015, Maharachchikumbura et al. 2016). Among them, the orders Lulworthiales and Koralionastetales comprise exclusively marine taxa. The majority of marine Dothideomycetes are intertidal, primarily from mangrove habitats and rely on the active discharge of their ascospores (Suetrong et al. 2009). According to the review by Suetrong et al. (2009) marine Dothideomycetes have representatives in the orders Capnodiales, Hysteriales, Jahnulales, Mytilinidiales, Patellariales and Pleosporales.

Marine fungi have been identified from different marine substrata such as wood and leaves of mangrove plants, algae (seaweeds), driftwood, saltmarsh plants (*Spartina*), dead coral and sand grains on beaches (Sutherland 1915a, 1916, Barghoorn & Linder 1944, Jones & Moss 1978, Kohlmeyer 1984, Kohlmeyer & Kohlmeyer 1993, Hyde et al. 1990, Jones 1995, Chatmala et al. 2004, Jones et al. 2006, 2015, Dayarathne et al. 2016), as well as extreme marine environments, such as hydrothermal vents, deep-sea trenches, cold methane seeps, the deep-sea subsurfaces and hypersaline, anoxic and suboxic waters (Raghukumar & Ravindram 2012, Xu et al. 2018). Marine fungi exhibit a distinct geographical distribution, generally being referred to as tropical/subtropical and temperate, while others are known to be cosmopolitan in their distribution e.g. *Aniptodera cheasapeakensis*, *Ceriosporopsis halima*, *Corollospora maritima*, *Lignicola laevis*, *Savoryella lignicola*, *Torpedospora radiata* and *Lulwoana uniseptata* (= as the asexual *Zalerion maritima*) (Hughes 1974) or cold water species (Pugh & Jones 1986). Schmit & Shearer (2004) investigated mangrove fungal diversity and concluded there were fewer mangrove taxa within a single ocean basin than between different ocean basins; although many mangrove-associated species have wide longitudinal distributions (Alias & Jones 2009, Pang et al. 2010). Any piece of wood, which is submerged for a certain period in marine or estuarine waters, can be colonized by marine fungi (Hyde et al. 2000a). A number of biological, chemical and physical factors can influence the diversity of marine fungi, including the availability of substrata for colonization, nature of the substratum, interference competition, water temperature, pH and salinity (Jones 2000). However, water temperature is regarded as the most significant physical factor effecting the occurrence of marine fungi at different geographical locations (Booth & Kenkel 1986, Hughes 1986). Booth & Kenkel (1986) also classified marine fungi into six groups based on temperature and salinity of

habitats, such as warm water homeothermic euryhaline, warm water euryhalothermic, cool water eurythermic homohaline, cool water euryhalothermic, cool water homeothermic euryhaline and eurytolerant species.

### **Taxonomic history of marine ascomycetes**

The first record of a marine fungus appears to date back to 1846, when Durieu de Monzonneuvo and Montagne described the ascomycete *Sphaeria posidoniae* (= *Halotthia posidonniae*) from the seagrass *Posidonia oceanica* (Montagne 1856). Subsequent reports on marine fungi was by the Crouan brothers (Kohlmeyer 1974), who described five marine fungi as early as 1867, and Sutherland (1915a, b, c, 1916) who published a series of papers dealing exclusively with marine fungi on marine algae. Marine mycology research was stimulated by the studies of Barghoorn & Linder (1944), wherein they described 25 species of lignicolous marine fungi. This motivated mycologists all over the world to undertake research on marine fungi. Hence 90 % of all recognized marine fungi have been described in the following five decades. The majority of literature on the higher marine fungi were initial contributions from Wilson (Great Britain), Höhnk (Germany), Meyers (USA), Cribb and Herbert (Australia), Johnson (USA), Feldmann (France), Kohlmeyer (Germany and later USA) and Jones (United Kingdom). Later, many researchers contributed to the study of marine fungi including their taxonomy, phylogeny, ontogeny, physiology, vertical and geographical distribution and ecological groups (Jones & Pang 2012). The first classification of marine fungi by Johnson & Sparrow (1961) and was amended by Kohlmeyer & Kohlmeyer (1979). Jones (1976) published the edited volume entitled “Recent advances in aquatic mycology” and Kohlmeyer published a key to marine species in 1971. Spatafora & Blackwell (1994) published the first sequence of a marine Sordariomycetes (*Halosphaeriopsis mediosetigera*). Jones et al. (2009) published the first formal classification of higher orders of marine fungi supported by phylogenetic data. Subsequently, there has been a dramatic increase in molecular phylogenetic data available for this group. For instance, reports by Sakayaroj et al. (2011) and Pang (2012) on Halosphaeriaceae, a family extensively studied with 75 % of species with sequence data; and major reviews of the marine Dothideomycetes by Suetrong et al. (2009) and Hyde et al. (2013). Other major studies, both on the morphology and phylogeny of marine fungi include those of Jones & Pang (2012), Boonyuen et al. (2011), Jones (2011), Raghukumar & Ravindram (2012), Singh et al. (2012), Orsi et al. (2013), Jones et al. (2014), Lepelletier et al. (2014), Manohar et al. (2014), Rämä et al. (2014) and Richards et al. (2015). These studies yielded sequences of marine fungi that were used in major revisions of the classification of Dothideomycetes and Sordariomycetes (Hyde et al. 2013, Wijayawardene et al. 2014, Ariyawansa et al. 2015a, Maharachchikumbura et al. 2015, 2016, Senanayake et al. 2015). These studies have highlighted many new lineages of marine fungi (Jones et al. 2015).

### **Studies on marine ascomycetes in Thailand**

This study focuses on the marine based taxa from India, Sweden Thailand and UK, especially the taxonomic history of marine ascomycetes. A number of studies have been carried out on collections of marine fungi made in Thailand, starting with Kohlmeyer (1984), Koch (1986), Hyde (1988, 1989a, b, 1992, 1995), Hyde et al. (1990), Chalermpongse (1991), Hyde & Jones (1992), Sundari et al. (1996) and Ito et al. (2001). Jones et al. (2006) introduced two novel species from mangroves in Thailand. Sakayaroj et al. (2010) reported on the diversity of marine fungi on the sea grass *Enhalus acoroides* from Thailand which includes Ascomycota (98%): Sordariomycetes (36%), Eurotiomycetes (33%) and Dothideomycetes (24%). Sakayaroj et al. (2011) also carried out a survey at Hat Khanom-Mu Ko Thale Tai National Park and listed 112 marine fungi representing 79 ascomycetes and 30 asexual morphs. Dayarathne et al. (2016) introduced a novel marine genus, *Halodiatrype* with two novel species from southern Thailand. The numbers of marine ascomycete species recorded from Thailand totals 154 (Jones et al. 2006), 125 (Suetrong et al. 2009, unpublished data), 152 (Dethoup & Manoch 2009), and 112 species (Sakayaroj et al. 2011), indicating a high species richness. Hence, it is worthy to conduct further investigations on Thai

marine ascomycetes for the identification of novel taxa, re-collecting of existing species to provide molecular data and to designate new types where necessary.

## Morphological diversity of marine ascomycetes

### Ascoma

Traditional ascomatal characters used in the classification of marine fungi include colour, shape, texture, neck length and size (Pang 2012). Ascomata may be cleistothecial, perithecial, apothecial or hysterothecial (Koch & Jones 1989, Jones et al. 2015, Dayarathne et al. 2018). Perithecial ascomata can be immersed, becoming erumpent or superficial. The most prominent ascomatal type is immersed perithecia (e.g. *Bathyascus*, *Halobyssothecium*, *Rhizophila*) (Hyde & Mouzouras 1988, Hyde & Jones 1989a, Dayarathne et al. 2018). Superficial perithecia are found in *Corollospora* species (Kohlmeyer 1986). In members of the Halosphaeriaceae, ascomata can be immersed or superficial (e.g. *Remispora*, *Thalassogena*). In addition, ascomata can be short to long necked or with an indistinct neck region (Suetrong et al. 2009, Jones et al. 2015, Dayarathne et al. 2019a, b). Orientation of ascomata varies among different species, such as vertical (in *Coronopapilla*), or horizontal (in *Savoryella* species and *Lautospora gigantea*). Ascoma shape and colour are mostly consistent in most families (Koch & Jones 1989, Jones et al. 2015). The presence of a pseudostroma or clypeus may also aid in identifying marine ascomycetes (Hyde et al. 2000a). Fig. 1 illustrates the different morphologies of ascomata of marine fungal species.

### Asci

Ascus morphology is particularly interesting and an important criterion for the discrimination of genera. Unitunicate asci can be different from each other in a number of ways including their apical structures. For example, the ascus apex may be thickened, or thickened with a refractive pore or plate (Fig. 2). In some genera (e.g. *Buergenerula*, *Linocarpon*) the ascus is composed of a subapical ring-like structure. They usually exist as two oval refractive bodies and a subapical apical apparatus in *Marinosphaera* (Hyde 1989). An ocular chamber or ocular chamber and a ring can be seen in bitunicate ascomycetes. The shape of the ascus may also be important, saccate, clavate or cylindrical asci are found among various marine taxa (Hyde et al. 2000a). The number of ascospores per ascus varies from 2-multi spores, two-spored asci (*Savoryella paucispora*), 4-spored (*Passeriniella savoryellopsis*), 6-spored asci (*Phycomelaina*), 8-spored (most marine ascomycetes), 16 spored asci (*Sedecimiella taiwanensis*) and polysporous (*Cryptovalsa*) (Hyde 1993b).

### Ascospores

The ascospores of marine taxa are remarkable in their morphologies and hence, important in defining the boundaries at the generic and species level. Ascospore colour (mostly hyaline, brown, greenish and yellow), shape and the septation are all useful taxonomic characters at the species level. In most genera ascospores are unevenly thickened, while *Lautospora* has muriform spores with very thick walls (Hyde & Jones 1989b). Diverse ascospore appendages and sheaths are fascinating characters of marine ascomycetes that are important in adhesion, dispersal and in colonizing marine substrata (Jones 1973, Kohlmeyer & Kohlmeyer 1979, Rees 1982, Hyde & Jones 1989b, Dayarathne et al. 2019a, b). The morphological characters mostly found among the marine ascomycete taxa include asci which are mostly deliquescing and mostly hyaline ascospores with appendages or sheaths (Jones & Moss 1978) (Fig. 3).

### Asexual morphs

Saccardo (1880) described the first asexual morph of a marine fungus, *Camarosporium roumeguerii* on the salt marsh plant *Salicornia herbacea*. Both hyphomycetes and coelomycetes have been reported from various marine habitats (Chatmala et al. 2004, Jones et al. 2009, 2015). Fifty species of native asexual marine fungi were recorded by Kohlmeyer & Kohlmeyer (1979). The number increased to 60 by Kohlmeyer & Volkmann-Kohlmeyer (1991) and later to 94 by

Jones et al. (2009). Abdel-Wahab & Bahkali (2012) reported 143 marine asexual morphic taxa while, Kurtzman et al. (2011a, b) reported 72 sexual and 14 asexual marine yeasts (Taphrinomycotina, Saccharomycotina) and Jones et al. (2015) listed 43 asexual filamentous fungi in 26 genera. Conidial fungi constitute approximately one fourth of all described filamentous marine fungi (Cole & Kendrick 1981). Jones et al. (2015) assigned asexual genera to their higher order position based on molecular studies. However, sequence data from conidial stages of fungi from marine habitats are lacking in GenBank and it is necessary to link asexual and sexual morphs.

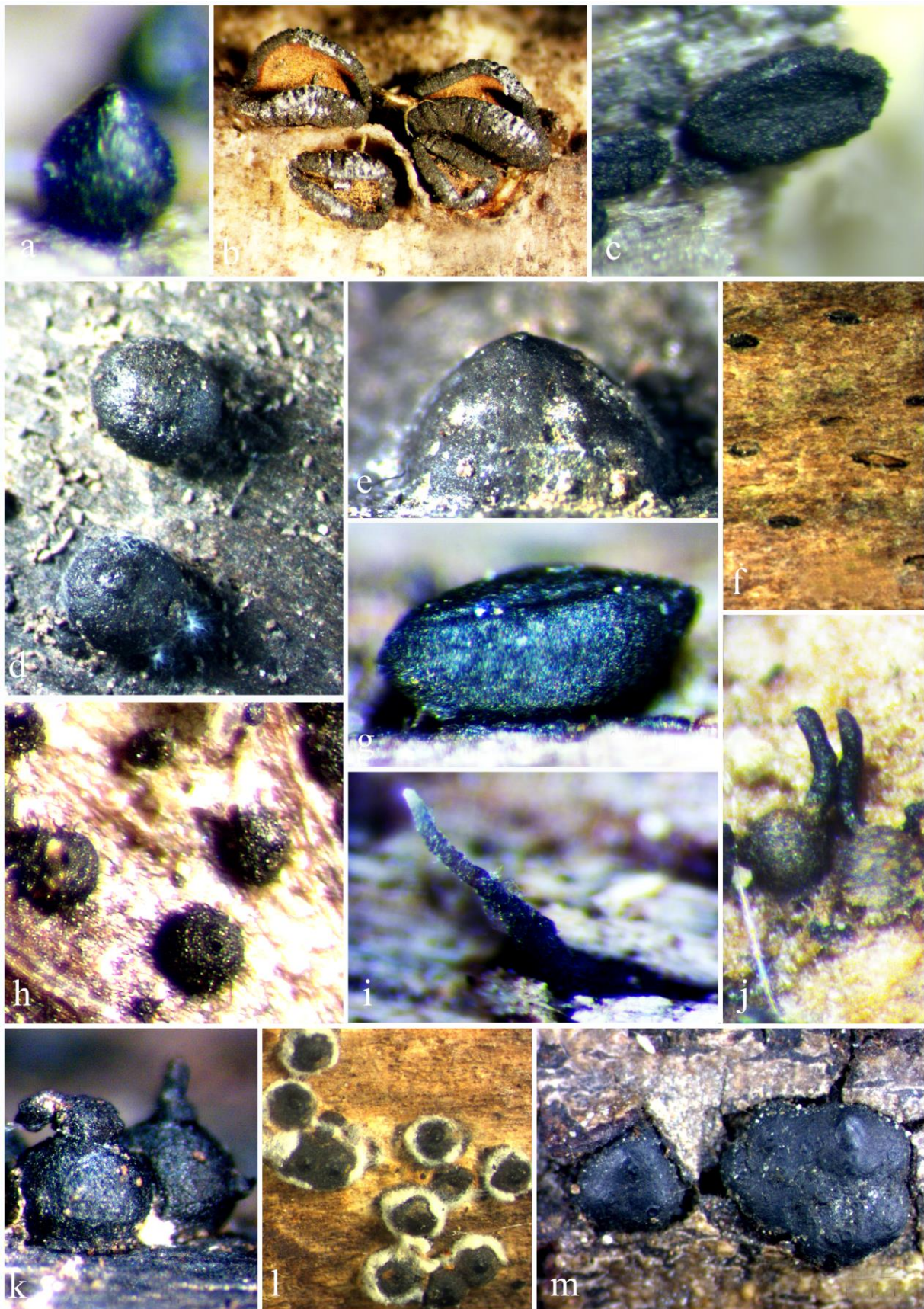
### **Ecological and economic significance of marine ascomycetes**

Marine ascomycetes are parasites, saprobes, endophytes or symbionts on a wide variety of substrates in oceans and estuaries (Hyde et al. 2000b, Chatmala et al. 2004). Hosts or substrates for marine fungi includes marine algae, submerged parts of tropical mangrove trees and temperate salt marsh halophytes, dead wood and other cellulosic substances, polyurethane panels (Jones & Le Campion-Alsumard (1970) and even animal chitin and conchyolin (Cole & Kendrick 1981, Hyde et al. 2000b, Chatmala et al 2004, Hyde et al 2015, Jones 2015, Li et al. 2016). They play an important role in decomposition and recycling nutrients of variety of substrates (Hyde et al. 2015, Jones et al. 2019). Saprobic marine ascomycetes are responsible for transformation of the polymeric compounds into dissolved or particulate organic matter utilizable by other consumers in the food web (Fell & Master 1973, Newell 1976, Nakagiri et al. 1996, Hyde et al. 1998, 2015), such as amino acids lysine and methionine, various vitamins and sterols (an important precursor for the manufacture of cholesterol in marine animals (Phillips 1984, Velmurugen & Lee 2012). Furthermore, some marine ascomycetes such as *Lignincola laevis* and *Stagonospora* spp. are important due to their ability to completely decolourize polymeric dyes by utilizing either glucose or cellulose as a primary carbon source (Pointing et al. 1998). Mangrove leaves are rich in lignocellulosic and cellulosic structural polymers, soluble organics, phenolics and tannins, making them highly resistant to degradation by many microbes. However, fungi such as *Pestalotiopsis* and *Cladosporium*, common primary saprotrophs, are often recovered from mangrove leaves (Raghukumar et al. 2004). The microbes also serve as food for some bottom dwelling organisms hence they are an essential trophic level in detritus marine food webs (Das et al. 2007). Cawthorn (2011) identified *Fusarium* spp. as a disease causal agent in American lobsters noting its opportunistic nature and propensity to invade through damaged or dead tissues. There has been a significant increase in the rate of discovery of natural products from marine and marine-derived fungi over the past decade with a total of over 1000 novel secondary metabolites (Rateb & Ebel 2011, Jones et al. 2019). Marine fungi are an untapped source of novel chemical structures and compounds for drug discovery and nutraceutical research (Pang et al. 2015, Liao et al. 2019). The additional stress of salinity might induce structurally diverse compounds from marine derived fungi. However, chemical structures produced by fungi *in vitro* are possibly different, implying direct extraction from tissues of plants or seaweeds (Pang et al. 2015). Examples of secondary metabolites produced by mangrove fungi includes a 14-membered resorcylic acid lactone, the known metabolite of hypothemycin and novel aigialomycins, isolated from *Aigialus parvus* with antiplasmodial activity (Isaka et al. 2002) and cytotoxicity against human cell lines (Barluenga et al. 2006).

### **Aim of the paper**

The taxonomic placement of numerous marine ascomycetes is still unclear and many of them have been tentatively placed in a higher rank until more sequence data are available to confirm their exact placement. Some 50% of described marine fungi have no sequence data and this seriously limits interpretation in taxonomic projects (Xu et al. 2018, Hassett et al. 2019 published on line). Furthermore, it has been estimated that the percentage of unexplored marine taxa is high (Jones 2011, Jones et al. 2019). Therefore, the objectives of this study are to evaluate the taxonomy and phylogeny of some marine fungal taxa with multiple loci sequence data (LSU, SSU, ITS rDNA and protein genes) and to introduce novel taxa along with comprehensive morpho molecular accounts.





**Figure 1** – Different morphological features of ascomata/stromata of marine based ascomycetes: a *Chaetosphaeria mangrovei* (MFLU 18-0146) – superficial, ovoid perithecia. b *Rhytidhysteron bruguierae* (MFLU 18-0571) – superficial, boat-shaped hysterothecia. c *Patellaria apiculatae* (MFLU 19-1236) – superficial, circular apothecia. d *Nemanium viridis* (MFLU 17-2600) –

superficial, sub-globose ascomata occur on black stroma. e *Acrocordiopsis patilii* (MFLU 18-0533) – semi immersed, hemispherical ascomata. f *Halodiatrype avicenniae* (MFLU 16-1185) – deeply immersed, globose to subglobose ascomata in a darkened pseudostroma. g *Hysterium thailandicum* (MFLU 18-0551) – superficial, elongate and depressed hysterothecia. h *Coniochaeta marina* (MFLU 17-2653) – semi-immersed to superficial, globose to ovoid perithecia with setae. i *Halosphaeria* sp. (MFLU 16-1223) – semi-immersed ascomata with a long neck. j *Jattaea mucronata* (MFLU 16-0534) – superficial, ascomata with a long neck. k *Caryospora rhizophorae* (MFLU 18-0536) – superficial, ascomata with a medium neck. l *Nemania maritima* (MFLU 16-1236) – immersed to half-erumpent stromata, coated with or surrounded by a waxy whitish tissue. m *Diatrype mangrovei* (MFLU 17-0412) – erumpent stromata arising through cracks on the bark.

## Material & Methods

### Fungal sampling and morphology

Fresh materials were collected from various marine habitats in Krabi, Phang-nga, Phetchaburi, Phuket and Ranong Provinces, in southern Thailand, Sweden and the UK. Specimens collected from Muthupet, Parangipettai and Pondicherry mangroves in India were examined and characterized as detailed in Devadatha et al. (2017). Specimens were brought to the laboratory in Zip-lock plastic bags and incubated at room temperature in the laboratory. Fungal specimens were examined with a Motic SMZ 168 stereomicroscope. Rehydrated fruiting bodies were used to observe characteristics of ascomata, asci, ascospores and other tissues and characters were photographed with a Canon 550D digital camera fitted to the Nikon ECLIPSE 80i compound microscope. Photomicrographs were arranged with Adobe Photoshop v. CS6 and all measurements were made with Tarosoft v. 0.9.0.7. Specimens were preserved and deposited at the Mae Fah Luang University Herbarium (MFLU), BIOTEC Bangkok Herbarium (BBH), Thailand and Ajrekar Mycological Herbarium (AMH), India.

Fruiting bodies were removed from the substrate using a sterilized needle and placed in a few drops of sterilized distilled water on a sterilized cavity slide and spore suspensions were prepared as described in Chomnunti et al. (2014). Germinating ascospores were aseptically transferred to Petri-dishes containing Potato Dextrose Agar (PDA) or Malt Extract Agar (MEA) media prepared in 50% or 100% concentrations of sterilized natural seawater (Atla 2006). Colonies were photographed and characters were recorded after two weeks. Living cultures were deposited at Mae Fah Luang University Culture Collection (MFLUCC) and Thailand, National Fungal Culture Collection of India (NFCCI), India. New species were established using modern criteria and standards (Taylor 2011, Seifert & Rossman 2010, Jeewon & Hyde 2016). Faces of fungi and Index Fungorum numbers were registered according to Jayasiri et al. (2015) and Index Fungorum (2019).

### DNA extraction, PCR amplification and phylogeny

Fresh fungal mycelia grown on MEA/PDA or seawater PDA/MEA for 4 weeks at 18–25 °C were scraped from the colony margin and sometimes perithecial contents of fresh specimens were used for genomic DNA extraction following the protocol outlined by Dayarathne et al. (2019c). DNA extraction kit (E.Z.N.A.® Forensic, DNA kit, D3591-01, Omega Bio-Tek) was used to extract DNA directly from ascomata following the manufacturer's instructions where necessary. PCR amplification and sequencing of the SSU nrDNA region using the primer pair NS1/NS4 (White et al. 1990). LSU nrDNA region using the primer pair LROR/LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994), ITS nrDNA region using primer pair ITS5/ITS4 (White et al. 1990) and TUB gene region using the primer pair Bt2a/Bt2b (Trouillas et al. 2011) were performed. Each amplification reaction contained 0.125 µL of 5 units/µL Ex-Taq DNA polymerase (TaKaRa), 2.5 µL of 10 × PCR buffer, 2 µL of 2mM MgCl<sub>2</sub>, 2.5 µL of 2 mM dNTPs, 1 µL of 0.2–1.0 µM primer, <500 ng DNA template and was adjusted with double-distilled water to a total volume of 25 µL.

Amplification of SSU, LSU, ITS, RPB2, TEF and TUB were accomplished as described in the Table 1.



**Figure 2** – Different morphological features of asci and apical structures of marine based ascomycetes: a *Rimaconus multiguttulatus* (MFLU 18-0535) – cylindrical, unitunicate asci. b

*Halodiatrype salinicola* (MFLU 17-2595) – clavate, unitunicate asci. c *Kamalomyces mangrovei* (MFLU 18-0550) – clavate bitunicate asci. d *Ascocratera manglicola* (MFLU 17-2588) – cylindrical bitunicate asci. e *Poaceascoma halophila* (MFLU 16-1178) – cylindrical to cylindrical-clavate asci. f *Lautospora simillima* (J.K. 5532) – 4-spored, short pedicellate asci. g *Salsuginea rhizophorae* (MFLU 18-0540) – 8-spored, long pedicellate asci. h *Halocryptovalsa salicorniae* (MFLU 16-0551) – multi-spored, long pedicellate asci. i. *Halosphaeria* sp. (MFLU 16-1223) – lacking an apical apparatus. j *Savoryella lignicola* (IMI 129784) – truncate apical thickening with a pore. k. *Diaporthe marina* (MFLU 17-2622) – refractive apical ring. l *Aniptodera chesapeakeensis* (MFLU 16-1189) – apically truncate, thin-walled, persistent apical ring with pore. m *Dyfrolomyces thailandicus* (MFLU 16-1173) – apically rounded, thickened obvious apical ring. n *Lautospora simillima* (J.K. 5532) – ocular chamber. o *Phaeoacremonium aureum* (MFLU 17-1581) – broadly rounded to obtuse, thickened apex. p, q *Nemania maritima* (MFLU 16-1236) – narrowly cuneiform, J+, sub-apical ring. r *Halorosellinia rhizophorae* (MFLU 17-2591) – rectangular, J+, apical apparatus.

All PCR products were visualized by electrophoresis at 120 V/cm for 20 min in 1 % agarose gel stained with ethidium bromide 0.5 mg/mL. The gel was visualized under a UV transilluminator to estimate the fragment size. PCR products were purified and sequenced with both forward and reverse primers at the Sunbiotech Company, Beijing, China.

### Phylogenetic analyses

Sequences generated from above mentioned primers of the different genes (LSU, SSU, ITS, BTUB, TEF and RPB2) were analyzed with other sequences retrieved from GenBank. Sequences with high similarity indices were determined from a BLAST search to find the closest matches with taxa in relevant families and relying on previous literature. Datasets were initially aligned by using MAFFT version 7 (Kato & Standley 2013, <http://mafft.cbrc.jp/alignment/server/>) and manually corrected where necessary using BioEdit sequence alignment editor (Hall 1999) to minimize the uninformative gaps. Gaps were treated as missing data. Nucleotide substitution models were determined with MrModeltest v. 2.2 (Nylander 2004). The GTR+I+G model was used in each analysis for phylogenetic studies.

Phylogenetic trees were generated using maximum likelihood (ML) and Bayesian inference analyses (BI). The ML trees were generated using the RAxML-HPC2 on XSEDE (v. 8.2.8) (Stamatakis et al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) using the GTR+I+G model of evolution. The Bayesian trees for various data sets were generated using MCMC sampling in MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001, Zhaxybayeva & Gogarten 2002). Ambiguously aligned regions were excluded and all characters were unordered and given equal weight. The Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed to determine whether the trees inferred under different optimality criteria were significantly different. The resulting trees were viewed with FigTree v.1.4.0 (Rambaut 2012) and the final layout was done with Microsoft power point (2016).

### Taxonomy

#### Ascomycota R.H. Whittaker

We use the recent treatments and updated accounts of Ascomycota in Wijayawardene et al. (2017a, 2018).

#### Class Dothideomycetes sensu O.E. Erikss & Winka

Dothideomycetes is considered to be largest and most phylogenetically diverse class in the phylum Ascomycota (Schoch et al. 2009, Hyde et al. 2013). Liu et al. (2017) provided divergence time estimates for the different taxonomic ranks of the class Dothideomycetes with multi-locus phylogeny. Our classification of different Dothideomycete taxa are based on Hyde et al. (2013),

Wijayawardene et al. (2014) and Liu et al. (2017). Dothideomycete subclasses and their families documented in this study are arranged alphabetically.



**Figure 3** – Different morphological features of ascospores of marine based ascomycetes: a *Acrocordiopsis patilii* (MFLU 18-0533) – broadly ellipsoidal, hyaline, 1-euseptate ascospore. b

*Neocamarosporium maritimae* (MFLU 19-1232) – ellipsoidal, muriform, yellowish brown ascospore. c *Halodiatrype avicenniae* (MFLU 16-1185) – oblong to allantoid light brown ascospore. d *Amarenographium ammophilicola* (MFLU 17-2571) – broadly fusiform, 6-septate, pale brown ascospore. e *Trematosphaeria pertusa* (MFLU 18-0526) -fusiform, 1-3-septate, dark brown ascospore. f *Halorosellinia xylocarpi* (MFLU 18-0545) – ellipsoidal, olive green ascospore. g *Nemania maritima* (MFLU 16-1236) – oblong, ellipsoidal, aseptate, brown ascospore. h *Diaporthe marina* (MFLU 17-2622) – ovoid, multi guttulate ascospore. i. *Koralionasteta ovalis* (NY 1317811) – ellipsoidal to oval ascospore with several cross septa. j *Kamalomyces mangrovei* (MFLU 18-0550) – elongate cylindrical to fusiform-clavate ascospore. k. *Aigialus grandis* (MFLU 16-1241) – muriform, ascospore with 16–18 transverse septa and 1–2 longitudinal septa. l *Halotestudina muriformis* (MFLU 17-2634) – muriform, with 7 transverse septa and 0–2 longitudinal septa. m *Dyfrolomyces thailandicus* (MFLU 16-1173) – ascospore surrounded by a sheath. n *Haiyanga salina* (MFLU 16-1191) – ascospore with 3-4 sub-polar spoon-shaped appendages. o *Halosphaeria appendiculata* (MFLU 17-2657) – ascospores with polar and/or lateral appendages. p *Amphitrite annulata* (UPS 10870) – ascospore with a ring-like collar encloses at the septum. q *Moana turbinulata* (NY 1347771) – ascospore with a single, uncoiling polar appendage. r *Pedumispora rhizophorae* (BRIP 19201) – filiform ascospores with a curved or hook-shaped end. s *Pontogeneia padinae* – filiform, curved, septate ascospore. t *Poaceascoma halophila* (MFLU 16-1178) – filiform septate ascospore with a wide base. u *Sammeyersia grandispora* (MFLU 16-1172) – long filamentous ascospore tapering at each end into an elongate apical chamber, filled with mucus.

### **Subclass Pleosporomycetidae** C.L. Schoch et al.

#### **Hysteriales** Lindau

The monotypic order Hysteriales was introduced by Lindau (1897) and has been accommodated within pyrenomycetes and discomycetes (Rehm 1896). Molecular analyses confirmed the placement of Hysteriales in Dothideomycetes (Boehm et al. 2009a, b, Shearer et al. 2009, Suetrong et al. 2009).

#### **Hysteriaceae** Chevall

Fungi classified in the family Hysteriaceae (Chevallier 1826) can be distinguished by thick-walled, hysterothecial ascomata which are navicular and characteristically dehiscing by an invaginated slit or sulcus (Zogg 1962, Clements 1909, Boehm et al. 2009a). Hysteriaceae currently comprises 14 genera, *Actidiographium*, *Coniosporium*, *Gloniopsis*, *Gloniella*, *Graphyllum*, *Hysterium*, *Hysterocarina*, *Hysteropycnis*, *Hysterobrevium*, *Hysterodiffractum*, *Oedohysterium*, *Ostreichnion*, *Psiloglonium* and *Rhytidhysterion* based on morphology and/or phylogenetic data (Boehm et al. 2009a, b, Wijayawardene et al. 2018, Jayasiri et al. 2018).

#### ***Hysterium*** Pers.

The genus *Hysterium*, typified by *H. pulicare* produces darkly pigmented and carbonaceous hysterothecia (Schoch et al. 2009, Hyde et al. 2017). Recent phylogenetic analyses showed that the hysterothecium has been derived number of times and there is an accountable genetic diversity within this group (Boehm et al. 2009b, Jayasiri et al. 2018, 2019).

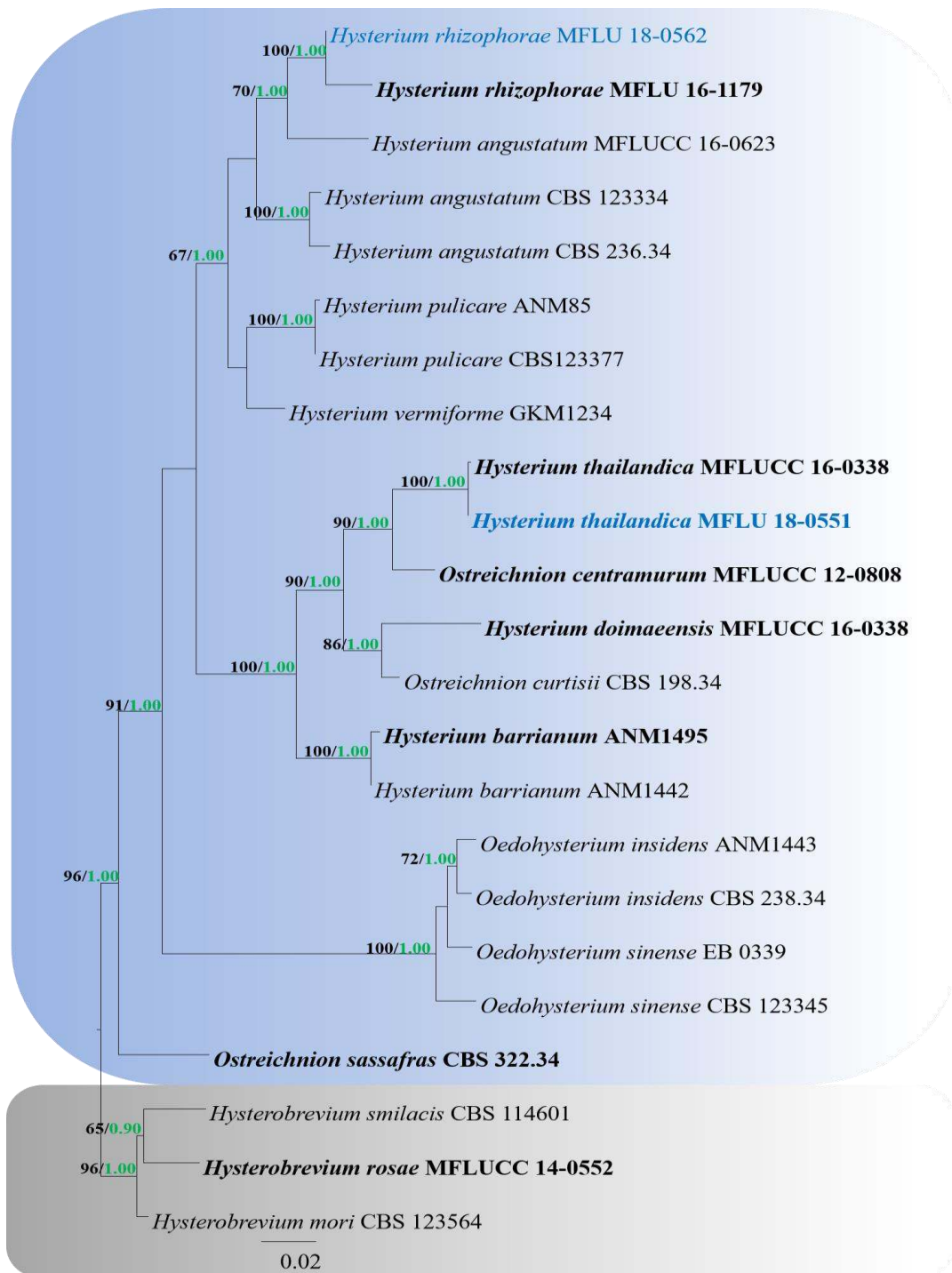
#### ***Hysterium rhizophorae*** Dayar. & K.D. Hyde, in Hyde et al., Fungal Diversity 87: 42 (2017) Fig. 5 Facesoffungi number: FoF 02911

*Saprobic* on bark attached to the living plant of *Avicennia* sp. Sexual morph: *Ascomata* 1.5–2.0 long × 0.15–0.25 mm wide × 0.2–0.25 high, hysterothecial, erumpent to superficial, with a immersed base, solitary to gregarious, straight to flexuous, ellipsoid or elongate, with pointed ends, opening by a depressed longitudinal slit, comprising orange cells, in vertical section subglobose to globose, carbonaceous, black. *Peridium* 38.5–52 µm wide, carbonaceous, comprising an outer layer of dark brown cells of *textura angularis* and inner layer of hyaline to pale brown cells of *textura*

*angularis*. *Hamathecium* comprises 1.5–2 µm wide *pseudoparaphyses*, cellular, septate, flexuous, branched. *Asci* 40.8–48.6 × 3.5–5 µm ( $\bar{x}$  = 45.2 × 3.7 µm, n = 15), 8-spored, uni to bi seriate, cylindric to claviform, apex thickened, short pedicellate. *Ascospores* 14.5–16 × 4–5.9 µm ( $\bar{x}$  = 15.2 × 5 µm, n = 20), overlapping biseriate, light brown, ellipsoidal, slightly curved, with 3 transverse septa, often slightly constricted at medium septum, with a guttule per cell, rounded at both ends. Asexual morph: Undetermined.

**Table 1** Genes/loci used in the study with their PCR primers and protocols.

Gene/loci	Primer		PCR protocol					References
	Forward	Reverse	Initial Denaturation	Denaturation	Annealing	Extension	Final extension	
Internal transcribed spacer (ITS)	ITS5	ITS4	94°C, 4 min 1 cycle	94°C, 45 sec 35 cycles	56°C, 45 sec	72°C, 1 min	72°C, 10 min 1 cycle	White et al. (1990)
Large subunit (LSU)	LROR	LR5	94°C, 4 min 1 cycle	94°C, 45 sec 35 cycles	56°C, 45 sec	72°C, 1 min	72°C, 10 min 1 cycle	Rehner & Samuels (1994) Vilgalys & Hester (1990)
Small subunit (SSU)	NS1	NS4	94°C, 4 min 1 cycle	94°C, 45 sec 35 cycles	56°C, 45 sec	72°C, 1 min	72°C, 10 min 1 cycle	White et al. (1990)
Elongation factor-1 alpha (EF 1- $\alpha$ )	EF1-728F	EF1- 986R	94°C, 3 min 1 cycle	94°C, 30 sec 35 cycles	55°C, 50 sec	72°C, 1 min	72°C, 10 min 1 cycle	Carbone & Kohn (1999)
RNA polymerase II second large subunit (RBP2)	fRPB2-5F	fRPB2-7cr	95°C, 5 min 1 cycle	95°C, 45 sec 40 cycles	55°C, 2 min	72°C, 1.5 min	72°C, 10 min 1 cycle	Liu et al. (1999)
Beta tubulin (BTUB)	Bt2a	Bt2b	94°C, 3 min 1 cycle	94°C, 30 sec 35 cycles	55°C, 50 sec	72°C, 1 min	72°C, 10 min 1 cycle	Glass & Donaldson (1995)



**Figure 4** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -12708.949995. The combined LSU, SSU, TEF and RPB2 sequence datasets comprised 23 strains of *Hysterium* and related taxa with *Hysterobrevium mori* (CBS 123564), *H. rosae* (MFLUCC 14-0552) and *H. smilacis* (CBS 114601) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 939 distinct alignment patterns, with 36.13% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.250302, C = 0.237232, G = 0.277079, T = 0.235387; substitution rates AC = 1.506537, AG = 3.785145, AT = 1.471121, CG = 1.308838, CT = 10.960968, GT = 1.000000; gamma distribution shape parameter  $\alpha = 0.148727$ . Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.02 changes. The ex-type strains are in bold and new isolates in blue bold.





**Figure 5** – *Hysterium rhizophorae* (MFLU 18-0562). a Appearance of hysterothecia on host. b Vertical section through hysterothecium. c Peridium. d, e Asci. f Pseudoparaphyses. g Ascospores. h Germinating ascospore. Scale bars: a = 200 µm, b = 100 µm, d, e, h = 50 µm, c, f, g = 20 µm.

Material examined – THAILAND, Phetchaburi Province, Cha-am beach, on bark attached to the living plant of *Avicennia* sp. (Rhizophoraceae), 30 November 2016, M.C. Dayarathne, MCD 090 (MFLU 18-0562, new host record).

GenBank numbers – LSU: MN017831; SSU: MN017899; TEF: MN433213

Notes – Our second sample collection, from Phetchaburi province, Thailand also yielded *H. rhizophorae* which indicates it is a relatively abundant species in southern Thailand. Devadatha et al. (2018) also reported this species from *Aegiceras corniculatum* (Primulaceae), collected from Muthupet mangroves in India. This is the first record of this species from *Avicennia* sp.

***Hysterium thailandicum*** Jayasiri & K.D. Hyde, in Jayasiri, Hyde, Jones, Peršoh, Camporesi & Kang, *Mycosphere* 9(4): 817 (2018) Fig. 6

Facesoffungi number: FoF04580

*Saprobic* on decaying, submerged mangrove wood. Sexual morph: *Hysterothecia* 430–500 µm high, 360–410 µm wide, 800–1100 µm long ( $\bar{x}$  = 490 × 400 × 1000 µm, n = 5), scattered, superficial, with a immersed base, elongate and depressed conchate, surface black, shiny, longitudinally striate, apex compressed, opening by longitudinal slit. *Peridium* 40–82 µm wide ( $\bar{x}$  = 60.6 µm, n = 5), comprising carbonaceous, brittle, heavily pigmented, small, prosenchymatous cells. *Hamathecium* 1–1.5 µm wide, persistent, trabeculate, hyaline, aseptate, branched, borne in a gel matrix, longer than asci. *Asci* 122–140 × 24.5–38 µm ( $\bar{x}$  = 125.2 × 28 µm, n = 20), 8-spored, bitunicate, arising from base, oblong to clavate, with a short pedicel, apex thickened, with refractive ring around cytoplasmic protrusion. *Ascospores* 45–70.2 × 8–13 µm ( $\bar{x}$  = 59 × 10.8 µm, n = 30), crowded to biseriate, fusiform when young, oblong at maturity, hyaline to olivaceous brown, 1-septate, highly constricted at the septum, wall greatly thickened, wall smooth. Asexual morph: *Conidiogenous cells* 7–9 × 1.8–3.2 µm, enteroblastic, phialidic, globose to flask-shaped, hyaline, thin-walled. *Conidia* 2–4 × 2–3.9 µm, fusiform to spherical, hyaline, with a central guttule.

Culture characteristics – Ascospores germinating on sea water PDA within 24 h and germ tubes produce from one or both ends. Colonies reaching on PDA 5 cm diam. After 1 week at 25 °C, flat, circular, initially white becoming greenish to reddish brown, smooth surface with undulate edge.

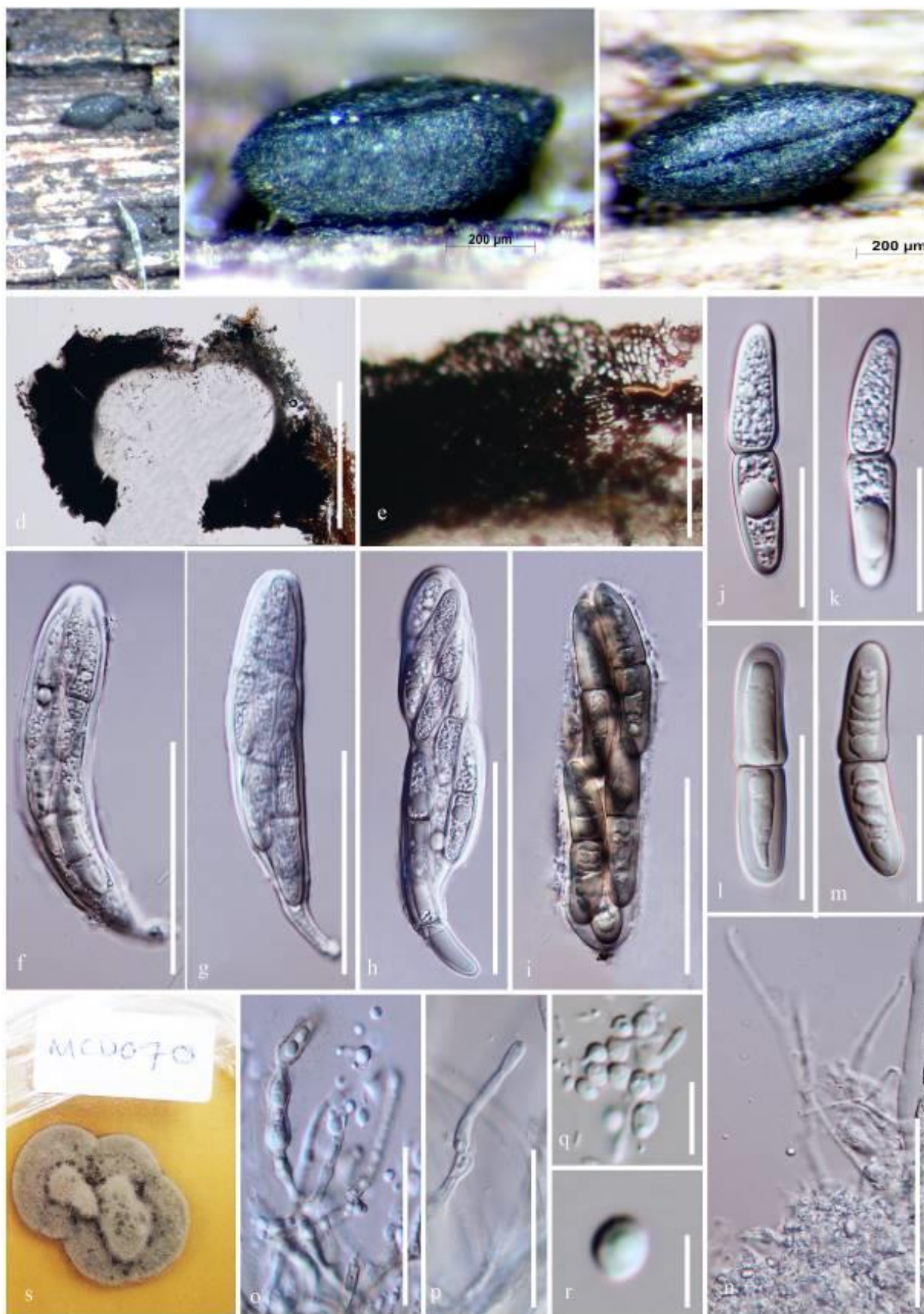
Material examined – THAILAND, Ranong Province, Ranong, on decaying, submerged mangrove wood (Rhizophoraceae), 07 December 2017, M.C. Dayarathne, MCD 70 (MFLU 18-0551).

GenBank numbers – LSU: MN017832; SSU: MN017900; TEF: MN077055; RPB2: MN077077

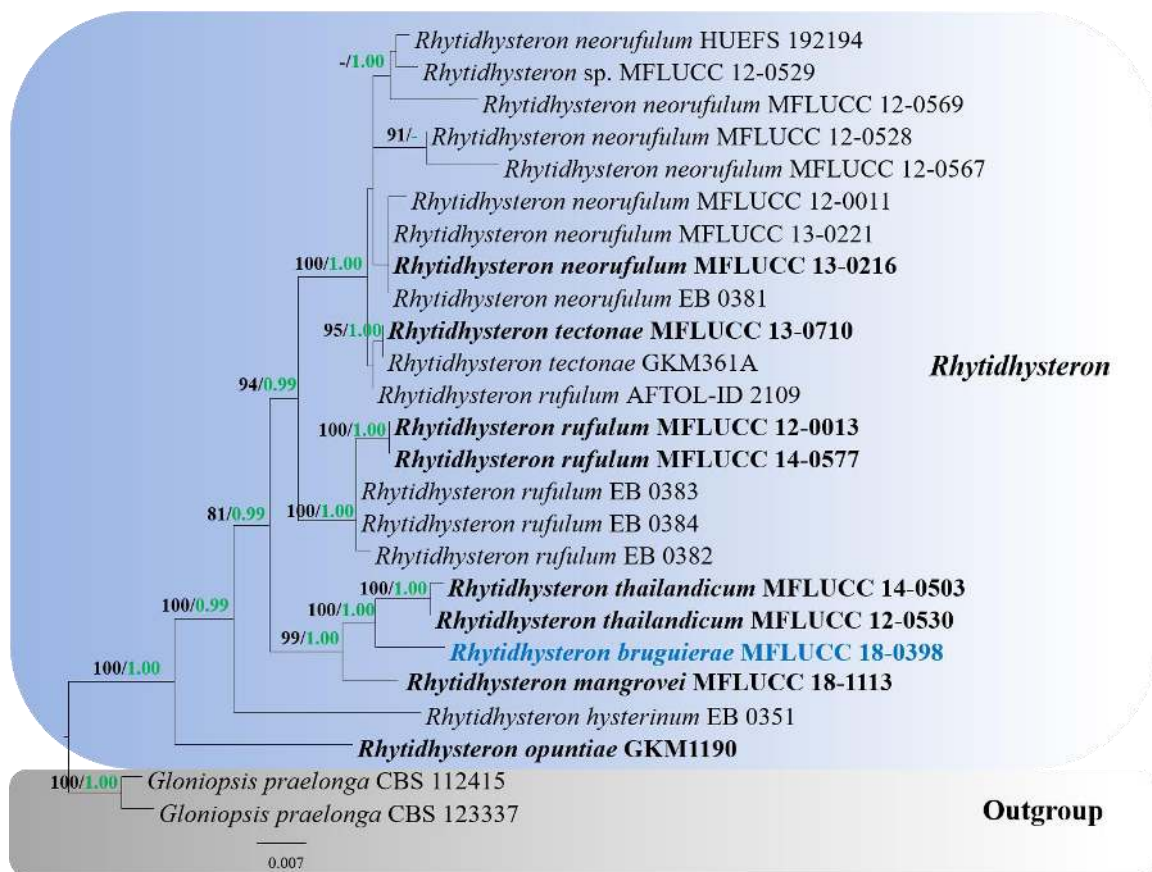
Notes – *Hysterium thailandicum* is morphologically reminiscent of ostreichnion-like *Hysterium* species. This is shown in our phylogram generated by combined LSU, SSU, TEF and RPB2 data where *H. thailandica* clustered together in a clade comprising ostreichnion-like *Hysterium* species (Fig. 4). Our new strain (MFLU 18-0551) morphologically resembles the holotype. However, a few morphological differences were observed among these two strains: asci of *H. thailandica* (MFLU 18-0551) are longer than those of the holotype (800–1100 µm vs 500–600 µm) and *H. thailandica* (MFLU 18-0551) has shorter asci than the holotype (122–140 µm vs 155–210 µm). Furthermore, ascospore colour at maturity is olivaceous brown in *H. thailandica* (MFLU 18-0551) and yellow in the holotype. Habitats of (MFLU 18-0551) and the holotype are also different where the habitat of our strain (MFLU 18-0551) is decaying, submerged mangrove wood and the holotype was found on dead stem of an unknown terrestrial plant. We also observed the asexual morph of this taxon in culture which morphologically resembles the asexual morphs reported for the genus *Hysterium* (Jayasiri et al. 2018, 2019).

### ***Rhytidhysterion*** Speg.

Spegazzini (1881) introduced the genus *Rhytidhysterion* to accommodate *R. brasiliense* and *R. viride* without designating a type species, while Clements & Shear (1931) designated *R. brasiliense* as the type species (Samuels & Müller 1979, Yacharoen et al. 2015). Subsequently, various authors broadened the concept of the genus by adding several species (Spegazzini 1921, Sharma & Rawla 1986, Barr 1990a, Magnes 1997, Doilom et al. 2016, Thambugala et al. 2016, Hyde et al. 2017) and currently *Rhytidhysterion* comprises 21 epithets (Kumar et al. 2019).



**Figure 6** – *Hysterium thailandicum* (MFLU 18-0551). a–c Hysterothecia on host surface. d Vertical section through hysterothecium. e Peridium. f–i Asci. j–m Ascospores. n Pseudoparaphyses. o, p Conidiophores. q, r Conidia. s Sporulating culture on MEA. Scale bars: b, c = 200  $\mu$ m, e–i = 50, j–n = 20  $\mu$ m.



**Figure 7** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -6729.544855. The combined LSU, SSU and TEF sequence datasets comprised 25 strains of *Rhytidhysterion* and related taxa with *Gloniopsis praelonga* (CBS 112415) and (CBS 123337) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 388 distinct alignment patterns, with 31.03% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.249183, C = 0.227333, G = 0.279667, T = 0.243816; substitution rates AC = 1.765891, AG = 2.355479, AT = 1.363094, CG = 0.813735, CT = 10.061233, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.020000. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.007 changes. The ex-type strains are in bold and new isolates in blue bold.

Multi-gene phylogenetic studies have shown that *Rhytidhysterion* should be placed in Hysteriaceae (Boehm et al. 2009a, b, De Almeida et al. 2014, Wijayawardene et al. 2014, Jayasiri et al. 2018, 2019). This genus is characterized by closed and navicular ascomata, later opening by a longitudinal slit to become irregularly apothecioid at maturity and heavily pigmented, and with thick-walled ascospores (Boehm et al. 2009a, Thambugala et al. 2016). An endophytic *Rhytidhysterion* sp. from mangroves has been reported by Pudhom & Teerawatananond (2014), while a recent study by Kumar et al. (2019) introduced *R. mangrovei* from dead twigs of a mangrove species. The present study introduces a novel taxon from submerged decaying wood of *Rhizophora* sp.

*Rhytidhysterion bruguierae* Dayarathne, sp. nov.

Fig. 8

Index Fungorum number: IF556574, Facesoffungi number: FoF 06154

Etymology – Epithet derived from the host genus where the fungus was isolated, *Bruguiera*

Holotype – MFLU 18-0571

*Saprobic* on decaying, submerged branches of *Bruguiera* sp. Sexual morph: *Ascomata* 400–950 mm long, 548–570 mm wide, 410–520 mm high (n = 5), hysterothecial, boat-shaped, crowded to aggregate, superficial, brown-black, with exposed, lenticular to irregular, brown-black disc, folded along the margins when dry and forming an elongate slit, perpendicularly striate. *Exciple* 148–162 µm wide (n = 5), composed of dark brown to black, thick-walled cells of *textura angularis*. *Hamathecium* comprised of dense, septate pseudoparaphyses, branched and forming a red epithecium above the asci, fused and slightly swollen at the apex and enclosed in a gelatinous matrix. *Asci* 128–148 × 10–14 µm ( $\bar{x}$  = 132 × 13 µm, n = 15), 6–8-spored, bitunicate, cylindrical, with short, furcate pedicel, rounded at the apex, with distinct ocular chamber, J-. *Ascospores* 14–26 × 6.2–9 µm ( $\bar{x}$  = 19.2 × 7.5 µm, n = 20), uniseriate, slightly overlapping, hyaline when immature, becoming yellowish-brown to reddish brown when mature, ellipsoidal to fusiform, rounded to slightly pointed at both ends, 1–3-septate, slightly constricted at the septa, with smooth wall. Asexual morph: Undetermined.

Culture characteristics – Ascospores germinating on sea water PDA within 24 h and germ tubes produced from one or both ends. Colonies growing on PDA 5 cm diam. after 1 week at 25 °C, flat, circular, initially white becoming greenish to reddish brown, smooth surface with undulate edge.

Material examined – THAILAND, Phetchaburi Province, Cha-am, on decaying *Bruguiera* sp. (Rhizophoraceae) wood, 12 January 2018, M.C. Dayarathne, MCD 183 (MFLU 18-0571, holotype); *ibid.*, (HKAS102064, isotype), ex type living culture MFLUCC 18-0398.

GenBank numbers – LSU: MN017833; SSU: MN017901, TEF: MN077056

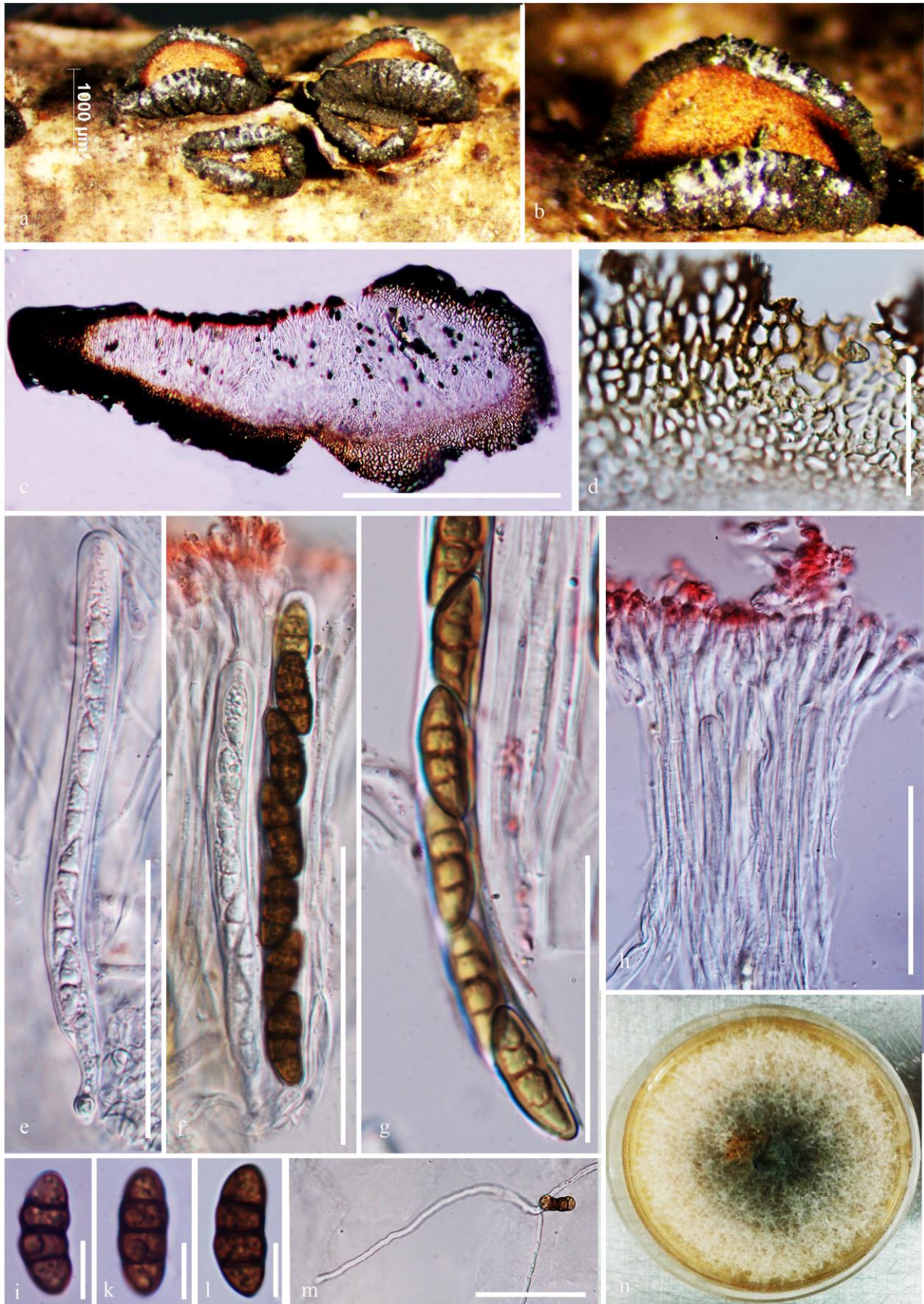
Notes – *Rhytidhysterion bruguierae* formed a separate (100% ML, 1.00 PP) and distinct lineage within the genus *Rhytidhysterion* (Fig. 7) in a clade comprising *R. thailandicum* (MFLUCC 14-0503, MFLUCC12-0530) and *R. mangrovei* (MFLUCC18-1113). *Rhytidhysterion bruguierae* shares similar morphological features with *R. mangrovei* and *R. thailandicum*. However, the colour of the epithecium in *R. bruguierae* is red, while in *R. mangrovei* and *R. thailandicum* it is mentioned only as dark (Thambugala et al. 2016, Kumar et al. 2019). The exciple of *R. bruguierae* is relatively thicker than that of *R. mangrovei* and *R. thailandicum* (148–162.8 µm vs 65–90 µm vs 72–130 µm). Hence, we compared the base pair differences of those three taxa following the guidelines of Jeewon & Hyde (2016). TEF base pair differences of *R. bruguierae* to *R. mangrovei* and *R. thailandicum* are listed in Table 2 and considered to be in the appropriate range for designation of a distinct species (Jeewon & Hyde 2016). Therefore, we establish our strain as a novel species in *Rhytidhysterion*.

**Table 2** Base pair comparison of *Rhytidhysterion bruguierae* to related taxa.

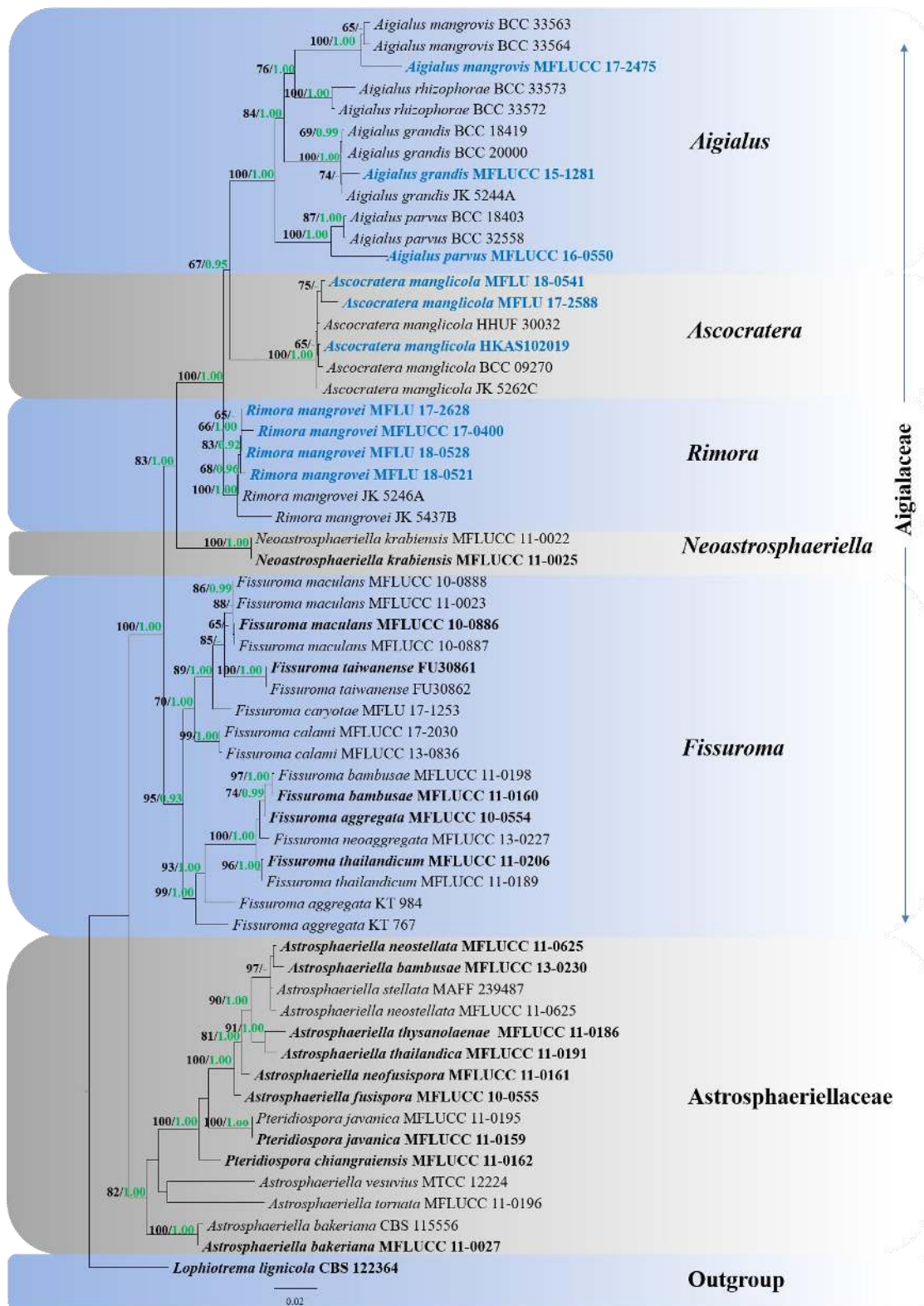
<b>Taxon</b>	<b>TEF</b>
<i>R. mangrovei</i>	3.4% (32 bp out of 932 bp)
<i>R. thailandicum</i>	3.2 % (30 bp out of 932 bp)

### **Pleosporales** Luttrell ex M.E. Barr

Luttrell (1955) introduced the order Pleosporales to accommodate Dothideomycetes with perithecial ascomata with pseudoparaphyses amongst the asci (Zhang et al. 2009). Among the orders of Dothideomycetes, Pleosporales is the most diverse and the largest that comprises more than 70 families (Wijayawardene et al. 2018). Pleosporales species are endophytes or epiphytes, parasites, lichenicolous or saprobes in terrestrial or aquatic environments including marine habitats (Zhang et al. 2009, Jones et al. 2015, Hyde et al. 2016).



**Figure 8** – *Rhytidhysterium brugiariae* (MFLU 18-0571, holotype). a, b Appearance of hysterothecia on host. c Vertical section through hysterothecium. d Exciple. e–g Young and mature asci. h Pseudoparaphyses with branched swollen reddish tips. i–l Ascospores. m Germinating ascospore. Scale bars: a = 1000 µm, c = 500 µm, d–f, h, m = 50 µm, g = 20 µm, i–k = 10 µm.



**Figure 9** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -13391.717934. The combined LSU, SSU and TEF sequence datasets comprised 59 strains of Aigialaceae and Astrosphaeriellaceae with *Lophiotrema lignicola* (CBS 122364) as the outgroup taxon. Tree topology of the ML analysis was similar to the BI and in congruent with previous study Tennakoon et al. (2018). The matrix had 947 distinct alignment patterns, with 22.69% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.246151, C = 0.245354, G = 0.282257, T = 0.226239; substitution rates AC = 1.037052, AG = 3.047760, AT = 0.967727, CG = 1.231952, CT = 10.474608, GT = 1.000000;

gamma distribution shape parameter  $\alpha = 0.177386$ . Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.02 changes. The ex-type strains are in bold and new isolates in blue bold.

### **Aigialaceae** Suetrong, Sakay., E.B.G. Jones, Kohlm., Volkm.-Kohlm. & C.L. Schoch

Suetrong et al. (2009) introduced the family Aigialaceae to accommodate species having carbonaceous ascomata that lack a papilla, trabeculate pseudoparaphyses (*sensu* Liew et al. 2000), cylindrical asci comprising an apical ring and ascospores with or without a sheath or gelatinous appendages around the apical cells (Read & Beckett 1996, Liu et al. 2011, Zhang et al. 2012, Hyde et al. 2013, Jones et al. 2015, Phookamsak et al. 2015, Tennakoon et al. 2018). Initially only marine genera, such as *Aigialus*, *Ascocratera* and *Rimora* were included in the Aigialaceae (Suetrong et al. 2009, Phookamsak et al. 2015, Wijayawardene et al. 2017a). However, later studies reported Aigialaceae species from different habitats including marine, terrestrial and mangroves (Hyde & Jones 1988, Hyde & Lee 1995, Liu et al. 2011, Phookamsak et al. 2015, Tennakoon et al. 2018). With the recent inclusion of *Fissuroma* and *Neoastrisphaeriella* (Liu et al. 2011), five genera are presently accepted in the Aigialaceae (Wijayawardene et al. 2017a, 2018). All genera in Aigialaceae have trabeculate pseudoparaphyses (*sensu* Liu et al. 2011).

### **Aigialus** Kohlm. & S. Schatz

*Aigialus*, the type genus of Aigialaceae is frequently found on mangrove wood in both tropical to subtropical regions (Suetrong et al. 2009). The genus *Aigialus* was initially accommodated in Melanommatales (*sensu* Barr 1979), based on the trabeculate pseudoparaphyses, but without assigning it to any family in the Dothideomycetes (Kohlmeyer & Schatz 1985), based on the possession of a refractive, J- apical ring in the ascus. Hawksworth et al. (1995) assigned the genus to Massariaceae in the order Pyrenulales. Liew et al. (2000) showed that trabeculate pseudoparaphyses were not important at the ordinal level. According to single gene sequence analysis, Tam et al. (2003), referred *Aigialus* to Pleosporales, and this was followed by Kirk et al. (2008). Classification of this genus is resolved with a higher number of taxon sampling of Dothideomycetes (Schoch et al. 2009, Suetrong et al. 2009, Zhang et al. 2012). There are five species accepted in the genus: *A. grandis*, *A. mangrovei*, *A. parvus*, *A. rhizophorae* and *A. striatispora* (Jones et al. 2009). During our investigation of marine mangrove fungi from Thailand we collected three species; *A. grandis*, *A. mangrovei* and *A. parvus*.

***Aigialus grandis*** Kohlm. & S. Schatz, Trans. Br. mycol. Soc. 85(4): 699 (1986) [1985] Fig. 10  
Facesoffungi number: FoF 06155

*Saprobic* on mangrove wood. Sexual morph: *Ascomata* 900–1200 × 420–1540 µm, subglobose to ellipsoidal, laterally compressed, completely or partially immersed in a black stroma, with a longitudinal furrow at the top, carbonaceous to coriaceous, black, gregarious, apapillate. *Peridium* 60–110 µm wide, coriaceous to carbonaceous, two-layered, outer layer composed of elongate cells, inner layer composed of smaller hyaline cells. *Hamathecium* comprising 1.5–2µm wide, trabeculate pseudoparaphyses, unbranched at the base, anastomosing above the asci, embedded in a gelatinous matrix. *Asci* 320–420 × 16–32 µm ( $\bar{x} = 405 \times 22.8$  µm, n = 10), 8-spored, bitunicate, fissitunicate, cylindrical, long-pedicellate, with a refractive non-amyloid, apical ring. *Ascospores* 62.6–70 × 12–18 µm ( $\bar{x} = 68 \times 17$  µm, n = 20), biseriate, ellipsoidal to broadly fusiform, muriform, 16–18 transverse septa and 1–2 longitudinal septa, dark brown except for hyaline to pale brown apical cells, glabrous, with a gelatinous cap or a mucilaginous sheath. Asexual morph: Unknown.

Culture characteristics – Colonies on MEA reaching 8 cm diam. after 2 weeks at 25 °C, dense, irregular, slightly raised, surface smooth with undulate edge, submerged, colony from above: grayish black; from below: yellowish at the margin, blackish gray at the center; mycelium hyaline.



Material examined – THAILAND, Krabi Province, Krabi, 8°25'52" N, 98°31'42" E, 0 m asl., on submerged root of *Rhizophora* sp., 7 December 2014, M.C. Dayarathne, KRB023 (MFLU 16-1241), living culture, MFLUCC 15-1281.

GenBank numbers – LSU: MN420684; SSU: MN420694

Notes – Our collection is similar to the type of *Aigialus grandis* in both morphological characters and molecular sequence data (Figs 9, 10).

*Aigialus mangrovei* Borse, Trans. Br. mycol. Soc. 88(3): 424 (1987)

Fig. 11

Facesoffungi number: FoF 06156

*Saprobic* on mangrove wood. Sexual morph: *Ascomata* 600–710 × 550–700.2 µm, subglobose, completely or partially immersed, carbonaceous to coriaceous, black, gregarious, ostiolate. *Peridium* 50–60 µm wide, coriaceous to carbonaceous, two-layered, outer layer composed of elongate cells, inner layer composed of smaller hyaline cells. *Hamathecium* comprising 0.8–1.4 µm wide, trabeculate pseudoparaphyses, unbranched at the base, anastomosing above the asci, embedded in a gelatinous matrix. *Asci* 288–400 × 20–30.4 µm ( $\bar{x}$  = 310 × 27 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical, long-pedicellate, with a refractive non-amyloid, apical ring. *Ascospores* 30–42.4 × 14–16.8 µm ( $\bar{x}$  = 35.5 × 13.1 µm, n = 20), biseriate, ellipsoidal to broadly fusiform, muriform, with 6–7 transverse septa and 1–2 longitudinal septa, dark brown, with hyaline to pale brown apical cells, glabrous, with a gelatinous cap or a mucilaginous sheath. Asexual morph: Unknown.

Culture characteristics – Colonies on PDA reaching 8 cm diam. after 2 weeks at 20–25 °C, dense, irregular, slightly raised, surface smooth with undulate edge, cottony, colony from above: white; from below: yellowish white at the margin, yellow to pale brown at the center; mycelium hyaline, not produced pigment on PDA media.

Material examined – THAILAND, Phetchaburi Province, Cha-am, on decaying *Rhizophora* sp. (Rhizophoraceae) wood, 30 August 2017, M.C. Dayarathne, MCD 127 (MFLU 17-2608); *ibid.*, HKAS102046, living culture MFLUCC 17-2475.

GenBank numbers – LSU: MN017834; SSU: MN017902

Notes – *Aigialus mangrovei* was introduced by Borse et al. (1987) from India. This fungus is also commonly found in Thailand (Suetrong unpublished data). Our strain MFLUCC 17-2475 is morphologically reminiscent of the protologue of *A. mangrovei* (Borse et al. 1987) and clustered in a clade comprising other *A. mangrovei* strains BCC 33563 and BCC 33564 (Fig. 9).

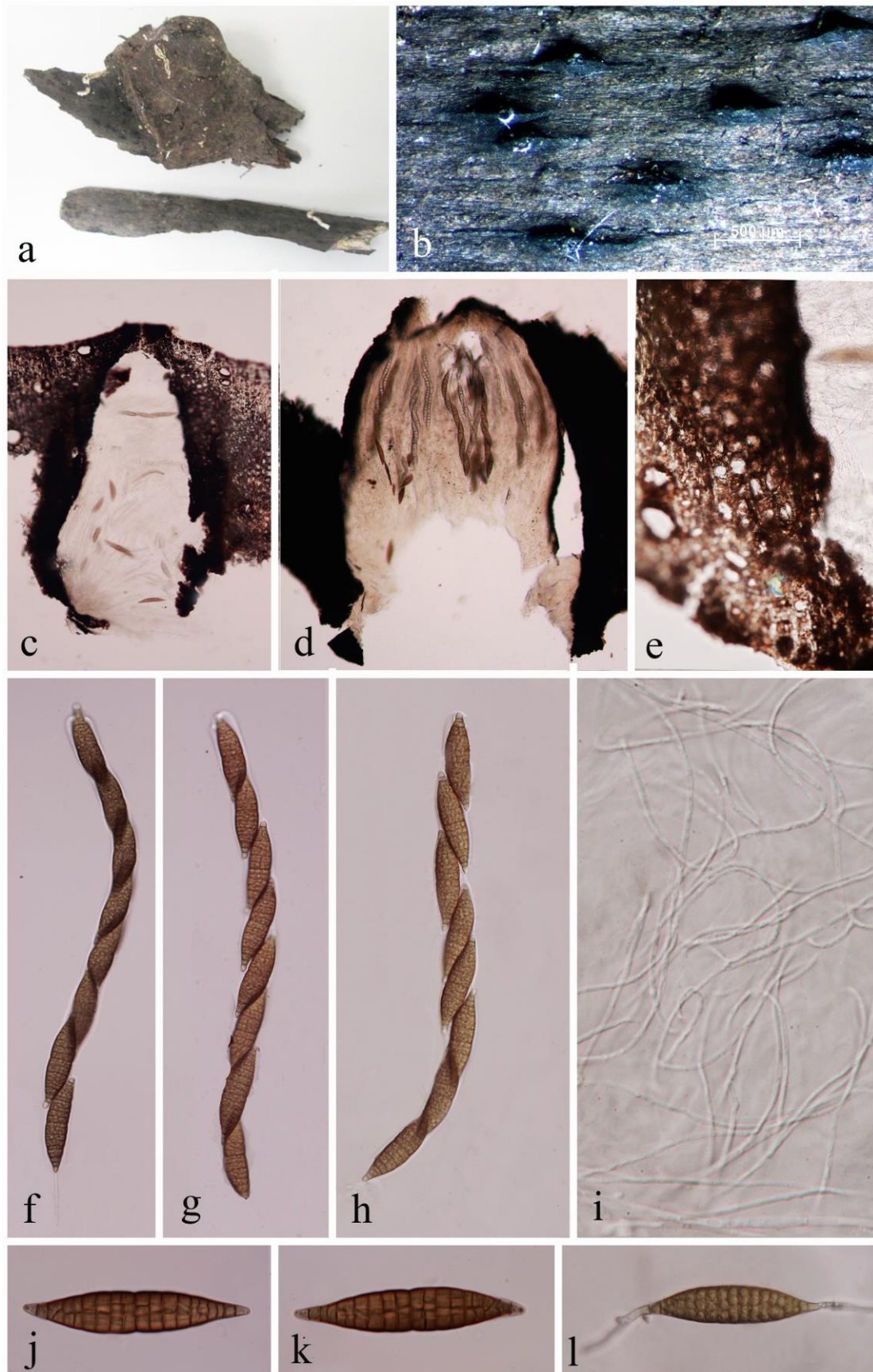
*Aigialus parvus* S. Schatz & Kohlm., in Kohlmeyer & Schatz, Trans. Br. mycol. Soc. 85(4): 704 (1986) [1985].

Fig. 12

Facesoffungi number: FoF 06201

*Saprobic* on mangrove wood. Sexual morph: *Ascomata* 500–1440 × 900–1500 µm, ellipsoidal to globose to subglobose, immersed to semi immersed, solitary to gregarious, carbonaceous, dark brown to black, ostiolate. *Peridium* 56–104 µm, carbonaceous, two-layered, outer layer composed of elongate cells, inner layer composed of smaller hyaline cells. *Hamathecium* comprising 1.2–2 µm wide, trabeculate pseudoparaphyses, unbranched at the base, extending above the asci, embedded in a gelatinous matrix. *Asci* 350–455 × 19–28 µm ( $\bar{x}$  = 400 × 24 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical, long-pedicellate, with a refractive non-amyloid, apical ring. *Ascospores* 42–56 × 12.6–16 µm ( $\bar{x}$  = 46.3 × 15.1 µm, n = 20), biseriate, ellipsoidal to broadly fusiform, muriform, with 8–12 transverse septa and 1–2 longitudinal septa, dark brown, with hyaline to pale brown apical cells, glabrous, with a gelatinous cap or a mucilaginous sheath. Asexual morph: Unknown.

Culture characteristics – Colonies on PDA reaching 6.5 cm diam. after 2 weeks at 25 °C, dense, irregular, slightly raised, surface smooth with undulate edge, submerged, colony from above: greenish black; from below: yellowish at the margin, blackish green at the center; mycelium hyaline, no pigmentation producing on PDA media.



**Figure 10** – *Aigialus grandis* (MFLUCC 15-1281). a Host. b Ascomata on host. c, d Sections of ascomata. e. Peridium. d. Trabeculate pseudoparaphyses. e–g. Immature and mature asci. j–j. Immature to mature ascospores. k. Germinating ascospore. Scale bars: b–d = 500 µm, f–h = 200 µm, e, i–l = 50 µm.



**Figure 11** – *Aigialus mangrovei* (MFLU 17-2608). a Ascomata on decaying wood. b Section of ascoma. c Peridium. d Trabeculate pseudoparaphyses. e–g Immature and mature asci. h–j Immature to mature ascospores. k Germinating ascospore. Scale bars: a = 1000 µm, b = 100 µm, c–q = 10 µm.



**Figure 12** – *Aigialus parvus* (MFLUCC 16-0550). a Ascomata on decaying wood. b, c Sections of ascomata. d. Ascus. e–g Ascospores. h Trabeculate pseudoparaphyses. i. Germinating ascospore. Scale bars: a = 1000  $\mu$ m, b = 100  $\mu$ m, c–q = 10  $\mu$ m.

Material examined – THAILAND, Phetchaburi Province, Hat Chao Samran, on submerged *Rhizophora apiculata* (Rhizophoraceae) wood, 28 July 2015, M. C Dayarathne, CHAM 008 (MFLU 16-1175), living culture MFLUCC 16-0550.

GenBank numbers – LSU: MN017835; SSU: MN017903

Notes – *Aigialus parvus* can be distinguished from other *Aigialus* species by the number of septa in ascospores, which comprises more than 7(8–12) transverse septa (Fig. 12), while *A. grandis* has more than 12 transverse septa in the ascospores and deeply constricted at the median septum. *Aigialus mangrovei* has 6–7 transversely septate and 1–2 vertically septate ascospores and *A. rhizophorae* has more than 12 transversely septate ascospores with a single vertical septum (Borse et al. 1987). *Aigialus striatisporus* is different from all of the above species by having vertical striations along the ascospore wall (Hyde 1992b, Jones et al. 2009).

### *Ascocratera* Kohlm.

The monotypic genus *Ascocratera* was established by Kohlmeyer (1986) with *A. manglicola* collected on dead wood of mangrove trees (*Avicennia germinans*, *Conocarpus erecta*, *Laguncularia racemosa* and *Rhizophora mangle*) in the Atlantic and Pacific Oceans. Kohlmeyer (1986) referred this genus to Massariaceae in Melanommatales and was subsequently studied by Suetrong et al. (2009) who accommodated this genus in the family Aigialaceae (Pleosporales).

*Ascocratera manglicola* Kohlm., Can. J. Bot. 64(12): 3036 (1986)

Fig. 13

Facesoffungi number: FoF 06157

*Saprobic* on mangrove wood. Sexual morph: *Ascomata* 752–890 × 1000–1300 µm, conical with a flat base, crater-like, erumpent from the substrate and superficial when mature, seated on a thin stroma, ostiole cells of the host enclosed in the lower part of the peridium. epapillate, carbonaceous, black, with irregular bulges or ridges around the ostiole, gregarious, single or sometimes two or more attached to each other. *Ostiole* circular, depressed, ostiolar canal filled with short hyaline branching periphyses, embedded in a gelatinous matrix. *Peridium* 84–150 µm thick on the sides, 45–75 µm, wide, composed of small irregular cells, encrusted with melanin particles, forming a *textura angularis*, cells of the host are incorporated in the peridium, especially in the basis of the lateral part; a *textura intricata*, composed of loose, thick hyaline hyphae, forms a wedge between the lateral and basal part of the peridium. *Pseudoparaphyses*: 1.5–3 µm in diam., trabeculate, septate, simple at the base and embedded in a gelatinous matrix, free at the tips, branched and anastomosing above the asci. *Asci* 300–430 × 20–28 µm ( $\bar{x}$  = 350 × 24.7 µm, n = 20), 8-spored, cylindrical, pedunculate, thick-walled, fissitunicate, arising from a basal ascogenous tissue, with a refractive apical ring in the endoascus, J-. *Ascospores* 40.8–60. × 14.2–20 µm ( $\bar{x}$  = 46 × 16.1 µm, n = 20), overlapping uni-seriate, ellipsoidal, at first one-, later three-septate, constricted at the central primary septum, hyaline, surrounded by a gelatinous evanescent sheath. Asexual morph: Unknown.

Material examined – THAILAND, Krabi Province, Phang Nga, on mangrove sp. (Rhizophoraceae) wood, 31 August 2017, M.C. Dayarathne MCD 105 (MFLU 17-2588); *ibid.*, HKAS102035. THAILAND, Krabi Province, Krabi, 8°25'52" N, 98°31'42" E, 0 m asl., on submerged root of *Rhizophora* sp., 7 December 2014, M.C. Dayarathne, KRB 012 (MFLU 19-1228); *ibid.*, HKAS102019. THAILAND, Ranong Province, Amphoe Maung, Mu 4 Tombol Ngao, Ranong Mangrove Research Center (GPS: 9°43' to 9°57'N; 98°29' to 98°39'E) on decaying wood of *Rhizophora* sp. (Rhizophoraceae), 7 December 2016, M.C. Dayarathne, MCD 056 (MFLU 18-0541); *ibid.*, HKAS102080.

GenBank numbers – LSU: MN017836, MN017837, MN017838; SSU: MN017904, MN017905, MN017906; TEF: MN077057, MN077058

Notes – *Ascocratera manglicola* is a unique genus in Aigialaceae by having carbonaceous, black, gregarious ascomata that are conical, crater-like, superficial on wood, on a black stroma, with trabeculate pseudoparaphyses, asci with a refractive apical ring, and hyaline ascospores, surrounded by a gelatinous evanescent sheath (Kohlmeyer 1986). This species has often been found

on mangrove wood in the intertidal zone, and known from various tropical localities (Schmit & Shearer 2003, Suetrong et al. 2009). Our strains MFLU 17-2588, MFLU 18-0541 and HKAS102019 nested together with other *A. manglicola* strains (HHUF 30032, BCC 09270 and JK 5262C). Our strains are mostly similar in characters to those described in the protologue (Kohlmeyer 1986).

***Rimora*** Kohlm., Volkm.-Kohlm., Suetrong, Sakay. & E.B.G. Jones

The genus *Rimora* was introduced by Suetrong et al. (2009) based on phylogeny to accommodate single species *R. mangrovei*.

***Rimora mangrovei*** (Kohlm. & Vittal) Kohlm., Volkm.-Kohlm., Suetrong, Sakay. & E.B.G. Jones, Stud. Mycol. 64: 166 (2009). Fig. 14

Facesoffungi number: FoF 06158

*Saprobic* on decaying mangrove wood. Sexual morph: *Ascomata* 213–432 × 268–535.5 µm, black, carbonaceous, gregarious, forming on a black stromatic crust, broadly oblong with a flat top in frontal view, opening with a cleft-like ostiolum at the flattened top, apapillate, immersed to erumpent or superficial. *Ostiole* cleft-like, central, periphysate. *Peridium* up to 138 µm wide, black, base comprising a mixture of fungal and host cells. *Pseudoparaphyses* up to 2 µm, trabeculate, numerous, branched. *Asci* 144–234 × 14.5–19 µm ( $\bar{x}$  = 191 × 16.6 µm, n=20), 8-spored, bitunicate, fission-tunicate, cylindrical, short pedicellate, thick-walled, without an apical apparatus. *Ascospores* 44.5–50 × 7.5–12 µm ( $\bar{x}$  = 48 × 8.5 µm, n=30), fusiform, hyaline, initially 1-septate, later becoming 3-septate, pale brown or brown, constricted at the central primary septum, biseriate, surrounded by an evanescent sheath. Asexual morph: undetermined.

Culture characteristics – Colonies on PDA reaching 8 cm diam. after 2 weeks at 25 °C, dense, irregular, slightly raised, surface smooth with circular edge, submerged, colony from above: greenish black; from below: blackish green; mycelium hyaline, not produced pigmentation on PDA media.

Material examined – THAILAND, Phetchaburi Province, Cha-am, on mangrove sp. (Rhizophoraceae) wood, 31 August 2017, M.C. Dayarathne MCD 154 (MFLU 17-2628); *ibid.*, HKAS102059. THAILAND, Ranong Province, Amphoe Maung, Mu 4 Tombol Ngao, Ranong Mangrove Research Center (GPS: 9°43' to 9°57'N; 98°29' to 98°39'E) on decaying wood of *Rhizophora* sp. (Rhizophoraceae), 07 December 2016, M.C. Dayarathne, MCD 041 (MFLU 18-0528); *ibid.*, HKAS102074, living culture MFLUCC 17-0400. THAILAND, Phetchaburi Province, Hat Chao Samran, on submerged *Rhizophora apiculata* (Rhizophoraceae) wood, 28 July 2015, M.C. Dayarathne, CHAM 031 (MFLU 18-0521).

GenBank numbers – LSU: MN017839, MN017840, MN420682, MN420683; SSU: MN017907, MN017908, MN420692, MN420693; TEF: MN077059, MN077060

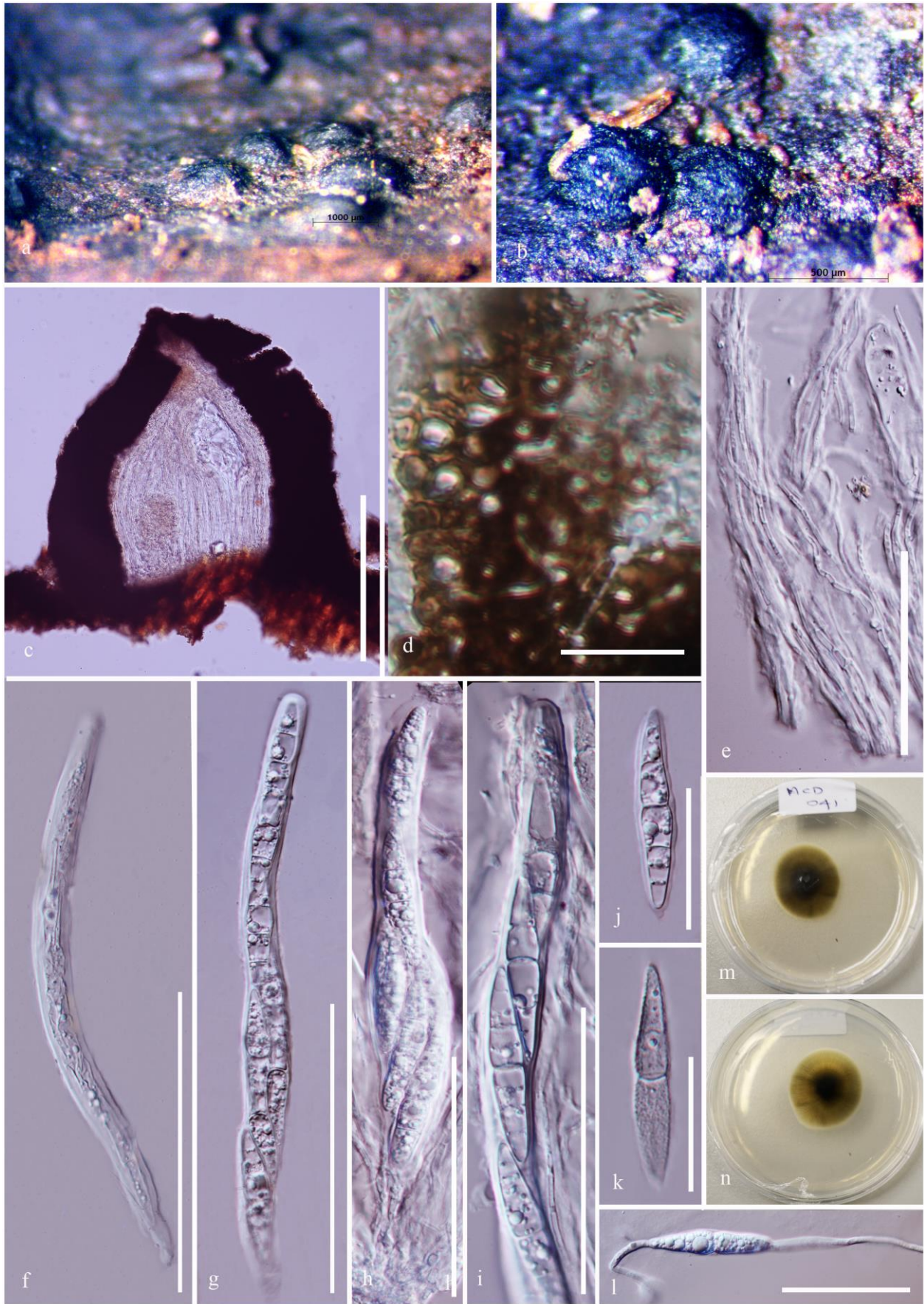
Notes – *Rimora mangrovei* was initially described from the bark and wood of mangrove trees from Belize and India by Kohlmeyer & Vittal (1986) as *Lophiostoma mangrovei*. Subsequently, Hyde et al. (2002) transferred *R. mangrovei* to *Astrosphaeriella* based on the trabeculate morphology of the pseudoparaphyses (*sensu* Liu et al. 2011). However, subsequent authors conceded that *A. mangrovei* (and *A. asiana*) differed from other *Astrosphaeriella* species by their round flattened ascomata, slit-like ostioles and dicotyledonous hosts (Liu et al. 2011). Suetrong et al. (2009) established the genus *Rimora* to accommodate this species based on its molecular phylogeny.

### **Dictyosporiaceae** Boonmee & K.D. Hyde

Dictyosporiaceae was introduced by Liu et al. (2015) and was formally accepted by Boonmee et al. (2016) along with *Dictyosporium* as the type genus. Species of Dictyosporiaceae are mostly saprobes found on decaying wood in aquatic and terrestrial habitats (Boonmee et al. 2016). Asexual morphs of this family are cheirosporous hyphomycetes (Boonmee et al. 2016). Twelve genera are currently accepted in this family (Wijayawardene et al. 2017a, 2018).



**Figure 13** – *Ascocratera manglicola* (MFLU 17-2588). a, b Ascomata on decaying wood. c Section of ascoma. d, e Asci. f Ascus apical ring. g Pseudoparaphyses. h–j Ascospores. k Germinating ascospore. Scale bars: a = 1000 µm, b = 100 µm, c–q = 10 µm.



**Figure 14** – *Rimora mangrovei* (MFLU 17-2628). a, b Ascomata on decaying wood. c Section of ascoma. d Peridium. e Pseudoparaphyses. f, i Asci. j, k Ascospores. l Germinating ascospore. m, n Cultures on PDA (m upper, n lower). Scale bars: b, c = 500  $\mu\text{m}$ , e, f–h = 100  $\mu\text{m}$ , d, i–k = 20  $\mu\text{m}$ .



## ***Dictyosporium* Corda**

*Dictyosporium* species occur on dead wood and plant litter in terrestrial and aquatic habitats (Ho et al. 2002, Pinnoi et al. 2006, Pinruan et al. 2007, Tibpromma et al. 2018). *Dictyosporium* was established by Corda (1836) with *D. elegans* as the type species. Members of this genus are saprobes mostly in terrestrial or aquatic environments (Hyde et al. 2011), while *D. pelagicum* has been reported from marine habitats (Jones et al. 2015). In this study, a novel species *Dictyosporium marinum* is described from a decaying wood buried in beach sand in the UK.

### ***Dictyosporium marinum* Dayarathne & E.B.G. Jones, sp. nov.**

Fig. 16

Index Fungorum number: IF556575; Facesoffungi number: FoF 06159

Holotype – MFLU 19-1229

*Saprobic* on buried wood in sandy beach. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. *Conidiomata* sporodochial on natural substrate in small groups, dark brown, with base attached on surface of substrate. *Conidiophores* micronematous, reduced to conidiogenous cell. *Conidiogenous cells* 1.5–3 × 1–2 µm ( $\bar{x}$  = 2.7 × 2.4 µm, n = 5), holoblastic, cylindrical, pale hyaline to light brown. *Conidia* 34–50 × 22–28 µm ( $\bar{x}$  = 43.5 × 24.5 µm, n = 20), solitary, oval to ellipsoid, cheiroid, not complanate, consisting of 35–40 cells arranged in 4–5 rows, with a basal conical shaped connecting cell, not easy to separate, brown, guttulate, subhyaline at the tip of peripheral rows, smooth-walled.

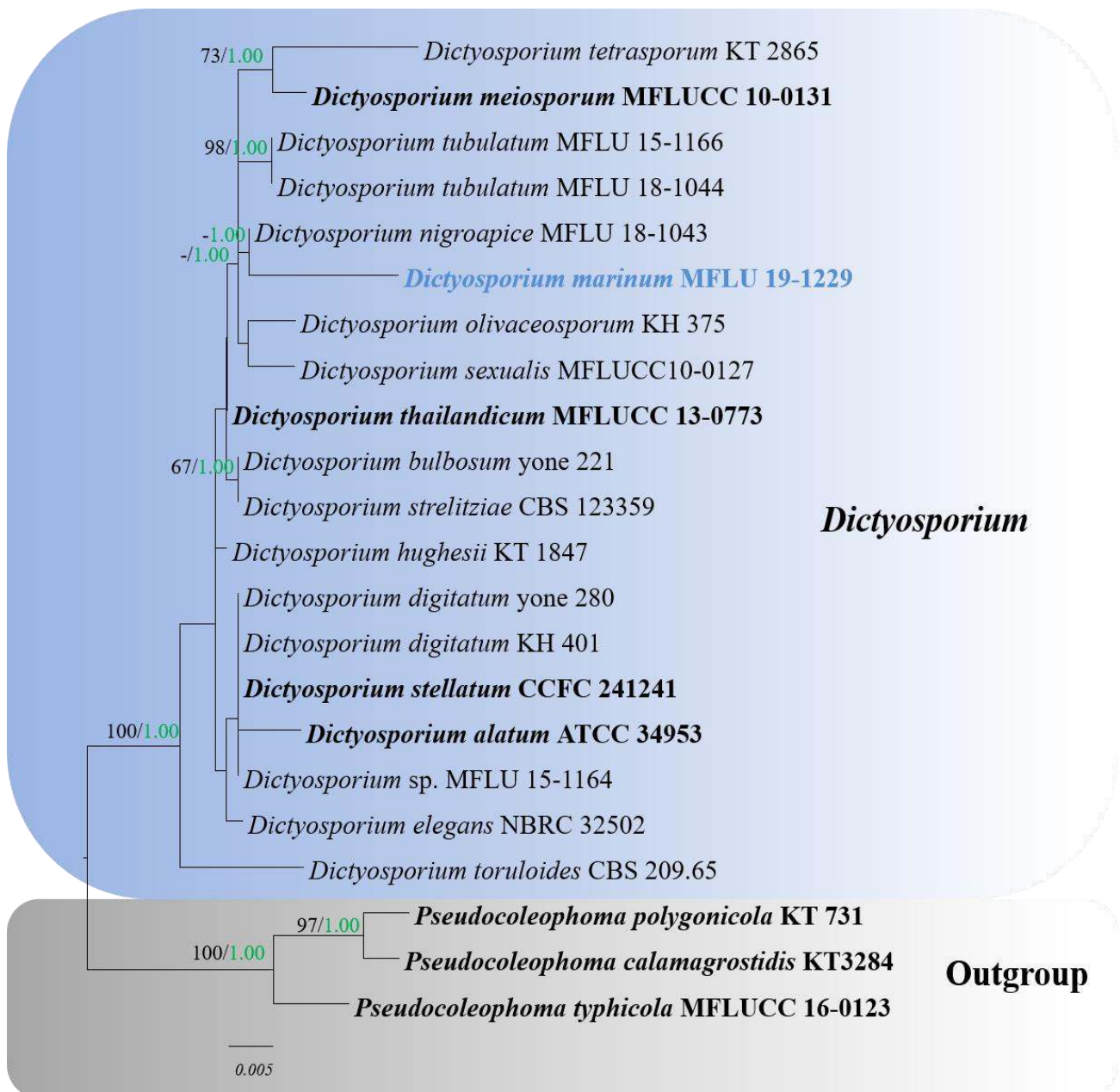
Material examined – UK, Carmarthenshire, Llanstephan beach, on buried wood in sand, 12 May 2017, E.B. Gareth Jones, GJ357 (MFLU 19-1229, holotype); *ibid.*, (BBH, isotype).

GenBank numbers – LSU: MN017841

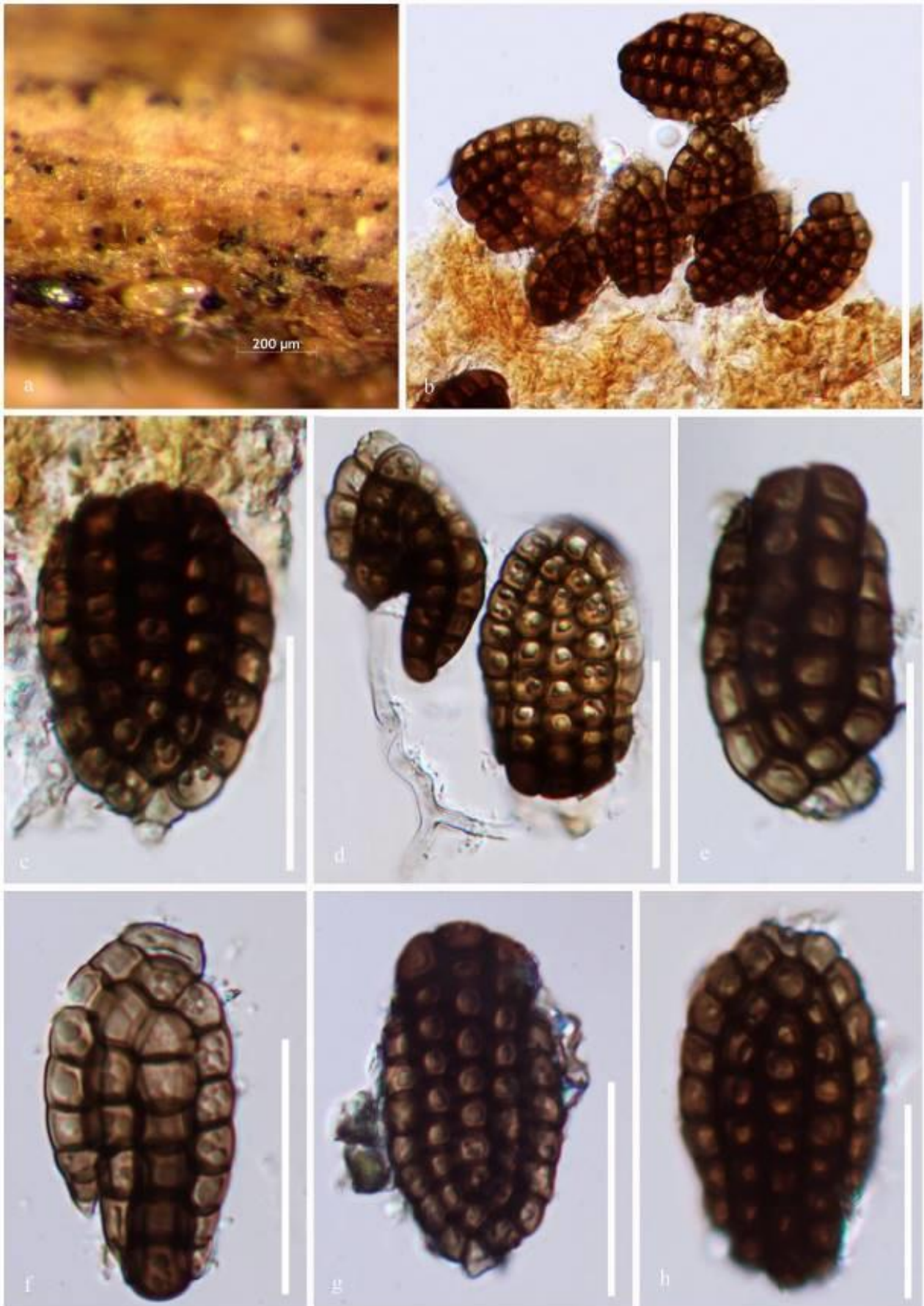
Notes – *Dictyosporium marinum* was isolated from buried wood in sand. It is characteristic of the genus *Dictyosporium*, such as having sporodochial colonies, micronematous to macronematous conidiophores and cheiroid, digitate complanate conidia with several parallel rows of cells (Goh et al. 1999). *Dictyosporium marinum* shows a close phylogenetic affinity to *Dictyosporium nigroapice* which was isolated from wood of *Machilus velutina* submerged in streams in Hong Kong (Fig. 15). Base pair difference between *D. marinum* and *D. nigroapice* is 1.5 % (786/798) of LSU. Furthermore, the two species are morphologically distinct especially in their conidial morphology. Conidia of *D. nigroapice* are narrower (15–20 µm) and comprise outer rows with a hyaline appendage (Goh et al. 1999), whereas *D. marinum* has relatively wider conidia (22–28 µm) that lacks appendages. A number of *Dictyosporium* species have been reported from the marine habitats including *D. pelagicum*, *D. inflatum* and *D. toruloides* (Kirschner et al. 2013, Jones et al. 2015). *Dictyosporium marinum* is different from *D. pelagicum* mainly by the conidial morphology, whereas conidia of *D. marinum* are larger than that of *D. pelagicum* (34–50 × 22–28 µm vs 12–66 × 9–28 µm) (www.marinfungi.org). Furthermore, cells of *D. marinum* are arranged in 4–5 rows, while cells of *D. pelagicum* are initially in three and become 3–8 irregular rows (www.marinfungi.org). We could not obtain any other gene sequences apart from LSU data for this species. We establish this collection as a novel species based on morphological differences and LSU phylogeny.

## **Didymellaceae Gruyter et al.**

The family Didymellaceae was introduced by De Gruyter et al. (2009) to accommodate *Didymella*, *Phoma* and phoma-like genera. Twenty-seven genera are currently assigned to Didymellaceae based on morphology and phylogenetic analyses (Chen et al. 2015, Jayasiri et al. 2017, Thambugala et al. 2017, Wijayawardene et al. 2018). Species of this family are known from a broad range of environments while the majority of Didymellaceae are economically important plant pathogens causing leaf and stem spots. *Didymella* and *Leptosphaerulina* species have previously been reported from marine habitats (Jones et al. 2015, Thambugala et al. 2017, Valenzuela-Lopez et al. 2018).



**Figure 15** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -1710.267074. LSU sequence dataset comprised 22 strains of Dictyosporiaceae and *Pseudocoleophoma calamagrostidis* (KT 731), *P. polygonicola* (KT 3284) with *P. typhicola* (MFLUCC 16-0123) as the outgroup taxon. Tree topology of the ML analysis was similar to the BI and in congruent with previous study Tribpromma et al. (2018). The matrix had 93 distinct alignment patterns, with 6.90% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.249746, C = 0.212290, G = 0.312691, T = 0.225274; substitution rates AC = 4.557369, AG = 7.848767, AT = 3.376704, CG = 0.304332, CT = 10.397771, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.020000. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.005 changes. The ex-type strains are in bold and new isolates in blue bold.



**Figure 16** – *Dictyosporium marinum* (MFLU 19-1229, holotype). a Colonies on submerged wood. b–d Conidia and conidiophores. e–h Conidia. Scale bars: a = 200 µm, e = 50 µm, c–h = 20 µm.

**Boeremia** Aveskamp, Gruyter & Verkley

Aveskamp et al. (2010) determined the boundaries of Didymellaceae and redefined the genera *Epicoccum*, *Peyronellaea* and *Stagonosporopsis*, and established the genus *Boeremia*. The genus *Boeremia* represents the species that are most similar to *Phoma exigua* (Aveskamp et al. 2010), but it has been phylogenetically shown that they are two distinct groups (Aveskamp et al. 2010, Chen et al. 2017). Identification of *Boeremia* species, and varieties of *Boeremia exigua*, are mainly based on host association (Berner et al. 2015) while Jayawardena et al. (2019) raised the most of *Boeremia exigua* varieties up to species level. There are no previous records of *Boeremia* from *Artemisia maritima* or any marine based habitats. We herein introduce a new species *Boeremia maritima* from a marine based habitat.

**Boeremia maritima** Dayarathne & E.B.G. Jones, sp.nov.

Fig. 18

Index Fungorum number: IF556576; Facesoffungi number: FoF 06160

Holotype – MFLU 19-1230

*Saprobic* on *Artemisia maritima*. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 140–280 × 100–260 µm, pycnidial conidiomata variable in shape and size, mostly globose to subglobose, glabrous, solitary. Ostioles apappillate. *Pycnidial wall* 8–14.8 µm wide, pseudoparenchymatous, comprising 2–3 cell layers of brown pigmented cells becoming light brown toward inner. *Conidiogenous cells* ca. 3–5.5 × 2–3.2 µm, phialidic, hyaline, simple, smooth, ampulliform to doliiform. *Conidia* 6–10.3 × 2–4 µm, ellipsoidal to oblong, hyaline, thin walled, smooth, 0–1-septate, with small guttules.

Culture characteristics – Colonies on PDA reaching 50–75 mm diam after 14 days, irregular. Immersed mycelium completely covered by a compact aerial mat, surface smooth, floccose, with fimbriate margins, upper smoke-grey with some mouse-grey zones; reverse black. Phylogenetic results obtained in the present study reveal genetic similarity between the present strain and the *Boeremia exigua* species complex.

Material examined – SWEDEN, Bohuslän, Tjärnö par., Koster Islands, Sydkoster, 0.7 km NW of Ekenäs, Rörvik, 58°53′54″; 11°02′23″, on shore line, subject to sea spray on *Artemisia maritima*, 20 June 2017, E.B. Gareth Jones, GJ 389b (MFLU 19-1230, holotype); *ibid.*, (BBH, isotype), ex-type living culture (MFLUCC 18-0411).

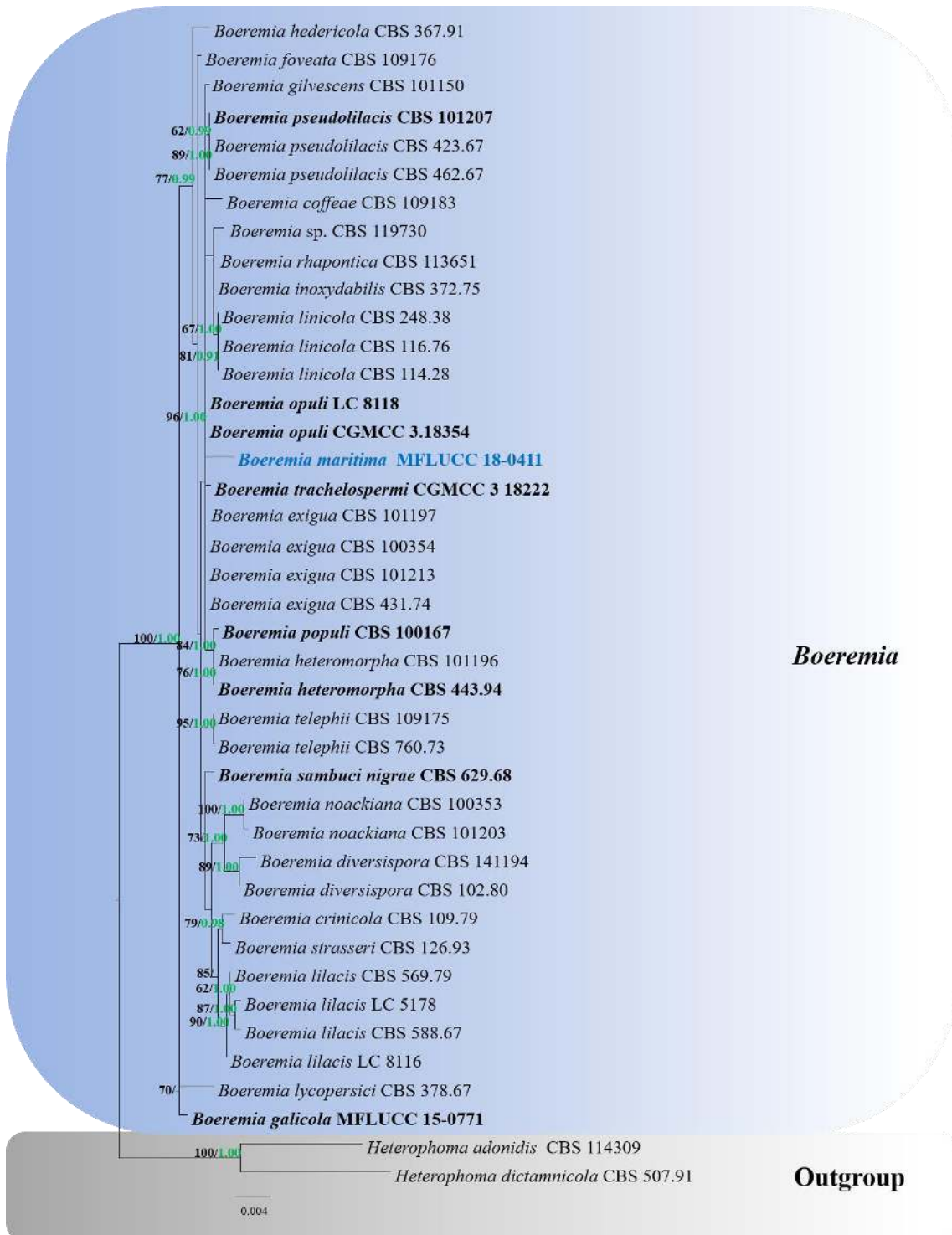
GenBank numbers – LSU: MN017842; ITS: MN047082; BTUB: MN431491

Notes – *Boeremia maritima* is similar to the *B. exigua* in having mostly globose to subglobose, pycnidial conidiomata, apappillate ostioles, phialidic, ampulliform to doliiform conidiogenous cells and ellipsoidal to oblong, 0–1-septate conidia with guttules (Boerema et al. 2004, Van der Aa et al. 2000, De Gruyter & Boerema 2002, Aveskamp et al. 2010). The new strain shows close phylogenetic affinities to *Boeremia opuli* (Fig. 17) and pairwise sequence alignment of LSU, ITS and BTUB reveals a difference of 6 bp (out of 1000 bp), 2 bp (out of 486 bp) and 3 bp (out of 330 bp) base pairs respectively. *Boeremia maritima* can be distinguished from *Boeremia opuli* by the measurements of conidiomata, conidiogenous cells and conidia (140–280 × 100–260 µm vs 245–360 × 200–305 µm, 3–5.5 × 2–3.2 µm vs 4–9(–10) × 4–7.5 µm, 6–10.3 × 2–4 µm vs 5.5–9.5 × 2.5–4 µm) (Chen et al. 2017). *Boeremia exigua* has a wide host range, while some species often appear to be host specific to a certain group of plants, such as *Coffea arabica* (Rubiaceae), *Forsythia* hybrids (Oleaceae), *Nerium oleander* and *Vinca* spp. (Apocynaceae), *Linum usitatissimum* (Linaceae), *Populus* and *Salix* (Salicaceae), and *Viburnum* spp. and occasionally *Lonicera* sp. (Caprifoliaceae) (Boerema et al. 2004, Van der Aa et al. 2000, De Gruyter & Boerema 2002, Aveskamp et al. 2010, Chen et al. 2017). *Boeremia exigua* has previously been reported from *Artemisia* species while *B. exigua* has been found from a marine habitat earlier (Jones et al. 2019).

**Neocamarosporiaceae** Wanas., Wijayaw., Crous & K.D. Hyde

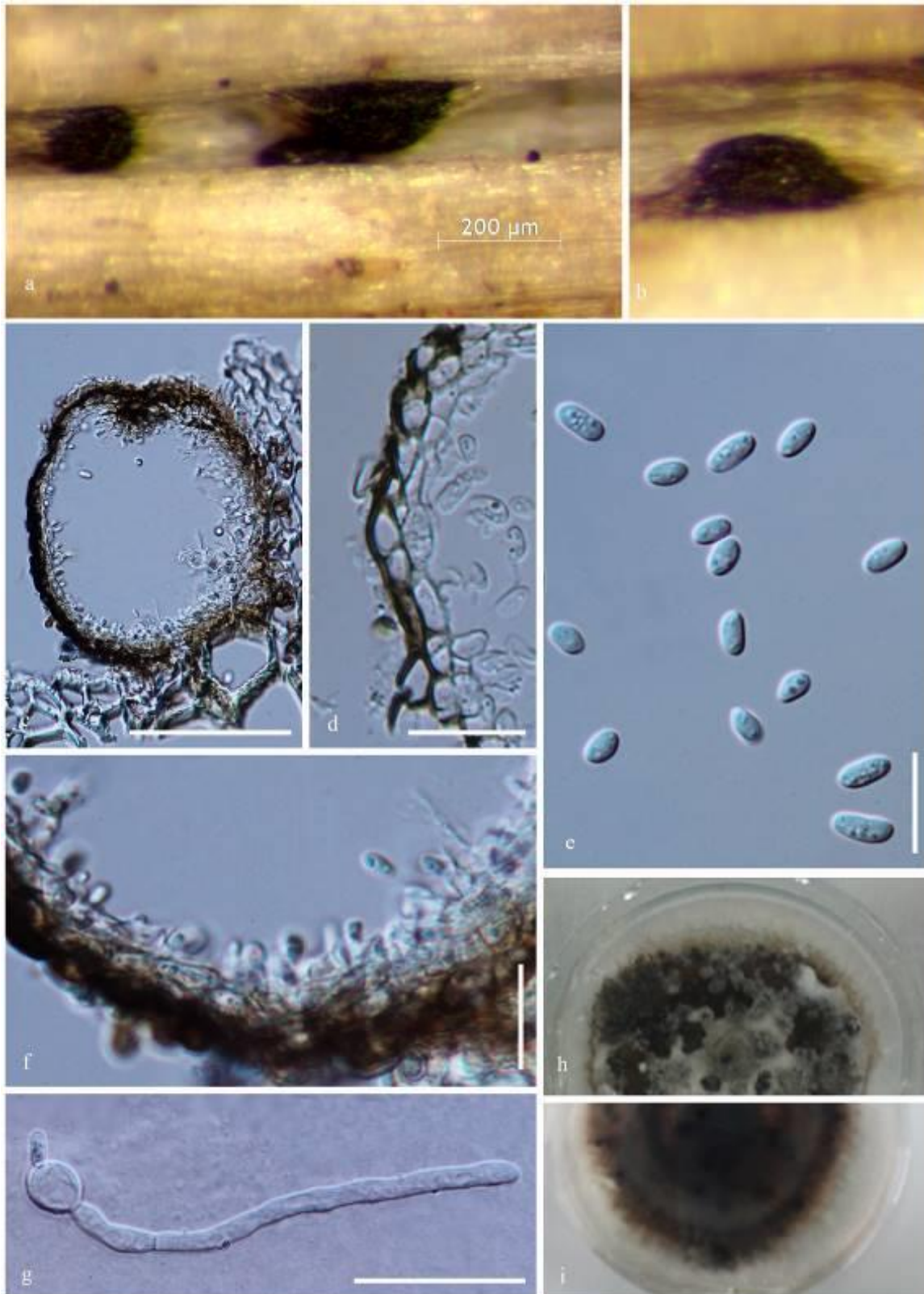
The family Neocamarosporiaceae was introduced by Wanasinghe et al. (2017) which comprises the genera *Dimorphosporicola* and *Neocamarosporium*. Here we introduce two new

*Neocamarosporium* marine species; *N. artemisiae* and *N. maritimae* which were collected from Sweden.



**Figure 17** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -4071.792778. The combined LSU, ITS and BTUB sequence datasets comprised 41 strains of *Boeremia* and related taxa with *Heterophoma adonidis* (CBS 114309) and *H. dictamnica* (CBS 507.91) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 141 distinct alignment patterns, with 9.23% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.242756, C = 0.237654, G = 0.269612, T = 0.249978; substitution rates AC = 1.780840, AG = 2.011469, AT = 1.626630, CG = 0.439694, CT = 9.798781, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.020000. Maximum

likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.004 changes. The ex-type strains are in bold and new isolates in blue bold.



**Figure 18** – *Boeremia maritima* (MFLU 19-1230, holotype). a, b Pycnidial conidiomata on host. c Section through conidioma. d Conidiomal wall. e Conidia. f Conidia attached to the conidiogenous

cells. g Germinating conidium. h, i Colonies on PDA (h-upper, i-lower). Scale bars: a, c = 200  $\mu\text{m}$ , g = 50  $\mu\text{m}$ , e, f = 20  $\mu\text{m}$ , d = 10  $\mu\text{m}$ .

***Neocamarosporium*** Crous & M.J. Wingf.

*Neocamarosporium* was introduced to accommodate *Neocamarosporium goegapense* collected from South Africa, that morphologically resembles *Camarosporium* (Crous et al. 2014). In a recent study, Wanasinghe et al. (2017) expanded this genus to include its sexual morphs. Currently there are 18 accepted species in *Neocamarosporium* (Wanasinghe et al. 2017).

***Neocamarosporium artemisiae*** Dayarathne & E.B.G. Jones, sp. nov.

Fig. 20

Index Fungorum number: IF556577; Facesoffungi number: FoF 06161

Etymology – Epithet derived from the host genus *Artemisia*.

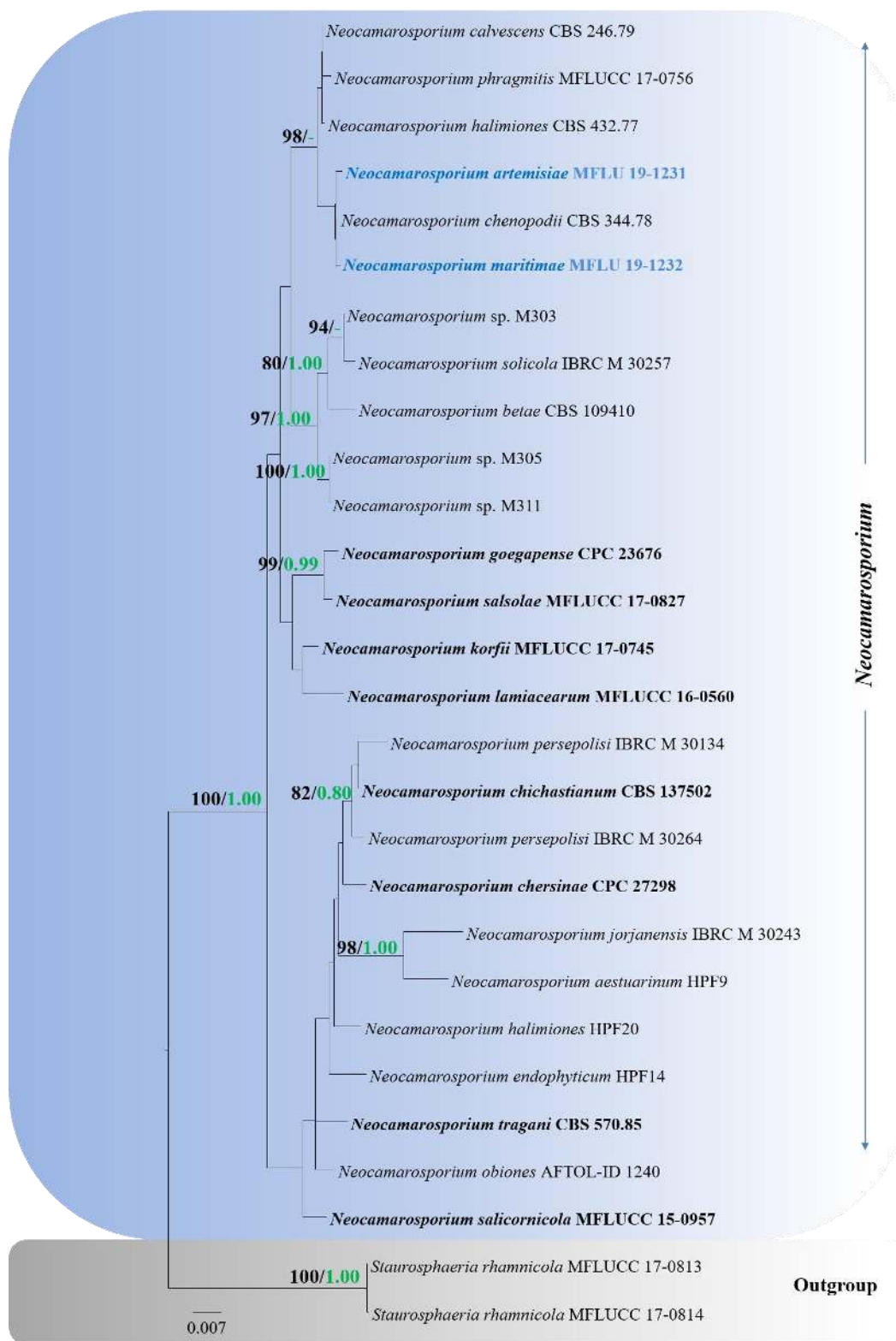
Holotype – MFLU 19-1231

*Saprobic* on woody branches. Asexual morph: *Conidiomata* pycnidial, 125–150  $\times$  200–225  $\mu\text{m}$  ( $\bar{x}$  = 138  $\times$  220  $\mu\text{m}$ , n = 10), solitary to gregarious, black, superficial, unilocular, with a papillate ostiole. *Pycnidial wall* 35–45  $\mu\text{m}$  wide, multi-layered, thick, comprising 4–6 layers, outer layer heavily pigmented, thick-walled, comprising blackish to dark brown cells of *textura angularis*, with setae 80–100  $\times$  3–5  $\mu\text{m}$ , cells towards the inside lighter, inner layer composed of 2–3 layers, hyaline, thin-walled cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, annellidic, doliiform, integrated, solitary, hyaline, smooth-walled, and formed from the inner layer of pycnidium wall. *Conidia* 12–16  $\times$  3–6  $\mu\text{m}$  ( $\bar{x}$  = 14  $\times$  4.5  $\mu\text{m}$ ; n = 30), oblong, straight to slightly curved, rounded at both ends, aseptate when immature becoming 1-septate, smooth-walled, initially hyaline, becoming yellowish brown at maturity. Sexual morph: Undetermined.

Material examined – SWEDEN, Bohuslän, Tjärnö par., Koster Islands, Sydkoster, 0.7 km NW of Ekenäs, Rörvik, 58°53'54''; 11°02'23'', on shore line, subject to sea spray on *Artemisia maritima*, 20 June 2017, E.B. Gareth Jones, GJ 392 (MFLU 19-1231, holotype); *ibid.*, (BBH, isotype).

GenBank submissions: LSU: MN017843; SSU: MN017910; ITS: MN047083; TEF: MN077062

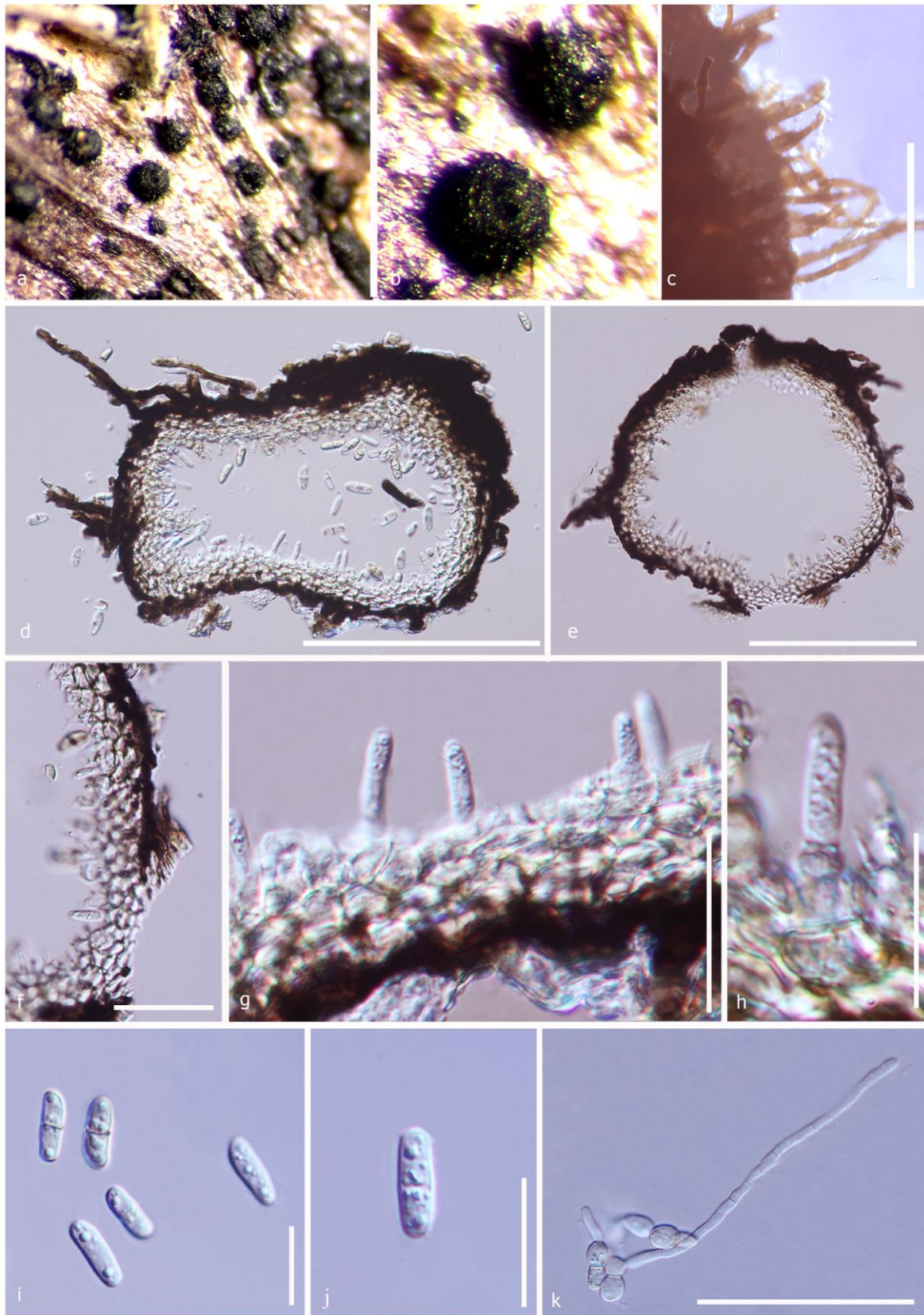
Notes – Phylogenetic results of the novel taxon *Neocamarosporium artemisiae* (MFLU 19-1231) shows it grouped in a clade comprising *N. calvescens* (CBS 246.79), *N. chenopodii* (CBS 344.78), *N. halimiones* (CBS 432.77), *N. obiones* (CBS 432.77), *N. phragmitis* (MFLUCC 17-0756) and *N. maritimae* sp. nov. with low statistical support (Fig. 19). However, the basepair differences of ITS of *N. artemisiae* to *N. obiones* and *N. phragmitis* are 0.81% (4 bp out of 489 bp) and 3.0% (16 bp out of 522 bp), respectively. While TEF base pair difference of *N. artemisiae* to *N. phragmitis* is 1.3% (12 bp out of 897 bp). There are no ITS and TEF DNA sequence data for *N. calvescens* and *N. chenopodii* for comparison. Asexual morphs of *Neocamarosporium* mostly produce muriform conidia, however, ascochyta-like and phoma-like asexual morphs have also been reported (Wanasinghe et al. 2017). Apart from our new strain, an asexual morph is only known from *N. obiones* among members of this clade. Conidial characteristics of *N. obiones*, such as, shape, colour and septation are similar to those of *N. artemisiae* (Verkley et al. 2014). However, the conidia of *N. obiones* are shorter (8–)9–12(–14)  $\mu\text{m}$  than those of *N. artemisiae* (12–16  $\mu\text{m}$ ). Furthermore, conidiomata of the new taxon comprises setae that are 80–100  $\mu\text{m}$  in length, a character not observed in other *Neocamarosporium* species. Several *Neocamarosporium* taxa have been reported from marine to saline habitats while only *N. obiones* and *N. salicorniicola* have been identified to species level (Wanasinghe et al. 2017). Only 6 bp out of 896 bp of TEF loci are different (0.7%) in between newly introducing *N. artemisiae* and *N. maritimae* species while, there are no base pair differences in ITS regions.



**Figure 19** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -7374.727686. The combined LSU, SSU, ITS and TEF sequence datasets comprised 28 strains of Neocamarosporiaceae and related taxa with *Staurosphaeria rhamnocola* (MFLUCC 17-0813) and (MFLUCC 17-0814) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 346 distinct alignment patterns, with 36.49% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.244178, C = 0.240851, G = 0.266682, T = 0.248290; substitution rates AC = 1.508884, AG = 1.935890, AT = 1.274607, CG = 0.778421, CT = 6.799020, GT = 1.000000; gamma distribution shape parameter  $\alpha$



= 0.020000. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.008 changes. The ex-type strains are in bold and new isolates in blue bold.



**Figure 20** – *Neocamarosporium artemisiae* (MFLU 19-1231, holotype). a, b Appearance of ascomata on host. c Setae. d, e Sections through pycnidial conidiomata. f Pycnidial wall. g, h

Conidia attached to the conidiogenous cells. i–k Conidia. l Germinating conidia. Scale bars: c–e = 100 µm, l = 50 µm, f–h = 20 µm, i–k = 10 µm.

*Neocamarosporium maritimae* Dayarathne & E.B.G. Jones, sp. nov.

Fig. 21

Index Fungorum number: IF556578; Facesoffungi number: FoF 06162

Etymology – Epithet derived from the host habitat epithet *maritima*.

Holotype – MFLU 19-1232

*Saprobic* on *Artemisia maritima* subjected to sea spray. Asexual morph: Undetermined. Sexual morph: *Ascomata* 200–250 µm high, 180–200 µm diam ( $\bar{x}$  = 240.1 × 190.4 µm, n = 5), black, superficial to semi-immersed, confluent, gregarious, cupulate when dry, globose, uniloculate, with an apapillate ostiole. *Ostiole* central, short, slightly sunken, minute and inconspicuous at the surface, smooth, ostiolar canal filled with hyaline cells. *Peridium* 20–35 µm wide, thin, comprising 2–3 layers, reddish brown to brown, cells towards the inside lighter, flattened, thin walled cells of *textura angularis*. *Hamathecium* comprising numerous, 2–3.6 µm (n = 40) wide, filamentous, branched, septate, pseudoparaphyses. *Asci* 75–100 × 11–16 µm ( $\bar{x}$  = 88.5 × 14 µm, n = 40), 8-spored, bitunicate, fissitunicate, cylindrical-clavate to cylindrical, pedicellate, apex rounded with a minute ocular chamber. *Ascospores* 12–20 × 6–8 µm ( $\bar{x}$  = 18 × 8.4 µm, n = 30), overlapping uniseriate, muriform, mostly ellipsoidal, with 3 transversely septate, with one longitudinal septum, deeply constricted at the middle septum, slightly constricted at remaining septa, initially hyaline, becoming yellowish brown at maturity, upper part wider than lower part, slightly paler, rounded at both ends, conical at lower end, surrounded by a mucilaginous sheath.

Material examined – SWEDEN, Bohuslän, Tjärnö par., Koster Islands, Sydkoster, 0.7 km NW of Ekenäs, Rörvik, 58°53'54''; 11°02'23'', on shore line, subject to sea spray on *Artemisia maritima*, 20 June 2017, E.B. Gareth Jones, GJ 389a (MFLU 19-1232 holotype); *ibid.*, (BBH, isotype).

GenBank submissions: LSU: MN017844; SSU: MN017911; ITS: MN047084; TEF: MN077061

Notes – *Neocamarosporium maritimae* best fits *Neocamarosporium* by having superficial to semi-immersed, confluent, gregarious ascomata with an apapillate ostiole, a hamathecium comprising numerous, filamentous, branched septate, pseudoparaphyses, cylindrical-clavate to cylindrical asci with an apex rounded with a minute ocular chamber, muriform ascospores that deeply constricted at the middle septum and slightly constricted at remaining septa, with a mucilaginous sheath (Wanasinghe et al. 2017). *Neocamarosporium maritimae* is phylogenetically closely related to *N. chenopodii* in our phylogenetic analyses with combined ITS, LSU, SSU and TEF data (Fig. 19) However, *N. maritimae* can be clearly distinguished from *N. chenopodii* by having muriform ascospores with 3-transverse septa and a one longitudinal septum whereas, *N. chenopodii* has only 3-transverse septa and lacks longitudinal septa (Ellis & Kellerm 1888). There are no ITS and TEF data generated for *N. chenopodii* (CBS 344.78) to compare the base pair differences following the guidelines of Jeewon & Hyde (2016). However, there are no base pair differences in LSU and SSU regions of those two species. *Neocamarosporium maritimae* shares morphological similarities to *N. lamiacearum* (Wanasinghe et al. 2017) by the presence of cylindrical-clavate to cylindrical asci and muriform ascospores. They can easily be distinguished by asci and ascospore measurements. Asci of *N. maritimae* are shorter than those of *N. lamiacearum* (75–100 µm vs 100–120 µm) and ascospores of *N. maritimae* are narrower than those of *N. lamiacearum* (6–8 µm vs 8–11 µm) (Wanasinghe et al. 2017). *Neocamarosporium calvescens* (= *Sphaeria calvescens*) also have similar morphologies to *N. maritimae*. *Neocamarosporium calvescens* can be clearly distinguished from *N. maritimae* in having ascomata covered with a velvety tomentum of stiff brown hyphae (Wehmeyer 1961).

### **Nigrogranaceae** Jaklitsch & Voglmayr

Jaklitsch & Voglmayr (2016) introduced the family Nigrogranaceae to accommodate the genus *Nigrograna* based on morphology and phylogeny.

### ***Nigrograna*** Gruyter, Verkley & Crous

*Nigrograna* was introduced by Jaklitsch & Voglmayr (2016) as a monotypic genus comprising the asexual species, *N. mackinnonii*. Ahmed et al. (2014) showed the sequence similarity of *N. mackinnonii* to the isolate labelled *Biatriospora marina* (type), hence, *Nigrograna* was considered a synonym of *Biatriospora* (Jaklitsch & Voglmayr 2016). Subsequently, four plant endophytic species (*B. antibiotica*, *B. carollii*, *B. peruviansis* and *B. yasuniana*) with unknown sexual states were introduced by Kolařík et al. (2017). Jaklitsch & Voglmayr (2016) observed several sexual morph strains that were phylogenetically related to *N. mackinnonii*, but produced entirely morphologically different ascospores from *Biatriospora marina*. Therefore, they questioned the validity of the *B. marina* strain used by Ahmed et al. (2014) and recognized that these two genera are distinct (Kolařík 2018). Following this generic concept, Tibpromma et al. (2017) introduced new sexual species, *N. cangshanensis*, as a saprobe on decaying wood in China. Phylogenetic analyses based on ITS DNA sequence data by Kolařík et al. (2017) showed that the species described by Kolařík et al. (2017), Borman et al. (2016), Jaklitsch & Voglmayr (2016), Travadon et al. (2016) and Tibpromma et al. (2017) formed distinct phylogenetic lineages. Therefore, this genus currently comprises 12 species (Kolařík 2018, Index Fungorum 2019). In this study, we are introducing two novel species to this genus from marine based habitats.

***Nigrograna samueliana*** Devadatha, V.V. Sarma & E.B.G. Jones sp. nov.

Figs 23, 24

Index Fungorum number: IF555779; Facesoffungi number: FoF 06163

Etymology – Specific epithet in honor of Dr. Samuel P. Meyers for his great contributions to marine mycology.

Holotype – AMH-10018

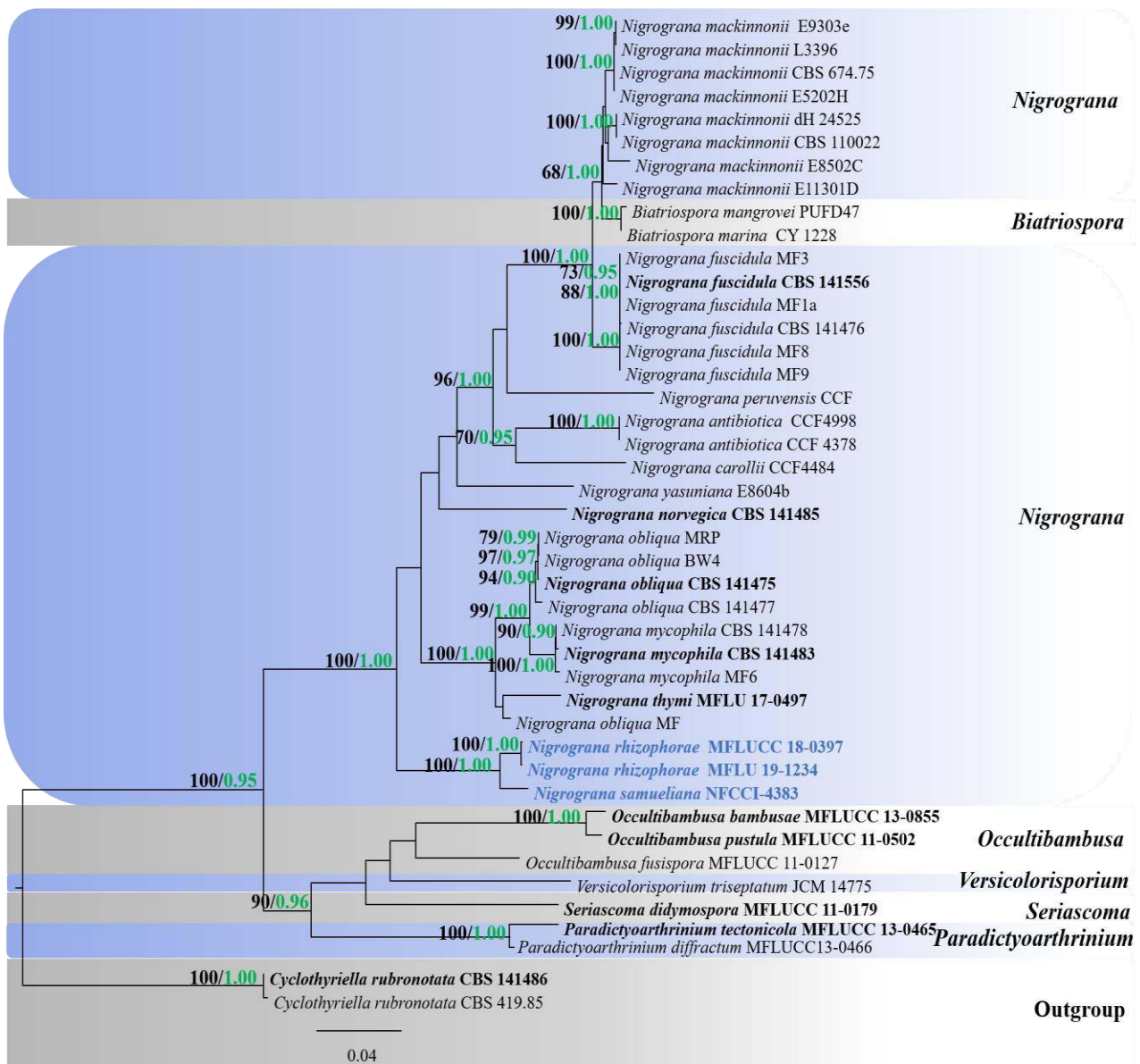
*Saprobic* on decaying mangrove wood. Sexual morph *Ascomata* 200–520 × 120–320 µm ( $\bar{x}$  = 340 × 230 µm, n = 5), sub-globose to oval, semi-immersed to immersed in bark and wood, black spots on host surface, subiculate, papillate, ostiolate, aggregated in small groups, brown to black often associated with pycnidia. *Ostirole* 75–120 × 60–80 µm ( $\bar{x}$  = 99 × 70 µm, n = 5), central, apex rounded. *Peridium* 15–35 µm wide ( $\bar{x}$  = 25 µm, n = 5), comprising several layers of thick-walled cells of *textura angularis*, brown. *Hamathecium* 1–2 µm wide, pseudoparaphyses, dense, filamentous, septate, branched, anastomosing between and above the asci, embedded in a mucilaginous matrix. *Asci* 65–75 × 6–8 µm ( $\bar{x}$  = 68 × 6 µm, n = 20), 8-spored, bitunicate, fissitunicate, thick-walled, clavate to cylindrical-clavate, short pedicellate, apically rounded with a small ocular chamber. *Ascospores* 9–13 × 2–4 µm ( $\bar{x}$  = 10 × 2 µm, n = 25), uniseriate, overlapping at the apex of asci, fusoid to ellipsoidal, with upper cells slightly wider than lower cells, straight, thick-walled, outer walls verrucose, initially reddish brown, becoming pale greyish-brown at maturity, 1–2 septate when young, becoming 3-septate with age, highly pigmented and constricted at the median septum in contrast to other septa. Asexual morph Coelomycetous on the natural host: *Pycnidia* similar to ascomata, internally white, but smaller, 95–175 × 125–300 µm ( $\bar{x}$  = 120 × 207 µm, n = 5), globose to sub-globose, immersed. *Pycnidial wall* 25–60 µm wide ( $\bar{x}$  = 35 µm, n = 5), pseudoparenchymatous, brown cells. *Conidiophores* 10–30 × 1–2.5 µm ( $\bar{x}$  = 20 × 1.5 µm, n = 10), arising from the wall in palisadic arrangement, filamentous, septate, hyaline, unbranched, with a terminal phialide. Solitary minute pegs and lageniform to sub-cylindrical phialides are arranged apically on the conidiophores. *Conidia* 2–4 × 1–2 µm ( $\bar{x}$  = 3 × 1.3 µm, n = 15), oblong to cylindrical, unicellular with 1–2 small granules, hyaline, smooth conidia are produced from the terminal ends of the conidiophores.

Culture characteristics – Ascospores germinating on 50% sea water agar within 24 hours, with germ tubes developed from both end cells and lateral sides of ascospores. Colonies on MEA slow growing, reaching 10–15 mm diam. after 15 days of incubation at 25 °C, surface pale greyish to light brown on the surface, reverse pale brown, filamentous, circular, umbonate.

Material examined – INDIA, Tamil Nadu, Parangipettai mangroves (11.59°N 79.5°E), on decaying wood of *Avicennia marina* (Acanthaceae), 23 April 2018, B. Devadatha, (AMH-10018, holotype) ex-type living culture, NFCCI-4383.



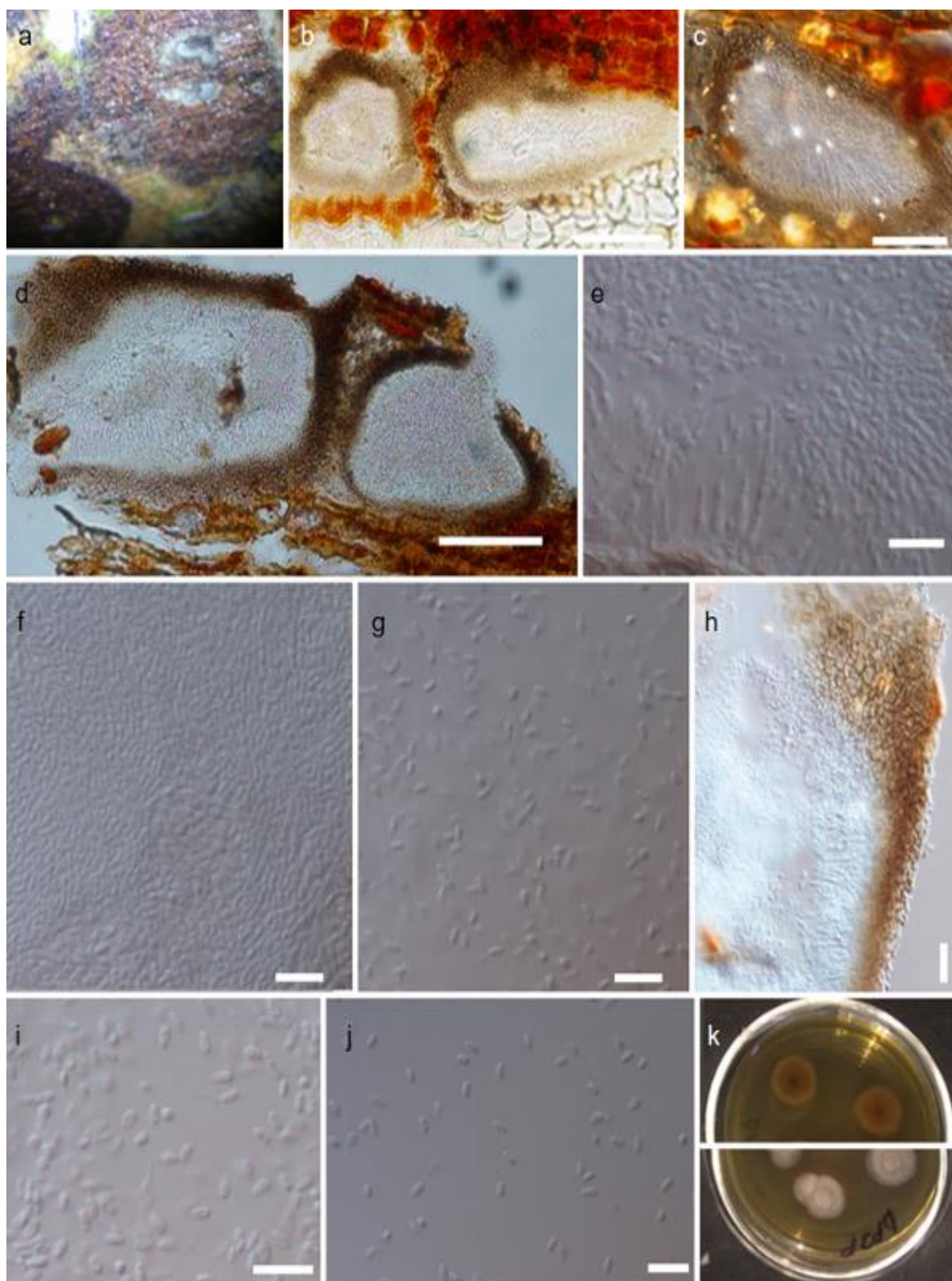
**Figure 21** – *Neocamarosporium maritimae* (MFLU 19-1232, holotype). a Ascomata on stems of *Artemisia maritima*. b Section of ascoma. c Peridium. d Pseudoparaphyses. e–g Asci. h–k Ascospores. l Ascospore stained with India ink to highlight the sheath. Scale bars: b = 100  $\mu$ m, d–g = 50, c = 20  $\mu$ m, h–l = 10  $\mu$ m.



**Figure 22** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -16401.828168. The combined LSU, ITS, TEF and RPB2 sequence datasets comprised 43 strains of Nigrogranaceae, Occultibambusaceae and Parathyridariaceae with *Cyclothyriella rubronotata* (CBS 141486 and CBS 419.85) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI and in congruent with previous studies (Jaklitsch & Voglmayr 2016, Ahmed et al. 2018, Hyde et al. 2013). The matrix had 1171 distinct alignment patterns, with 26.12% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.248016, C = 0.252034, G = 0.270020, T = 0.229930; substitution rates AC = 1.619520, AG = 4.018583, AT = 1.649857, CG = 1.110080, CT = 11.161648, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.550382. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.04 changes. The ex-type strains are in bold and new isolates in blue bold.



**Figure 23** – Sexual morph of *Nigrograna samueliana* (AMH-10018, holotype). a Ascomata semi-immersed in decaying wood. b Vertical section of ascomata. d Peridial wall layers. e Filamentous pseudoparaphyses. g–i Immature and mature asci. c, f, k–p Immature and mature ascospores. q Germinating ascospore. Scale bars: b = 100 µm, c–q = 10 µm.



**Figure 24** – Asexual morph of *Nigrograna samueliana* (AMH-10018). a Asexual morph on the natural host showing pycnidia. b–d Pycnidia in vertical section. e Conidiophores with phialides. h Peridium. f, g, i, j Immature and mature conidia. k Reverse and front view of culture. Scale bars: b–d = 100  $\mu$ m, e–j = 10  $\mu$ m.

GenBank numbers – ITS: MG947599; LSU: MG947595; SSU: MG947598; TEF: MG968955; RPB2: MG948547

Notes – Most *Nigrograna* species have indistinguishable morphological characters such as subiculate ascomata and apically free paraphyses among immature asci and hence, Jaklitsch & Voglmayr (2016) treated them as cryptic species. Blast search results of LSU and ITS sequence data revealed that our taxon belongs to *Nigrograna*. Furthermore, combined LSU, ITS, TEF and RPB2 phylogeny, including taxa from *Nigrograna* and *Biatriospora*, showed that *Nigrograna samueliana* clusters together with the novel species *Nigrograna rhizophorae* in a strongly supported phylogenetic clade (ML 100% and 1.00 PP) (Fig. 22). *Nigrograna samueliana* shares similar ascomata, asci and ascospore characters in addition to sharing pycnidial and conidial characters with the existing *Nigrograna* species. However, *Nigrograna samueliana* is distinct from *N. cangshanensis*, *N. fuscidula*, *N. mycophila*, *N. novergica*, *N. obliqua* and *N. thymi* in lacking a subiculum and ostiolar necks and by its occurrence in marine habitats (Jaklitsch & Voglmayr 2016, Tibpromma et al. 2017). *Nigrograna novergica* differs from *N. samueliana* as it occurs on pseudostromata of *Diaporthe* sp., and has ascospores that are fusoid to clavate with acute ends (Jaklitsch & Voglmayr 2016). *Nigrograna thymi* can be easily distinguished from *N. samueliana* and other species in having 4–5 septate, dark brown ascospores (Hyde et al. 2017). Both *N. obliqua* and *N. mycophila* are distinct from *N. samueliana* in having dark brown ascospores (Jaklitsch & Voglmayr 2016). *Nigrograna fuscidula* has immersed ascomata in contrast to *N. samueliana* that has semi-immersed and erumpent ascomata (Jaklitsch & Voglmayr 2016). *Nigrograna cangshanensis* has smaller, immersed ascomata, a thicker peridium and ascospores that are olive gray to yellowish-brown when compared to *N. samueliana* (Hyde et al. 2017). The asexual morph of *Nigrograna rhizophorae* is different from *N. samueliana* by having unbranched, relatively shorter (10–30  $\mu\text{m}$  vs 20–35  $\mu\text{m}$ ) conidiophores and smaller conidia (2–4  $\mu\text{m}$  vs 3–4.5  $\mu\text{m}$ ). (See notes under *N. rhizophorae* for the comparison of asexual morph). Based on the distinct morphology, habitat and phylogenetic relationships, *Nigrograna samueliana* could be delineated from the other species reported. Hence, we introduce it as a novel species under *Nigrograna* in this paper. Furthermore, ITS, TEF and RPB2 base pair differences of *N. samueliana* and *N. rhizophorae* are 3.5% (out of 542 bp), 2.06% (out of 1000 bp) and 9.8 % (out of 750 bp) which are indicative of two different species according to the guidelines of Jeewon & Hyde (2016).

***Nigrograna rhizophorae*** Dayarathne, E.B.G. Jones & K.D. Hyde, sp. nov.

Fig. 25

Index Fungorum number: IF556579, Facesoffungi number: FoF 06164

Etymology – Specific epithet derived from the host genus

Holotype – MFLU 19-1233

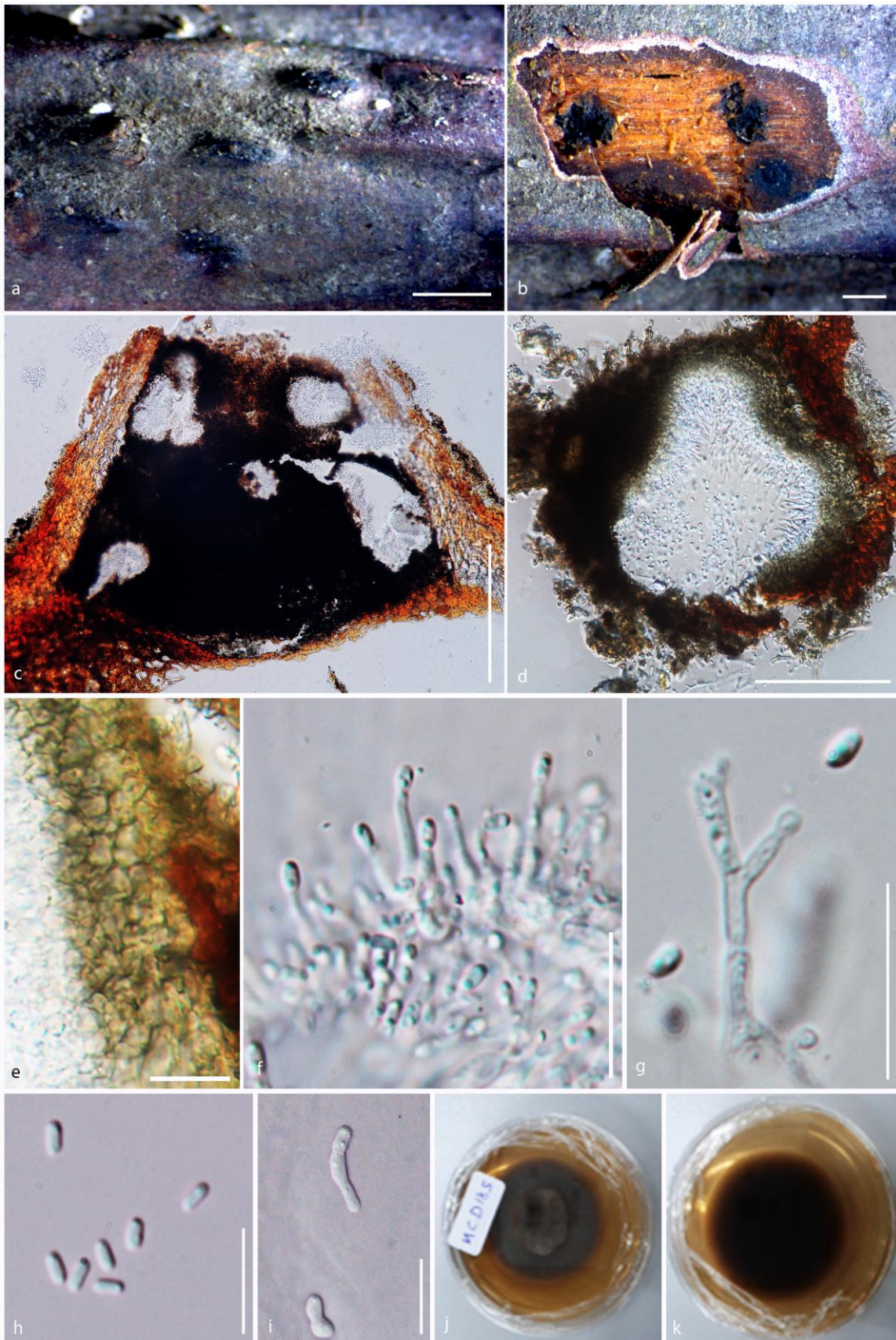
*Saprobic* on decaying wood of *Rhizophora* sp. Sexual morph: Undetermined. Asexual morph: Coelomycetous. *Pycnidia* 0.9–1 mm  $\times$  0.6–0.8 mm ( $\bar{x}$  = 0.92  $\times$  0.85 mm, n = 5), globose to subglobose, immersed to semi immersed, scattered or aggregated in small groups, appearing as black spots on host surface. *Peridium* 60–80  $\mu\text{m}$  diam. wide ( $\bar{x}$  = 72  $\mu\text{m}$ , n = 5), pseudoparenchymatous, brown. *Conidiophores* 20–35  $\times$  1.5–2.0  $\mu\text{m}$  diam. ( $\bar{x}$  = 25  $\times$  1.5  $\mu\text{m}$ , n = 10), arising from the wall in palisadic arrangement, filamentous, septate, hyaline, sparsely branched, with a terminal phialide. Solitary minute pegs and lageniform to sub-cylindrical phialides are arranged apically on the conidiophores. *Conidia* 3–4.5  $\times$  1–2  $\mu\text{m}$  ( $\bar{x}$  = 3.5  $\times$  1.5  $\mu\text{m}$ , n = 30) diam., ellipsoidal to cylindrical, unicellular with 1–2 small granules, subhyaline, smooth conidia are produced from the terminal ends of the conidiophores.

Culture characteristics – Ascospores germinating on 50% sea water agar within 24 hours, with germ tubes developed from lower side of ascospores. Colonies on MEA slow growing, reaching 10–20 mm diam., after 15 days of incubation at 25 °C, circular, grey at the center becoming black, black at the margins, spreading, flattened, fluffy, sparse, aerial, surface smooth with filamentous edge, reverse black, filamentous, circular, umbonate.

Material examined – THAILAND, Phetchaburi Province, Cha-am District, on submerged propagule of *Rhizophora* sp. (Rhizophoraceae), 18 January 2018, M.C. Dayarathne, MCD 180 (MFLU 19-1233, holotype); *ibid.*, (HKAS102066, isotype), ex-type living culture (MFLUCC 18-0397). THAILAND, Phetchaburi Province, Cha-am District, on decaying wood of *Rhizophora* sp.



(Rhizophoraceae) wood, 18 January 2018, M.C. Dayarathne, MCD 185 (MFLU 19-1234); *ibid.*, (HKAS102066).



**Figure 25** – *Nigrograna rhizophorae* (MFLU 19-1233, holotype). a, b Pycnidia on host surface. c Pycnidia in vertical section. e Pycnidial wall. f, g Conidiophores with phialides. h Conidia.

i Germinating conidia. j. Front and reverse views of culture. Scale bars: a, b = 1000  $\mu\text{m}$ , c, d = 200  $\mu\text{m}$ , e, f, h = 20  $\mu\text{m}$ , g, i = 10  $\mu\text{m}$ .

GenBank numbers – LSU: MN420686, MN017845; ITS: MN047085; ITS: TEF: MN077063, MN077064; RPB2: MN431490, MN431489

Notes – *Nigrograna rhizophorae* best fits the genus *Nigrograna* by being coelomycetous and comprising globose to sub-globose, immersed to semi immersed pycnidia with a brown, pseudoparenchymatous peridium, filiform, simple to sparsely branched conidiophores with pegs along one or two sides and solitary terminal, lageniform to sub-cylindrical phialides and oblong, cylindrical, subhyaline, 1-celled conidia. Phylogenetic analyses based on LSU, ITS, TEF and RPB2 data showed that *N. rhizophorae* (MFLU 19-1234, MFLUCC 18-0397) formed a well separated (ML100% and 1.00 PP) sister clade to *N. samueliana* (Fig. 22). The asexual morph of *N. samueliana* can be easily distinguished from *N. rhizophorae* by having unbranched, relatively shorter (10–30  $\mu\text{m}$  vs 20–35  $\mu\text{m}$ ) conidiophores and smaller conidia (2–4  $\mu\text{m}$  vs 3–4.5  $\mu\text{m}$ ). Furthermore, ITS, TEF and RPB2 base pair differences of *N. samueliana* and *N. rhizophorae* are 3.5% (19 out of 542 bp), 2.06% (20 out of 1000 bp) and 9.8 % (74 out of 750 bp) which are indicative of two different species according to the guidelines of Jeewon & Hyde (2016).

### **Periconiaceae** Nann.

The family Periconiaceae (Nannizzi 1934) has long been ignored and placed as a member of Massarinaceae (Zhang et al. 2012, Hyde et al. 2013, Liu et al. 2017). However, Tanaka et al (2015) revised *Massarineae* and established it as a distinct family based on their phylogenetic analysis (Phukhamsakda et al. 2016). Liu et al. (2017) showed that *Massarineae* and Periconiaceae are sister taxa to Massarinaceae which diverged in the Cretaceous period (around 70 Mya) (Liu et al. 2017).

### **Periconia** Tode

*Periconia* was introduced by Tode (1791) and is typified by *P. lichenoides* and has been shown to produce valuable bioactive compounds (Li et al. 1998, Kim et al. 2004, Harnpicharnchai et al. 2009, Zhang & Wang 2015, Zhang et al., 2016). There are 187 species epithets in Index Fungorum (2019) and more than 20 have been transferred to other genera. Currently ca. 50 species are recognized as truly belonging to *Periconia* (Carmaran & Novas 2003, Seifert et al. 2011, Markovskaja & Kačergius 2014, Tanaka et al. 2015, Liu et al. 2017, Thambugala et al. 2017, Hyde et al. 2018). *Periconia* species are common plant pathogens on various crops (Leukel 1948) and they are cosmopolitan in distribution as saprobes and endophytes colonizing herbaceous plants in various ecosystems including marine environments (Mason & Ellis 1953, Ellis 1976, Rao & Rao 1964, Matsushima 1996, Minter et al. 2001, Carmaran & Novas 2003, Cantrell et al. 2007, Markovskaja & Kačergius 2014, Liu et al. 2017).

### **Periconia salina** Dayarathne & E.B.G. Jones, sp. nov.

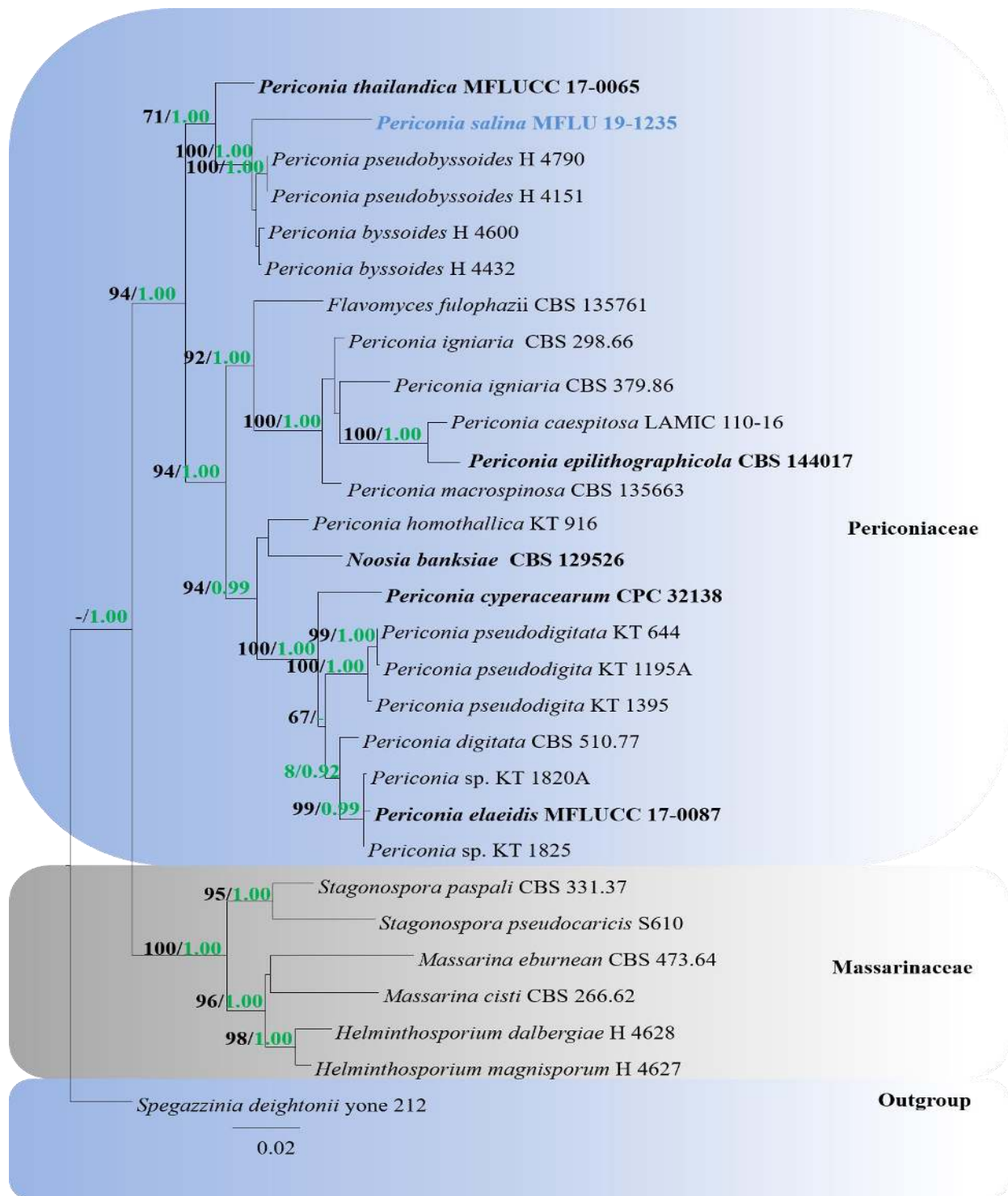
Fig. 27

Index Fungorum number: IF556580; Facesoffungi number: FoF 06165

Etymology – Epithet refers to the saline environment where the species was found

Holotype – MFLU 19-1235

*Saprobic* on unidentified bark, buried in sand dune. Sexual morph: Undetermined. Asexual morph: *Colonies* on substrate numerous, effuse, dark brown to black, floccose. *Mycelium* partly superficial composed of branched, septate, hyaline hyphae 1–2  $\mu\text{m}$  wide. *Conidiophores* 385–400  $\times$  20–25  $\mu\text{m}$ , macronematous, mononematous, unbranched, erect, straight or slightly flexuous, forming spherical heads at apex, 80–98  $\mu\text{m}$  diam ( $\bar{x}$  = 82.5  $\mu\text{m}$ , n = 10), single, grayish brown to dark brown, 5–6-septate, smooth to minutely verruculose, thick-walled. *Conidiogenous cells* holoblastic, polyblastic, acropetally proliferating, terminal, integrated or discrete, ovoid to globose, pale brown, smooth, collapsing when old. *Conidia* 16.4–18.4  $\times$  15–18  $\mu\text{m}$  diam ( $\bar{x}$  = 16.8  $\times$  16.5  $\mu\text{m}$ , n = 30), solitary, subglobose to globose, dark brown, verruculose, aseptate.



**Figure 26** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -7756.450937. The combined LSU, SSU and ITS sequence datasets comprised 29 strains of Periconiaceae and Massarinaceae and *Spegazzinia deightonii* (yone 212) as the outgroup taxon. Tree topology of the ML analysis was similar to the BI. The matrix had 467 distinct alignment patterns, with 18.89% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.248100, C = 0.227570, G = 0.275219, T = 0.249111; substitution rates AC = 2.574509, AG = 3.950406, AT = 2.257360, CG = 1.199888, CT = 10.821629, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.127888. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.02 changes. The ex-type strains are in bold and new isolates in blue bold.



**Figure 27** – *Periconia salina* (MFLU 19-1235, holotype). a Substrate. b Colonies on substrate. c, d Conidiophores. e Conidial masses at the apex of conidiophores. f–h Conidia. Scale bars: a–c = 200 µm, d, e = 100 µm, f–h = 10 µm.

Material examined – UK, Carmarthenshire, Wales, Llanstephan, on unidentified bark, buried in sand dune, 12 May 2017, E.B. Gareth Jones, GJ374 (MFLU 19-1235, holotype); *ibid.*, (BBH, isotype).

GenBank numbers – LSU: MN017846; SSU: MN017912; ITS: MN047086

Notes – *Periconia salina* best fits the genus *Periconia* in having pale to dark brown, unbranched, smooth or verruculose, macronematous conidiophores, monoblastic or polyblastic conidiogenous cells and spherical, aseptate, catenate or solitary, verruculose, pale to dark brown conidia (Carmarán & Novas 2003, Markovskaja & Kačergius 2014). *Periconia salina* shows close phylogenetic affinities to *P. byssoides* and *P. pseudobyssoides*, but is distinct as a new species (Fig.

26). Base pair differences of *P. byssoides* and the new species are 4% (34 bp out of 855 bp) LSU, 3.5% (30 bp out of 871 bp) SSU and 0.8% (42 bp out of 526 bp) ITS. The new species is morphologically different from *P. byssoides* and *P. pseudobyssoides*. The conidia of *P. salina* are larger than those of *P. byssoides* (15–18 µm diam. vs 10–15 µm diam). (Ellis 1971). Spherical heads at the apex of conidiophores of *P. pseudobyssoides* are smaller than those of *P. salina* (30–70 µm vs 80–98 µm diam.) (Markovskaja & Kačergius 2013).

### **Phaeoseptaceae** S. Boonmee, Thambugala & K.D. Hyde

The family Phaeoseptaceae was established by Hyde et al. (2018) to accommodate *Phaeoseptum*, *Lignosphaeria* and *Neolophiostoma*. Multigene phylogenetic analyses by Phukhamsakda et al. (2019) revealed that *Neolophiostoma* forms a distinct lineage and is also distantly related to both Halotthiaceae and Phaeoseptaceae. Members of the Phaeoseptaceae are saprobic on decaying wood and are distributed across different habitats such as terrestrial, freshwater and marine (Zhang et al. 2013, Hyde et al. 2018, Phukhamsakda et al. 2019).

### **Phaeoseptum** Ying Zhang, J. Fourn. & K.D. Hyde

*Phaeoseptum* was introduced by Zhang et al. (2013) to accommodate *P. aquaticum* which is most comparable to the maritime fungus *Mauritiana rhizophorae*. Zhang et al. (2013) showed that the genera *Halothia*, *Mauritiana*, *Pontoporeia* and *Phaeoseptum aquaticum* formed a monophyletic clade in phylograms generated from LSU sequence analyses. The genera *Halothia*, *Mauritiana*, *Phaeoseptum*, *Pontoporeia* initially placed within Halotthiaceae but *Phaeoseptum* has now been transferred to Phaeoseptaceae (Hyde et al. 2018). This genus is characterized by semi-immersed to immersed ascomata, narrowly cellular, anastomosed pseudoparaphyses, cylindrical-clavate, pedicellate asci and fusiform to ellipsoidal or vermiform, asymmetrical and brown muriform ascospores (Zhang et al. 2013, Hyde et al. 2018). Currently three species are accepted in this genus. The phylogram, constructed based on combined LSU, SSU, TEF, ITS and RPB2 marker gene data sets in this study, also strongly supports the establishment of the family Phaeoseptaceae by Hyde et al. (2018). Furthermore, we introduce two novel species to the genus *Phaeoseptum*.

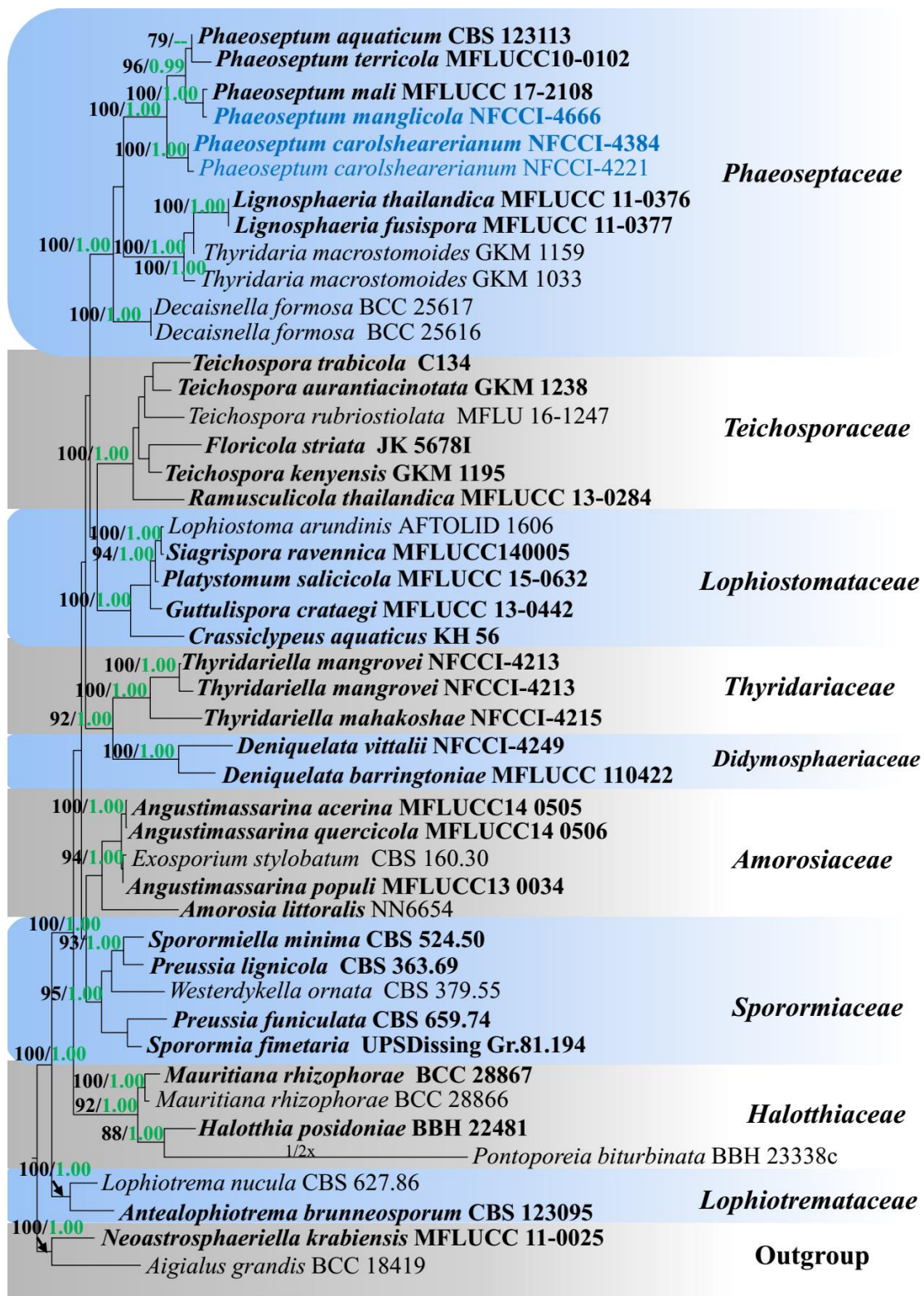
### **Phaeoseptum carolshearerianum** Devadatha, V.V. Sarma & E.B.G. Jones sp. nov. Fig. 29

Index Fungorum number: IF555721; Facesoffungi number: FoF 06166

Etymology – Specific epithet in honour of the mycologist Dr. Carol A. Shearer on her great contributions to aquatic mycology.

Holotype – AMH-9909

Saprobic on decaying wood of *Avicennia marina*. Sexual morph Ascomata 290–620 µm high × 295–400 µm in diam. ( $\bar{x}$  = 392 × 339 µm, n= 10), semi-immersed to immersed, aggregate to solitary, globose to subglobose, emerging as dark brown to black prolonged parts on surface of the host. Peridium unequal in thickness 35–80 µm ( $\bar{x}$  = 54 µm, n=10) wide at the base, 30–70 µm ( $\bar{x}$  = 48 µm, n =10) at the sides, comprising several, inner layers of hyaline to light brown cells of *textura angularis*, outer layers of thick-walled pale brown to reddish brown cells of *textura angularis* and fusing at the outside with the host tissues. Hamathecium 1–2.5 µm wide, numerous rarely branched, septate pseudoparaphyses anastomosing above the asci, embedded in a gelatinous matrix. Asci 103–195 × 18–23 µm diam. ( $\bar{x}$  = 139 × 21 µm, n=20), 8-spored bitunicate, fissitunicate, long pedicellate, cylindrical to cylindrical clavate, apically rounded and thickened at the apex with a small ocular chamber. Ascospores 29–37.5 × 8.5–12.5 ( $\bar{x}$  = 33.4 × 10 µm, n=50), uni- to bi-seriate, muriform, asymmetric oblong to broadly fusiform, with convex ends, enlarged at the 4th and 5<sup>th</sup> cell, tapering towards the end cells, 1–2 longitudinal or vertical septa in each segment except in the end cells, 8–10-transversely septate, slightly constricted at the 5<sup>th</sup> septum, initially hyaline, turning into pale brown at maturity, guttulate, flattened, roughened surface, often moderately twisted from apical region to the ends, sometimes straight and rounded at the apex, lacking mucilaginous sheath. Asexual Morph: Undetermined.



**Figure 28** – Phylogenetic analyses is carried out using combined LSU, SSU, TEF, ITS, and RPB2 sequence datasets composed of 46 strains of taxa in Phaeoseptaceae and other representative genera of the families in Pleosporales with *Aigialus grandis* BCC 18419 and *Neoastrospheariella krabiensis* MFLUCC 11-0025 as outgroup taxa. Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -32398.659815. The matrix had 1977 distinct alignment patterns, with 37.40 % of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.246116, C = 0.252481, G = 0.273616, T = 0.227787; substitution rates AC = 1. 276855, AG =2.793720, AT = 1.537700, CG = 1.158693, CT = 6.112503, GT = 1.000000; proportion of invariable sites I = 0.326300; gamma distribution shape

parameter  $\alpha = 0.470687$ . Phylogenetic trees obtained from ML and BI were topologically similar and congruent with previous studies (Hyde et al. 2018, Phukhamsakda et al. 2019). Maximum likelihood bootstrap (ML, black) values > 70% and Bayesian posterior probabilities (PP, green) > 0.95% are given above the nodes. The scale bar indicates 0.08 changes. The ex-type strains are in bold and new isolates in blue bold.



**Figure 29** – *Phaeoseptum carolshearerianum* (AMH-9909, holotype). a Ascomata on decaying wood of *Avicennia marina*. b, c Longitudinal sections of ascomata. d Section of peridium. e Pseudoparaphyses. f–j Immature and mature asci. k–o Ascospores. p Germ tubes developing from

terminal ends of ascospore. Scale bars: b, c = 100  $\mu\text{m}$ , f, h, i = 50  $\mu\text{m}$ , d, e, j–p = 10  $\mu\text{m}$ .

Culture characteristics – Ascospores germinating on 50% sea water agar within 24 hours, with germ tubes developed from both end cells of ascospores. Colonies on MEA slow growing, reaching 10–15 mm diam. on 7th day, after 15 days of incubation reach 40–55 mm at 25 °C, surface pale olive grey in centre, dark grey at the margin, olivaceous to black stromata on the surface, olivaceous exudates. Reverse deep mouse grey, convex, granular, circular. Mycelium 2–7.5  $\mu\text{m}$  diam., dense, branched, septate, broad, hyaline to pale brown, smooth-walled. *Chlamydoconidia* 8–15  $\times$  7–12.5  $\mu\text{m}$ , catenate, highly intact, hyaline to pale brown, oval to ellipsoidal, smooth to rough-walled.

Material examined – INDIA, Tamil Nadu, Tiruvarur, Muthupet mangroves (10.4°N 79.5°E), on decaying wood of *Avicennia marina* (Acanthaceae), 28 March 2015, B. Devadatha (AMH-9908, holotype), ex-type living culture NFCCI-4221. *ibid.*, Parangipettai mangroves, on decaying wood of *Avicennia marina* (Acanthaceae), 28 April 2018, B. Devadatha PUHD57, living culture NFCCI-4384.

GenBank numbers – NFCCI-4221, NFCCI-4384: ITS: MK307810, MK307812; LSU: MK307813, MK307815, MK307818; SSU: MK307816, MK307818; TEF: MK309874, MK309876; RPB2: MK309877, MK309879

Notes – Morphological studies suggest that the new species, *Phaeoseptum carolshearerianum* and *P. manglicola*, share similar characters of the genus *Phaeoseptum*. Furthermore, the combined LSU, SSU, TEF, ITS and RPB2 phylogeny also revealed that they group together with *Phaeoseptum aquaticum*, *P. mali* and *P. terricola* with significant support (100% ML, 1.00 PP) (Fig. 28). *Phaeoseptum carolshearerianum*, *P. aquaticum*, *P. manglicola*, *P. mali* and *P. terricola* share similar dark pigmented, immersed ascomata, cylindrical to clavate, pedicellate asci and muriform brown ascospores. *Phaeoseptum carolshearerianum*, however, is distinct from other *Phaeoseptum* species in having larger ascomata, lacking a pseudoclypeus and asymmetric oblong to broadly fusiform ascospores, enlarged at 4<sup>th</sup> and 5<sup>th</sup> septa, 8–10 trans-septate. Both morphological and phylogenetic studies suggest that this marine species is clearly distinct from *P. aquaticum*, *P. manglicola*, *P. mali* and *P. terricola*. Furthermore, ITS base pair differences of *P. carolshearerianum* and *P. manglicola* resulted in 9.9% (43 bp out of 443 bp) which indicates both are different species (Jeewon & Hyde 2016). Hence, we introduce the two strains as a novel species in *Phaeoseptum*.

***Phaeoseptum manglicola*** Devadatha, V.V. Sarma & E.B.G. Jones sp. nov.

Fig. 30

Index Fungorum number: IF555722; Facesoffungi Number: FoF 06167

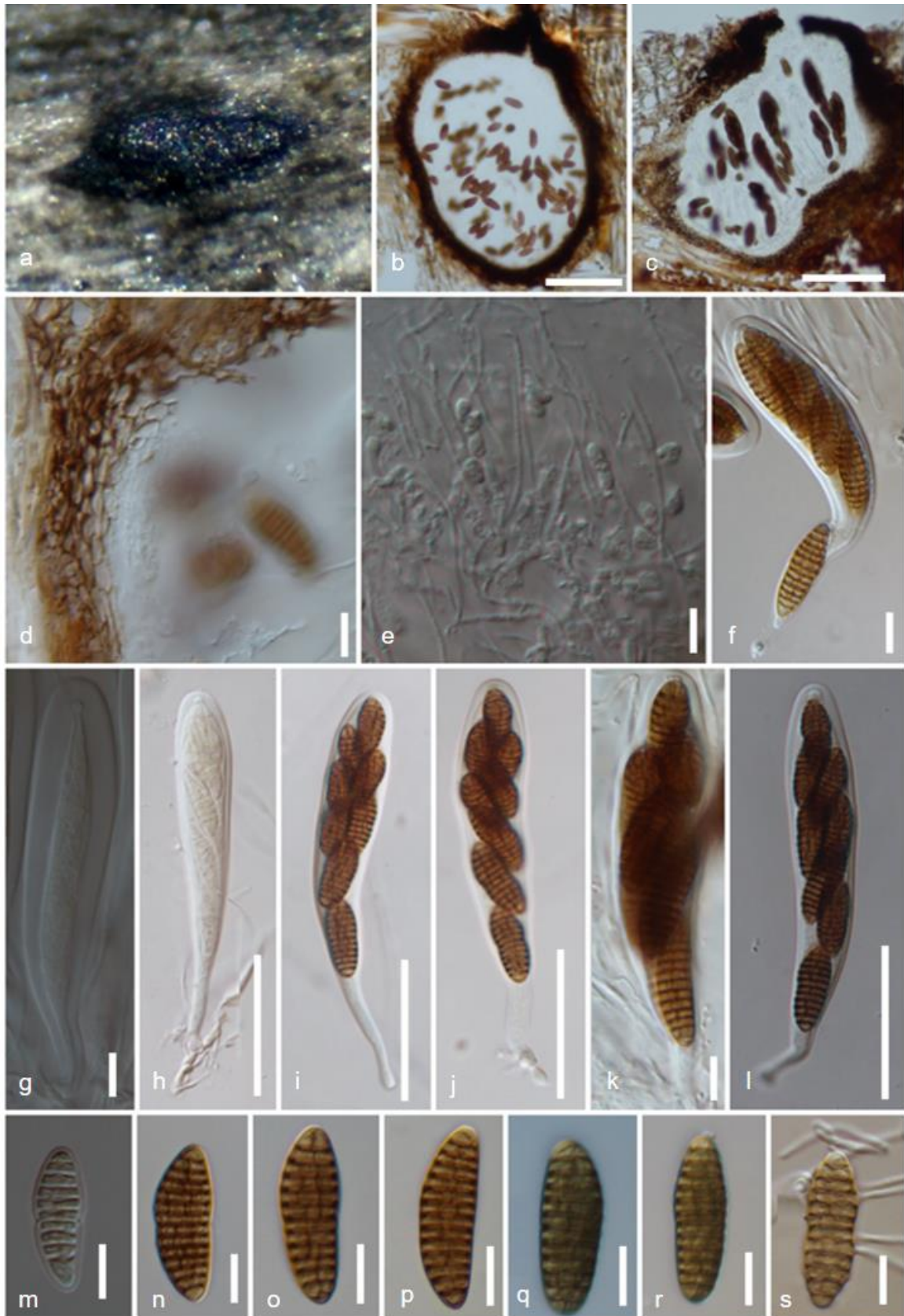
Etymology – In reference to its mangrove habitat

Holotype – AMH-9908

*Saprobic* on decaying mangrove wood. Sexual morph *Ascomata* 210–420  $\mu\text{m}$  high  $\times$  170–374  $\mu\text{m}$  in diam. ( $\bar{x}$  = 321  $\times$  238, n=10), aggregate to solitary, semi-immersed to immersed, erumpent through the host tissue, ostiolate, short papillate, globose to subglobose, irregular, clypeate, raised as dark brown to black spots on surface of the host. *Ostiole* 28–94  $\mu\text{m}$  high, 39–96  $\mu\text{m}$  diam., short, pale brown. *Peridium* 30–85  $\mu\text{m}$  ( $\bar{x}$  = 56  $\mu\text{m}$ , n=7) wide at the base, 24–69  $\mu\text{m}$  ( $\bar{x}$  = 48  $\mu\text{m}$ , n=7) on the sides, comprising 4–6 layers, of outer thick-walled and brown cells and inner thin-walled layer of hyaline to pale brown cells of *textura angularis*, fusing at the outside with the host tissues. *Hamathecium* composed of 1–2.5  $\mu\text{m}$  wide, several, branched, cellular, septate, narrow pseudoparaphyses, anastomosing above the asci, embedded in a gelatinous matrix. *Asci* 102–212  $\times$  17–27.5  $\mu\text{m}$  ( $\bar{x}$  = 138  $\times$  23  $\mu\text{m}$ , n=25), 8-spored, bitunicate, fissitunicate, cylindrical to clavate, long pedicellate, apically rounded and thickened, with a refractive plate in the ectoascus and a refractive apical plate in the endoascus. *Ascospores* 27–36  $\times$  7.5–13  $\mu\text{m}$  ( $\bar{x}$  = 32  $\times$  11  $\mu\text{m}$ , n=50), uniform, 2–3-seriate at the apex, 1-seriate at the base, overlapping, initially hyaline, becoming pale brown to yellowish brown at maturity, smooth-walled, flattened, lacking a mucilaginous sheath, often straight, sometimes slightly curved, rarely constricted at the middle septum, oblong to broadly



fusiform, with rounded to convex ends, 9–13 transverse septa, 1–2 longitudinal septa in each row, Y-shaped septum present or absent in the end cells, end cells hyaline to pale brown. Asexual morph: Undetermined.



**Figure 30** – *Phaeoseptum manglicola* (AMH-9908, holotype). a Ascomata on decaying wood of *Avicennia marina*. b–c Longitudinal sections of ascomata. d Section of peridium. e

Pseudoparaphyses. f–l Immature and mature asci. m–r Ascospores. s Germ tubes developed from terminal ends of ascospore. Scale bars: b–c = 100  $\mu\text{m}$ , h–j, l = 50  $\mu\text{m}$ , d–f, g, k, m–s = 10  $\mu\text{m}$ .

Culture characteristics – Ascospores germinating on 50% sea water agar producing germ tubes from both ends of the ascospores within 24 hours. Colonies on MEA slow growing, surface pearl grey to dawn grey, reaching 9–15 mm in diam. on the 7<sup>th</sup> day, after incubation for 25 days attains 41–50 mm in diam. and turn into pale brown to dark brown, stromatic, reverse warm blackish brown with olive buff at margins, moderate growth, cottony, undulate, umbonate, and irregular. Mycelium 0.5–4  $\mu\text{m}$ , aerial, dense, stromatic, pale brown to dark brown, septate, branched, smooth-walled, hyaline to pale brown. *Chlamydospores* 13–28  $\times$  10–16  $\mu\text{m}$ , obovoid to ellipsoidal, hyaline to dark brown, smooth-walled when young becoming verrucose at maturity, highly pigmented on the walls and at the septum.

Material examined – INDIA: Tamil Nadu, Tiruvarur, Muthupet mangroves (10.4°N 79.5°E), on decaying wood of *Avicennia marina* (Acanthaceae) (AMH-9908, holotype), 28 March 2015, *Suaeda monoica* (Amaranthaceae) 24 December 2016 (PUHD9, paratype), B. Devadatha (AMH-9908, holotype), ex-type living culture NFCCI-4666.

GenBank numbers – ITS: MK307811; LSU: MK307814; SSU: MK307817; TEF: MK309875; RPB2: MK309878.

Notes – *Phaeoseptum manglicola* clusters with *P. mali* with significant support from ML 100% and 1.00 PP. *Phaeoseptum manglicola* resembles *P. aquaticum* in having similar ascomata, asci and ascospores that are broadly fusoid with broadly rounded ends, multi-transverse septa and longitudinal septa with a Y-shaped septum in the end cells. However, *P. manglicola* differs from *P. aquaticum* in having ascomata that are narrower (170–374  $\mu\text{m}$  vs 400–600  $\mu\text{m}$ ), with a thick-walled peridium (30–85  $\mu\text{m}$  vs 20–30  $\mu\text{m}$ ), slightly shorter ascospores (30.5–38.3  $\times$  9.5–12  $\mu\text{m}$  vs 27–36  $\times$  7.5–13  $\mu\text{m}$ ) and 1–2 longitudinal septa in many cells, producing obovoid to ellipsoidal chlamydospores in culture and in its occurrence in a marine habitat (Zhang et al. 2013). *Phaeoseptum manglicola* and *P. mali* share similarities in their ascomata, asci and ascospore characters and their overlapping dimensions. However, *P. manglicola* is distinguishable from *P. mali* in having globose to subglobose or irregular vs globose ascomata, thick-walled peridium (30–85  $\mu\text{m}$  vs 5–19  $\mu\text{m}$ ) and ascospores that are oblong to broadly fusiform vs broadly cylindrical with (9–13 vs 11–14) longitudinal septum in many cells and habitat marine vs terrestrial (Phukhamsakda et al. 2019). Furthermore, a notable nucleotide differences of 1.48% (out of 405 bp) were found within ITS gene region of *P. manglicola* and *P. mali* which was in agreement with guidelines outlined by Jeewon & Hyde (2016) to establish new species based on DNA sequence data. *Phaeoseptum terricola* is different from *P. manglicola* in having smaller ascomata, asci and ascospores, ascospores that are allantoid or cylindrical and in its occurrence in terrestrial habitats (Hyde et al. 2018). *Decaisnella formosa* has fusiform to elongated ascospores that are distoseptate when young, with (8–) 9–13 transverse septa and 1–6 longitudinal septa in all cells, slightly constricted at the septa in contrast to *Phaeoseptum carolshearerianum* and *P. manglicola* (Abdel-Wahab & Jones 2003). *Mauritiana rhizophorae*, another marine species, can be distinguished from *P. carolshearerianum* and *P. manglicola* as it produces phragmosporous ascospores (Poonyth et al. 2000). *Platystomum scarbidisporum* is a muriformed marine fungus which differs from *Phaeoseptum carolshearerianum* and *P. manglicola* in producing smaller ascospores, fusiform, with 5–7 (–8) transverse septa and 1–2(–3) longitudinal septa constricted at the central septum, brown with a rough or verrucose ascospores (Abdel-Wahab & Jones 2000). Therefore, based on both morphological and phylogenetic evidence we established this species as a novel taxon within *Phaeoseptum*.

### **Phaeosphaeriaceae** M.E. Barr

Barr (1979) introduced the family Phaeosphaeriaceae, which was initially characterized by immersed to superficial, globose to subglobose ascomata, short papilla, bitunicate asci and hyaline, yellowish or brown, fusiform to ellipsoidal, filiform, or muriform, septate ascospores (Barr 1979,

Shoemaker 1984, Zhang et al. 2012, Hyde et al. 2013, Phookamsak et al. 2014). Phaeosphaeriaceae comprise more than 60 sexual and asexual genera (Phookamsak et al. 2014, 2017, 2019, Wijayawardene et al. 2014, 2017a, 2018, Hyde et al. 2016, Yang et al. 2019, Bakhshi et al. 2019, Maharachchikumbura et al. 2019, Marin-Felix et al. 2019). Species in this family are often necrotrophic pathogens or saprobes on plants (Shoemaker & Babcock 1989, Carson 2005, Stukenbrock et al. 2006, Cannon & Kirk 2007, Tibpromma et al. 2017).

***Amarenographium*** O.E. Erikss

Eriksson (1982) introduced the genus *Amarenographium* to accommodate *Camarosporium metableticum* whose morphology differed from the definition of *Camarosporium*. The conidiophores are much longer and branched, with phialidic conidiogenesis with solitary conidia that are provided with a characteristic polar gelatinous appendage. Eriksson (1982) described the morphological similarity between *Amarenographium metableticum* and *Amarenomyces ammophilae* and suggested these taxa might be the same biological species. Nag Raj (1993) also considered *Amarenomyces* as the sexual morph of *Amarenographium*. However, these genera were not connected by molecular data or culture-based methods, thus Phookamsak et al. (2014) and Wijayawardene et al. (2014) treated *Amarenographium* and *Amarenomyces* as distinct genera in Phaeosphaeriaceae. Taylor et al. (2003) described a second species of *Amarenographium*, *A. sinense* from a dead petiole of *Trachycarpus fortunei* from Hubei Province, China. Sequence data is available only for *A. solium* and *A. ammophilae*, while the type species of *Amarenographium* lacks sequence data. However, *A. solium* did not group within Phaeosphaeriaceae in previous studies by Hodhod et al. (2012), Wijayawardene et al. (2014) and Wanasinghe et al. (2018), hence, *Amarenographium* was considered as a polyphyletic genus in Pleosporales (Wijayawardene et al. 2014, Wanasinghe et al. 2018). In this study, we introduce the first sexual morph for *Amarenographium*, which was collected from a marine based habitat.

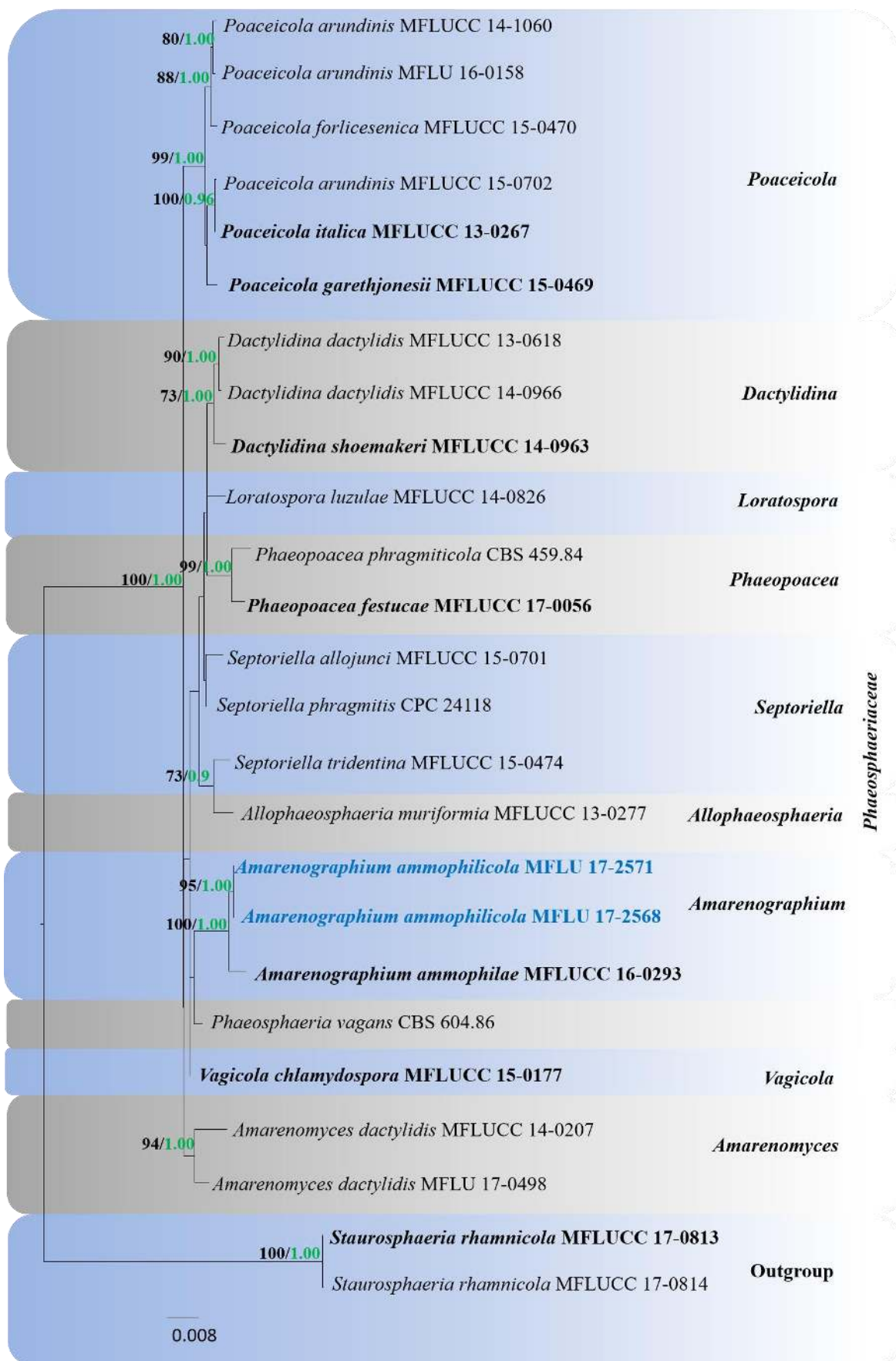
***Amarenographium ammophilicola*** Dayarathne, E.B.G. Jones & K.D. Hyde, sp. nov. Figs 32, 33

Index Fungorum number: IF556581; Facesoffungi Number: FoF 06168

Etymology – The species epithet based on the host *Ammophila arenaria* on which the fungus was collected.

Holotype – MFLU 17-2571

*Saprobic* on leaf of Marram grass (*Ammophila arenaria*) in sand dune. Sexual morph: *Ascomata* 300–320 × 150–170 µm semi-immersed to erumpent through host tissue, solitary or scattered, uniloculate, coriaceous, globose to subglobose, black. *Ostiole* central, with short papilla. *Peridium* 25–30 µm wide, comprising of 3–4 layers of light brown cells of *textura angularis*. *Hamathecium* comprising 1–2 µm wide, cylindrical to filiform, septate, branched, pseudoparaphyses. *Asci* 130–138 × 30–35 µm ( $\bar{x}$  = 132 × 32 µm, n = 10), 8-spored, bitunicate, cylindrical, short pedicellate, with small ocular chamber. *Ascospores* 40–45 × 15–18 µm ( $\bar{x}$  = 42.16 µm, n = 20), overlapping, broadly fusiform, 6-septate, widest at the middle cells, hyaline to pale brown, constricted at the septa. Asexual morph: *Conidiomata* 161–185 × 150–170 µm, pycnidial, scattered, solitary, immersed to erumpent, visible as black spots on host surface, uniloculate, globose to subglobose, glabrous, dark brown, ostiole central, with short papilla. *Conidiomata wall* 22–30 µm wide, thin walled, of equal thickness, composed of 3–5 layers of flattened, brown to dark brown, pseudoparenchymatous cells, arranged in a *textura angularis*. *Conidiophores* arising from basal cavity of conidioma, mostly reduced to conidiogenous cells, hyaline to brown, septate. *Conidiogenous cells* 5–9 × 3.5–5.5 µm ( $\bar{x}$  = 7 × 4.6 µm, n=10), holoblastic, phialidic, discrete, oblong to ampulliform, hyaline to brown, 1-septate at the base, smooth-walled. *Conidia* 20–48 × 7–16 µm ( $\bar{x}$  = 46 × 15.4 µm, n = 30), solitary, dictyosporous, muriform, ellipsoidal, ovoid, or lageniform, with obtuse ends or sometimes truncate at the basal cell, initially hyaline, becoming brown to yellowish-brown at maturity, mostly 7–8 transverse septa, 0–2 longitudinal septa, with several segments, smooth and thick-walled, with a mucoid appendage.



**Figure 31** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -7756.450937. The combined LSU, SSU, ITS and TEF sequence dataset comprised 26 strains and *Staurosphaeria rhamnocola* (MFLUCC 17-0813, MFLUCC 17-0814). as

the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 18.89% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.248100, C = 0.227570, G = 0.275219, T = 0.249111; substitution rates AC = 2.574509, AG = 3.950406, AT = 2.257360, CG = 1.199888, CT = 10.821629, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.127888. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.008 changes. The ex-type strains are in bold and new isolates in blue bold.

Culture characteristics – Culture on MEA slow growing, reaching 40 mm diam., after three weeks, circular, olivaceous-grey at the center, yellowish white at the margins, spreading, flattened, felt-like, sparse, aerial, surface, smooth with filamentous edge; reverse blackish-grey at the center, yellowish white at the margins.

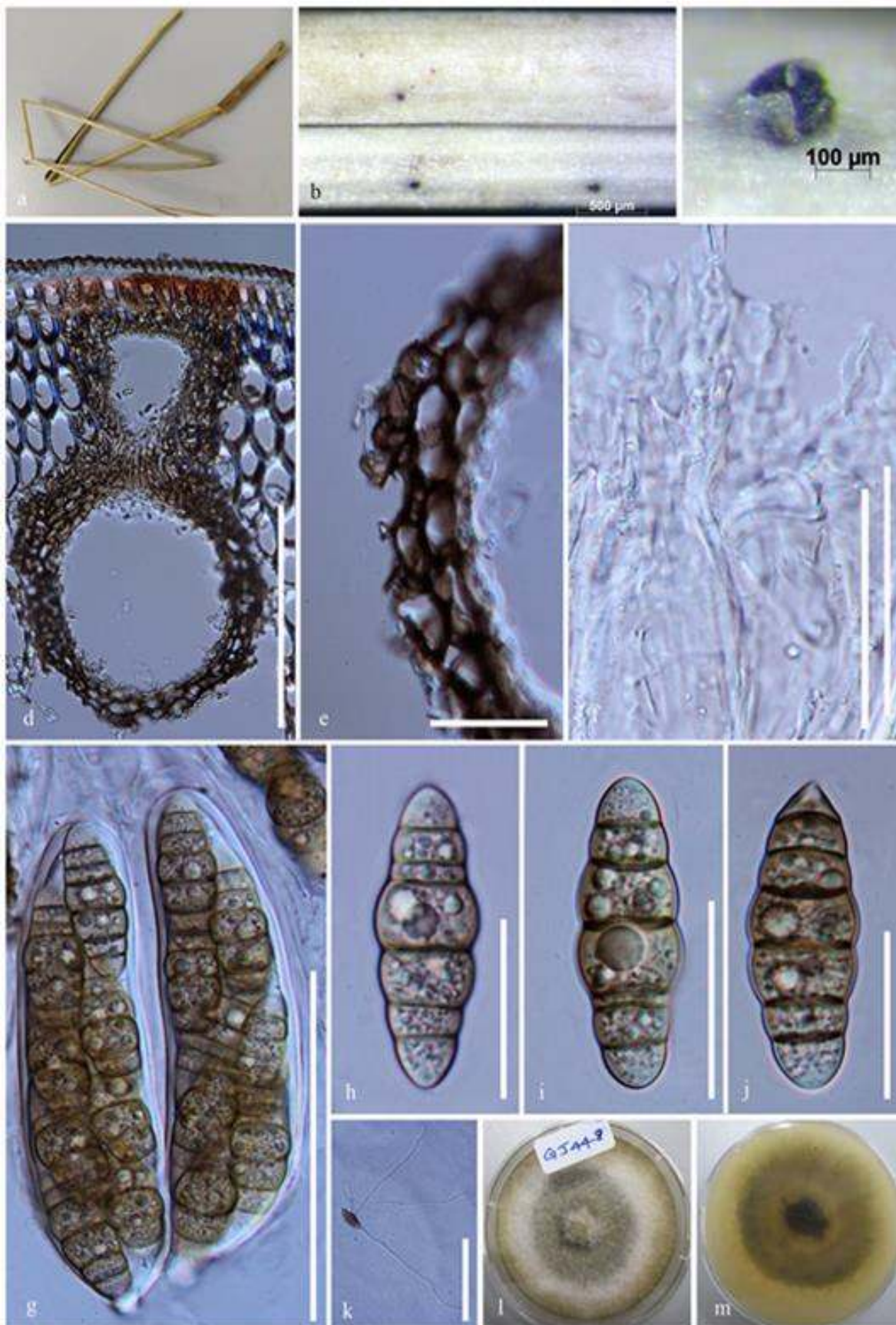
Material examined – UK, Wales, Carmarthenshire, Cefn Sidan, on leaf of Marram grass (*Ammophila arenaria*) in sand dune, 15 October 2017, E.B. Gareth Jones, GJ448 (MFLU 17-2571, holotype); *ibid.*, on leaf of Marram grass (*Ammophila arenaria*) in sand dune, 15 October 2017, E.B. Gareth Jones, GJ445a (MFLU 17-2568, paratype).

GenBank numbers – LSU: MN017847, MN017848; SSU: MN017913, MN017914; ITS: MN047087, MN047088; TEF: MN077065, MN077066

Notes – In a phylogenetic analysis based on concatenated LSU, SSU, ITS and TEF sequence data, *Amarenographium ammophilicola* groups within Phaeosphaeriaceae and sister to *A. ammophilae* in a well-separated clade (100% ML, 1.00 PP) (Fig. 31). We obtained sexual and asexual morphs of this species from the same host but from different specimens. They formed sister lineages in our phylogram and there are no significant base pair differences among those two strains. *Amarenographium ammophilicola* is morphologically somewhat similar to *Amarenomyces ammophilae*, the type species of *Amarenomyces* which was earlier considered as the sexual morph of *Amarenographium*. Our phylogenetic analyses showed that *A. ammophilicola* does not group with any of the *Amarenomyces* species. *Amarenographium ammophilicola* is phenotypically different from *Amarenomyces ammophilae* by the relatively larger ascospores (300–320  $\mu\text{m}$  vs 170–290), and the absence of hyaline gelatinous sheath surrounding ascospores and the ascospore guttulation pattern (Nag Raj 1993). This is further confirmed by their base pair differences in ITS and TEF gene loci (Table 3). The asexual morph of *A. ammophilicola* shows typical asexual morph characteristics of *Amarenographium* such as, pycnidial, papillate, ellipsoidal to subglobose, glabrous, brown to dark brown conidiomata, hyaline to brown, septate, branched, ampulliform conidiogenous cells, dictyosporous, brown to dark brown, muriform, conidia which are paler at the end cells with a mucoid appendage at the apex (Phookamsak et al. 2014). The asexual morph of *A. ammophilicola* is morphologically reminiscent of the type *Amarenographium metableticum* but can be easily distinguish by measurements of conidiogenous cells and ascospores septation. Conidiogenous cells and conidia (3–4  $\mu\text{m}$   $\times$  1.5–2.5  $\mu\text{m}$  and 24–27  $\mu\text{m}$   $\times$  12–15  $\mu\text{m}$ ) of *A. metableticum* are smaller than those of *A. ammophilicola* (5–9  $\times$  3.5–5.5  $\mu\text{m}$  and 20–48  $\times$  7–16  $\mu\text{m}$ ) (Phookamsak et al. 2014). Furthermore, *A. metableticum* has 3–4 transverse septa, 1–4 longitudinal septa in their conidia while, *A. ammophilicola* has 7–8 transverse septa, 0–2 longitudinal septa (Phookamsak et al. 2014).

**Table 3** Base pair differences of *Amarenographium ammophilicola* to related taxa.

Taxon	ITS	TEF
<i>Amarenographium ammophilae</i>	1.8% (10 bp out of 538 bp)	-
<i>Amarenomyces ammophilae</i>	4.3% (23 bp out of 538 bp)	3.2% (28 bp out of 869 bp)



**Figure 32** – Sexual morph of *Amarenographium ammophilicola* (MFLU 17-2571, holotype). a Host. b, c Appearance of ascomata on host. d Section of ascomata. e Peridium. f Pseudoparaphyses. g Asci. h–j Ascospores. k Germinating ascospore. l, m Cultures on MEA (l-upper, m-lower). Scale bars: b = 500 µm, c, d, f, g = 100 µm, k = 50 µm, e, h–j = 20 µm.

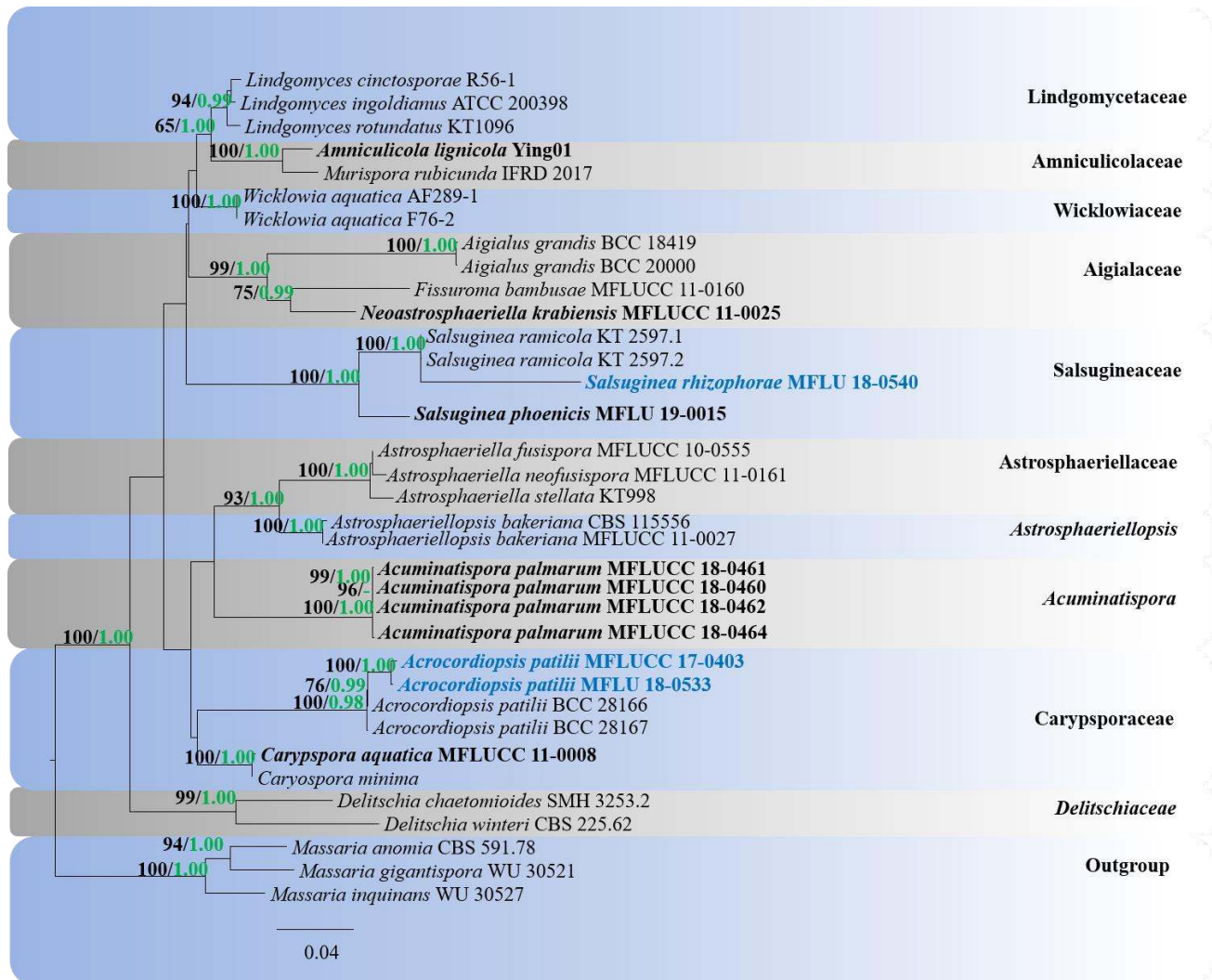


**Figure 33** – Asexual morph of *Amarenographium ammophilicola* (MFLU 17-2568). a Host. b Appearance of conidioma on host. c Section of pycnidium. d Pycnidial wall. e–i Conidia attached to the conidiogenous cells. j–m Immature to mature conidia. Scale bars: a = 500 µm, c, d, f, g = 100 µm, k = 50 µm, e, h–j = 20 µm.

**Caryosporaceae** Huang Zhang, K.D. Hyde & Ariyaw.

Ariyawansa et al. (2015a) introduced the new family Caryosporaceae to accommodate *Acrocordiopsis* and *Caryospora* based on phylogenetic analyses. Wijayawardene et al. (2018) referred *Acrocordiopsis* in Salsugineaceae following Hyde et al. (2013). Jones & Pang (2012)

considered the phylogenetic placement of *A. patilii* as unresolved. Zhang et al. (2018) showed that the phylogenetic placement of the taxa *Acrocordiopsis*, *Astrosphaeriella*, *Astrosphaeriellopsis* and *Caryospora* are not well resolved.



**Figure 34** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -16446.792988. The combined LSU, SSU, TEF and RPB2 sequence datasets comprised 29 strains of Salsugineaceae and related taxa with *Massaria inquinans* (WU 30527), *M. anomia* (CBS591.78) and *M. gigantispora* (WU 30521) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI and. The matrix had 1170 distinct alignment patterns, with 39.14% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.253912, C = 0.231759, G = 0.277414, T = 0.236915; substitution rates AC = 1.596685, AG = 4.432406, AT = 1.238993, CG = 1.541040, CT = 9.176903, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.178423. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.04 changes. The ex-type strains are in bold and new isolates in blue bold.

### *Acrocordiopsis* Borse & K.D. Hyde

*Acrocordiopsis patilii* was phylogenetically first treated by Suetrong et al. (2009) who demonstrated that *A. patilii* grouped as a residual paraphyletic assemblage within the class Dothideomycetes and could not be assigned to any of the families in this class. Subsequently, this was supported by Jones & Pang (2012). Hyde et al. (2013) assigned *Acrocordiopsis* to Salsugineaceae based on sequence data of Suetrong et al. (2009). Ariyawansa et al. (2015b) accommodated *Acrocordiopsis* in the family Caryosporaceae along with *Caryospora*, while,



Wijayawardene et al. (2018) listed *A. patilii* in Salsugineaceae following Hyde et al. (2013). There are two accepted species in this genus (Index Fungorum 2019). However according to our phylogenetic analyses *Acrocordiopsis* grouped within Caryosporaceae. Hence, we reinstate *Acrocordiopsis* within Caryosporaceae. However, type species of *Caryospora*, *C. putaminum* has not been sequenced.

***Acrocordiopsis patilii*** Borse & K.D. Hyde, Mycotaxon 34(2): 536 (1989) Fig. 35

Index Fungorum number – IF 135664, Facesoffungi Number – FoF 00787

*Saprobic* on submerged wood of *Rhizophora apiculata* and *Xylocarpus* sp. Sexual morph: *Ascomata* up to 2.5 mm diam., conical or semi-globose, occur on black stroma, gregarious, black, carbonaceous, superficial, axis vertical, base flattened, epapillate, ostiolate. *Peridium* 160–200 µm wide, composed of several layers of thick-walled, melanized cells, appearing black. *Pseudoparaphyses* 1.2–3 µm wide, numerous, filiform, anastomosing and septate, trabeculate, branched. *Asci* 175–200 × 13.5–15 µm ( $\bar{x}$  = 178 × 14.2 µm, n = 30), 8-spored, cylindrical, bitunicate, thick-walled, pedicellate, with an apical thickening and an ocular chamber. *Ascospores* 15–20 × 10–15 µm ( $\bar{x}$  = 16.2 × 13.4 µm, n = 30), uni-seriate, bi-celled, broadly ellipsoidal, hyaline, 1-euseptate, slightly constricted at the septum, with a large guttule in each cell, with small granules concentrated near the septum, thick walled. Asexual morph: Undetermined.

Culture characteristics – Ascospores germinating on 50% sea water agar within 24 hours, with germ tubes developed from lower side of ascospores. Colonies on PDA slow growing, reaching 10–20 mm diam., after 15 days of incubation at 25 °C, filamentous, greenish black at the center becoming black, yellowish at the margins, spreading, flattened, fluffy, sparse, reverse black, yellowish at the margins.

Material examined – THAILAND, Ranong Province, Ranong District, on decaying, submerged wood of *Xylocarpus* sp. (Meliaceae), 07 December 2016, M.C. Dayarathne, MCD 64 (MFLU 18-0547); *ibid.*, (HKAS102085), living cultures MFLUCC 17-0403). THAILAND, Ranong Province, Ranong, on decaying, submerged wood of *Rhizophora apiculata* (Rhizophoraceae) 06 December 2016, M.C. Dayarathne, MCD 46 (MFLU 18-0533).

GenBank numbers – LSU: MN017849, MN017850; SSU: MN017915, MN017916; TEF MN077067; RPB2: MN077078

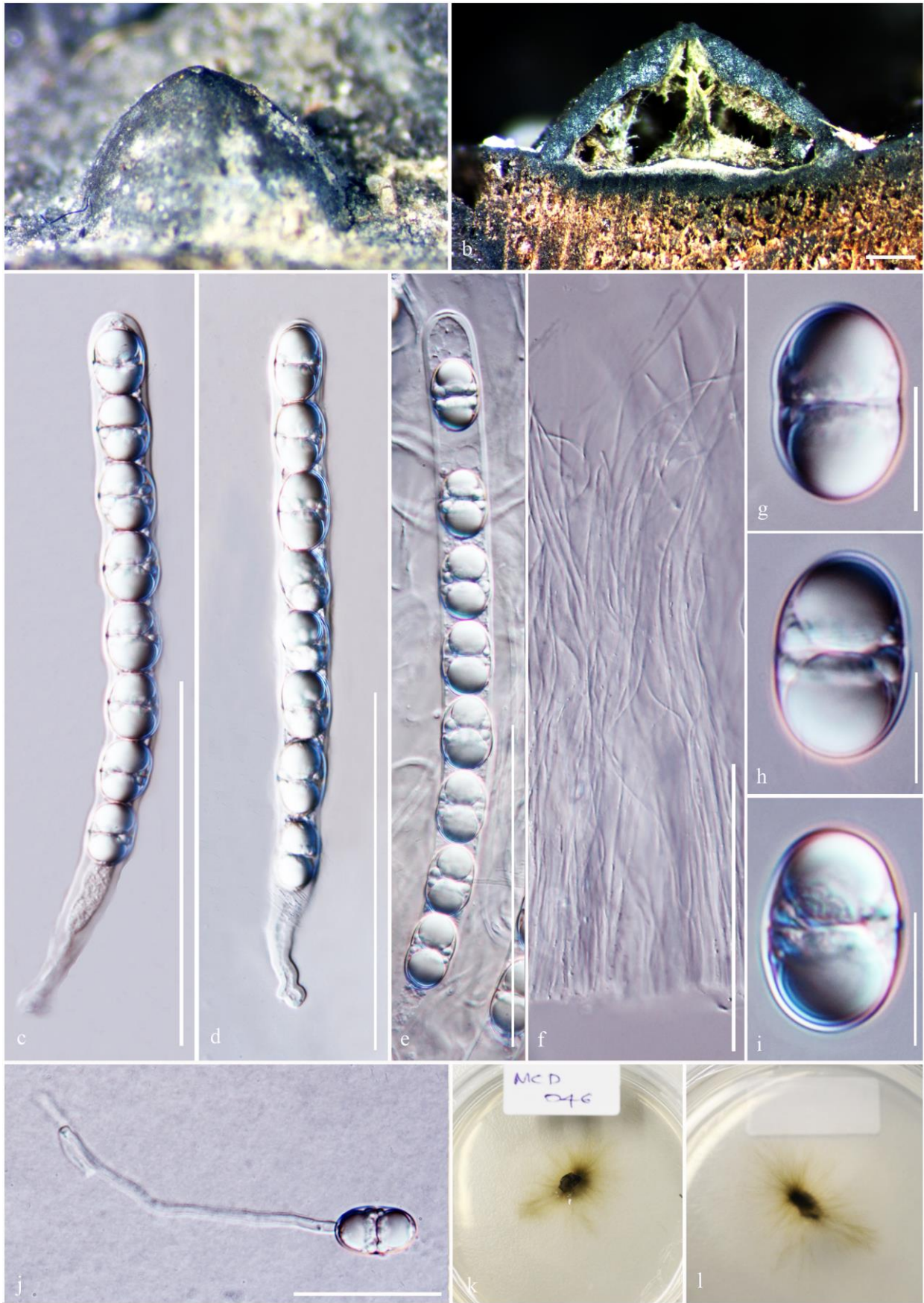
Notes – Our two strains (MFLUCC 17-0403, MFLU 18-0533) are morphologically and phylogenetically (Fig. 34) similar to *Acrocordiopsis patilii* and there are no accountable base pair differences of LSU, SSU, and RPB2 data. *Acrocordiopsis patilii* phenotypically closely resembles *A. sphaerica* but differs in having smaller and narrower asci (Table 4). Furthermore, ascospores of *A. sphaerica* are often spherical and wider in diameter and not constricted at the septum, while in *A. patilii* ascospores are narrower (Table 4) and slightly constricted at the septum. However, no sequence data are available for *A. sphaerica* to determine their phylogenetic relationships. In this study we provide a new host record of this species from *Xylocarpus* sp.

### **Salsugineaceae** K.D. Hyde & Tibpromma

Hyde et al. (2013) introduced the family Salsugineaceae to accommodate the genera *Acrocordiopsis* and *Salsuginea* (family type). In the phylogenetic analyses of Hyde et al. (2013) *Salsuginea ramicola* clusters with *Acrocordiopsis patilii* in a clade within the Pleosporales with weak support. However, Zhang et al. (2018) showed that these two genera were distantly placed and this is supported by our data.

### ***Salsuginea*** K.D. Hyde, Bot. Mar. 34(4): 315 (1991)

*Salsuginea* was introduced with *S. ramicola* as the type species on mangrove wood of intertidal *Aegiceras* sp. collected in Thailand (Hyde 1991a). Zhang et al. 2009 and in Jones et al. (2019) introduced the second species, *S. phoenicis* to the genus. In this study we introduce a novel species *S. rhizophorae* from decayed mangrove wood.



**Figure 35** – *Acrocordiopsis patilii* (MFLU 18-0547). a Appearance of ascomata on host. b Vertical section of ascomata. c–e Asci f Pseudoparaphyses. g–i Ascospores. j Germinating ascospore. k, l Cultures on PDA. (k-upper, l-lower). Scale bars: b = 200  $\mu\text{m}$ , c–f = 100  $\mu\text{m}$ , g–i = 10  $\mu\text{m}$ , j = 50  $\mu\text{m}$ .

**Table 4** Synopsis of the morphological characteristics of *Acrocordiopsis* species.

Species	Ascomata (mm)	Asci (µm)	Ascospores (µm)	Reference
<i>A. patilii</i>	2.5 diam.	175–198 × 13.5–14.8	15.8–20.5 × 11.2–14.4	This study
<i>A. sphaerica</i>	1–2 high	180–270 × 19.5–34	18–27 × 15–24	Alias et al. (1999)

*Salsuginea rhizophorae* Dayarathne, E.B.G. Jones & K.D. Hyde, sp.nov.

Fig. 36

Index Fungorum number: IF556582; Facesoffungi Number: FoF 06169

Etymology – Species epithet refers to the host genus where the fungus was isolated

Holotype – MFLU 18-0540

*Saprobic* on decaying wood submerged in brackish waters in mangroves. Sexual morph: *Ascomata* 590–725 × 620–680 µm ( $\bar{x}$  = 684 × 653 µm, n = 5), immersed beneath a raised dark brown to black pseudoclypeus, brown to black, carbonaceous, comprising host cells and dark fungal hyphae, solitary, in section flask-shaped, with a protruding papilla. *Papilla* conspicuous, central, cone-shaped, brown to black, ostiolate. *Peridium* 84–115 µm wide, comprising a single layer of light brown cells of *textura globulosa*, emerging at the outside with the host, where cells of fungus become irregular and contorted and intermingle with host cells. *Hamathecium* 0.9–1.8 µm wide, comprising numerous, filiform, trabeculate, branched, septate, hyaline pseudoparaphyses. *Asci* 175–200 × 10–15 µm ( $\bar{x}$  = 178 × 14.2 µm, n = 30), 8-spored, bitunicate, fissitunicate, cylindrical to clavate, with an apical apparatus, rounded, with an ocular chamber. *Ascospores* 80–95 × 28–42 µm ( $\bar{x}$  = 178 × 30 µm, n = 30), partially overlapping uni-seriate, fusciform, symmetrical, tapering toward acute ends, 1-septate, constricted at the septum, hyaline when immature becoming brown to dark brown with age, with subhyaline germ pores at both ends, smooth-walled, with a large guttule per each cell and numerous small granules. Asexual morph: Undetermined.

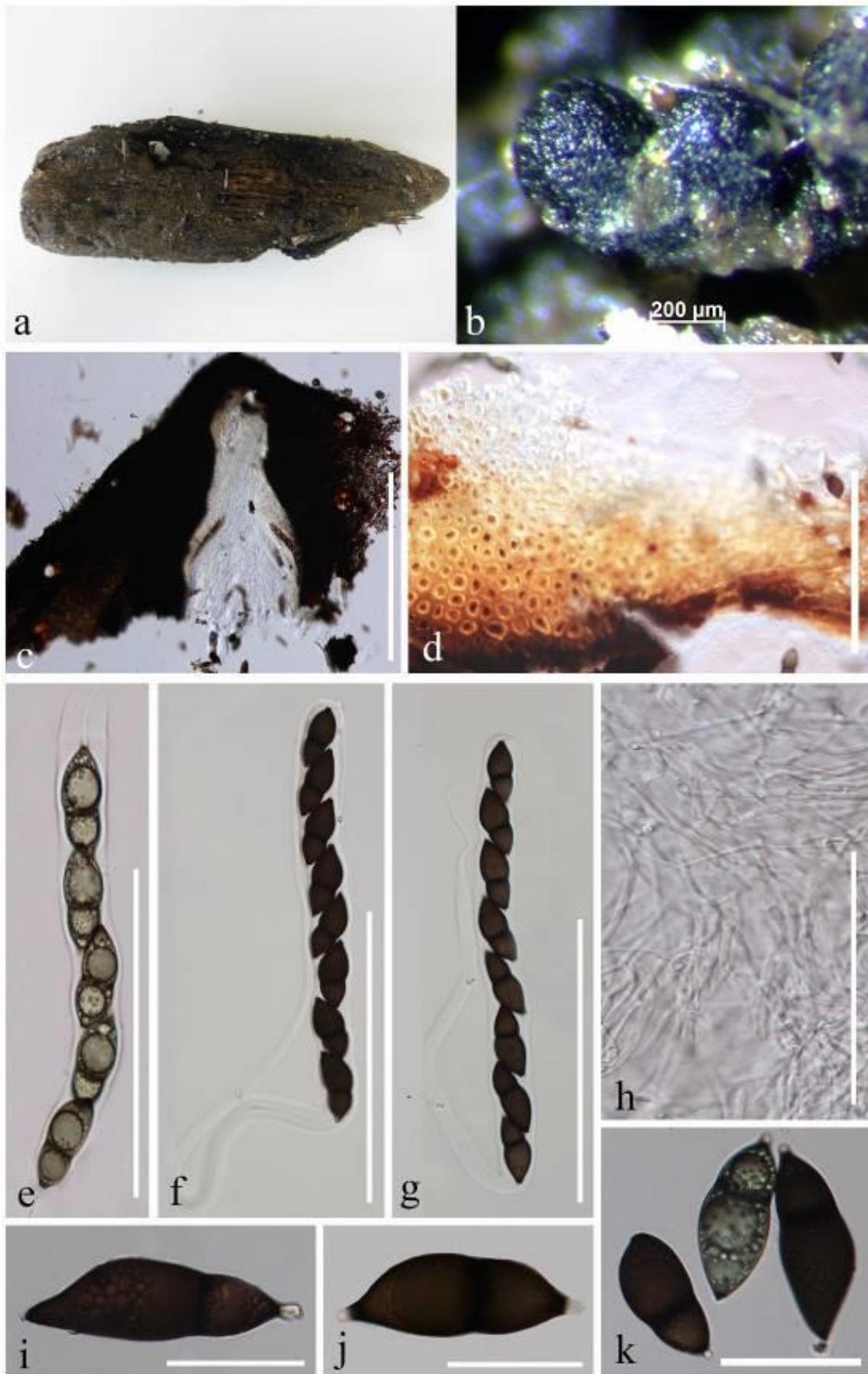
Material examined – THAILAND, Ranong Province, Ranong District, on decaying, submerged wood of *Rhizophora apiculata* (Rhizophoraceae), 07 December 2016, M.C. Dayarathne, MCD 055 (MFLU 18-0540, holotype); *ibid.*, (BBH, isotype).

GenBank numbers – LSU: MN017851

Notes – *Salsuginea rhizophorae* is morphologically comparable to *S. ramicola* and their characters summarized in Table 5. Both species having conical ascomata but the ascomatal base of *S. ramicola* is considerably wider (elongated horizontally than vertical axis) (Hyde 1991a) and ascomata of *S. rhizophorae* are flask shaped which is elongated in vertical axis rather than the horizontal axis. Base pair differences between *S. rhizophorae* and *S. ramicola* are 22 bp of LSU out of 769 bp (2.8%) and SSU 3.8% (36 bp out of 936 bp). Base pair differences of LSU of *S. phoenicis* and *S. rhizophorae* are 5.7% (44 bp out of 769 bp) for LSU.

### Testudinaceae Arx

von Arx (1971) introduced the family Testudinaceae to include the genera *Testudina* (type), *Neotestudina*, *Lepidosphaeria*, *Argynna* (later transferred to Argynnaceae) and *Pseudophaeotrichum* (synonymized as *Neotestudina*). Testudinaceae is characterized by astomatous ascomata with a dark peridium, bitunicate asci and dark, 1-septate ascospores (about 10 µm long) (Phookamsak et al. 2015, Wanasinghe et al. 2017). Hawksworth & Booth (1974) synonymized Testudinaceae under Zopfiaceae. Subsequently, Hawksworth (1979), considered Zopfiaceae as a synonym of Testudinaceae on the basis of the size and more especially the shape and type of sculpturing of the ascospores in SEM studies. Eriksson (1981) accepted both families and Zopfiaceae was validated by Eriksson & Hawksworth (1992). Hyde et al. (2013) considered Testudinaceae as a family in Dothediomycetes and included five genera: *Lepidosphaeria*, *Neotestudina*, *Testudina*, *Ulospora* and *Verruculina* (Suetrong et al. 2009).



**Figure 36** – *Salsuginea rhizophorae* (MFLU 18-0540, holotype). a Host. b Appearance of ascomata on *Rhizophora apiculata*. c Section through ascoma. d Peridium. e–g Asci.

h Pseudoparaphyses. i–k Ascospores. Scale bars: b = 200  $\mu\text{m}$ , c = 500  $\mu\text{m}$ , e–h = 100  $\mu\text{m}$ , d, i–k = 50  $\mu\text{m}$ .

Currently, Testudinaceae includes the genera *Angustospora*, *Lepidosphaeria*, *Lojkania*, *Muritestudina*, *Neotestudina*, *Testudina*, *Ulospora* and *Verruculina* (Hyde et al. 2013, Wanasinghe et al. 2017). This paper introduces a novel genus *Halotestudina* and a novel species to the family Testudinaceae which was collected from mangrove habitats.

**Table 5** Measurements of the ascomata, asci and ascospores of *Salsuginea* species.

Species	Ascomata (mm)	Asci ( $\mu\text{m}$ )	Ascospores ( $\mu\text{m}$ )	Reference
<i>S. ramicola</i>	1040–2600 $\times$ 455–1430	440–512 $\times$ 29–34	59–72 $\times$ 24–30	Hyde 1991a
<i>S. rhizophorae</i>	590–725.4 $\times$ 620–680	175–198 $\times$ 13.5–14.8	80–94 $\times$ 28–42	This study
<i>S. phoenicis</i>	410–480 $\times$ 680–980	140–275 $\times$ 23–30	46–62 $\times$ 16–24	Jones et al. (2019)

***Halotestudina*** Dayarathne & K.D. Hyde, gen. nov.

Index Fungorum number: IF 556584; Facesoffungi number: FoF 06170

*Saprobic* on mangrove wood. Sexual morph: *Ascomata* subglobose, completely or partially immersed, carbonaceous to coriaceous, black, gregarious, ostiolate. *Peridium* coriaceous to carbonaceous, two-layered, outer layer composed of elongate cells, inner layer composed of smaller hyaline cells. *Hamathecium* comprising trabeculate pseudoparaphyses, unbranched at the base, anastomosing above the asci, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical, long-pedicellate, with a refractive non-amyloid, apical ring. *Ascospores* biseriate, ellipsoidal to broadly fusiform, muriform, pale brown to brown, with hyaline to pale brown apical cells, glabrous, with a gelatinous cap or a mucilaginous sheath. Asexual morph: Unknown.

Type – *Halotestudina muriformis* Dayarathne & K.D. Hyde

Notes – *Halotestudina* can be easily distinguish from other Testudinaceae genera by its brown muriform ascospores that are constricted at each septum. The genus *Muritestudina* resembles *Halotestudina* by having muriform ascospores but it can be clearly distinguished as it has hyaline ascospores surrounded by a thick, mucilaginous sheath (Wanasinghe et al. 2017). *Verruculina* is clearly different form *Halotestudina* in ascospore morphology which are 1-septate, while ascospores of *Halotestudina* are muriform. In our phylogenetic analyses they formed a moderately-supported (73% ML) clade (Fig. 37). *Halotestudina* and *Verruculina* are two marine genera referred to Testudinaceae. *Halotestudina* shows a close phylogenetic placement to the genera *Trematosphaeria* and the *Angustospora*. *Trematosphaeria* species are different form *Halotestudina* in having a trabeculate hamathecium embedded in a gelatinous matrix and reddish-brown ellipsoid to fusoid transversely septate ascospores sometimes with paler end cells (Boise 1985, Barr 1990b, Mckeown et al. 2001). *Angustospora* is morphologically quite different from *Halotestudina* in having large ascospores with a median septum and additional septa near the poles of the ascospores (Li et al. 2016).

***Halotestudina muriformis*** Dayarathne & K.D. Hyde, sp. nov.

Fig. 38

Index Fungorum number: IF556583; Facesoffungi number: FoF 06171

Holotype – MFLU 17-2634

*Saprobic* on mangrove wood. Sexual morph: *Ascomata* 750–990  $\times$  700–750  $\mu\text{m}$  ( $\bar{x}$  = 880  $\times$  740  $\mu\text{m}$ , n = 5), globose to subglobose, semi immersed, carbonaceous to coriaceous, black, gregarious, ostiolate. *Peridium* 70–90  $\mu\text{m}$  wide, coriaceous to carbonaceous, two-layered, outer

layer composed of elongate cells, inner layer composed of smaller hyaline cells. *Hamathecium* comprising 0.6–1.5 µm wide, hyaline, branched, aseptate, pseudoparaphyses, anastomosing above the asci, embedded in a gelatinous matrix. *Asci* 180–240 × 15–27 µm ( $\bar{x}$  = 200 × 22 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical, short-pedicellate, with a non-amyloid, apical ring. *Ascospores* 30–45 × 10–17 µm ( $\bar{x}$  = 34 × 12 µm, n = 20), uniseriate, ellipsoidal to broadly fusiform, muriform, with 7 transverse septa and 0–2 longitudinal septa, brown, with pale brown apical cells, constricted at the septa, smooth-walled. Asexual morph: Unknown.

Material examined – THAILAND, Krabi Province, Krabi, on decaying wood of *Rhizophora* sp. (Rhizophoraceae) wood, 31 August 2017, M.C. Dayarathne, MCD 163 (MFLU 17-2634, holotype), ex type living culture MFLUCC 18-0392; THAILAND, Phetchaburi Province, Cha-am beach, on bark attached to the living plant of *Bruguiera* sp. (Rhizophoraceae), 06 December 2016, M.C. Dayarathne, MCD 035 (MFLU 18-0524), living culture MFLUCC 17-0395.

GenBank numbers – LSU: MN017852, MN017894, MN017853, MN017854; SSU: MN017918, MN017947, MN017919, MN017920; TEF: MN077068; RPB2: MN077079

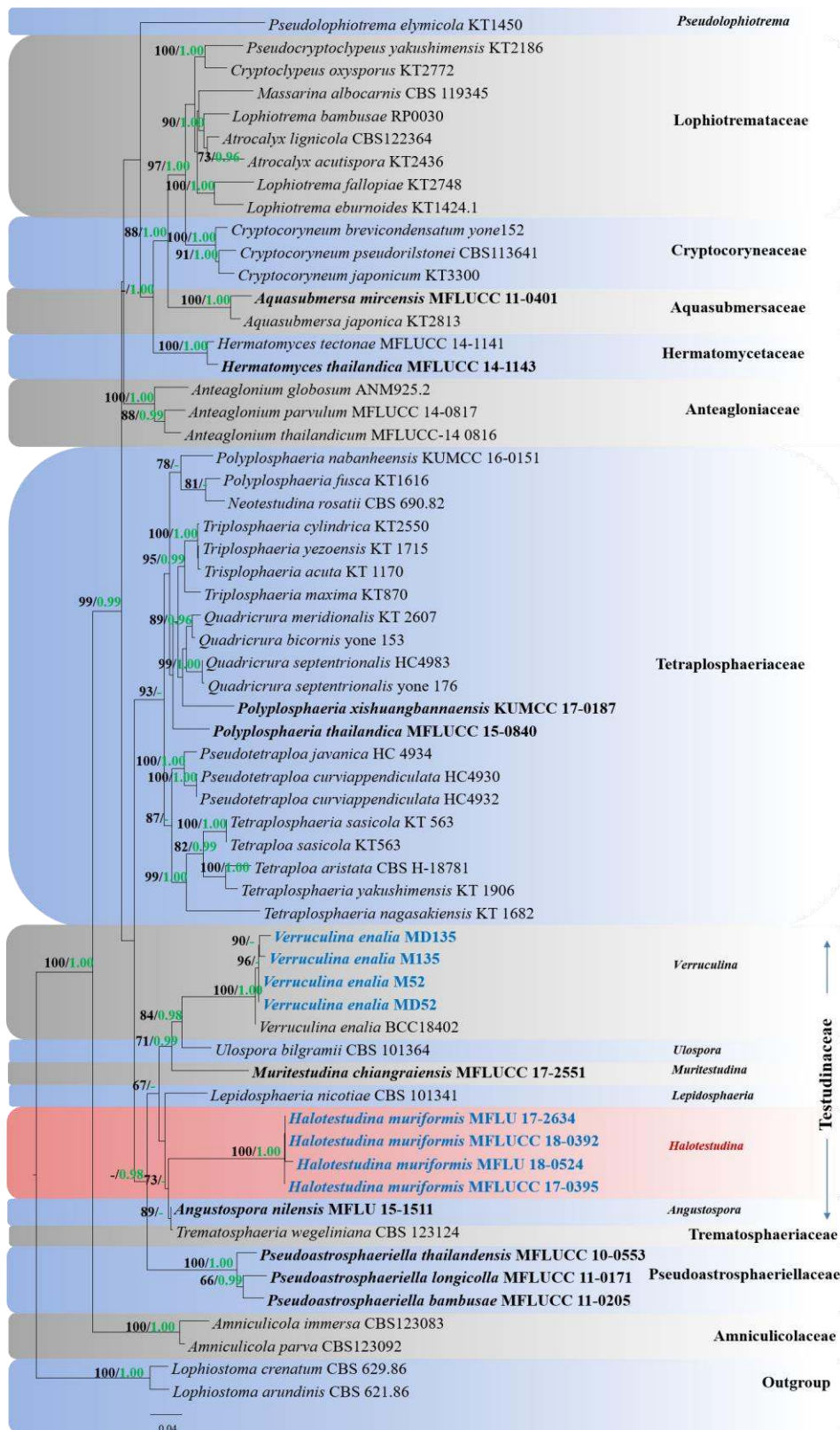
Notes – *Halotestudina muriformis* is characterized by globose to subglobose, semi immersed, carbonaceous to coriaceous ascomata, branched, aseptate, pseudoparaphyses, anastomosing above the asci and ellipsoidal to broadly fusiform, brown, muriform ascospores with 7 transverse septa and 0–2 longitudinal septa, with pale brown apical cells, constricted at the septa. Four isolates of *H. muriformis* formed a moderately supported (73% ML) clade within Testudinace sister to *Angustospora nilensis* (MFLU 15-1511) and *Trematosphaeria wegeliniana* (CBS 123124) (Fig. 37). Morphologically, genera of Testudinaceae are highly diverse especially by having different ascospore morphologies and this is the first record of brown muriform ascospores in Testudinaceae. TEF and RPB2 base pair differences of *Halotestudina muriformis* to *Muritestudina chiangraiensis* and *Verruculina enalia* are given in Table 6.

### ***Verruculina* (Kohlm.) Kohlm. & Volkm-Kohlm.**

Kohlmeyer & Volkmann-Kohlmeyer (1990) introduced the obligate marine monotypic genus *Verruculina* with *V. enalia* as the type species. The type species was originally described as *Didymosphaeria enalia*. Later, Barr (1990b) assigned it to *Lokjania* and finally to *Verruculina* (Kohlmeyer & Volkmann-Kohlmeyer 1990). Molecular studies confirmed its placement in Testudinaceae (Suetrong et al. 2009, Hyde et al. 2013, Phookamsak et al. 2015, Wanasinghe et al. 2017).

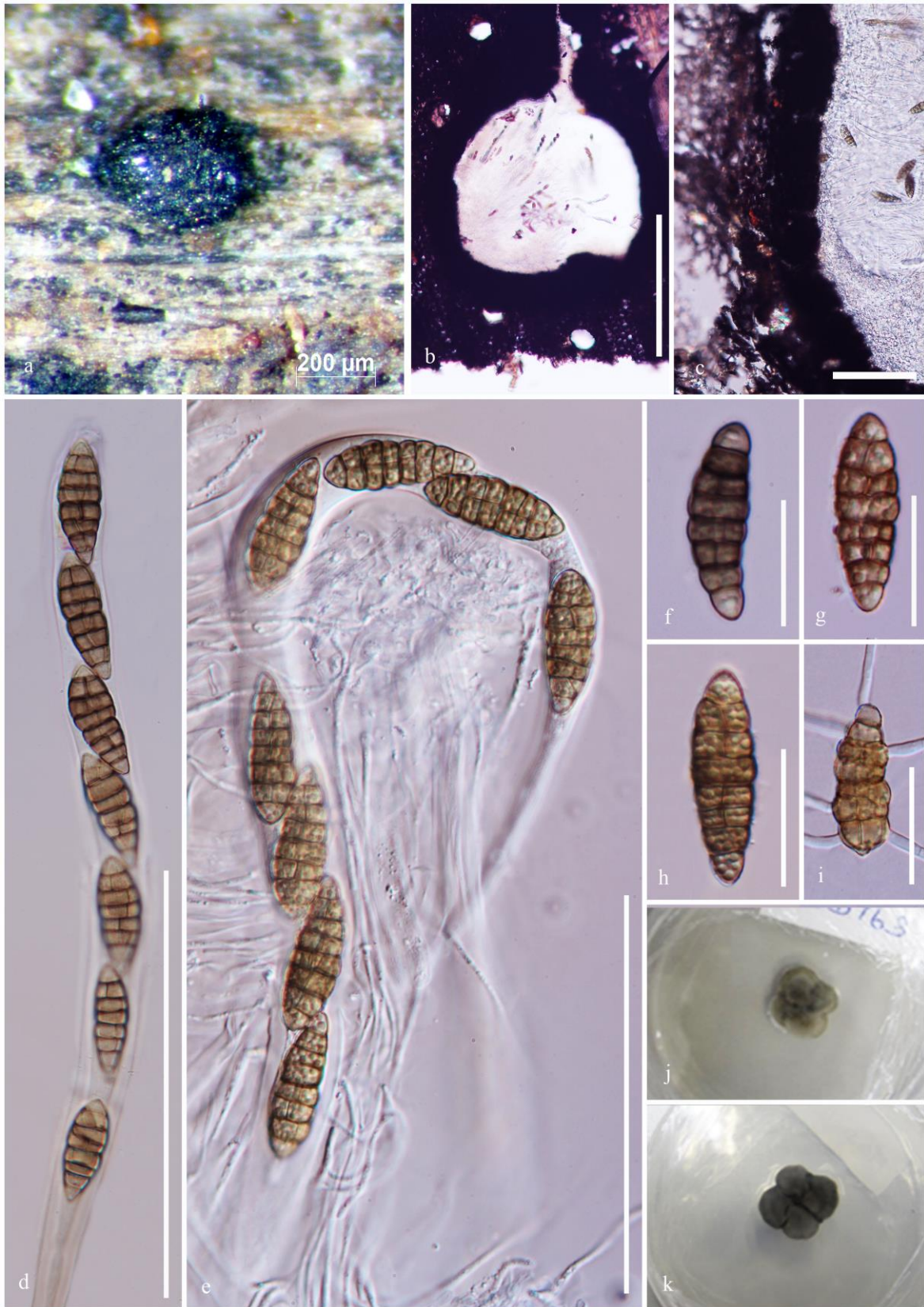
### **Key to genera of Testudinaceae**

- |   |                       |
|---|-----------------------|
| 1. Ascospores muriform.....   | 2                     |
| 1. Ascospores only transversely septate .....   | 3                     |
| 2. Ascospores hyaline .....   | <i>Muritestudina</i>  |
| 2. Ascospores brown.....  | <i>Halotestudina</i>  |
| 3. Ascomata perithecial, peridium lacking plates .....  | 4                     |
| 3. Ascomata cleistothecial, peridium composed of plates .....                                 | 6                     |
| 4. Pseudoparaphyses trabeculate .....   | 5                     |
| 4. Pseudoparaphyses not trabeculate .....   | <i>Verruculina</i>    |
| 5. Ascospores one septate .....   | <i>Lojkania</i>       |
| 5. Ascospores (3)–5–(7) septate .....   | <i>Angustospora</i>   |
| 6. Ascospores ornamented .....  | 7                     |
| 6. Ascospores glabrous .....  | 8                     |
| 7. Asci obovate or broadly clavate, ascospores reticulate .....                               | <i>Testudina</i>      |
| 7. Asci clavate, with a long stalk, ascospores finely echinulate, darker near the septum..... | <i>Lepidosphaeria</i> |
| 8. Asci obovate or nearly sphaerical, ascospores not darker near the septum .....             | <i>Neotestudina</i>   |
| 8. Ascospores with germ-slits.....  | <i>Ulopora</i>        |



**Figure 37** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with likelihood value of -31642.769668. The combined LSU, SSU, TEF and RPB2 sequence datasets comprised 61 strains with *Lophiostoma crenatum* (CBS 629.86) and *L. arundinis* (CBS 621.86) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI and in congruent with previous study Wanasinghe et al. (2017). The matrix had 1742 distinct alignment patterns, with 33.77% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.247462, C = 0.247183, G = 0.271298, T = 0.234057; substitution rates AC = 1.697785, AG =

4.459687, AT = 1.614825, CG = 1.267576, CT = 9.339520, GT = 1.000000; gamma distribution shape parameter  $\alpha = 0.184250$ . Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.80% are given above the nodes. The scale bar indicates 0.04 changes. The ex-type strains are in bold and new isolates in blue bold.



**Figure 38** – *Halotestudina muriformis* (MFLU 17-2634, holotype). a Ascoma on host surface. b



Section of ascoma. c Peridium. d, e Asci and pseudoparaphyses. f–h Immature and mature ascospores. s Germinating ascospore. n, o Cultures on sea water PDA (n-upper, o lower). Scale bars: a, b = 200  $\mu\text{m}$ , d, e = 100  $\mu\text{m}$ , c = 50  $\mu\text{m}$ , f–i = 20  $\mu\text{m}$ .

**Table 6** Base pair differences of *Halotestudina muriformis* and related taxa.

Taxon	LSU	TEF	RPB2
<i>Muritestudina chiangraiensis</i>	6.8% (60 bp out of 874 bp)	26% (136 bp out of 515 bp)	21% (202 bp out of 1011 bp)
<i>Verruculina enalia</i>	7.2% (63 bp out of 874 bp)	111.3% (103 bp out of 912)	19.6% (156 bp out of 792 bp)

*Verruculina enalia* (Kohlm.) Kohlm. & Volkm.-Kohlm., Mycol. Res. 94(5): 689 (1990) Fig. 39  
Facesoffungi number: FoF 05281

*Saprobic* on *Rhizophora* sp. Sexual morph: *Ascomata* 800–1200  $\mu\text{m}$  high, 410–520  $\mu\text{m}$  diam., ( $\bar{x}$  = 780  $\times$  460  $\mu\text{m}$ , n = 10) subglobose, ampulliform or depressed ellipsoidal, black, carbonaceous, partly or completely immersed, clypeate, solitary, ostiolate, papillate. *Ostiole* periphysate. *Peridium* 80–142  $\mu\text{m}$  thick, one–stratum, composed of about six or more layers of irregular roundish or elongate, thick–walled cells, forming a *textura angularis*. *Pseudoparaphyses* 2–2.6  $\mu\text{m}$  diam., septate, rarely branched. *Asci* 130–156  $\times$  10.2–12.4  $\mu\text{m}$  ( $\bar{x}$  = 146  $\times$  10.8  $\mu\text{m}$ , n = 15), thick–walled, bitunicate, 8-spored, cylindrical, persistent, pedicellate, fissitunicate, with a faint apical apparatus. *Ascospores* 17.0–21  $\times$  8.3–10  $\mu\text{m}$  ( $\bar{x}$  = 18.5  $\times$  9.4  $\mu\text{m}$ , n = 30), overlapping uniseriate, hyaline when immature becoming dark-brown, ellipsoidal, 1-septate, constricted at the septum, verrucose to verruculose, with a large guttule in each cell.

Culture characteristics – Ascospores germinating on sea water agar within 24 h with germ tubes produced from terminal ends. Colonies reaching 6 cm in diam. in 25 days at room temperature. Colonies on sea water PDA fast growing, grayish white, brown at margins, reverse yellowish brown, filamentous, lobate.

Material examined – THAILAND, Phetchaburi Province, Cha-am beach, on bark attached to the living *Rhizophora* sp. (Rhizophoraceae), 06 December 2016, M.C. Dayarathne, MCD 052 (MFLU 18-0538); *ibid.*, HKAS102078. living culture MFLUCC 17-0406, THAILAND, Krabi Province, Krabi, on decaying wood of *Rhizophora* sp. (Rhizophoraceae), 30 August 2017, M.C. Dayarathne, MCD 135 (MFLU 17-2614), living culture (MFLUCC17-2478).

GenBank numbers – LSU: MN017855, MN017877, MN017856, MN017895; SSU: MN017921, MN017935, MN017922, MN017948; ITS: MN047089, MN047090, MN047091, MN047092

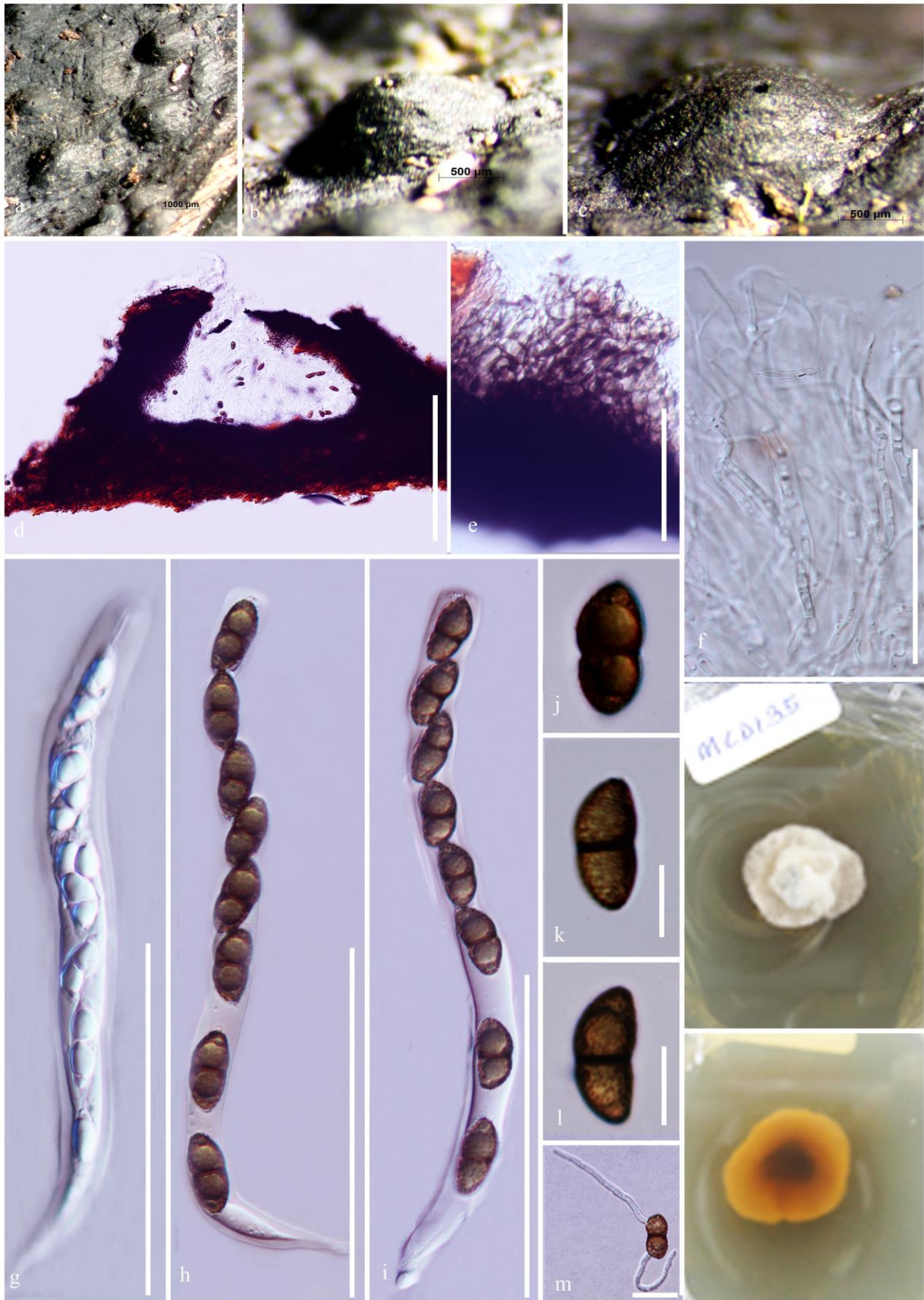
Notes – *Verruculina enalia* is distinct in having papillate ascomata and ascospores that are verrucose or verruculose (Suetrong et al. 2009). The new isolates MFLUCC 17-0406 and MFLUCC17-2478 have similar morphologies to *Verruculina enalia* described by Kohlmeyer & Volkmann-Kohlmeyer (1990) and clustered in a single clade in our phylogram (Fig. 37). However, we could not observe hyaline tubercle at each apex which is sometimes present in ascospores of this species.

### Pleosporomycetidae genera *incertae sedis*

**Botryosphaeriales** C.L. Schoch et al.

**Botryosphaeriaceae** Theiss. & H. Syd.

Theissen & Sydow (1918) erected the family Botryosphaeriaceae with *Botryosphaeria* as the type genus. Botryosphaeriaceae species are well-known as saprobes, endophytes and opportunistic pathogens and many species are causative agents of disease on ecologically and economically important plants (Slippers & Wingfield 2007, Mehl et al. 2014, Tibpromma et al. 2018) and cosmopolitan in distribution but absent in polar regions (Hyde et al. 2013, 2014, Phillips et al. 2013, 2019, Slippers et al. 2013, Dissanayake et al. 2016).



**Figure 39** – *Verrucilina enalia* (MFLU 18-0538). a–c Ascomata on host surface. d Longitudinal section of ascoma. e Peridium. f Pseudoparaphyses. g–i Immature and mature asci. j–i Verrucose to verruculose ascospores. s. Germinating ascospore. n, o Cultures on sea water PDA. Scale bars: a = 1000  $\mu\text{m}$ , b = 500  $\mu\text{m}$ , d = 100  $\mu\text{m}$ , g–i = 50  $\mu\text{m}$ , c = 20  $\mu\text{m}$ , j–i = 10  $\mu\text{m}$ .

According to Wijayawardene et al. (2018), there are 28 genera included in this family. The family has been revised by many authors both on morphological and phylogenetic basis (Phillips et al. 2008, 2013, 2019, Slippers et al. 2013, Hyde et al. 2014, Dissanayake et al. 2017, Tibpromma et al. 2018). There are several reports on the presence of Botryosphaeriaceae species from terrestrial mangroves (Osorio et al. 2016) such as *Botryosphaeria ribis* from *Hibiscus tiliaceus*, a mangrove associate, in the Hawaiian Islands (Stevens & Shear 1929), *Fusicoccum* sp. from symptomatic branches and stems of *Rhizophora mangle* in South Florida (Rayachhetry et al. 1996), *Botryosphaeria* sp., *Lasiodiplodia* sp. and *Neofusicoccum* sp. from mangroves in Brazil (De Souza et al. 2013), *Neofusicoccum* sp. from *Sonneratia ovata* in China (Xing et al. 2011), 14 taxa *Diplodia estuarina*, *D. sapinea*, *Lasiodiplodia avicenniae*, *L. bruguierae*, *L. gonubiensis*, *L. theobromae*, *N. cryptoaustrale*, *N. kwambonambiense*, *N. lumnitzeriae*, *N. luteum*, *N. mangroviorum*, *N. parvum*, *N. umdonicola* and a *Botryosphaeria* sp. as endophytes of mangroves from South Africa (Osorio et al. 2016) and *Lasiodiplodia mangrovei* from aerial dead twigs of *Rhizophora apiculata* in Thailand (Hyde et al. 2019).

### ***Lasiodiplodia* Ellis & Everh.**

*Lasiodiplodia* was introduced by Ellis & Everhart (1894) without a description. Clendenin (1896) provided a description and attributed the genus to Ellis & Everhart. The type species is *L. theobromae* (Phillips et al. 2013). Most members of this genus are plant pathogens that cause cankers, die-back, fruit or root rot, branch blight or discoloration on a wide range of woody hosts that are mostly distributed in tropical and subtropical regions (Punithalingam 1980, Ismail et al. 2012, Phillips et al. 2013). We herein introduce the second species associated with *Bruguiera* sp., but as the first saprobic species from submerged, decaying *Bruguiera* wood, whereas, the previous species was reported as an endophyte (Osorio et al. 2016).

### ***Lasiodiplodia krabiensis* Dayarathne, sp. nov.**

Fig. 41

Index Fungorum number: IF556586; Facesoffungi number: FoF 06173

Etymology – Epithet refers to the geographical region, Krabi, Thailand, where the species was found.

Holotype – MFLU 17-2617

*Saprobic* on submerged wood of *Bruguiera* sp. Sexual morph: unknown. Asexual morph: *Conidiomata* pycnidial 680–740 × 300–360 μm ( $\bar{x}$  = 718 × 326.5 μm, n = 5), scattered to gregarious, immersed to semi immersed, conspicuous on host surface with a short neck, dark brown, solitary, uniloculate, globose to subglobose, ostiolate, papillate. *Pycnidial wall* 64–82 μm wide, composed of several layers of thick-walled, hyaline to dark brown cells of *textura angularis*. *Paraphyses* 20–51 μm wide, aseptate, hyaline, straight *Conidiogenous cells* 10–14 × 3–5.3 μm ( $\bar{x}$  = 13 × 3.6 μm, n = 20), annellidic, cylindrical, thick-walled, smooth. *Conidia* 24–26.8 × 13.2–16.6 μm ( $\bar{x}$  = 25. × 14 μm, n = 20), subglobose to oval, aseptate, hyaline to subhyaline with age, guttulate, without longitudinal striations and mucilaginous sheath.

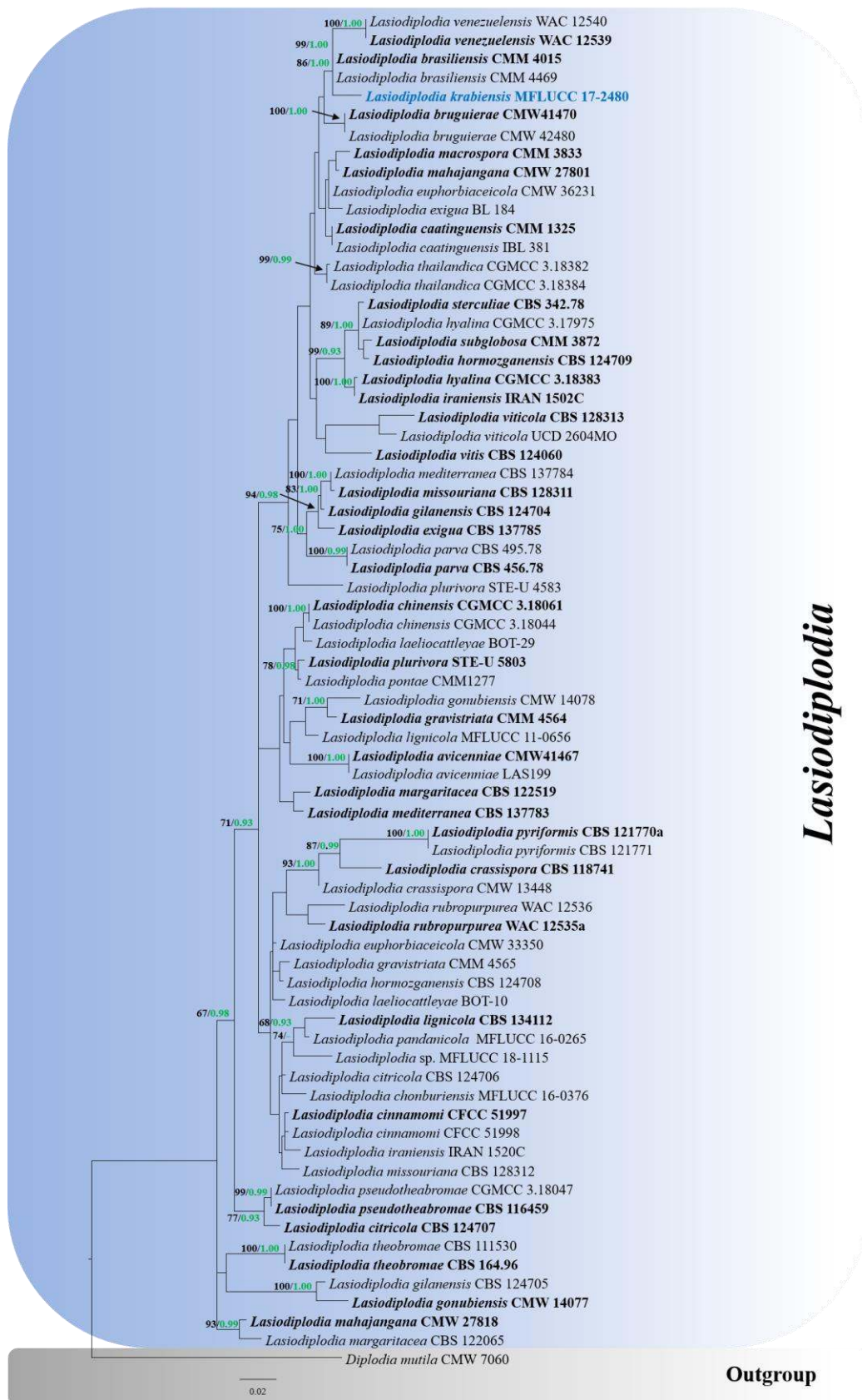
Culture characteristics – Conidia germinating on sea water PDA within 12 h. Colonies on seawater PDA, white at first, becoming black with age, circular, with entire edge, raised, velvety, black in reverse, with smooth margin.

Material examined – THAILAND, Krabi Province, Phang Nga, on decaying submerged wood of *Bruguiera* sp. (Rhizophoraceae), 30 August 2017, M.C. Dayarathne, MCD 142 (MFLU 17-2617, holotype); *ibid.*, (HKAS102051, isotype), ex-type living culture (MFLUCC 17-2480).

GenBank numbers – ITS: MN047093; TEF: MN077070

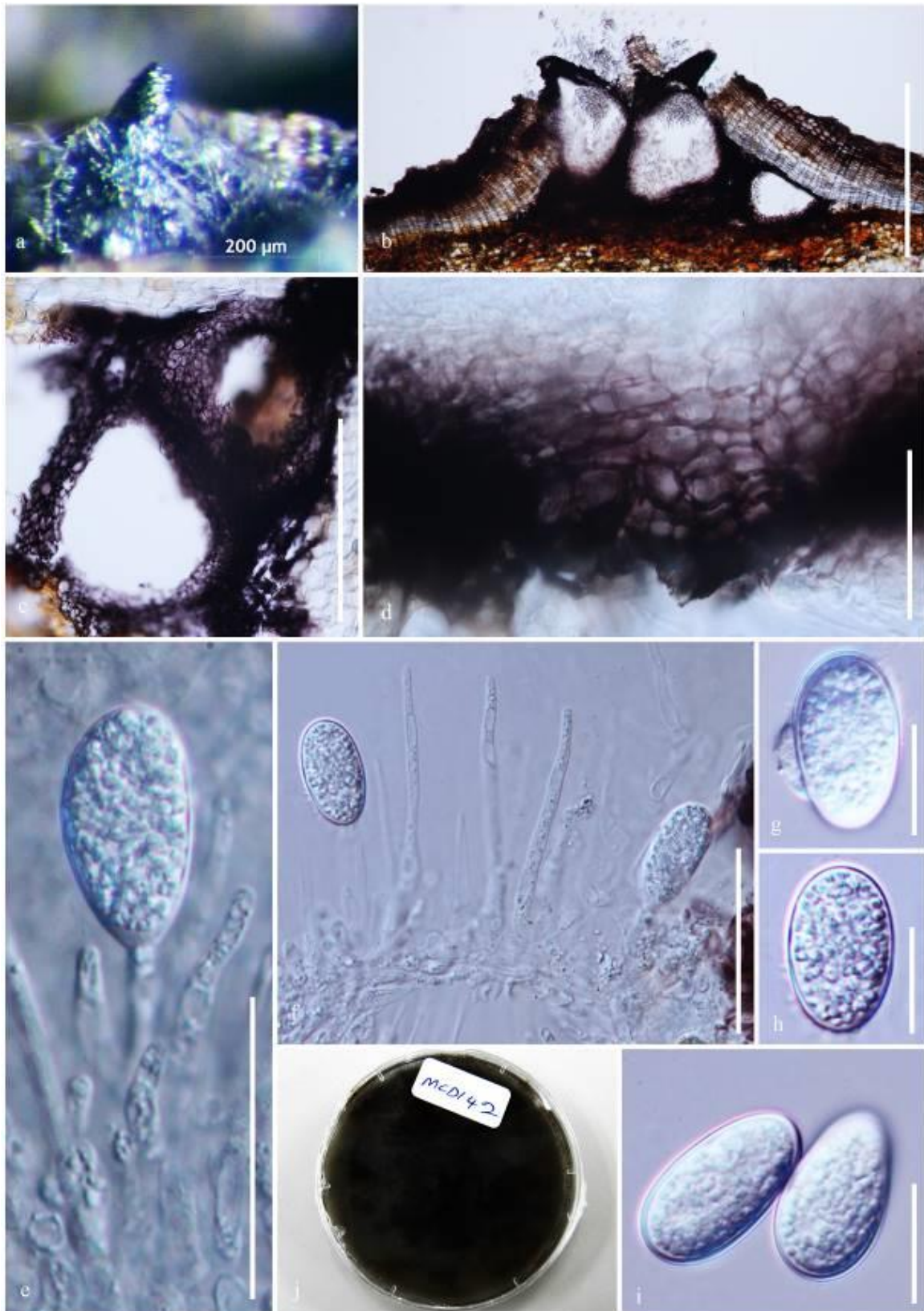
Additional GenBank numbers – LSU: MN017859; SSU: MN017924

Notes – *Lasiodiplodia krabiensis* differs from its closest phylogenetic neighbor *L. brasiliense* (Fig. 40) by subglobose to oval conidia with rounded ends, while *L. brasiliense* comprises conidia mostly with a truncate base. Additionally, conidia of *L. krabiensis* are hyaline throughout the life cycle and conidia of *L. brasiliense* become brown with the age (Netto et al. 2014).



**Figure 40** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -4346.694438. The combined ITS and TEF sequence datasets comprised 72 strains of *Lasiodiplodia* with *Diplodia mutila* (CMW 7060) as the outgroup taxon. Topology of the ML tree was similar to the BI. The matrix had 258 distinct alignment patterns, with 46.36% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.213332, C =

0.288271, G = 0.257830, T = 0.240567; substitution rates AC = 1.221116, AG = 5.177782, AT = 2.334654, CG = 1.157416, CT = 7.150251, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.139994. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.02 changes. The ex-type strains are in bold and new isolates in blue bold.



**Figure 41** – *Lasiodiplodia krabiensis* (MFLU 17-2617, holotype). a Conidiomata on host tissue. b,

c Vertical sections through conidiomata. d Pycnidial wall. e, f Conidia attached to the conidiogenous cells and paraphyses. g–i Conidia. j Mature culture on sea water PDA. Scale bars: a, b = 200  $\mu\text{m}$ , c = 100  $\mu\text{m}$ , d–f = 50  $\mu\text{m}$ , g–i = 10  $\mu\text{m}$ .

The base pair difference of the TEF locus is in the range of 2.5% (7 bp out of 280) and they can be considered as two distinct species using the guidelines of Jeewon & Hyde (2016). *Lasiodiplodia bruguierae*, which has been reported as an endophyte of *Bruguiera gymnorhiza*, is clearly different from this novel taxon by having brown pigmented conidia with striations (Osorio et al. 2016). We believe that both phenotypic and phylogenetic distinctness of this strain is sufficient to introduce it as a novel taxon within the genus *Lasiodiplodia*.

**Dyfrulomycetales** K.L. Pang et al.

**Pleurotremales** Walt. Watson

Pleurotremales was controversial and reinstated with re-observation of the isotype of *Pleurotrema polysemum*, and eradication of Dyfrulomycetales from the class Sordariomycetes (Maharachchikumbura et al. 2016). Sequence data of the type of *Pleurotrema* is still inadequate (Maharachchikumbura et al. 2016). Pleurotremales comprises three genera *Dyfrulomyces*, *Melomastia* and *Pleurotrema* (Pang et al. 2013, Norphanphoun et al. 2017, Zhang et al. 2017). In this study, a new *Dyfrulomyces* species collected from unidentified mangrove wood in Krabi, Thailand and a new host record of *D. thailandica* are provided with detailed morphological information and support from combined multi-gene phylogenetic analysis of SSU, LSU and TEF data.

**Dyfrulomyces** K.D. Hyde et al.

*Dyfrulomyces* was established by Pang et al. (2013) with species characterized by a clypeus on the substrate, immersed ascomata and multi-septate ascospores with/without a sheath in bitunicate/fissitunicate asci, to accommodate *D. tiomanensis*. Nine species are recognized within this genus, which have mostly been collected from marine habitats on mangrove wood (Hyde 1992b, Pang et al. 2013, Hyde et al. 2017, Norphanphoun et al. 2017, Zhang et al. 2017, Wijayawardene et al. 2017a, Hyde et al. 2018).

***Dyfrulomyces neothailandicus*** Dayarathne, Jones E.B.G. & K.D. Hyde, sp. nov.

Fig. 43

Index Fungorum number: IF556585; Facesoffungi number: FoF 06172

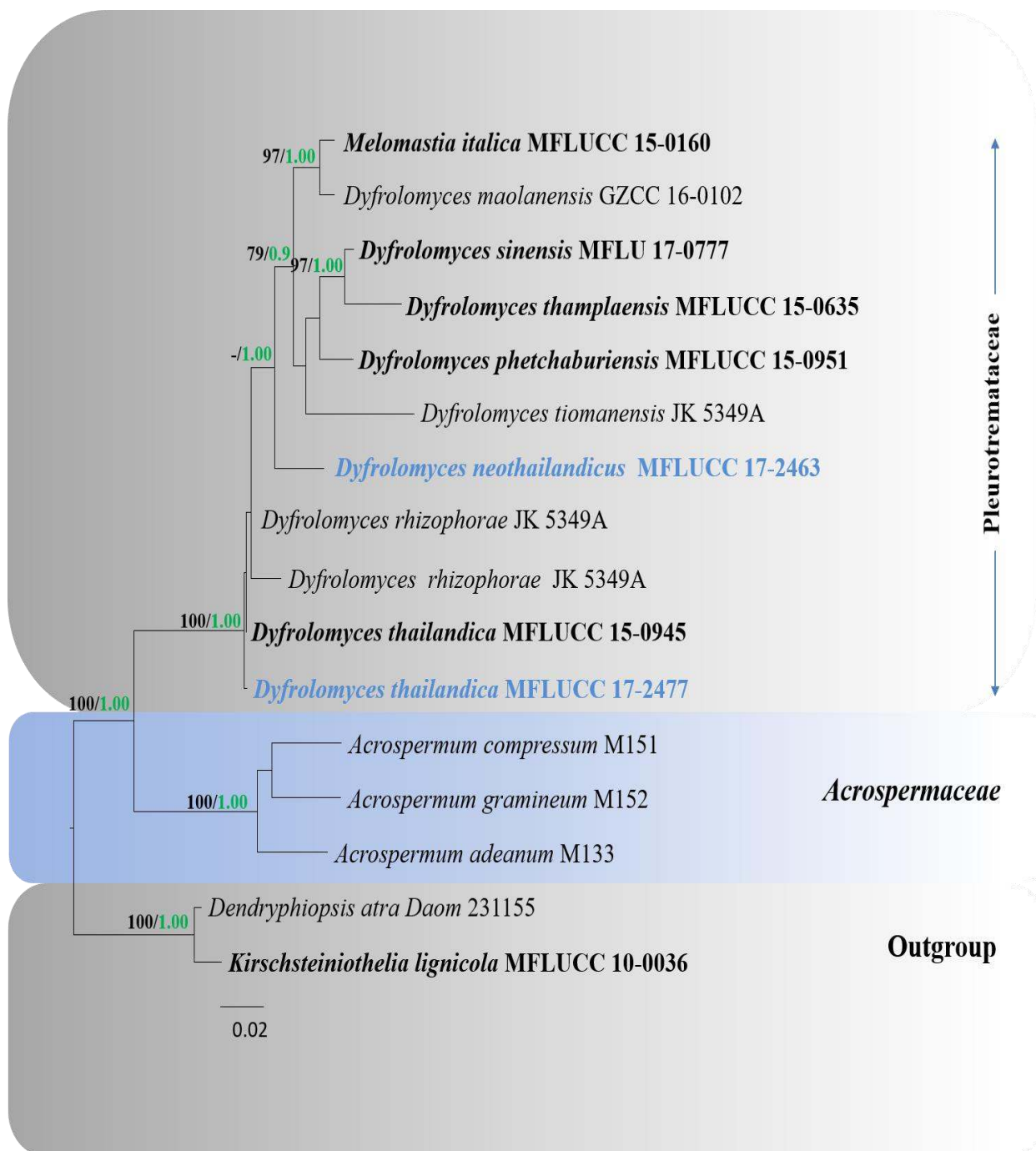
Etymology – Name reflects the similarity to *Dyfrulomyces thailandicus*

Holotype – MFLU 17-2589

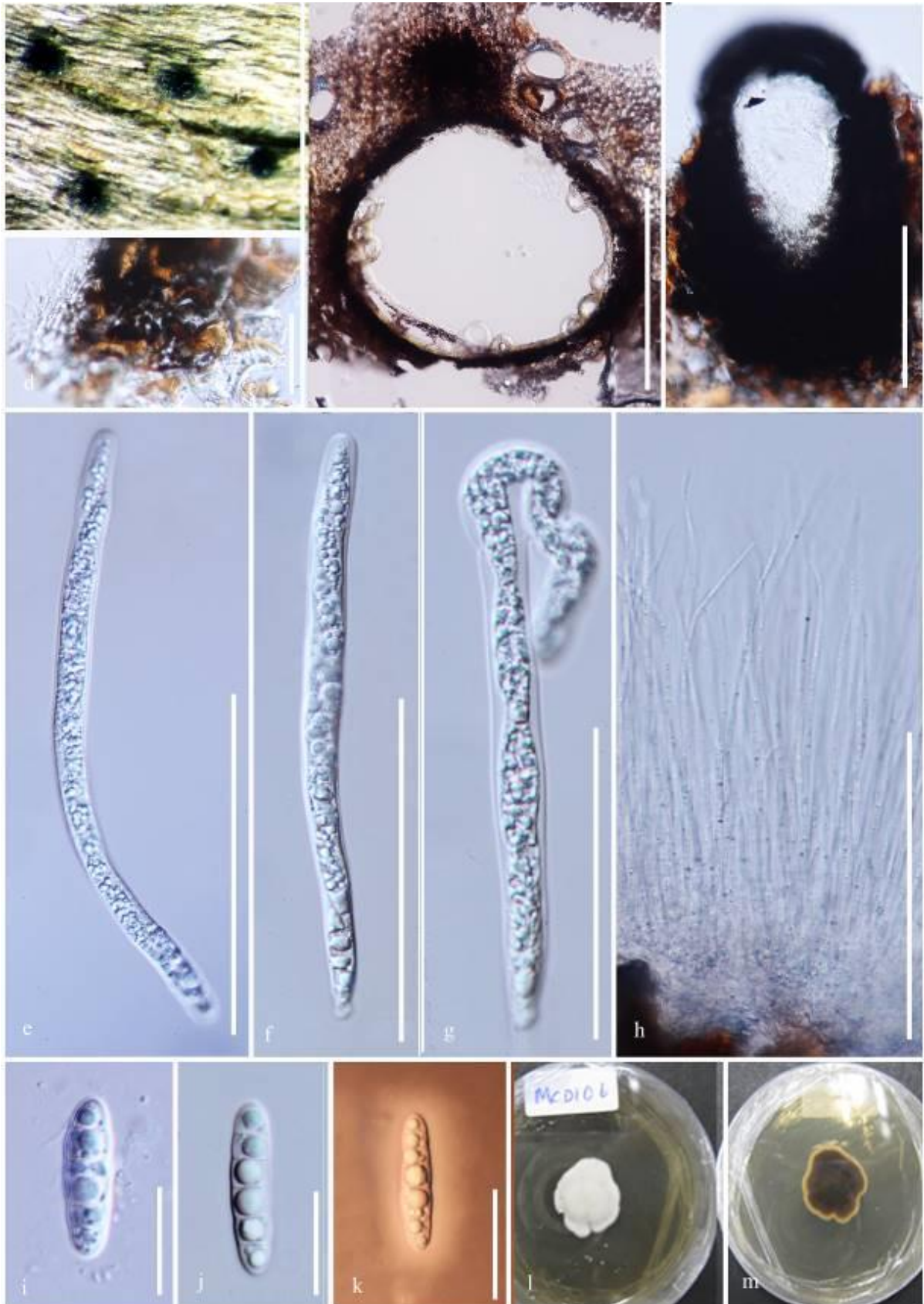
*Saprobic* on submerged decaying wood of *Rhizophora* sp. in mangrove vegetation. Sexual morph: *Ascomata* 480–496  $\mu\text{m}$   $\times$  580–620  $\mu\text{m}$  ( $\bar{x}$  = 485  $\times$  585  $\mu\text{m}$ , n = 10), solitary, semi-immersed, clypeate, globose to subglobose, dark brown to black, ostiolate, ostiolar canal filled with dark cells, apapillate. *Peridium* 26–35  $\mu\text{m}$  wide, comprising an outer layer of dark brown cells of *textura angularis*, becoming lighter inwardly. *Hamathecium* 0.4–0.8  $\mu\text{m}$  wide, comprising numerous, septate pseudoparaphyses embedded in a gelatinous matrix. *Asci* 165–190  $\times$  10–12  $\mu\text{m}$  ( $\bar{x}$  = 168  $\times$  10.8  $\mu\text{m}$ , n = 10), 8-spored, bitunicate, cylindrical, short-pedicellate, with a thickened apex. *Ascospores* 26–28  $\times$  7.2–8  $\mu\text{m}$  ( $\bar{x}$  = 28  $\times$  6.5  $\mu\text{m}$ , n = 20), slightly overlapping uniseriate, hyaline, ellipsoidal narrower towards the ends, 5-septate, with a large guttule in each cell, slightly constricted at septa, surrounded by a thick gelatinous sheath, 3.6–4  $\mu\text{m}$  thick. Asexual morph: Undetermined.

Culture characteristics – Colonies on sea water PDA slow growing at 25–28  $^{\circ}\text{C}$ , reaching 5 cm in 14 days, white at first, becoming yellowish when mature, undulate and reverse yellowish white.

Material examined – THAILAND, Krabi Province, Phang Nga, on decaying submerged wood of *Rhizophora* sp. (Rhizophoraceae), 31 August 2017, M.C. Dayarathne, MCD 106 (MFLU 17-2589, holotype); *ibid.*, (HKAS102031, isotype), ex type living culture (MFLUCC 17-2463).



**Figure 42** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -8949.656165. The combined SSU, LSU and TEF sequence datasets comprised 16 strains including *Dendryphiopsis atra* (Daom 231155) and *Kirschsteiniothelia lignicola* (MFLUCC 10-0036) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 560 distinct alignment patterns, with 29.47% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.245187, C = 0.247290, G = 0.292101, T = 0.215422; substitution rates AC = 1.089829, AG = 2.835107, AT = 1.002128, CG = 1.479574, CT = 8.823891, GT = 1.000000. Maximum likelihood bootstrap (ML, black) values >65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.02 changes. The ex-type strains are in bold and new isolates in blue bold.



**Figure 43** – *Dyfrolomyces neothailandicus* (MFLU 17-2589, holotype). a Appearance of ascomata on host surface. b Vertical sections through ascoma. c Section through neck region. d Peridium. e–g Asci. h Pseudoparaphyses. i, j Ascospores with sheath. k Ascospore stained with Indian ink to show gelatinous sheath. l, m Cultures on PDA (l-upper, m-lower). Scale bars: b = 200  $\mu\text{m}$ , e–h = 100  $\mu\text{m}$ , c = 50  $\mu\text{m}$ , d, i–k = 20  $\mu\text{m}$ .



Notes – *Dyfrolomyces neothailandicus* closely resembles *D. thailandica* and only differs in the dimensions of the asci (165–190 × 10–12 µm vs 146–158 × 7–9 µm), ascospores (26–28 × 7.2–8 µm vs 24–32 × 6–8 µm) and pattern of ascospore guttulation (Hyde et al. 2016). Ascospores of *D. neothailandicus* comprise a large guttule per each cell and *D. thailandica* has small numerous guttules in the ascospores (Hyde et al. 2016). Furthermore, they have distant placement in our phylogram (Fig. 42). Furthermore, 22 bp of LSU sequences are different out of 824 bp (2.7%). *Dyfrolomyces neothailandicus* differs from all the other species by ascospore characteristics, in particular ascospore septation, and measurements. Ascospores of *D. neothailandicus* are 5-septate, those of *D. tiomanensis*, *D. marinospora*, *D. rhizophorae*, *D. thamplaensis*, *D. phetchaburiensis* and *D. maolanensis* have 20–24, 3, 4–6, 8–10 and 3 septa, respectively (Hyde 1992b, Pang et al. 2013, Hyde et al. 2017, Norphanphoun et al. 2017, Zhang et al. 2017, Hyde et al. 2018). While the terrestrial species *D. sinensis* is different from *D. neothailandicus* in lacking a gelatinous sheath around the ascospores (Hyde et al. 2018). However, we could not obtain SSU and TEF data for this species, therefore further taxon sampling is recommended.

***Dyfrolomyces thailandicus*** Dayar., E.B.G. Jones & K.D. Hyde, in Hyde et al., Fungal Diversity: 78(1): 1–237 (2016) Fig. 44

Facesoffungi number: FoF02445

*Saprobic* on pneumatophores of *Avicennia marina* in mangrove vegetation. Sexual morph: *Ascomata* 340–428 µm × 200–267 µm ( $\bar{x}$  = 380 × 250 µm, n = 5), solitary, semi-immersed, clypeate, globose to subglobose, dark brown to black, ostiolate, ostiolar canal filled with dark cells, apapillate. *Peridium* 22–30 µm wide, comprising an outer layer of dark brown cells of *textura angularis*, becoming lighter inwardly. *Hamathecium* comprising numerous, 2.1–3.0 µm wide, septate pseudoparaphyses embedded in a gelatinous matrix. *Asci* 140–156 × 7.4–9 µm ( $\bar{x}$  = 150 × 8.2 µm, n = 10), 8-spored, bitunicate, cylindrical, short-pedicellate, with a thickened apex. *Ascospores* 24.2–34 × 6.1–7 µm ( $\bar{x}$  = 28.2 × 6.5 µm, n = 20), slightly overlapping uniseriate, hyaline, ellipsoidal, mostly 3–5-septate, with several small guttules, slightly constricted at septa, surrounded by a thick gelatinous sheath, 1.2–2.4 µm thick. Asexual morph: Undetermined.

Culture characteristics – Colonies on sea water PDA slow growing at 25 °C, reaching 2 cm in 14 days, white at first, becoming yellowish when mature, undulate and reverse yellowish white.

Material examined – THAILAND, Ranong Province, Ranong, on decaying, submerged *Rhizophora* sp. wood (Rhizophoraceae) wood, 29 August 2018, M.C. Dayarathne, MCD 131 (MFLU 17-2610); *ibid.*, (HKAS102048), living culture MFLUCC 17-2477.

GenBank numbers – LSU: MN017858; SSU: MN017923; TEF: MN077069

Notes – Our new isolate (MFLUCC 17-2477) resembles *Dyfrolomyces thailandicus* which was introduced by Hyde et al. (2016). According to our phylogenetic analysis based on combined LSU, SSU and TEF sequenced data, *D. thailandica* formed distinct lineage sister to *D. thailandica* (Fig. 42). SSU and LSU base pairs sequences of our new isolate and *D. thailandica* are exactly similar to each other. However, we are herein provided new TEF data for this species and this is the first record of this species from *Rhizophora* sp. While the earlier record was from *Avicennia marina* (Hyde et al. 2016).

**Patellariales** D. Hawksw. & O.E. Erikss.

**Patellariaceae** Corda

Based on the structure of the apothecial fruiting bodies Patellariaceae was introduced by Corda (1838) to accommodate three genera, *Cryptodiscus*, *Mellitiosporium* and *Patellaria* (generic type). Kutorga & Hawksworth (1997) accepted 12 genera in Patellariaceae. Based on morphology, Zhang & Hyde (2009) transferred the genus *Pseudoparodia* to Patellariaceae. Yacharoen et al. (2015) monographed the family accepting 13 genera, while Wijayawardene et al. (2018) listed 21 genera. Patellariaceae species are saprotrophs or weak pathogens on wood, and bark of a range of trees, and shrubs and are characterized by superficial apothecioid ascomata, cupulate or discoid, exciple pseudoparenchymatous or prosenchymatous hypothecium, with 8-spored, bitunicate,

fissitunicate, clavate to cylindrical asci and usually ascospores varying in shape (Yacharoen et al. 2015, Hernández-Restrepo et al. 2016). So far only two marine Patellariaceae species (*Banhegyia setispora* and *Patellaria atrata*) have been reported while we introduce the third Patellariaceae species collected from mangroves in southern Thailand.

***Patellaria*** Fr., Syst. mycol. (Lundae) 2(1): 158 (1822)

*Patellaria* is characterized by superficial, black, apothecioid ascomata, with a greenish-black epithecium formed from the branched and swollen paraphyses, bitunicate, fissitunicate asci, and hyaline, clavate to cylindrical, phragmo-septate ascospores (Kutorga & Hawksworth 1997). However, *Patellaria* species are vary in the number of asci, spore size, and number of ascospores within the asci. Sequence data are available only for *Patellaria atrata* and *P. quercus* (Boehm et al. 2009a, b, Schoch et al. 2009, Suetrong & Jones 2006, Suetrong et al. 2009). *Patellaria* cf. *atrata* has been reported from various mangrove woody species collected in Hong Kong and Thailand (Suetrong et al. 2009).

***Patellaria apiculatae*** Dayarathne & K.D. Hyde, sp. nov.

Fig. 46

Index Fungorum number: IF556587; Facesoffungi number: FoF 06174

Etymology – Epithet refers to the host epithet “*apiculata*”

Holotype – MFLU 19-1236

*Saprobic* on fallen decaying *Rhizophora apiculata* wood. Sexual morph: *Ascomata* 450–540 × 230–270 μm ( $\bar{x}$  = 505 × 248 μm; n = 5), apothecial, superficial, scattered, sessile, closed at first and opening at maturity, longitudinally wide, exposing the dark hymenium at the center, circular, flattened, with a carbonaceous rim, black. *Exciple* 30–42 μm wide ( $\bar{x}$  = 35 μm; n = 5), 2-layered, outer layer pseudoparenchymatous, bluish-black, inner layer composed of thick-walled cells of *textura prismatica*, gelatinous, blue-black at the base (hypotheceum) with cells of *textura angularis*, greenish-blue to colourless. *Hamathecium* composed of 1–5 μm wide, thick, cylindrical, hyaline, septate, branched paraphyses, slightly swollen and rounded at the apex, forming a bluish-black and thick epithecium over the asci. *Asci* 60–75 × 12–18 μm ( $\bar{x}$  = 62 × 16 μm; n = 10), 8-spored, bitunicate to fissitunicate, cylindrical, clavate, with a short and slightly curved pedicel, apically rounded, with an ocular chamber. *Ascospores* 25–30 × 6–7.5 μm ( $\bar{x}$  = 26 × 7 μm; n = 20), seriate overlapping, clavate to fusiform, slightly curved, narrowed at the lower end, 5–6-septate, hyaline. Asexual morph: Unknown.

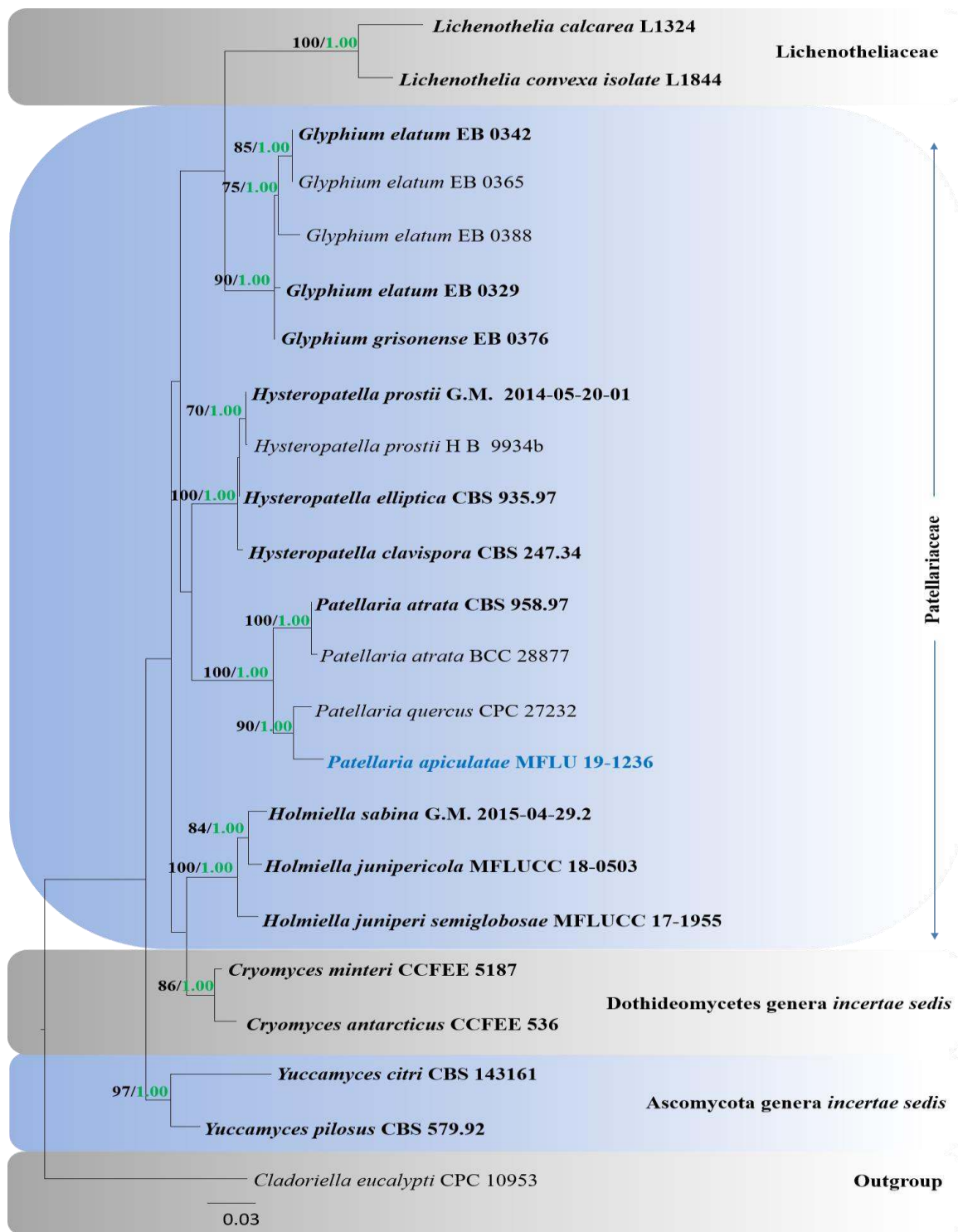
Material examined – THAILAND, Phetchaburi Province, Cha-am, on decaying *Rhizophora apiculata* (Rhizophoraceae) wood, 31 November 2016, M.C. Dayarathne, MCD 096 (MFLU 19-1236, holotype); *ibid.*, (HKAS102097, isotype).

GenBank numbers – LSU: MN017860; SSU: MN017925; ITS: MN047094

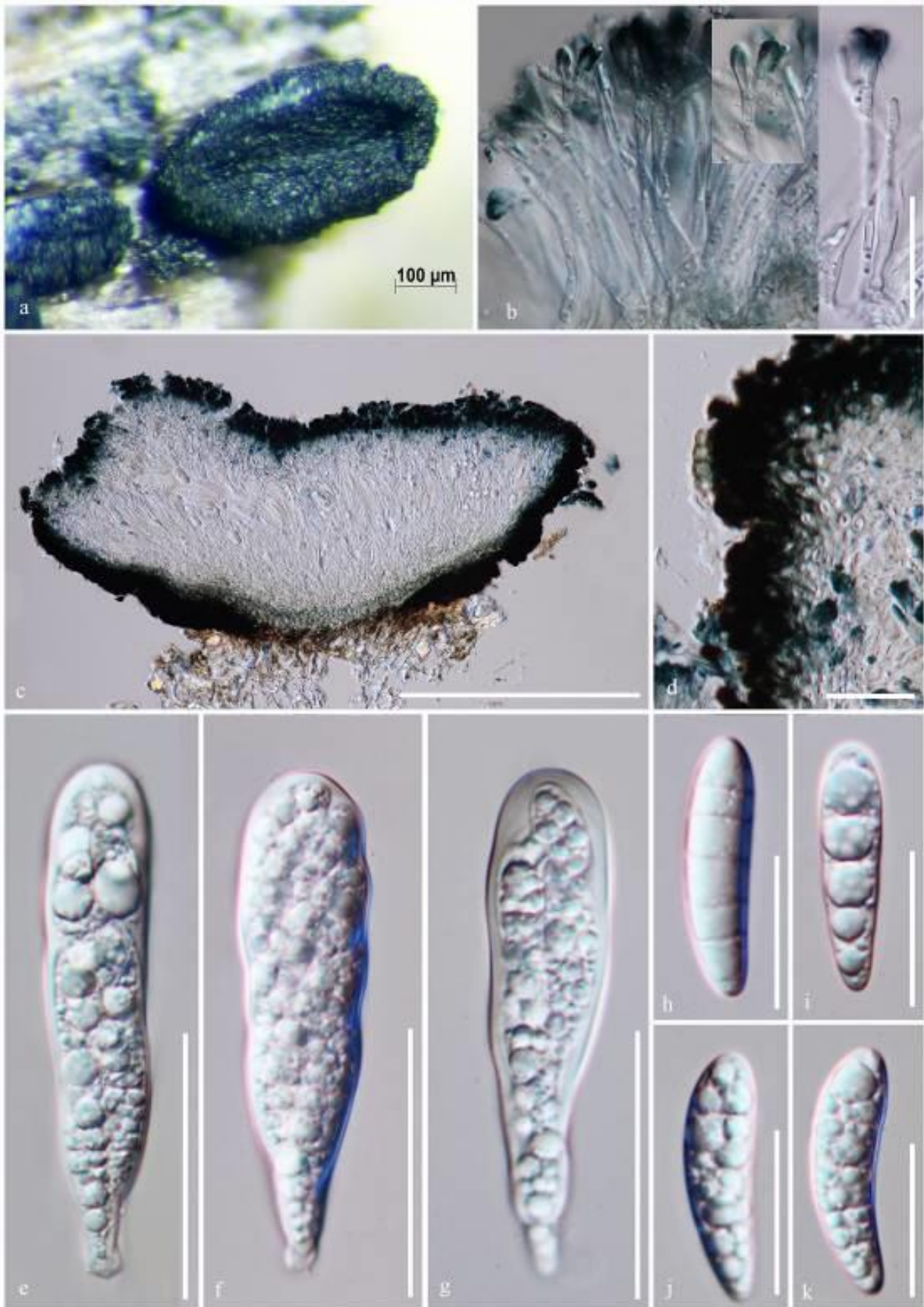
Notes – This saprotrophic fungus preferentially colonizes decaying *Rhizophora apiculata* wood. *Patellaria apiculatae* resembles *Patellaria* species by having superficial, black, apothecioid ascomata, with a greenish-black epithecium formed from the branched and swollen paraphyses, bitunicate, fissitunicate asci, and hyaline, clavate to cylindrical, phragmo-septate ascospores (Kutorga & Hawksworth 1997). Phylogenetic analyses of combined LSU, ITS and SSU data established the placement of this novel species within the genus *Patellaria* (ML 90% and 1.00 PP) (Fig. 45). *Patellaria apiculatae* showed close phylogenetic affinities to *Patellaria atrata* (BCC 28877, CBS 958.97) and *Patellaria quercus* (CPC 27232). *Patellaria apiculatae* can be clearly distinguish from *Patellaria atrata* by the size of ascomata (450–540 μm vs 675–1160 μm) shorter asci (60–75 μm vs 98–135 μm) and ascospore septation (5–6 vs 5–11-septate) (Yacharoen et al. 2015). *Patellaria quercus* is only known from its asexual morph and Hernández-Restrepo et al. (2016) showed that it has a 99 % similarity of LSU sequence data to *Patellaria atrata* (BCC28876). Considering the base pair differences of *P. apiculatae* and *P. quercus*, 45 bp of ITS are different out of 568 (7.9%) and 7 bp of LSU are different out of 830 bp (1%) hence, depending on both morphological, phylogenetic and differences of molecular sequence data, we establish our strain as a novel taxon within the genus *Patellaria*.



**Figure 44** – *Dyfrolomyces thailandicus* (MFLU 17-2610). a Appearance of ascomata on host surface. b Vertical sections through ascoma e Immature asci attached with pseudoparaphyses. d, e Asci. f, g Ascospores with sheath. h Ascospore stained with Indian ink. i Germinating ascospore. j, k Cultures on sea water PDA (j-upper, k-lower). Scale bars: a = 500 µm, b = 200 µm, c, d = 50 µm, e–i = 10 µm.



**Figure 45** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -16392.233800. The combined LSU, ITS, and SSU sequence datasets comprised 25 strains of *Patellariales* and related taxa with *Cladoriella eucalypti* (CPC 10953) as the outgroup taxon. Tree topology of the ML analysis was similar to the BI. The matrix had 505 distinct alignment patterns, with 46.36% of undetermined characters or gaps, with 26.87% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.251078, C = 0.225448, G = 0.286828, T = 0.236646; substitution rates AC = 1.704084, AG = 3.719431, AT = 1.560107, CG = 1.583619, CT = 9.108690, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.139994. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.03 changes. The ex-type strains are in bold and new isolates in blue bold.



**Figure 46** – *Patellaria apiculatae* (MFLU 19-1236, holotype). a Apothecia on host tissue. b Hamathecium tissue; note: pseudoparaphyses branching at their tips. c Hand section of apothecium. d Peridium with elongated and rounded cells. e–g Young and mature asci. h–k Ascospores. Scale bars: c = 200 µm, a = 100 µm, e–g = 50 µm, b, d, h–k = 20 µm.

## **Stigmatodiscales** Voglmayr & Jaklitsch

The order Stigmatodiscales was introduced by Voglmayr et al. (2016) for a new family Stigmatodiscaceae to encompass asteromassaria-like and stigmatomassaria-like taxa. A phylogenetic analysis by Voglmayr et al. (2016) showed that Stigmatodiscales grouped within Dothideomycetes, but their closest relatives remain obscure and unresolved. Phylogenetically Stigmatodiscales forms a poorly supported relationship to the orders Acrospermales, Dyfrolomycetales and Monoblastiales, but are morphologically and ecologically clearly distinguishable (Voglmayr et al. 2016).

## **Stigmatodiscaceae** Voglmayr & Jaklitsch

A dothideomycetous family with apothecial ascomata embedded in host tissue lacking an excipulum, saccate fissitunicate asci, large, 3-septate ascospores with an eccentric euseptum and two additional distosepta, surrounded by a large gelatinous sheath, characterizes Stigmatodiscaceae (Voglmayr et al. 2016). This family comprises the genera *Asterodiscus* and *Stigmatodiscus* reported from dead corticated twigs of *Acer*, *Carpinus* and *Tamarix*.

## ***Asterodiscus*** Voglmayr, Gardiennet & Jaklitsch

*Asterodiscus* is a monotypic genus which is found on corticated dead twigs of *Tamarix* spp. with ascospores matching the genus *Asteromassaria* (Barr 1982). However, it is unique by having apothecial ascomata with apically swollen paraphyses (Voglmayr et al. 2016). We introduce a second species to this genus collected from a mangrove strand in Krabi Province, Thailand.

## ***Asterodiscus mangrovei*** Dayarathne, E.B.G. Jones & K.D. Hyde, sp. nov.

Fig. 48

Index Fungorum number: IF556588; Facesoffungi number: FoF 06175

Etymology – Epithet refers to the mangrove environment where the species found

Holotype – MFLU 16-1216

*Saprobic* on submerged mangrove wood. Asexual morph: *Ascomata* 220–300 µm high, apothecial, embedded in cortex of dead twigs, lifting the bark as a black bump, globose, apically covered by black cells of *textura intricata*, dark brown to black, without distinct margin. *Hamathecium* of hyaline, septate paraphyses, 1–2.8 µm wide, often branched and anastomosing above, with swollen free apical ends, embedded in a gel matrix and covered by a yellowish-brown amorphous matrix forming an epithecium. *Asci* 60–72 × 12–16 µm ( $\bar{x}$  = 66 × 13.5 µm; n = 5), 8-spored, seriate, bitunicate, fissitunicate, J-, broadly fusoid to saccate, with thin ecto- and thick endotunica; with a wide ocular chamber. *Ascospores* 20–26 × 6–10 µm ( $\bar{x}$  = 22.5 × 9.5 µm; n = 10), asymmetric, upper portion slightly larger than the lower part, ends rounded to subacute, hyaline to light brown with age, 1-euseptate when immature, becoming 3-septate at maturity, distinctly constricted at the septa, wall thick, smooth to slightly verruculose, surrounded by a sheath widely expanding in water after spore discharge. Sexual morph: Undetermined.

Material examined – THAILAND, Krabi Province, Tha Pom Khlong Nam, on decaying wood at a mangrove strand, 16 December 2015, M.C. Dayarathne, KLA025 (MFLU 16-1216, holotype); *ibid.*, (BBH, isotype).

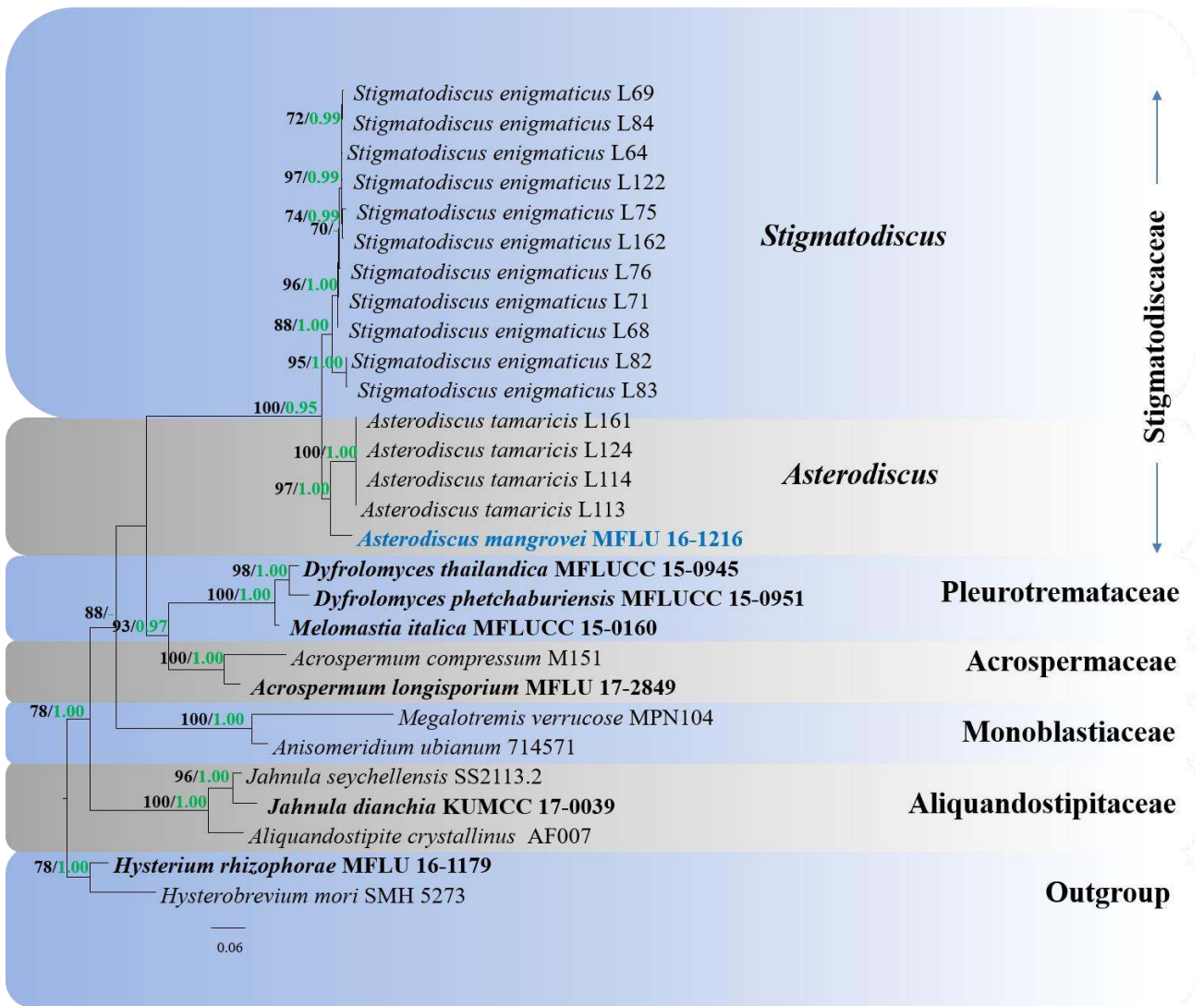
GenBank numbers – LSU: MN017861; SSU: MN017926; ITS: MN047095

Notes – *Asterodiscus mangrovei* comprises characteristics of the genus *Asterodiscus* such as apothecial ascomata with apically swollen paraphyses, broadly fusoid to saccate asci, asymmetric ascospores with upper portion slightly larger than the lower part and surrounded by a gelatinous sheath (Voglmayr et al. 2016). This novel taxon can be clearly distinguished from *A. tamaricis* (type) by its paraphyses, covered by a yellowish-brown amorphous incrustation while paraphyses of *A. tamaricis* are covered by an olivaceous to deep blue amorphous incrustation (Voglmayr et al. 2016). Their ITS base pair difference is 7.1% (43 bp out of 537 bp) which is adequate to consider them as two different species according to the guidelines of Jeewon & Hyde (2016). The habitat of the new species is different from the existing members of the family Stigmatodiscaceae. Hence, by

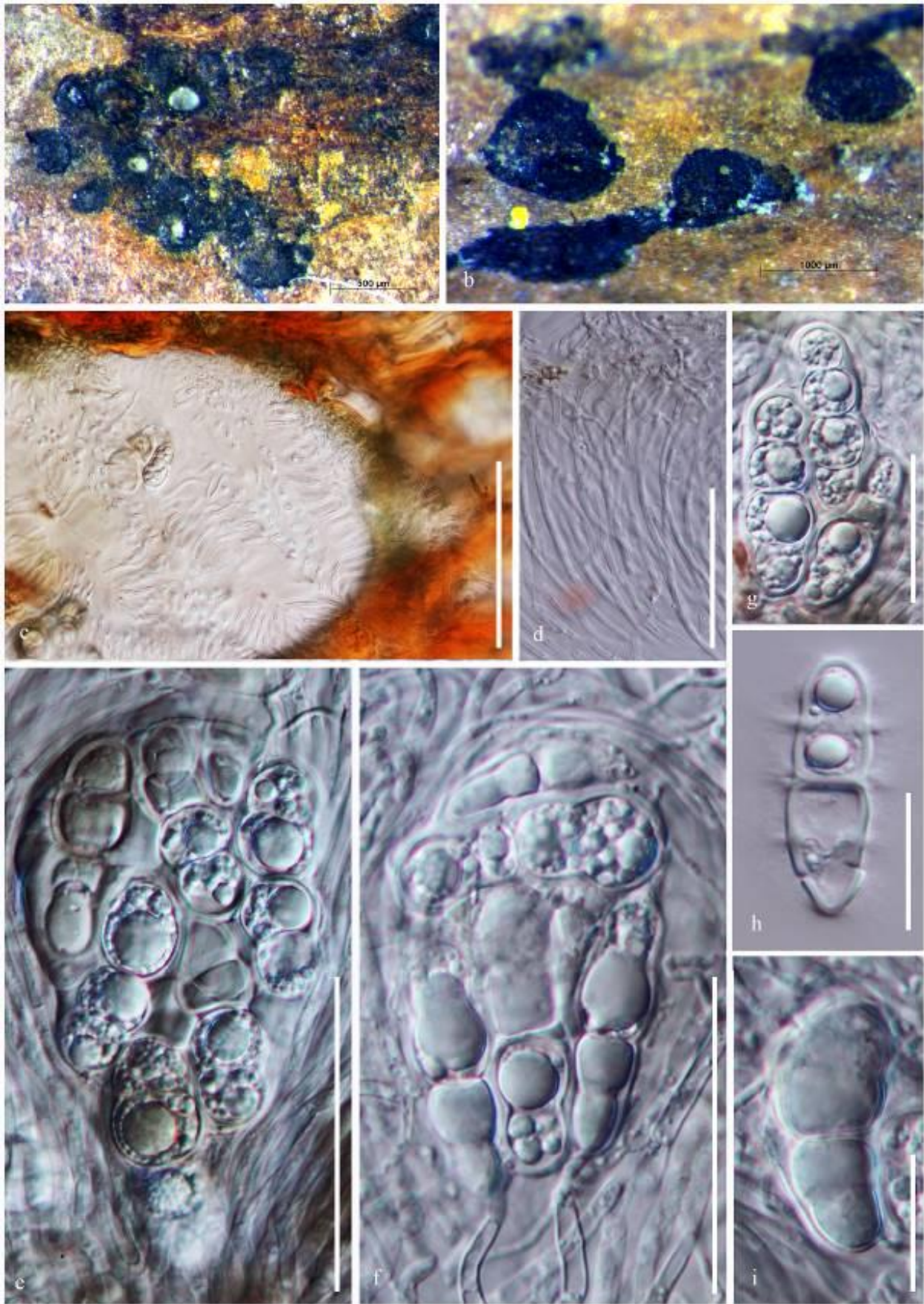
considering all these factors we establish this new strain as a novel taxon in the genus *Asterodiscus* (Fig. 47).

**Tubeufiales** Boonmee, Rossman & K.D. Hyde

Boonmee et al. (2014) introduced the monotypic order Tubeufiales to accommodate family Tubeufiaceae. Subsequently, Suetrong et al. (2014) established the family Wiesneriomycetaceae into Tubeufiales. However, Bezerra et al. (2017) excluded the family Wiesneriomycetaceae and included in Tubeufiales based on morphological and phylogenetic analyses and transferred the family to a new order Wiesneriomycetales.



**Figure 47** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -32147.923018. The combined LSU, SSU and ITS sequence datasets comprised 28 strains of *Stigmatodiscaceae* including *Hysterium rhizophorae* (MFLU 16-1179) and *Hysterobrevium mori* (MFLUCC 14-0520) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 1449 distinct alignment patterns, with 54.72% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.222854, C = 0.275339, G = 0.300056, T = 0.201750; substitution rates AC = 0.879400, AG = 1.729549, AT = 1.351867, CG = 0.987520, CT = 5.944616, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.318529. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 90% are given above the nodes. The scale bar indicates 0.06 changes. The ex-type strains are in bold and new isolates in blue bold.



**Figure 48** – *Asterodiscus mangrovei* (MFLU 16-1216, holotype). a, b Apothecial ascomata on host. c Section through apothecium (partial). d Apically inflated septate paraphyses, covered by a



yellowish-brown amorphous incrustation. e, f Asci embedded in gel matrix with paraphyses. g–i Hyaline ascospores. Scale bars: b = 1000  $\mu\text{m}$ , a = 500  $\mu\text{m}$ , c = 100  $\mu\text{m}$ , d–f = 50  $\mu\text{m}$ , g–i = 10  $\mu\text{m}$ .

### **Tubeufiaceae** M.E. Barr

Tubeufiaceae is typified by *Tubeufia* and was introduced by Barr (1979). Lu et al. (2018b) recently revised this family and listed 42 genera under this family. Tubeufiaceae species are characterized by superficial ascomata, pseudoparaphysate hamathecium, bitunicate asci and multi-septate, hyaline to pale brown cylindrical ascospores (Brahmanage et al. 2017, Lu et al. 2017, 2018a, b, Liu et al. 2018, Phookamsak et al. 2018). Asexual morphs of Tubeufiaceae are hyphomycetes and are often helicosporous, while some are chlamydosporous and phragmosporous (Boonmee et al. 2011, 2014, Brahmanage et al. 2017, Doilom et al. 2017, Lu et al. 2018a, b, Liu et al. 2018).

### **Kamalomyces** R.K. Verma, N. Sharma & Soni

Verma et al. (2008) introduced the genus *Kamalomyces* which is typified by *K. indicus*. Ascomata on a subiculum of black hyphae, with solitary, gregarious, subglobose to lemoniform ascomata with short stalks and lacking ostioles, bitunicate, broadly cylindrical to clavate asci and hyaline, vermiform, crowded, septate ascospores are the characteristic features of *Kamalomyces* (Verma et al. 2008, Boonmee et al. 2011, 2014, Phookamsak et al. 2018, Lu et al. 2018a, b). *Kamalomyces* currently comprises six species and phylogenetic relationship of *Kamalomyces* was reported by Phookamsak et al. (2018). In this study, we introduce a new record for the recently introduced *K. mangrovei* collected from *Xylocarpus* sp.

***Kamalomyces mangrovei*** Dayarathne & K.D. Hyde, in Hyde et al., *Fungal Divers.*: 96:1–242 (2019) Fig. 50

Facesoffungi number: FoF 04946

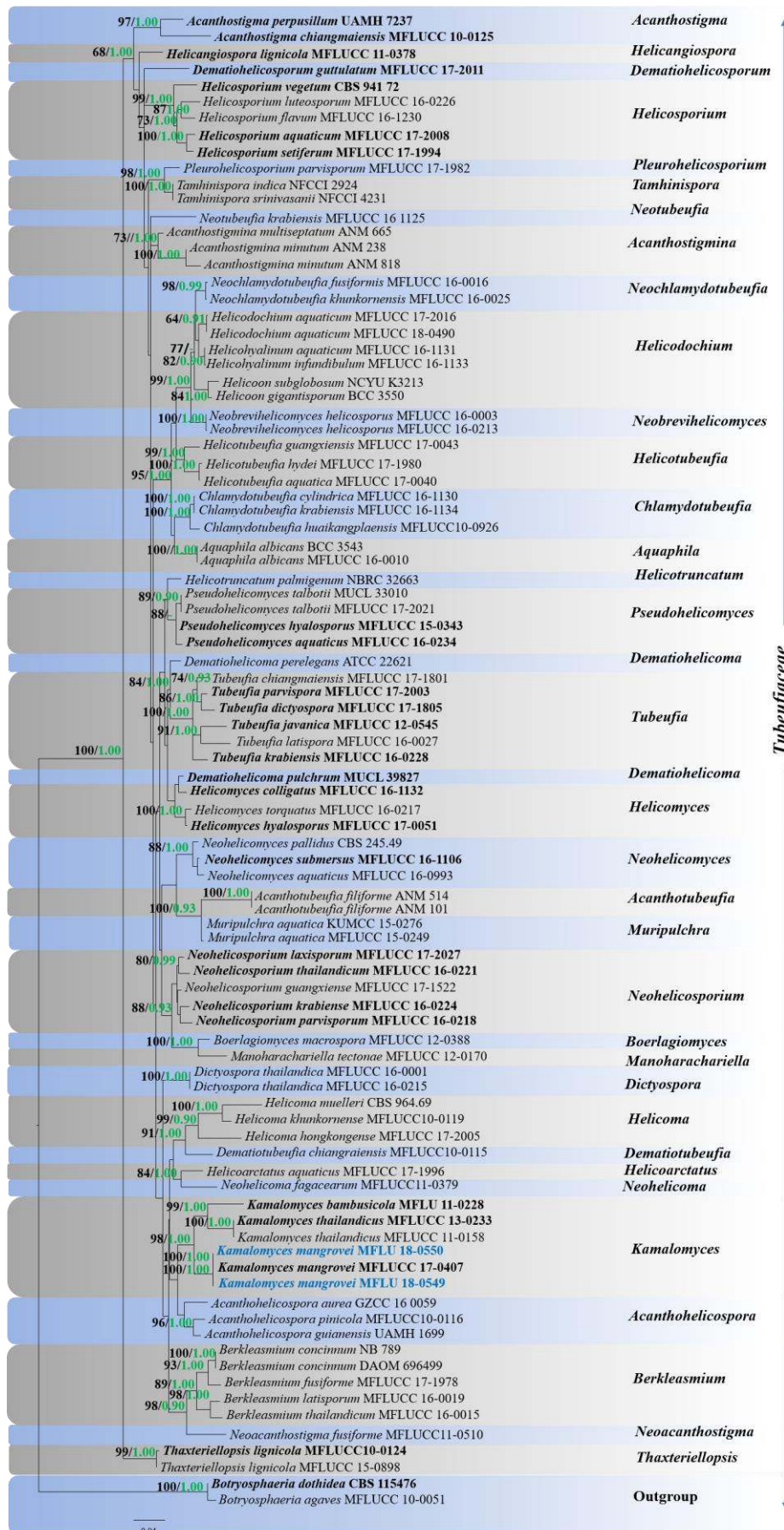
*Saprobic* on decaying wood of *Xylocarpus* sp. Sexual morph: *Ascomata* 278–300  $\times$  240–280  $\mu\text{m}$ , superficial, solitary to gregarious, embedded in a subiculum of crowded, black, septate, thick-walled hyphae, superficial, solitary, gregarious, globose to subglobose, glabrous, short-stalked, apapillate, with indistinct ostiolate. *Peridium* 30–42  $\mu\text{m}$  wide, comprising light brown cells of *textura angularis*, and inwardly small, subhyaline cells of *textura prismatica*. *Hamathecium* comprising numerous, 1.5–2  $\mu\text{m}$  wide, filiform, septate, branched, hyaline pseudoparaphyses. *Asci* 140–175  $\times$  16.2–20  $\mu\text{m}$  ( $\bar{x}$  = 162  $\times$  17.5  $\mu\text{m}$ , n = 20), 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short pedicellate, apically rounded, with an ocular chamber. *Ascospores* 50–64  $\times$  6.4–7.5  $\mu\text{m}$  ( $\bar{x}$  = 62  $\times$  7  $\mu\text{m}$ , n = 30), 2–3-seriate, hyaline becoming light brown when mature, elongate cylindrical to fusiform-clavate, tapering towards the lower cells, enlarged at the 4<sup>th</sup> and 5<sup>th</sup> cell, straight or slightly curved, 8–9 septa, distoseptate. Asexual morph: Undetermined.

Culture characteristics – Colonies on PDA reaching 3 cm diam. after 30 days at 25 °C, circular, smooth margin white at first, dark gray to black after 6 weeks, flat on the surface, without aerial mycelium, reverse brownish black.

Material examined – THAILAND, Ranong Province, Amphoe Maung, Mu 4 Tombol Ngao, Ranong Mangrove Research Center (GPS: 9°43' to 9°57'N; 98°29' to 98°39'E) on decaying wood of *Xylocarpus* sp. (Meliaceae), 06 December 2016, M.C. Dayarathne, MCD 066 (MFLU 18-0549); *ibids.*, MCD 068 (MFLU 18-0550); (HKAS102087).

GenBank numbers – LSU: MN017862, MN017863; SSU: MN017927, MN017928; ITS: MN047096, MN047097; TEF: MN077071, MN077072

Notes – Solitary to gregarious, globose to subglobose, short-stalked ascomata, lacking ostioles embedded in a subiculum of black hyphae, bitunicate, cylindrical to clavate asci and hyaline, elongate cylindrical to fusiform-clavate, septate ascospores are the characteristic features of *Kamalomyces mangrovei*. Our new collection MFLU 18-0549 and MFLU 18-0550 are morphologically and phylogenetically referred to *K. mangrovei* (Figs 48, 49) (Hyde et al. 2019).



**Figure 49** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -32147.923018. The combined LSU, ITS and TEF sequence datasets

comprised strains of Tubeufiaceae including *Botryosphaeria agaves* (MFLUCC 10-0051) and *Botryosphaeria dothidea* (CBS 115476) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 1449 distinct alignment patterns, with 54.72% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.222854, C = 0.275339, G = 0.300056, T = 0.201750; substitution rates AC = 0.879400, AG = 1.729549, AT = 1.351867, CG = 0.987520, CT = 5.944616, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.318529. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.04 changes. The ex-type strains are in bold and new isolates in blue bold.

**Class Sordariomycetes** O.E. Erikss. & Winka

**Subclass Diaporthomycetidae** Senan. et al.

**Diaporthales** Nannf.

Species in *Diaporthales* are saprobes and soil inhabitants, plant and animal pathogens, parasites or endophytes in plants (Zhang et al. 2006, Rossman et al. 2007, Maharachchikumbura et al. 2015). There are 21 families within this order (Senanayake et al. 2017, 2018, Xavier et al. 2019, Guterres et al. 2019). Diaporthalean species are characterized by yellowish brown to black valsoid or diatrypoid pseudo- or asco-stromata, brown to black perithecial fruiting bodies, regularly immersed in stromata of leafy or woody substrates with papillate ostioles swollen at the apex. The mature 2–32-spored asci with a J-, refractive apical apparatus, float freely and become dehiscent at maturity (Barr 1978, Samuels & Blackwell 2001, Senanayake et al. 2017, Hyde et al. 2019). Coelomycete or rarely hypomycete asexual morphs of this order comprises conidiophores which arise from the top most cell layer of basal or parietal tissue or from under the developing scutellum or else reduced to enteroblastic, holoblastic, phialidic or annellidic hyaline or olivaceous conidiogenous cells (Senanayake et al. 2017, 2018, Hyde et al. 2019).

**Diaporthaceae** Höhn. ex Wehm.

The family Diaporthaceae includes numerous pathogenic, endophytic, and saprobic species associated with terrestrial habitats, while there are few reports on marine species (Maharachchikumbura et al. 2016, Senanayake et al. 2017). A recent study by Senanayake et al. (2017) introduced the genera *Chiangraiomyces*, *Hyaliappendispora* and *Paradiaporthe* to this family based on phylogeny and morphology in addition to the 11 accepted genera in Maharachchikumbura et al. (2016) and Wijayawardene et al. (2018) who list 15 genera under this family.

**Diaporthe** Nitschke

*Diaporthe* was established by Nitschke (1870) to accommodate a group of species with stromatic ascomata, ellipsoid to fusiform spores and enclosed asexual morphs with unilocular pycnidia (Wehmeyer 1933). *Diaporthe* is an economically significant genus with plant pathogenic, endophytic and saprobic fungi (Udayanga et al. 2011, 2012, 2014, 2015, Hyde et al. 2016, Dissanayake et al. 2017). Rossman et al. (2015) proposed the protection of the older name *Diaporthe* over *Phomopsis* for application of a single name for one fungus. *Diaporthe* (= *Phomopsis*) *mangrovei* is reported from intertidal prop roots of *Rhizophora apiculata* in Thailand as a marine derived Diaporthaceae species (Hyde 1991b, Jones et al. 2015). *Diaporthe* (= *Phomopsis*) *pittospori* also has been reported from marine habitat (Jones et al. 2019). However, there are no sequence data available for any marine derived *Diaporthe* species. An updated phylogenetic tree for *Diaporthe* species with three novel species is presented in this paper (Fig. 51).

**Diaporthe salinicola** Dayarathne, sp. nov.

Fig. 52

Index Fungorum number: IF556589; Facesoffungi number: FoF 06176

Etymology – Name reflects the saline environment where the taxon was found and “-cola” means inhabiting.

Holotype – MFLU 18-0553

*Saprobic* on decaying submerged *mangrove* wood. Sexual morph: Stroma absent. *Ascomata* 2100–3150 × 680–790 μm, globose to subglobose, black, coriaceous, semi-immersed, single to clustered. *Neck* 1100–2200 × 100–130 μm, cylindrical, black, ostiole canal lined with periphyses. *Peridium* 35–55 mm thick, comprising compressed cells of *textura angularis*, outer layers composed of black brown, thick-walled cells, inner layers composed of pale brown to hyaline thin-walled cells. *Paraphyses* 1–2.5 μm wide, longer than asci, hyaline, cylindrical, septate. *Asci* 80–115 × 14–16.4 μm ( $\bar{x}$  = 90.6 × 16, n = 10), 8-spored, unitunicate, thin-walled, apedicellate, broad cylindrical to obclavate, with a minute apical ring, ca. 1 mm high and 2 mm diam. *Ascospores* 16–18.2 × 5.8–7 μm ( $\bar{x}$  = 16.4 × 7, n = 20), overlapping biseriate, Obpyriform to ovoid, hyaline, one-septate, constricted at the septum, thin-walled, smooth-walled, with numerous guttules. Asexual morph: Undetermined.

Culture characteristics – Colonies on sea water added PDA reaching 10 cm diam. after 2 weeks at 25 °C, dense, circular, slightly raised, surface smooth with entire edge, fluffy, colony from above: gray at the center reddish gray at the margin; from below: black at the center ash at the margin, not produced pigmentation on PDA media.

Material examined – THAILAND, Ranong Province, Amphoe Maung, Mu 4 Tombol Ngao, Ranong Mangrove Research Center (GPS: 9°43' to 9°57'N; 98°29' to 98°39'E) on decaying wood of *Xylocarpus* sp. (Meliaceae), 06 December 2016, M.C. Dayarathne, MCD 072 (MFLU 18-0553, holotype); *ibid.*, (BBH, isotype), ex-type living culture, MFLUCC 17-0413. THAILAND, Krabi Province, Krabi beach, on decaying submerged wood, 31 August 2017, M.C. Dayarathne, MCD 109 (MFLU 17-2592).

GenBank numbers – ITS: MN047098, MN047099, TEF: MN077073, MN077074

Additional GenBank numbers – LSU: MN017864, MN017865

Notes – *Diaporthe salinicola* is the first sexual species of *Diaporthe* isolated as a true marine species from Thailand. Long necks (1100–2200 × 100–130 μm) of the ascomata is a significant character for this species among other *Diaporthe* species. *Diaporthe aquatica*, which was isolated from wood submerged in a small ditch in China, shows close morphological characteristics in ascomatal morphology such as absence of stromata and having long necks. However, *D. salinicola* is different from *D. aquatica* by the size (16–18.2 × 5.8–7 μm vs 10–12.3 × 3–4 μm) and the shape of the ascospores. Whereby, ascospores of *D. salinicola* are obpyriform while the ascospores of *D. aquatica* are ellipsoidal to fusiform (Hu et al. 2012). This is confirmed as they group distantly in our phylogram (Fig. 51). According to our phylogenetic analysis *Diaporthe salinicola* showed close affinities to *D. lithocarpus* which is only known in its asexual morph. We compared the ITS and TEF base pair differences of *D. salinicola* to *D. lithocarpus* and are 3.04 % (16 bp out of 525 bp) and 3.01 % (9 bp out of 299). Therefore, according to the guidelines of Jeewon & Hyde (2016), basepair differences of *D. salinicola* and *D. lithocarpus* are in the recommended range to consider them as two distinct taxa. However, cultures did not get sporulate to observe asexual morph.

***Diaporthe krabiensis*** Dayarathne, sp. nov.

Fig. 53

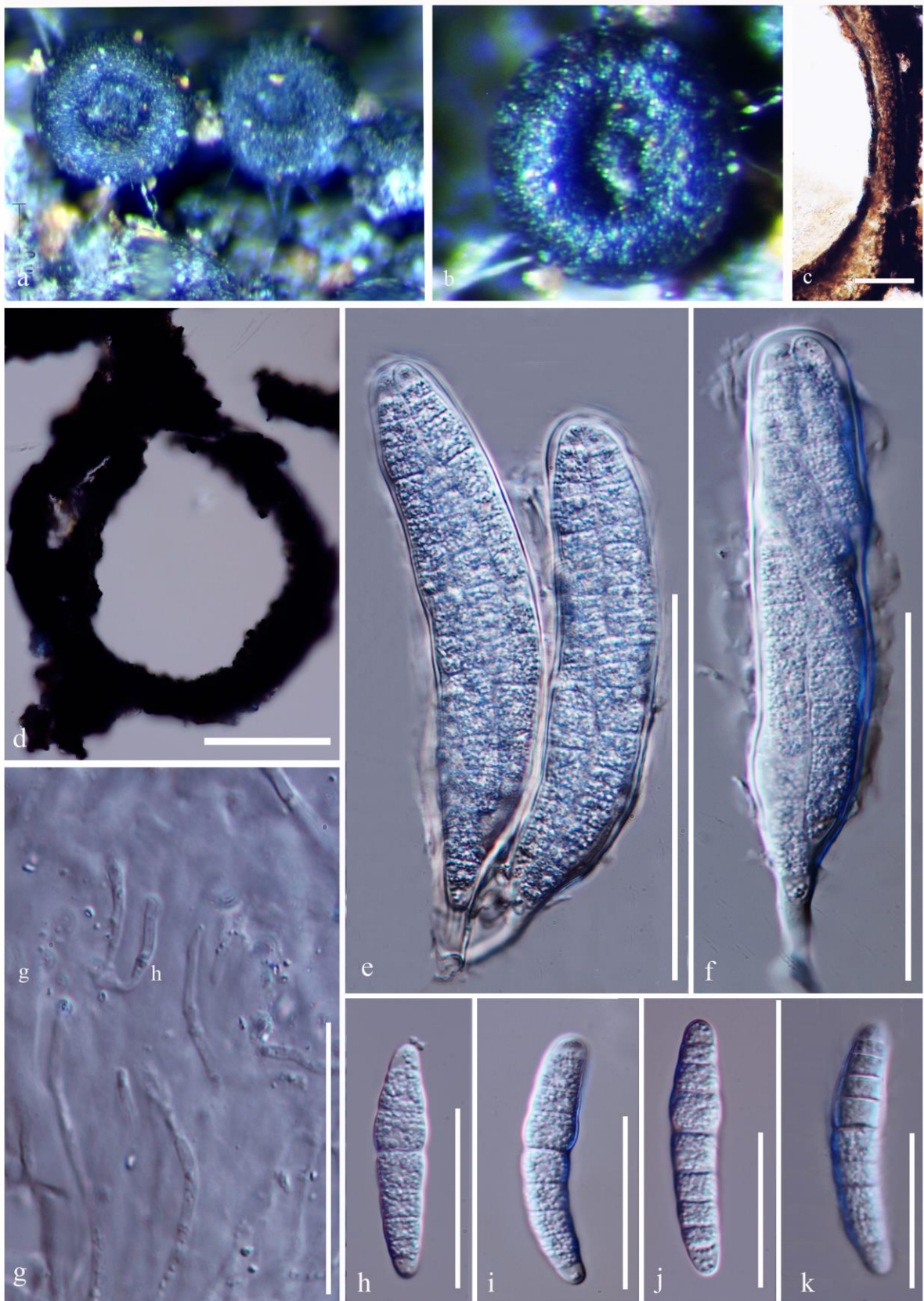
Facesoffungi number: FoF 06177

Etymology – Epithet refers to the marine environment where the species was collected.

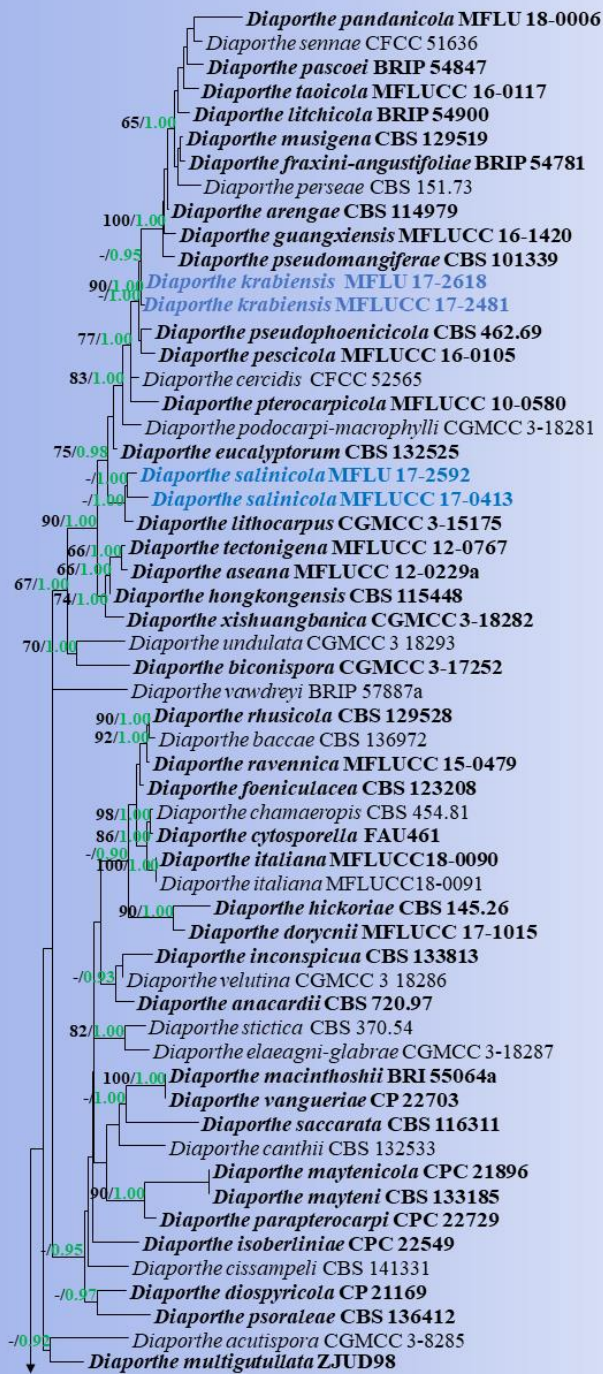
Holotype – MFLU 17-2618

*Saprobic* on submerged wood of *Bruguiera* sp. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 117–145 × 130–140 μm, pycnidial or multiloculate, scattered, globose or irregular, black. *Peridium* 15–18 μm wide consisting of brown cells of *textura angularis* in surface view. *Paraphyses* 1.5–18 μm wide, hyaline, septate, branched. *Conidiophores* 2–3-septate, branched, densely aggregated, cylindrical, straight to sinuous rarely reduced to conidiogenous cells, hyaline, smooth-walled. *Conidiogenous cells* 8–16 × 0.5–2 μm ( $\bar{x}$  = 8 × 1.8, n = 10), phialidic, subcylindrical, tapering towards the apex, hyaline, with periclinal thickening, and a flared

collarete. *Beta conidia* 15–32 × 0.9–1.4 μm ( $\bar{x}$  = 28.5 × 1.2, n = 20), fusiform to hooked, aseptate, hyaline, smooth-walled. (alpha and gamma conidia not observed)



**Figure 50** – *Kamalomyces mangrovei* (MFLU 18-0550). a, b Ascomata. c Peridium. d Section through ascoma. e–f Asci. g Pseudoparaphyses. h–k Ascospores. Scale bars: d = 100 μm, e–f = 50 μm, c, g–j = 20 μm.



*Diaporthe*

**Figure 51** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -26084.946068. The combined ITS, TEF, BTUB sequence datasets comprised 206 strains of *Diaporthe* with *Diaporthella corylina* (CBS 121124) as the outgroup taxon. Tree topology of the ML analysis was similar to the BI. The matrix had 806 distinct alignment patterns, with 46.36% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.229079, C = 0.298623, G = 0.236340, T = 0.235958; substitution rates AC = 1.382163, AG = 3.309173, AT = 1.349227, CG = 0.945554, CT = 4.934047, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.449392. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.2 changes. The ex-type strains are in bold and new isolates in blue bold.

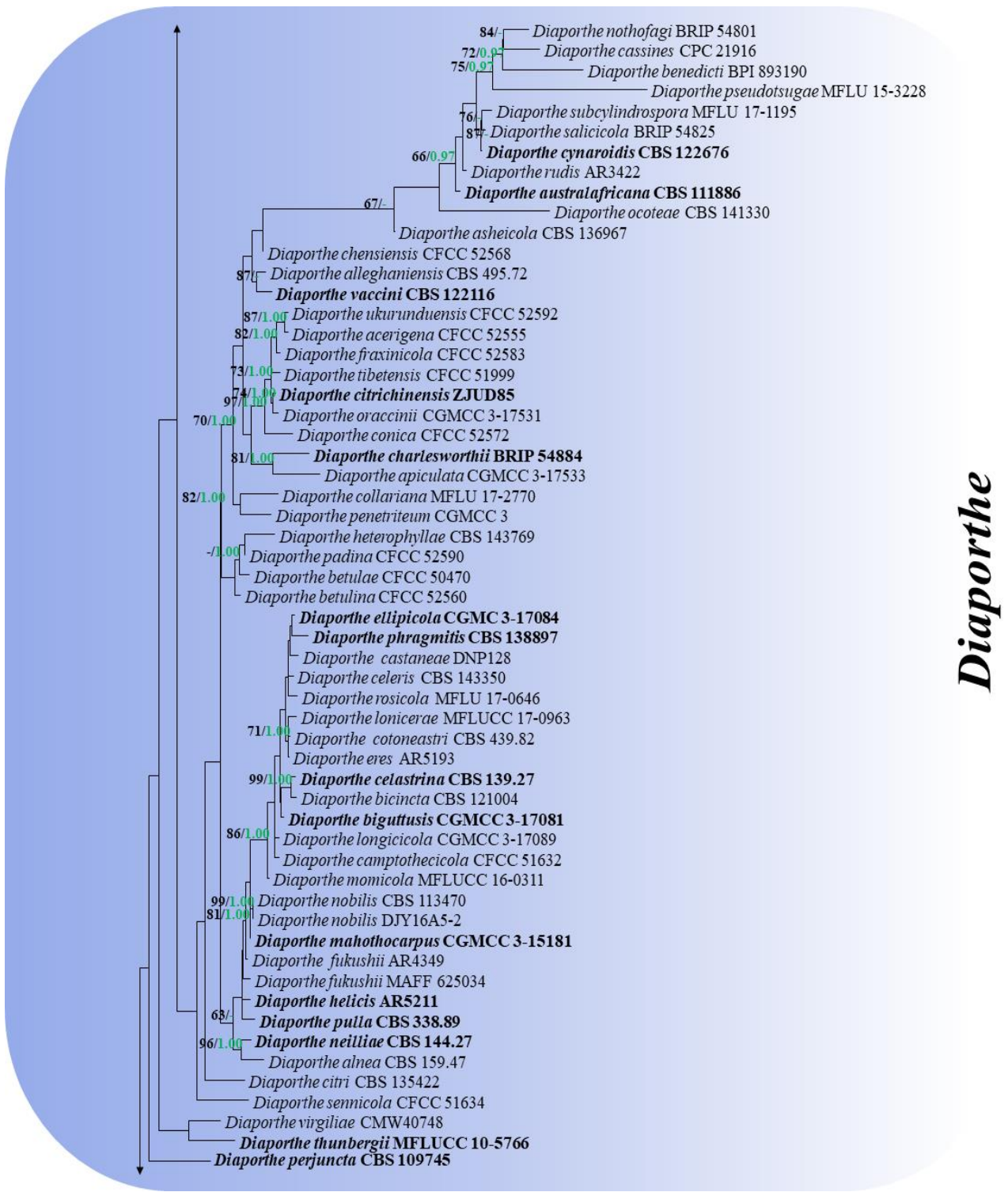


Figure 51 – Continued.

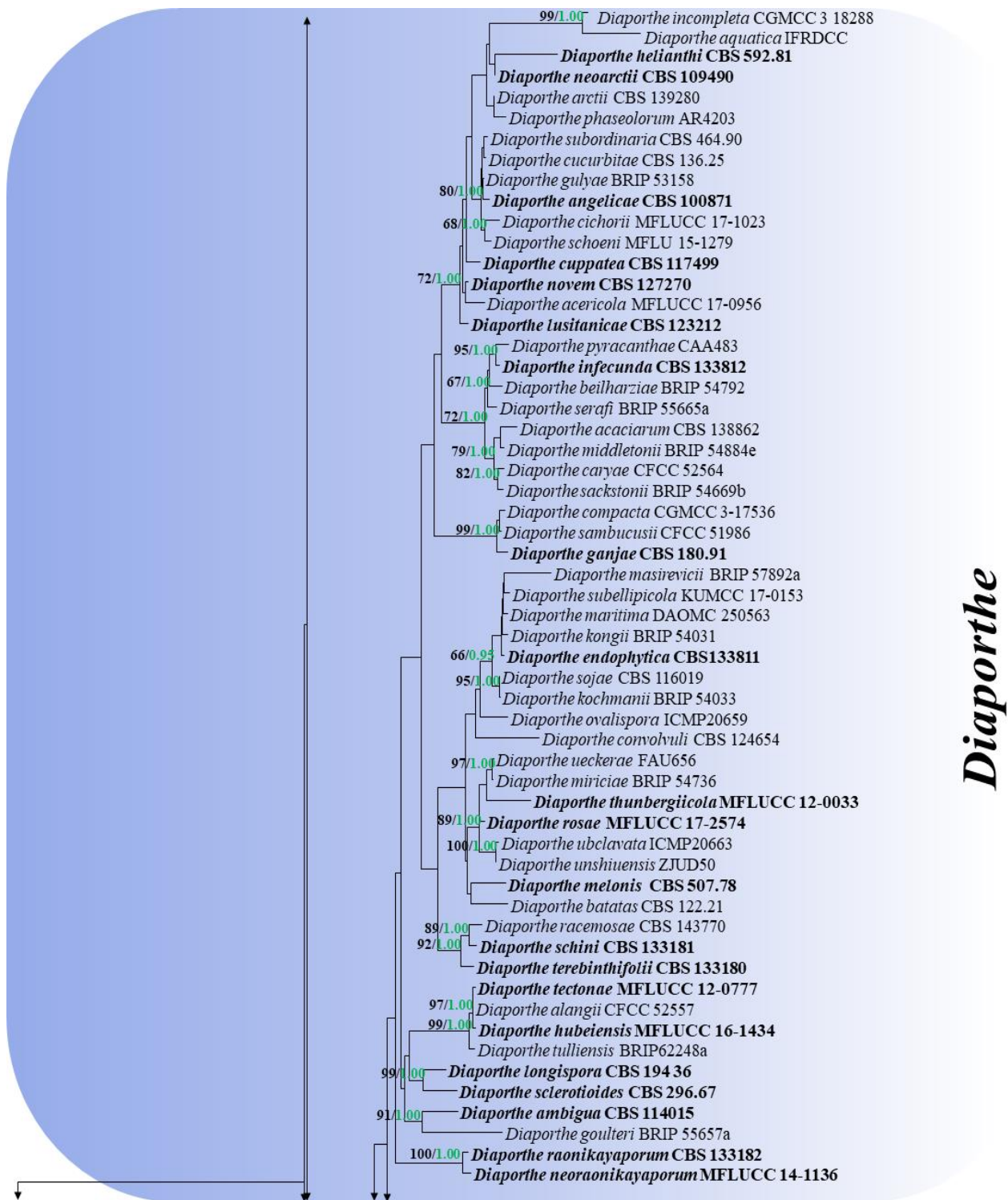
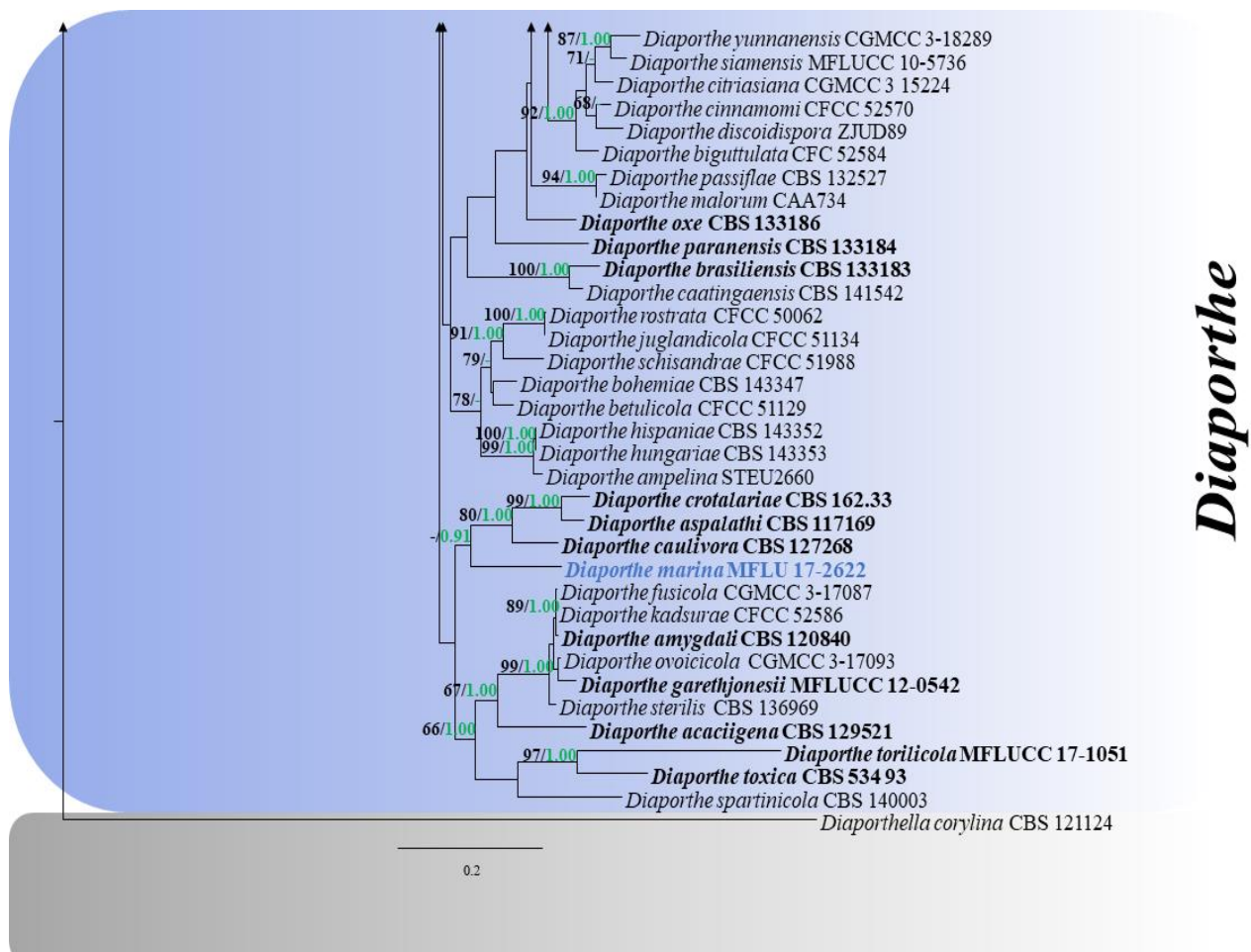


Figure 51 – Continued.





**Figure 51** – Continued.

Culture characteristics – Colonies on sea water MEA reaching 15 cm diam. after 2 weeks at 25 °C, dense, circular, slightly raised, surface smooth with entire edge, fluffy, colony from above: white; from below: yellowish white, not produced pigmentation on MEA media.

Material examined – THAILAND, Krabi Province, Phang Nga, on decaying submerged wood of *Bruguiera* sp. (Rhizophoraceae), 30 August 2017, M.C. Dayarathne, MCD 143 (MFLU 17-2618, holotype); *ibid.*, (HKAS102052, isotype), ex-type living culture MFLUCC 17-2481.

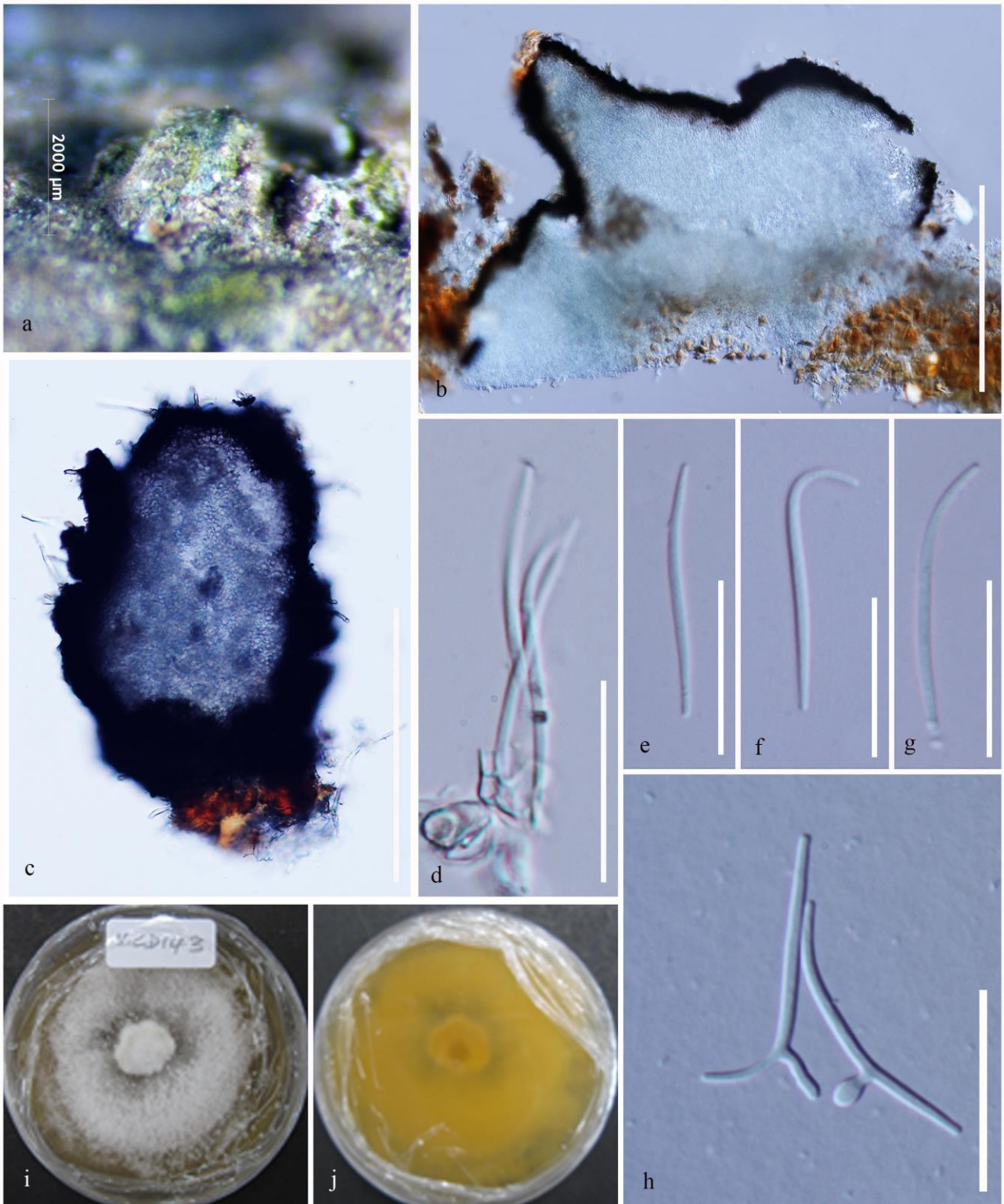
GenBank numbers – ITS: MN047100, MN047101; TEF: MN433215; BTUB: MN431495

Additional GenBank numbers – LSU: MN017866; SSU: MN017929

Notes – Asexual morphs of *Diaporthe* are difficult to differentiate as most have similar morphologies (Santos et al. 2011, Dissanayake et al. 2017, Perera et al. 2018). A new taxon collected from Phang Nga, Thailand proved difficult to identify as alpha conidia were also not observed (Dissanayake et al. 2017, Perera et al. 2018). We observed conidial germination of beta conidia. However, beta conidia are sterile tissues and usually do not germinate and produce cultures. We could not observe any other type (alpha or gamma) conidia. Cultures did not sporulate with any of methods we used. Furthermore, sequence data obtained from both mycelial scraps and direct sequencing of the fruiting bodies are identical. However, more taxon sampling is needed confirm this observation. Based on phylogenetic differences, we establish this as a novel species, *D. krabiensis*. *Diaporthe pseudophoenicicola* and *D. pescicola* showed close phylogenetic relationship to *Diaporthe krabiensis*. Base pair differences of ITS regions of *D. krabiensis* to *D. pescicola* and *D. pseudophoenicicola* are 1.4% (7 bp out of 518 bp) and 1.9% (10 bp out of 524 bp). *Diaporthe pseudophoenicicola* and *D. pescicola* have relatively wider conidiomata than *D. krabiensis* (400 µm, 300 µm, 130–140 µm) (Gomes et al. 2013, Dissanayake et al. 2017). There are no beta conidia described for *D. pseudophoenicicola*.



**Figure 52** – *Diaporthe salinicola* (MFLU 18-0553, holotype). a, b Ascoma with a long neck. c Vertical sections through ascoma. d Section through neck region. e Peridium. f Paraphyses. g–i Asci. j, k Ascospores. l Germinating ascospore. m, n Mature culture on sea water PDA (m lower, n upper). Scale bars: b, c = 200  $\mu\text{m}$ , d, g, h = 50  $\mu\text{m}$ , f, i = 20  $\mu\text{m}$ , j–k = 10  $\mu\text{m}$ .



**Figure 53** – *Diaporthe krabiensis* (MFLU 17-2618, holotype). a Pycnidia on host. b, c Section through the pycnidia. d, f Conidiophores with beta conidia. d Conidiophores with beta conidia. e-g Beta conidia. h Germinating beta conidium. i, j Cultures on sea water PDA (i upper, j lower). Bars: b, c = 100 µm, d–h = 10 µm.

***Diaporthe marina*** Dayarathne, sp. nov.

Index Fungorum number – IF556591, Facesoffungi number – FoF 06178

Etymology – Name reflects the marine environment where the taxon was found

Holotype – MFLU 17-2622

Fig. 54

*Saprobic* on decaying submerged *Rhizophora* sp. (Rhizophoraceae) wood. Sexual morph: Stroma absent. *Ascomata* 390–500 × 200–300 μm, globose to subglobose, black, coriaceous, semi-immersed, single to clustered. *Neck* 180–220 × 78–85 μm, cylindrical, black, ostiole canal filled with periphyses. *Peridium* 35–55 mm thick, comprising compressed cells of *textura intricata*, outer layers composed of black, thick-walled cells, inner layers composed of black to hyaline thin-walled, *textura intricata*. *Paraphyses* not observed. *Asci* 150–164.5 × 15–18 μm ( $\bar{x}$  = 160 × 16, n = 10), 8-spored, unitunicate, thin-walled, apedicellate, cylindrical to clavate, with a apical ring, ca. 1 mm high and 2 mm diam. *Ascospores* 16–18 × 10–13 μm ( $\bar{x}$  = 17 × 12.5, n = 25), overlapping uniseriate, ovoid, initially hyaline becoming light brown to brown at maturity, one-septate, upper cell small lower cell enlarged (ratio upper/lower 1:2), constricted at the septum, thin-walled, smooth-walled, with numerous guttules becoming non guttulate at maturity. Asexual morph: Undetermined.

Material examined – THAILAND, Krabi Province, Phang Nga, on decaying submerged wood of *Rhizophora* sp. (Rhizophoraceae), 30 August 2017, M.C. Dayarathne, MCD 147 (MFLU 17-2622, holotype); *ibid.*, (HKAS102054, isotype).

GenBank numbers – ITS: MN047102

Additional GenBank numbers – LSU: MN017867; SSU: MN017930

Notes – *Diaporthe marina* is characterized by having long necked ascomata which lack stromatic tissue, peridium with *textura intricata*, cylindrical asci and ovoid, one-septate, ascospores with a small upper cell and an enlarged lower cell. *Diaporthe marina* differs from *D. aquatica* and *D. salinicola*, by having a peridium of *textura intricata* and ascospores with a small upper cell and an enlarged lower cell with a prominent septum, whereas the other two species have a peridium of *textura angularis* and ellipsoidal to obpyriform ascospores (Hu et al. 2012). Furthermore, the phylogenetic placements of these three species are distant to each other (Fig. 51). *Diaporthe caulivora* shows a close phylogenetic affinity to *D. marina* but morphologically they differ from each other by ascomatal, asci and ascospore morphologies (Santos et al. 2011). However, no culture could be obtained for this species therefore, DNA was extracted directly from the sexual morph on the host

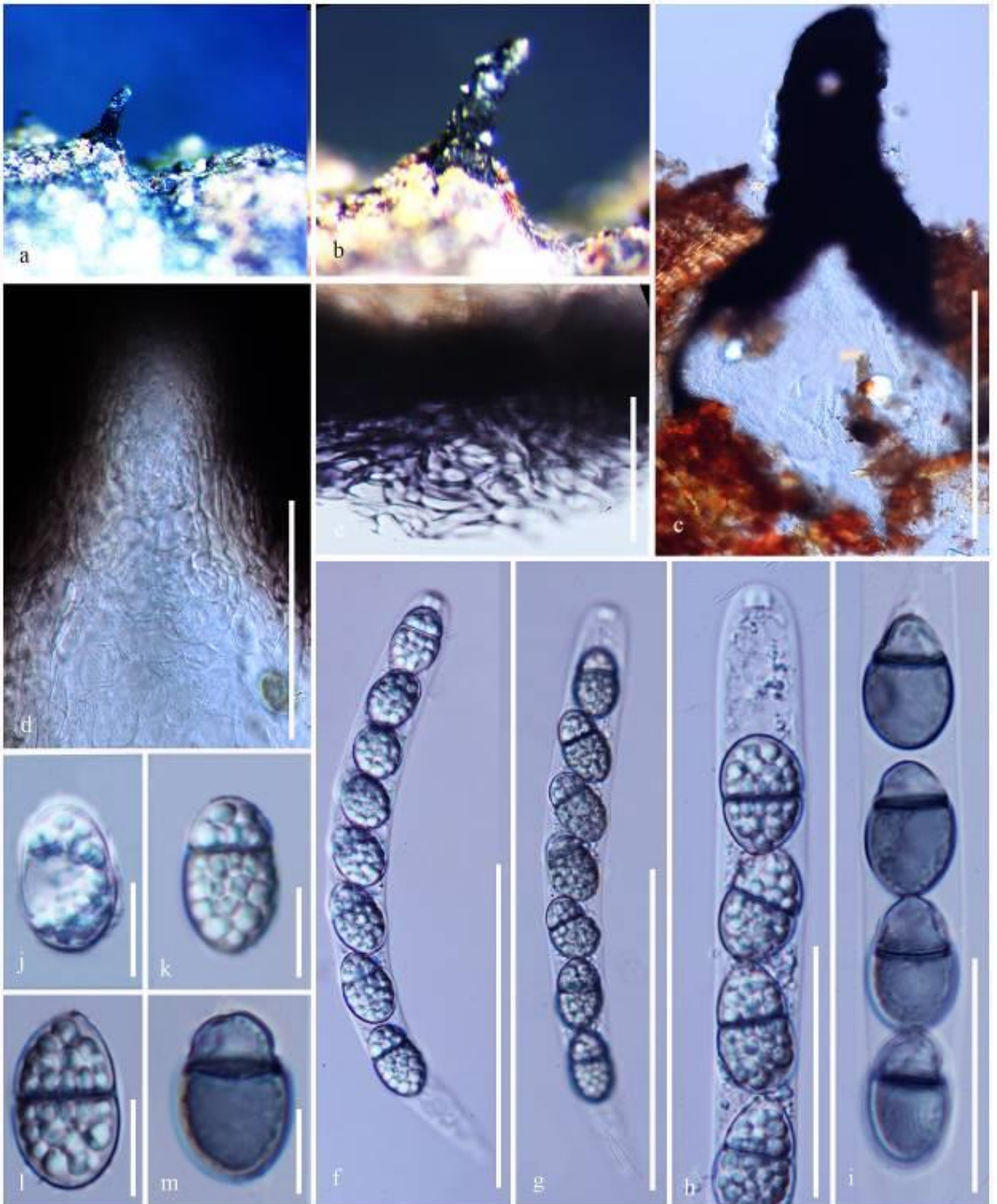
## **Subclass Hypocreomycetidae** O.E. Erikss. & Winka

### **Hypocreales** Lindau

Currently, Hypocreales comprises nine families: Bionectriaceae, Clavicipitaceae, Cordycipitaceae, Hypocreaceae, Nectriaceae, Niessliaceae, Ophiocordycipitaceae, Stachybotriaceae and Tilachliaceae (Wijayawardene et al. 2017a, 2018).

### **Nectriaceae** Tul. & C. Tul.

Tulasne & Tulasne (1865) erected the family Nectriaceae with *Nectria* as the type genus. Nectriaceae comprises uniloculate, pigmented ascomata and phialidic amerosporous to phragmosporous conidia and includes pathogens, saprobes, or fungicolous or insecticolous fungi (Rossman et al. 1999, Rossman 2000, Chaverri et al. 2011, Hyde et al. 2014, Lombard et al. 2015, Tribpromma et al. 2018). Petch (1938) recognized Nectriaceae as a family of Hypocreales but subsequently Nectriaceae was synonymized under Hypocreaceae (Miller 1949, von Arx 1954, Rogerson 1970, Barr 1990a). However, Lumbsch & Huhndorf (2010), Maharachchikumbura et al. (2015) and Lombard et al. (2015) reinstated Nectriaceae and included 66 genera (Wijayawardene et al. (2018). We herein introduce five novel marine Nectriaceae taxa based on morphology and sequence data analyses (Figs 55, 56, 57).



**Figure 54** – *Diaporthe marina* (MFLU 17-2622, holotype). a, b Ascomata with long necks. c Vertical sections through ascoma. d Section through neck region. e Peridium. f–i Asci. j–m Aseptate to septate ascospores. Scale bars: c = 200  $\mu\text{m}$ , f–g 100  $\mu\text{m}$ , d, h, i = 50  $\mu\text{m}$ , f, i = 20  $\mu\text{m}$ , j–m = 10  $\mu\text{m}$ .

***Chaetopsina* Rambelli**

*Chaetopsina* is a dematiaceous hyphomycete which was introduced by Rambelli (1956) to

include *C. fulva* and this genus remained monotypic for 25 years. Later Matsushima (1971) introduced *C. ramifera*. Later, several other species have been added by Sutton & Hodges (1976), Rambelli & Lughini (1976, 1979), Morgan-Jones (1979), Crane & Schoknecht (1982), Kirk & Sutton (1985), Kirk & Sutton (1985), Samuels (1985), Wingfield (1987), Merli et al (1992), Zucconi & Rambelli (1993), and Holubova-Jechova (1990). Hence, currently the genus comprises 19 species. *Chaetopsina* is characterized by mononematous, erect, septate, pigmented, setiform, apically sterile conidiophores (Rambelli 1956). The conidiogenous cells are discrete, ampulliform or lageniform, phialidic, and produce unicellular, hyaline, smooth conidia, which are aggregated in slimy masses (Ellis 1971). Samuels (1985) proposed that *Chaetopsina* should be restricted to asexual morphs of *Nectria sensu lato* with reddish-brown setose conidiophores that turn yellow in lactic acid. This was accepted by Kirk & Sutton (1985), who further restricted *Chaetopsina* to species with unicellular, fusiform, smooth-walled conidia, and fertile regions situated terminally or along the axes of the setiform conidiophores. Based on these amended generic concepts, only five species were accepted in the genus by Kirk & Sutton (1985). Only six *Chaetopsina* species have sequence data in GenBank (2019 June).

***Chaetopsina aurantisalinicola*** Dayarathne, E.B.G. Jones & K.D. Hyde, sp. nov. Fig. 56

Index Fungorum number: IF556592; Facesoffungi number: FoF 06179

Etymology – Epithet refers to the orange (*'aurantiaco'* in Latin) conidiophores and culture and the *"salinicola"* means salt inhabiting.

Holotype – MFLU 18-0566

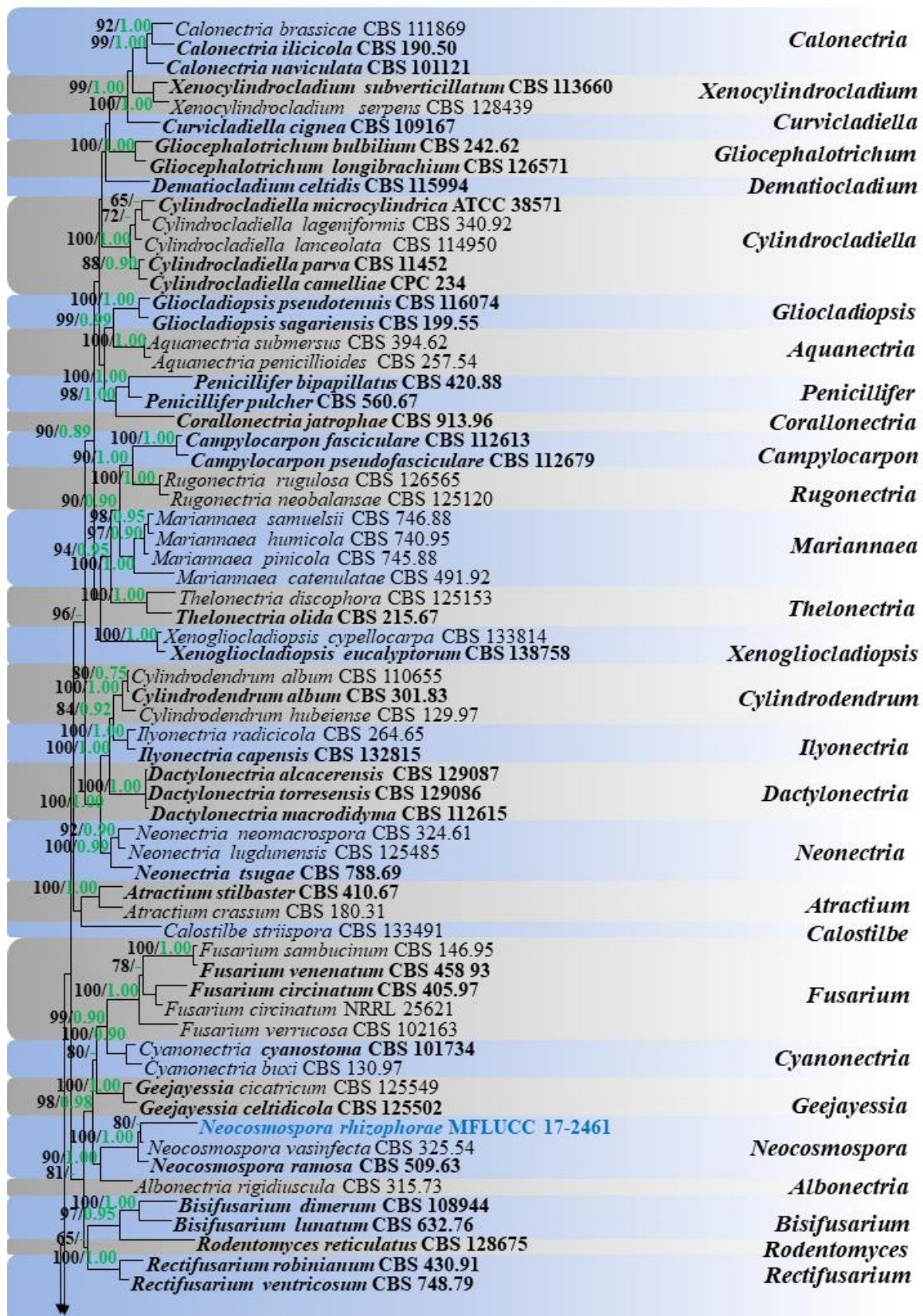
*Saprobic* on submerged *Rhizophora* wood. Sexual morph: Undetermined. Asexual morph: Colonies tomentose, pale yellow-orengish brown. Mycelium partly superficial and partly immersed in the substratum, composed of irregularly branches, septate, smooth, subhyaline to yellowish or pale red-brown hyphae. *Conidiophores* 150–200 µm long, macronematous, mononematous, orengish brown, paler towards the apex, erect, straight or slightly curved, thickwalled, smooth, 6–8-septate, with a bulbose base 35–45 µm wide, subulate, 12–14.8 µm wide just above the bulbose base, tapering gradually to 4.2–5 µm wide at the apex. *Fertile region* yellow to orengish brown, apical, comprising loosely and regularly arranged penicillate, smooth-walled, 2-septate branches bearing conidiogenous cells, arranged in several whorls. *Conidiogenous cells* 3–16.5 µm long, discrete, ampulliform, hyaline, blastic. *Conidia* 10–26 × 3.5–6.8 ( $\bar{x}$  = 18.7 × 5 µm, n = 30), solitary, unicellular, cylindrical to cylindro-fusoid or ellipsoidal, hyaline, smooth.

Culture characteristics – Colonies on sea water MEA reaching 10 cm diam. after 2 weeks at 25 °C, dense, circular, slightly raised, surface smooth with entire edge, fluffy, colony from above: red, yellow and brown whorls; from below reddish yellow.

Material examined – THAILAND, Ranong Province, Ranong, on decaying submerged wood of *Rhizophora* sp. (Rhizophoraceae), 02 December 2017, M.C. Dayarathne, MCD 21Y (MFLU 18-0566, holotype); *ibid.*, (BBH, isotype), ex type living culture MFLUCC 17-0414.

GenBank numbers – LSU: MN017868; ITS: MN047103

Notes – *Chaetopsina aurantisalinicola* is the only marine species of this genus. *Chaetopsina aurantisalinicola* is characterized by having tomentose, pale yellow-oraengish brown colonies, oraengish brown, 6–8-septate conidiophores. *Chaetopsina aurantisalinicola* differs from other *Chaetopsina* species by orangish brown, smooth, 6-8 septate conidiophores and relatively larger conidia (10–26 × 3.5–6.8 µm). *Chaetopsina aurantisalinicola* forms a strongly supported distinct clade (100% ML, 1.00 PP) neighboring *C. penicillata* in our phylogram (Fig. 54). However, *C. penicillata* is different from *C. aurantisalinicola* by having setose conidiophores, phialides with a periclinal thickening and a flared collarete and elliptic to sub-fusoid conidia with a protuberant abscission scar at the base (Samuels 1985), whereas, *C. aurantisalinicola* has smooth conidiophores, phialides without periclinal thickening and a flared collarete and cylindrical to cylindro-fusoid or ellipsoidal conidia without a protuberant abscission scar at the base. In addition, *Chaetopsina aurantisalinicola* and *C. penicillata* have 1.3% (11 bp out of 840 bp and 9% (52 bp out of 568 bp) base pair differences in LSU and ITS loci, respectively.



**Figure 55** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -18535.484456. The combined ITS, LSU, TEF and RPB2 dataset comprised 206 strains of Nectriaceae with *Bionectria ochroleuca* (AFTOL-ID 187) and *Clonostachys buxi* (CBS 696.93) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 1104 distinct alignment patterns, with 60.52% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.228206, C = 0.270125, G = 0.302346, T =

0.199324; substitution rates AC = 0.975608, AG = 1.608474, AT = 1.168311, CG = 0.986748, CT = 5.376529, GT = 1.000000; gamma distribution shape parameter  $\alpha = 0.318529$ . Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.3 changes. The ex-type strains are in bold and new isolates in blue bold.

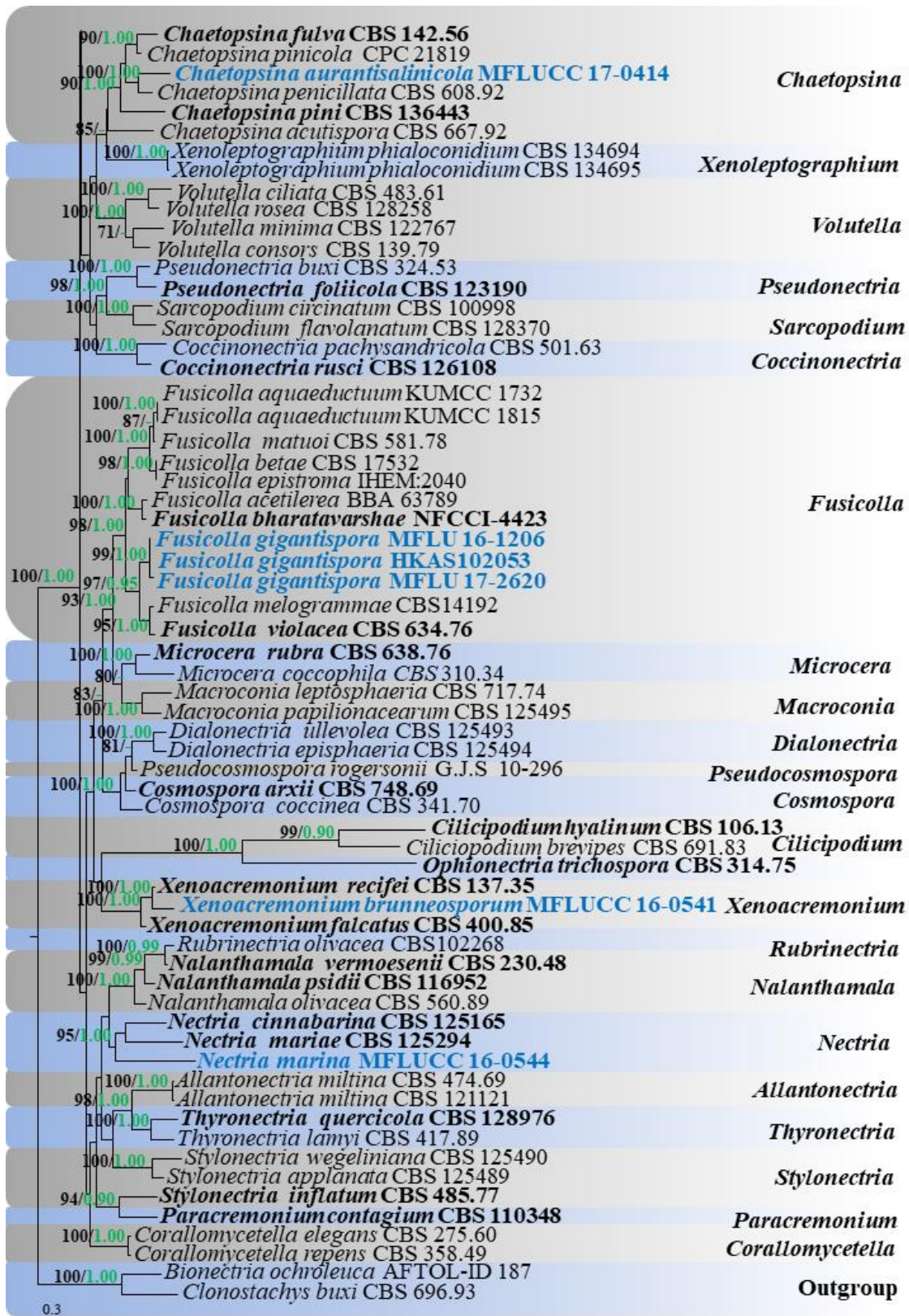


Figure 55 – Continued.





**Figure 56** – *Chaetopsina aurantisalinicola* (MFLU 18-0566, holotype). a–c Colonies on substrate. d Conidiophores bearing conidia. e, f Conidia attached to the conidiogenous cells. g–j Conidia. k Germinating conidium. l Culture on sea water PDA (upper). Scale bars: a, d = 100 µm, e, f = 50 µm, j–k = 10 µm.

### ***Fusicolla* Bonord.**

The genus *Fusicolla*, typified by *Fusicolla betae*, comprises nine accepted species as listed in Index Fungorum (2019). *Fusicolla ossicola*, *F. aquaeductuum*, *F. bharatavarshae* and *F. melogrammae* were reported to have both sexual and asexual states while the other species were reported only as asexual morphs (Gräfenhan et al. 2011). *Fusicolla* is characterized by superficial, nonstromatic, globose to sub-globose, pale yellow to pale orange ascomata, lacking reaction to KOH, 1-septate, ellipsoidal, hyaline and straight or slightly curved ascospores (Lechat & Rossman 2017). Jones et al. (2019) introduced a marine species *F. bharatavarshae* which was collected from India.

### ***Fusicolla gigantispora* Dayarathne & K.D. Hyde, sp. nov.**

Fig. 57

Index Fungorum number: IF556593; Facesoffungi number: FoF 06180

Etymology – Specific epithet refers to the relatively bigger size of ascospores

Holotype – MFLU 16-1206

*Saprobic* on dead bark of *Avicennia marina* and *Bruguiera* sp. subjected to sea water spray. Sexual morph: *Ascomata* 280–300 × 235–280 μm, perithecial, superficial, solitary to gregarious, non-stromatic, globose to sub-globose, coriaceous pale yellow to pale orange, not developing colour in 3% KOH, ostiolate. *Peridium* 20–25 μm, thick, two-layered, outer layer of 2–4 layers of *textura angularis*, inner layer of 3–4 layers of hyaline cells. *Paraphyses* 4–5 μm wide, septate, deliquescent. *Asci* 205–250 × 20–24 μm, ( $\bar{x}$  = 225 × 22 μm, n = 20), unitunicate, cylindrical to narrowly clavate, short pedunculate, thin-walled, 8-spored, uniseriate, persistent, apically truncate when immature, with a J- apical ring. *Ascospores* 20–35 × 20–28 μm, ( $\bar{x}$  = 24 × 24 μm, n = 50), rounded or ellipsoidal, sometimes flat at the lower end, aseptate, hyaline when immature becoming dark brown at maturity, slightly constricted at the septum when young, rough walled, without appendage or sheath.

Material examined – THAILAND, Krabi Province, Tha pom Khlong Nam, N 08°12'50.4", E 98°46'42.7" N, 0 m asl., on dead bark of *Avicennia marina* at a mangrove stand subjected to sea water spray, 17 December 2015, M.C. Dayarathne, KLA013 (MFLU 16-1206 holotype); *ibid.*, (HKAS102053 isotype). THAILAND, Krabi Province, Tha pom Khlong Nam, on dead bark of *Bruguiera* sp. subjected to sea water spray at a mangrove stand, 30 August 2017, M.C. Dayarathne, MCD145 (MFLU 17-2620); *ibid.*, (HKAS101990).

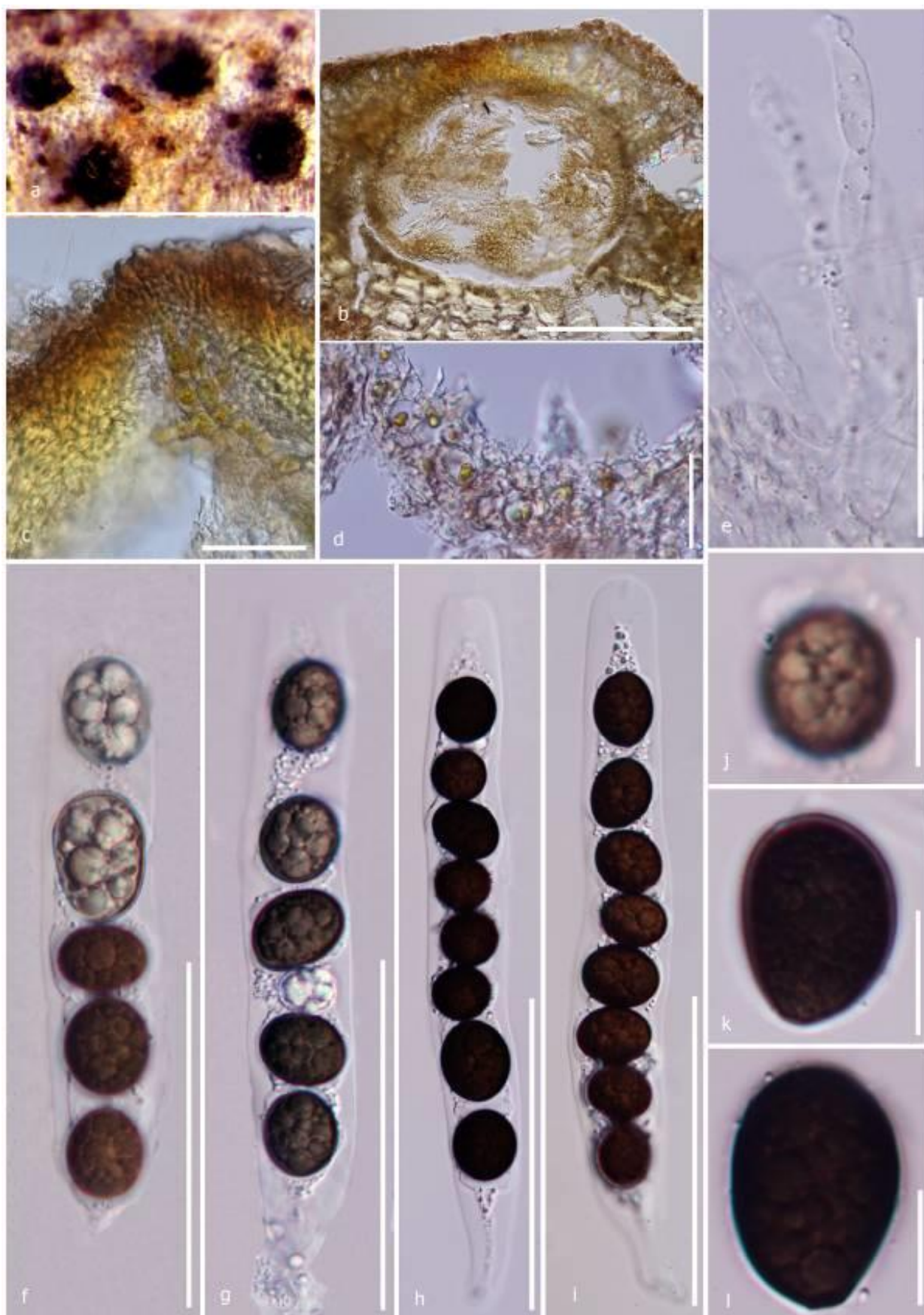
GenBank numbers – LSU: MN017869, MN017876, MN017870, ITS: MN047104, MN047105, MN047106

Additional GenBank number – SSU: MN017931

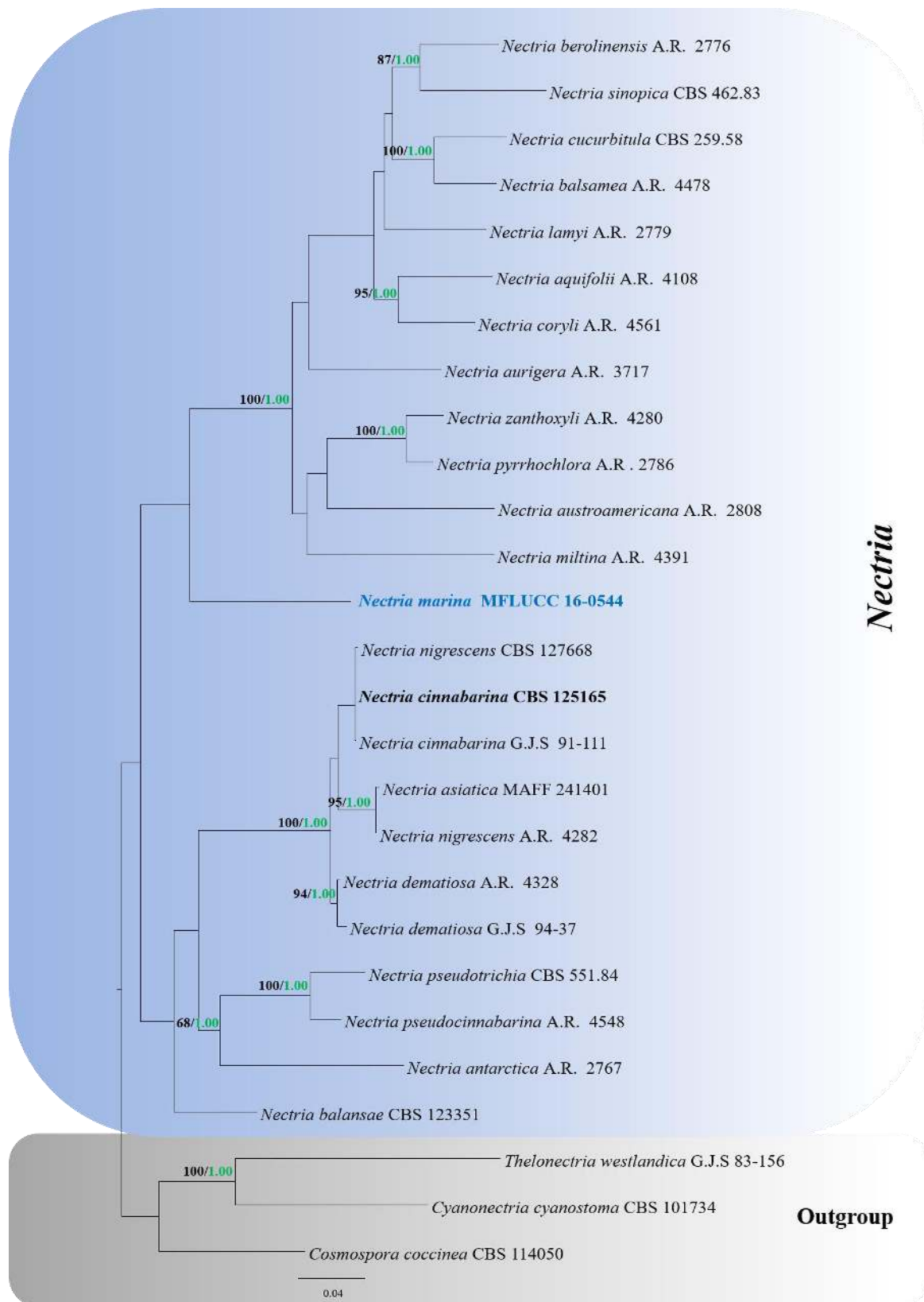
Notes – Phylogenetic analyses indicated that the three strains of the novel *Fusicolla gigantispora* (MFLU 16-1206 and MFLU 17-2620) clustered with *Fusicolla melogrammae* (CBS14192) with high statistical support (99% ML, 1.00 PP) (Fig. 55). However, *F. gigantispora* is significantly different from other species of *Fusicolla* in having rounded or ellipsoidal aseptate, relatively larger ascospores (20–35 × 20–28 μm), that are hyaline when immature and becoming dark brown when mature. Furthermore, the ascospores of *F. melogrammae* are ellipsoidal, rounded at ends, 1-septate, (12–14 × 4.5–5 μm), spinulose, hyaline to pale golden brown when mature and slightly constricted at the septum. Base pair differences of *F. melogrammae* and *F. gigantispora* are 12 bp out of 776 bp (1.6%) of LSU and 25 bp out of 545 bp (4.6%) of ITS which indicates they are two distinct species. The recently introduced asexual species *F. septimanifiniscientiae* also differed in 23 bp out of 526 bp (4.3 %).

### ***Nectria* (Fr.) Fr.**

The genus *Nectria* is typified by *N. cinnabarina*, a wood-inhabiting fungus common in temperate regions of the Northern Hemisphere (Hirooka et al. 2011). This genus is characterized by red, globose, fleshy, warted perithecia that often become cupulate upon drying, 0–3-septate ascospores (Rossman et al. 1999). We here introduce a novel marine species to *Nectria* from Thailand.



**Figure 57** – *Fusicolla gigantispora* (MFLU 16-1206, holotype). a Ascomata superficial on the dead bark of *Avicennia marina*. b Section through ascoma. c Section through osteolar region. d Peridium. e Paraphyses. f–i Immature and mature asci. j–l Ascospores. Scale bars: b = 100  $\mu\text{m}$ , f–i = 50  $\mu\text{m}$ , j–l = 20  $\mu\text{m}$ .



**Figure 58** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -14641.704613. The combined ITS and LSU dataset comprised 27 strains of *Nectria* with *Thelonectria westlandica* (G.J.S 83-156), *Cyanonectria cyanostoma* (CBS 101734) and *Cosmospora coccinea* (CBS 114050) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 747 distinct alignment patterns, with 8.56% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.235998, C = 0.272392, G = 0.263123, T = 0.228487; substitution rates AC = 0.997494, AG = 1.911235, AT = 1.488006, CG =

0.908697, CT = 4.445539, GT = 1.000000; gamma distribution shape parameter  $\alpha = 0.168504$ . Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.04 changes. The ex-type strains are in bold and new isolates in blue bold.

*Nectria marina* Dayarathne & K.D. Hyde, sp. nov.

Fig. 59

Index Fungorum number: IF556594; Facesoffungi number: FoF 06181

Etymology – Name refers to the marine environment where the species found.

Holotype – MFLU 16-1180

*Saprobic* on submerged mangrove wood. *Stromata* 1.5 mm high and 3.0 mm diam, superficial or erumpent, globose to sub globose, orangish red, produce pigment with KOH+, solitary to gregarious, pseudoparenchymatous, cells forming *textura angularis*, intergrading with ascomatal wall. *Ascomata* 640–800 × 500–650  $\mu\text{m}$ , ( $\bar{x} = 720 \times 600 \mu\text{m}$ ,  $n = 5$ ), superficial on well-developed stromata, solitary, sub-globose to globose, scarlet to sienna, slightly cupulate upon drying, apical region slightly darker, KOH+ reddish brown, smooth to roughened. *Peridium* 25–40  $\mu\text{m}$  thick, of three regions: outer region intergrading with stroma, cells forming *textura angularis*, walls pigmented; middle region cells forming *textura prismatica*, containing scarlet to bay, pigmented droplets, walls pigmented to gold; inner region, of elongate, thin-walled, hyaline cells, forming *textura prismatica*. *Paraphyses* 1.2–1.8 wide, hyaline, branched, septate. *Asci* 165–180 × 12–20  $\mu\text{m}$ , ( $\bar{x} = 175 \times 18 \mu\text{m}$ ,  $n = 30$ ), 8-spored, clavate, with inconspicuous ring at apex. *Ascospores* 25–38 × 7–9.5  $\mu\text{m}$ , ( $\bar{x} = 30 \times 8.2 \mu\text{m}$ ,  $n = 30$ ), biseriate, ellipsoidal to fusiform, straight, 1-septate, deeply constricted at the septa, hyaline, guttulate, thick walled.

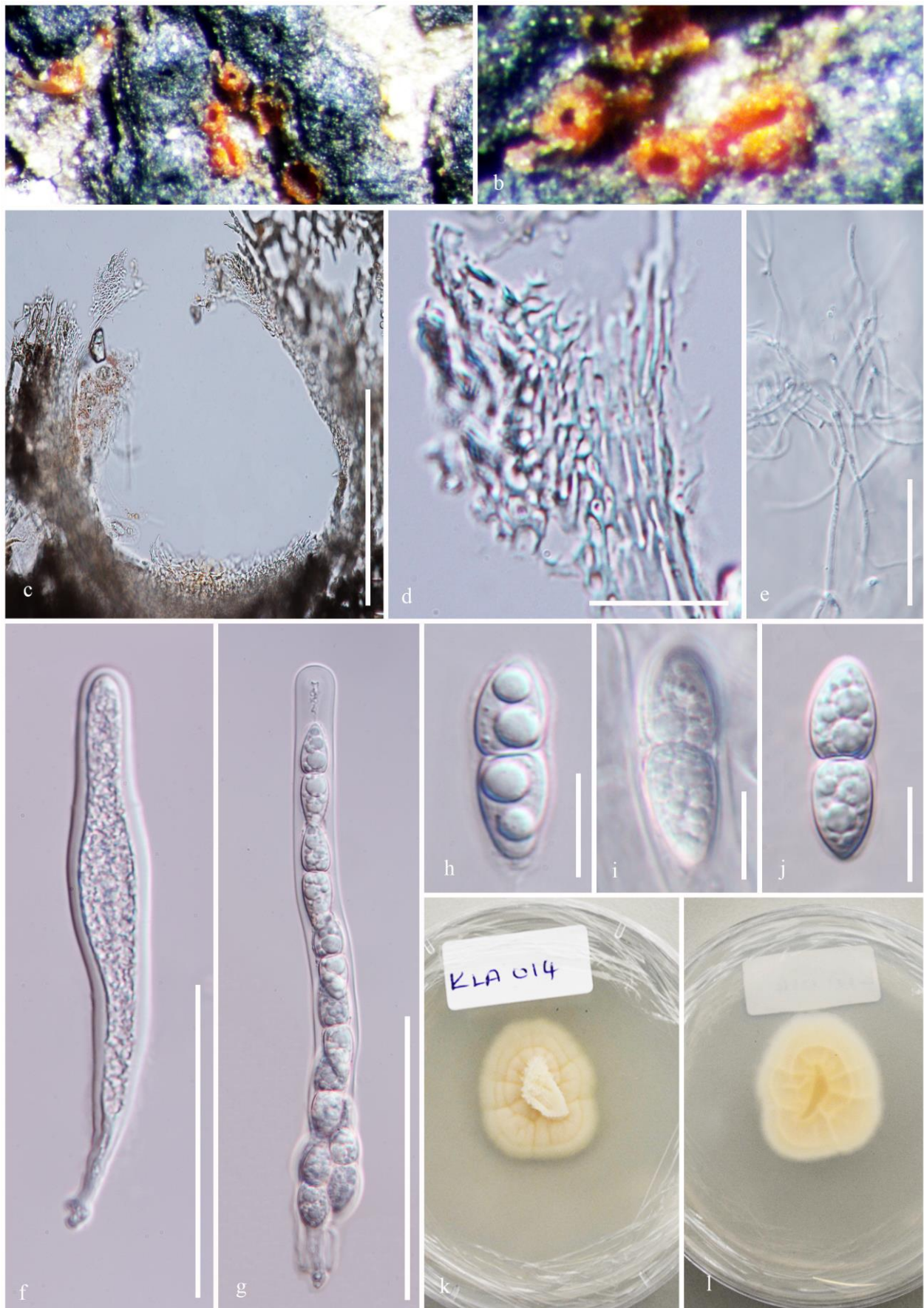
Material examined – THAILAND. Krabi Province: Tha Pom Khlong Song Nam (N 08°12'50.4", E 98°46'42.7"), submerged mangrove wood, 16 December 2015, M.C. Dayarathne KLA014 (MFLU 16-1180, holotype); *ibid.*, (HKAS102021, isotype), ex-type living cultures MFLUCC 16-0544.

GenBank numbers – LSU: MN017871; ITS: MN047108; TEF: MN433214

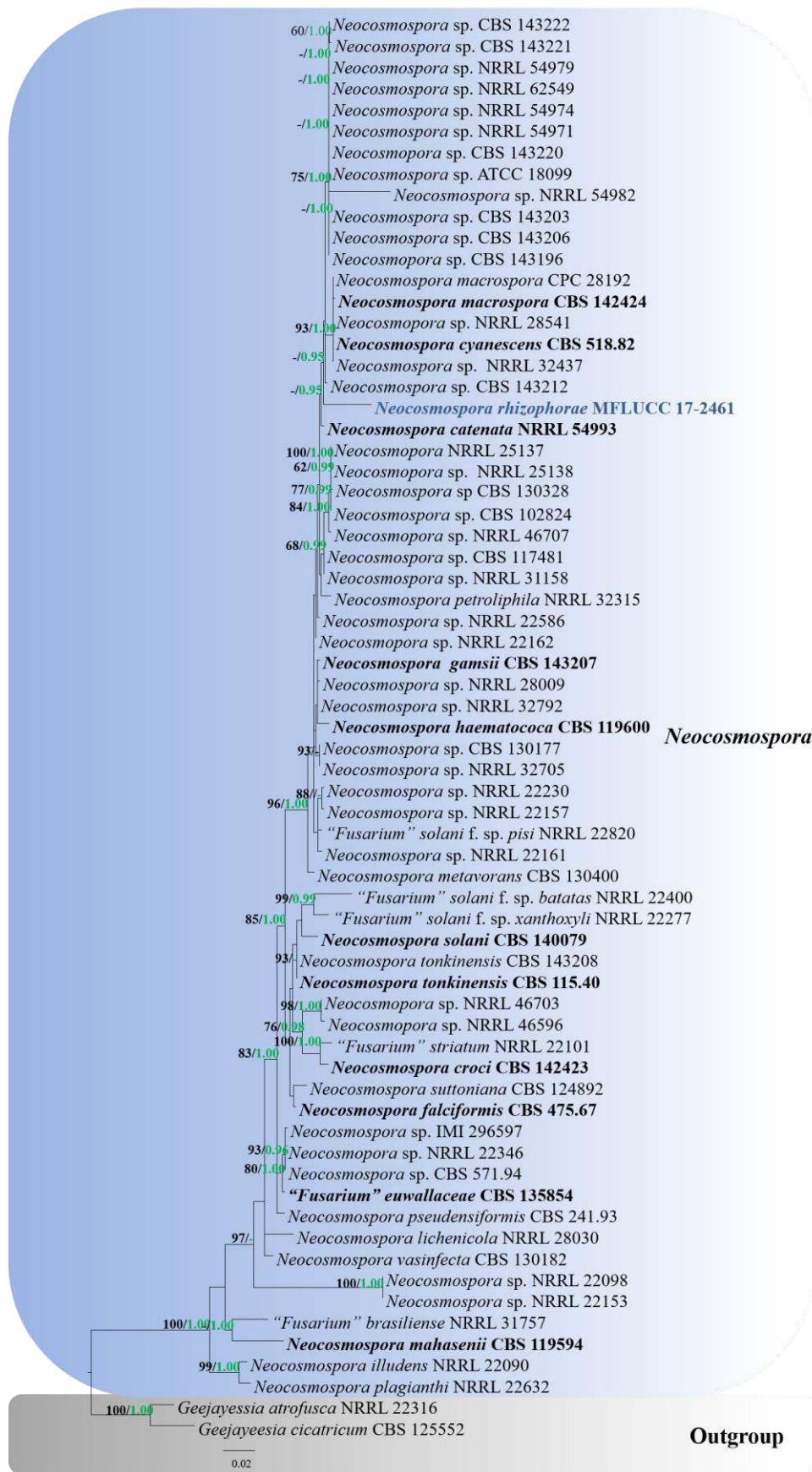
Notes – *Nectria marina* is characterized by red, globose, fleshy ascomata and 1-septate ascospores which are in common with other *Nectria* species. In our phylogram, *Nectria marina* formed a distinct basal lineage to a clade comprising, *N. austroamericana*, *N. aquifolii*, *N. aurigera*, *N. balsamea*, *N. berolinensis*, *N. coryli*, *N. cucurbitula*, *N. lamyi*, *N. miltina*, *N. pyrrochlorella*, *N. sinopica* and *N. zanthoxyli* (Fig. 58). *Nectria miltina* is different form *N. marina* by having allantoid to short-cylindrical, aseptate, smaller ascospores (4.3–5.9 × 1.3–1.9  $\mu\text{m}$ ) (Hirooka et al. 2011) while, ascospores of *N. marina* are ellipsoidal to fusiform, 1-septate, and comparatively larger (25–38 × 7–9.5  $\mu\text{m}$ ). However, ascospores of our novel species is well distinguished from all the other *Nectria* species by being deeply constricted at the septa and sharply pointed at their apices.

*Neocosmospora* E.F. Sm.

The genus *Neocosmospora* was established by Smith (1899) for a single species, *Neocosmospora vasinfecta*. Since then, a number of other species have been added to the genus (Cannon & Hawksworth 1984). *Neocosmospora* is characterized by perithecial, orange to red, rarely dark brown, globose to pyriform, ostiolate ascomata with a short neck lined with periphyses, peridium composed of an outer pigmented layer, sometimes covered with rhizoidal hyphae, and hyaline inner layers, mostly with periphysoids, 8-spored, cylindrical, rarely clavate asci with a short stalk and usually lacking apical structures and uniseriate, rarely biseriate, pale brown to almost hyaline, 1-(2-) celled, globose to ellipsoidal, ornamented ascospores (Cannon & Hawksworth 1984). The genus *Neocosmospora* has been a highly renowned fungal group for more than 100 years, mainly because it contains significant number of plant pathogenic species (Sandoval-Denis & Crous 2018, Sandoval-Denis et al. 2018). It includes causative agents of fruit-rot, root-rot and seedling damping-off, affecting diverse plant hosts (Leslie & Summerell 2006, Domsch et al. 2007, Nalim et al. 2011).



**Figure 59** – *Nectria marina* (MFLU 16-1206, holotype). a, b Stromata superficial on the dead wood. c Section through stroma. d Peridium. e Paraphyses. f, g Immature and mature asci. h–j Ascospores. k, l Cultures on PDA. Scale bars: c = 100  $\mu$ m, e–g = 50  $\mu$ m, d = 20  $\mu$ m, h–j = 10  $\mu$ m.



**Figure 60** Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -4264.829108. The combined ITS and LSU dataset comprised 95 strains of *Neocosmopora*, several fusarium-like taxa with *Geejayessia atrofusca* (CBS 125552) and *G. cicatricum* (NRRL 22316) as the outgroup taxa. Tree topology of the ML analysis was similar to

the BI. The matrix had 308 distinct alignment patterns, with 7.06% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.250273, C = 0.258917, G = 0.272267, T = 0.218543; substitution rates AC = 1.866807, AG = 2.127266, AT = 2.318509, CG = 0.912700, CT = 6.511334, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.168504. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.02 changes. The ex-type strains are in bold and new isolates in blue bold.

***Neocosmospora rhizophorae*** Dayarathne sp. nov.

Fig. 61

Index Fungorum number: IF556595; Facesoffungi number: FoF 06182

Etymology – Epithet refers to the host, *Rhizophora* sp., where the species found.

Holotype – MFLU 17-2588

*Saprobic* on submerged wood of *Rhizophora* sp. *Ascomata* 330–345 × 300–325  $\mu\text{m}$  ( $\bar{x}$  = 340 × 320  $\mu\text{m}$ , n = 5), orange to dark brown-red, globose to pyriform, semi immersed, solitary or gregarious, coarsely warted, glabrous. *Peridium* 50–68  $\mu\text{m}$  wide, composed of thick-walled cells of *textura angularis*. *Paraphyses* 1.2–3  $\mu\text{m}$  wide, persistent, narrow, branched, tapering towards the apex. *Asci* 80–90 × 15–18  $\mu\text{m}$  ( $\bar{x}$  = 85 × 16  $\mu\text{m}$ , n = 30), 8-spored, clavate, unitunicate, with a broad and somewhat flattened and simple apex. *Ascospores* 15–25 × 6–9.2  $\mu\text{m}$ , ( $\bar{x}$  = 20 × 8  $\mu\text{m}$ , n = 50), obliquely uniseriate or irregularly biseriate, obovoid to subfusiform, 1-septate, hyaline, thick-walled, longitudinally finely striated, guttulate, often slightly constricted at the septum.

Material examined – THAILAND, Krabi Province, Phang Nga, on *Rhizophora* sp. (Rhizophoraceae) wood, 31 August 2017, M.C. Dayarathne, MCD 102 (MFLU 17-2586, holotype); *ibid.*, (HKAS102030, isotype), ex-type living culture (MFLUCC 17-2461).

GenBank numbers – LSU: MN017872; ITS: MN047109

Additional GenBank number – SSU: MN017932

Notes – *Neocosmospora rhizophorae* is morphologically similar to other members of *Neocosmospora* by its reddish membranous ascomata and 1-septate, longitudinally finely striate ascospores (Sandoval-Denis & Crous 2018). *Neocosmospora rhizophorae* shows close phylogenetic affinities to an unidentified *Neocosmospora* spp. (CBS 143212), which are important veterinary pathogens, and currently under study (Geiser pers. comm.) (Sandoval-Denis & Crous 2018). *Neocosmospora rhizophorae* is morphologically reminiscent of *N. gamsii* but can be clearly distinguish by the size of asci (80–90 × 15–18  $\mu\text{m}$  vs 72–87.5 × 7.5–9  $\mu\text{m}$ ) and ascospores (15–25 × 6–9.2  $\mu\text{m}$  vs 10.5–11.5 × 5.0–6.5  $\mu\text{m}$ ) (Sandoval-Denis & Crous 2018). They are distantly placed in our phylogram (Fig. 60). Somewhat similar morphologies are visible in *Kallichroma* to this novel taxon. However, *Kallichroma* species can be distinguished from *N. rhizophorae* by epapillate or clypeoid ascomata and ascospores surrounded by mostly a gelatinous sheath (Kohlmeyer & Kohlmeyer 1965, 1979, Kohlmeyer 1984, Abdel-Wahab et al. 2017).

***Xenoacremonium*** L. Lombard & Crous

Facesoffungi Number: FoF 06183

*Saprobic* on wood. Sexual morph: *Stromata* erumpent, orange to brown, pseudoparenchymatous, cells forming *textura angularis*, intergrading with ascomatal wall. *Ascomata* superficial, aggregated in groups of 2–3, brown, subglobose to globose. *Peridium* 50–90  $\mu\text{m}$  thick, comprises two regions, outer region intergrading with stroma, cells forming *textura angularis*, inner region elongate, thin-walled, hyaline cells, forming *textura prismatica*. *Paraphyses* 2–3  $\mu\text{m}$  wide, persistent, aseptate, not branched, tapering towards the apex. *Asci* 8-spored, unitunicate, mainly uniseriate to partly biseriate, with inconspicuous ring at apex, clavate. *Ascospores* ellipsoidal to fusiform with rounded ends, straight or slightly curved, aseptate, hyaline, smooth walled. Asexual morph: *Mycelium* consisting of hyaline, septate, branched hyphae. *Conidiophores* either as lateral phialidic pegs or arising laterally from somatic hyphae, erect, cylindrical to subcylindrical, unbranched or rarely branched, aseptate or septate, smooth, hyaline to pale brown. *Conidiogenous cells* terminal, monophialidic, hyaline, smooth, elongate-ampulliform



or subcylindrical, tapering towards the apex, with periclinal thickening and inconspicuous collarete. *Conidia* aseptate, fusiform to ellipsoidal to cylindrical, slightly or strongly curved, forming slimy heads on the conidiophore (Asexual morph description from Lombard et al. 2015).

Notes – The genus *Xenoacremonium* was established by Lombard et al. (2015) for a group of fungi previously known as *Acronium recifei* (Gams 1971). The second species *X. falcatus* was introduced by Lombard et al. (2015). Members of this genus are presently known only as asexual morphs. Here we introduce the first sexual morph species to *Xenoacremonium* which was collected from a marine habitat.

***Xenoacremonium brunneosporum*** Dayarathne, E.B.G. Jones & K.D. Hyde, sp. nov. Fig. 62

Index Fungorum number: IF556596; Facesoffungi number: FoF 06184

Etymology – Epithet refers derived Latinization of brow ascospores

Holotype – MFLU 16-1204

*Saprobic* on submerged *Rhizophora* wood. Sexual morph: *Stromata* up to 230 × 570 µm, erumpent, orange to brown, pseudoparenchymatous, cells forming *textura angularis*, intergrading with ascomatal wall. *Ascomata* 261–300 × 124–180 µm, superficial, aggregated in groups of 2–3, brown, subglobose to globose. *Peridium* 30–50 µm thick, comprises two regions, outer region 30–45 µm thick, intergrading with stroma, cells forming *textura angularis*, inner region 15–18 µm thick, of elongate, thin-walled, hyaline cells, forming *textura prismatica*. *Paraphyses* 2–3 µm wide, persistent, aseptate, not branched, tapering towards the apex. *Asci* 90–110 × 12–17 ( $\bar{x}$  = 92.4 × 16 µm, n = 20), 8-spored, unitunicate, mainly uniseriate to partly biseriate, with inconspicuous ring at apex, clavate. *Ascospores* 15.7–20.3 × 6–8.5 ( $\bar{x}$  = 17 × 7.3 µm, n = 30), ellipsoidal to fusiform with rounded ends, straight or slightly curved, aseptate, hyaline to pale brown, smooth walled. Asexual morph: Undetermined

Culture characteristics – Reaching 2 cm within 10 days on sea water PDA, when incubated at 25 °C, circular, effuse, with diffuse margin, white, and becoming yellowish-white, to dull yellow with age.

Material examined – THAILAND, Krabi Province, Tha Pom Khlong Song Nam (N 08°12'50.4", E 98°46'42.7"), on aerial bark of *Rhizophora mucronata*, 16 December 2015, M.C. Dayarathne KLA011 (MFLU 16-1204, holotype); *ibid.*, (HKAS101988, isotype), ex-type living cultures MFLUCC 16-0541.

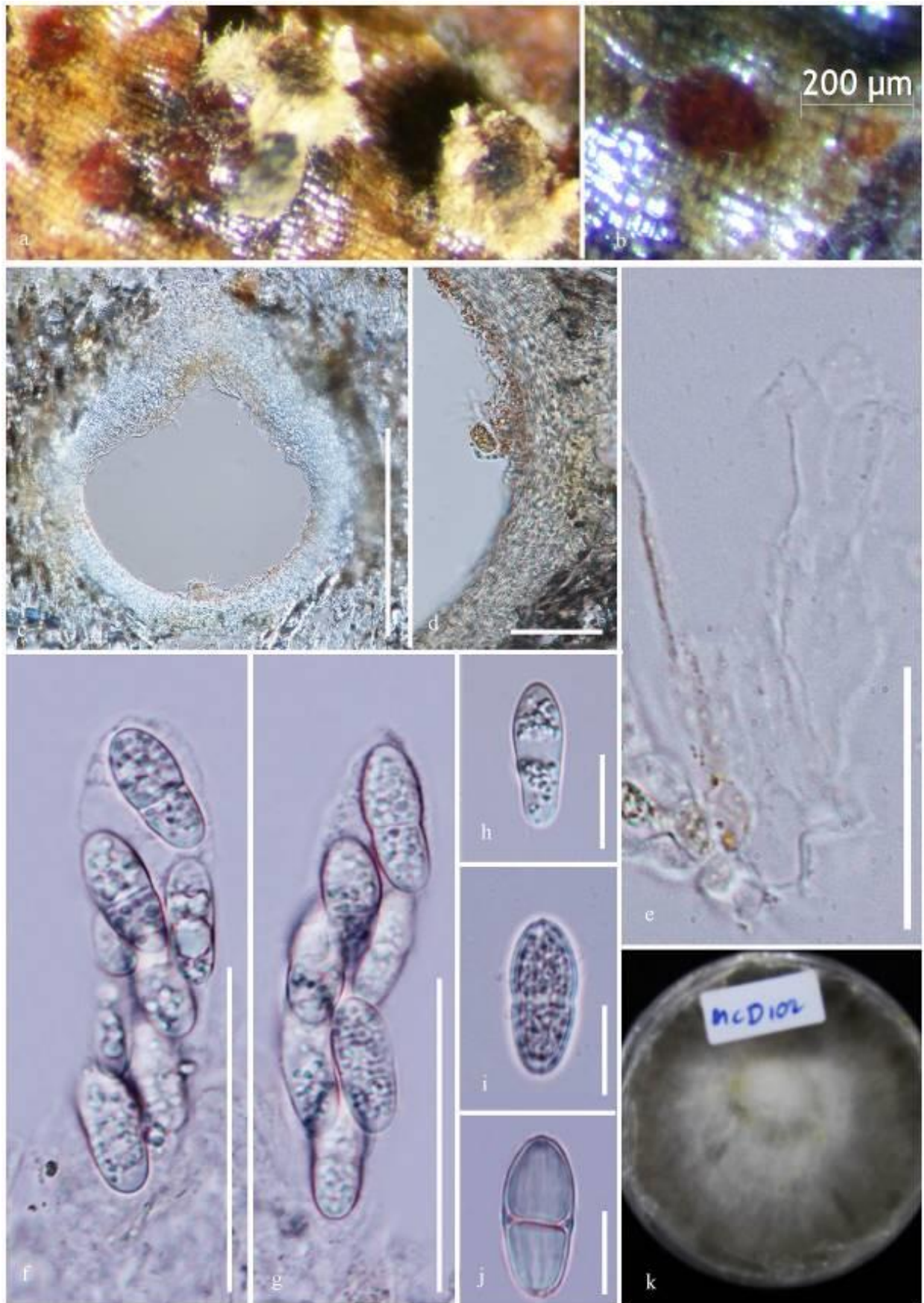
GenBank numbers – LSU MN017873; ITS: MN047107; RPB2: MN077080; TEF: MN077075

Additional GenBank numbers – SSU: MN017933

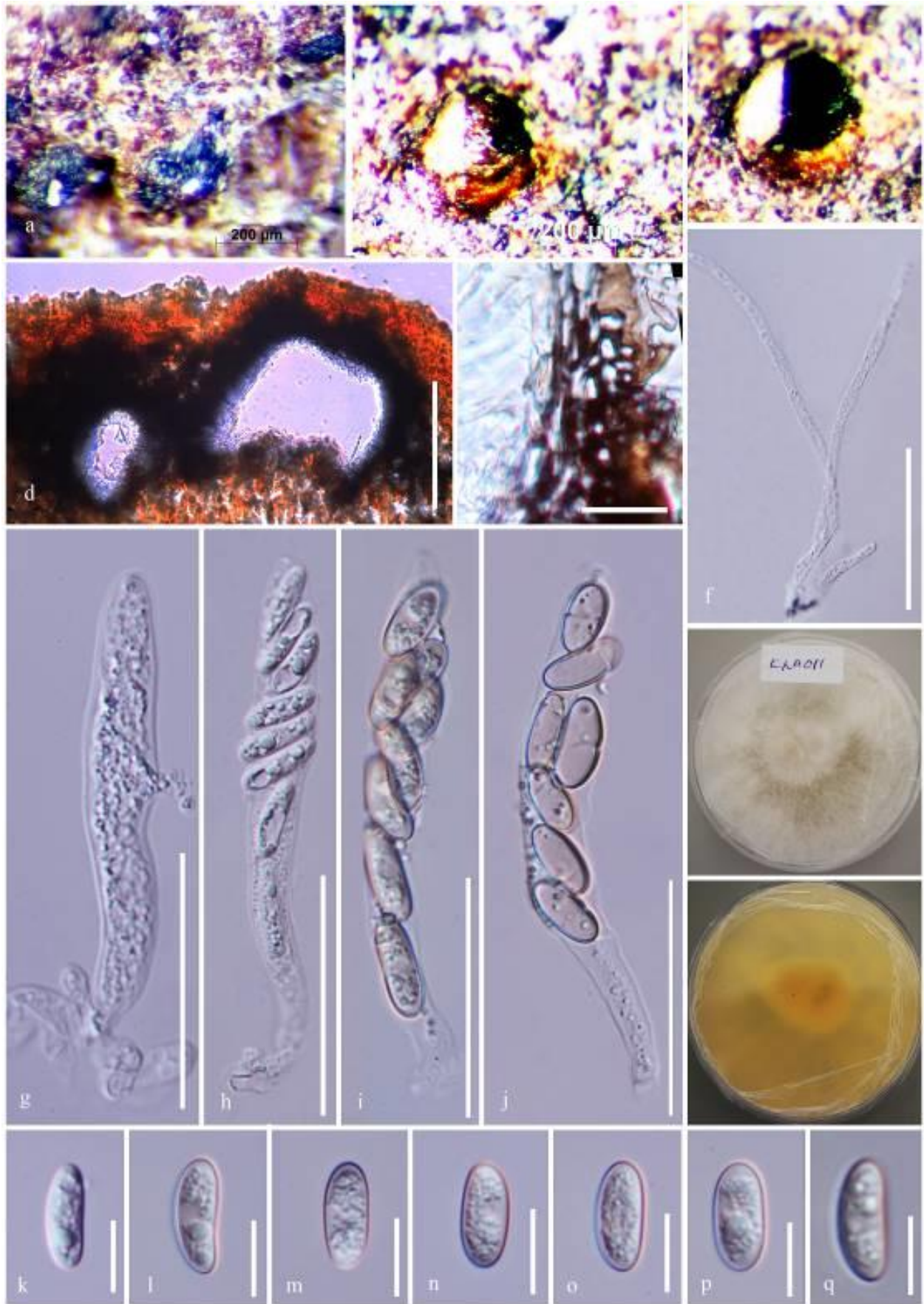
Notes – Our new isolate MFLU 16-1204 grouped within *Xenoacremonium* with strong statistical support (100% ML, 1.00 PP) (Fig. 55). Therefore, we establish this taxon as the first sexual species of this genus. *Xenoacremonium brunneosporum* has orange to brown stromata, clavate asci and ellipsoidal to fusiform ascospores with rounded ends that are characteristic features of Nectriaceae i.e. *Thelonectria lucida*. *Xenoacremonium brunneosporum* shows close phylogenetic affinities to *X. recifei* with 6 bp of ITS loci out of 540 bp (1.1%) and 16 bp of RPB2 loci out of 726 bp (2.3%) differences between them. Further studies are needed to discover the asexual morph of this species to confirm the morphological relationships of this species to existing taxa in the genus.

### **Ophiocordycipitaceae** G.H. Sung et al.

Ophiocordycipitaceae was introduced by Sung et al. (2007) and comprises seven genera, *Drechmeria*, *Harposporium*, *Hymenostilbe*, *Ophiocordyceps*, *Polycephalomyces*, *Purpureocillium* and *Tolypocladium* (Quandt et al. 2014, Wijayawardene et al. 2017a, 2018). Species accommodated in this family are phylogenetically distinct from Cordycipitaceae and Clavicipitaceae (Quandt et al. 2014). Asexual morphs of Ophiocordycipitaceae exhibit a vast range of variation, some of which are confined in phylogenetic distribution while the rest are often found in disparate lineages (Zare et al. 2000, Gams & Zare 2001, Sung et al. 2001, 2007).



**Figure 61** – *Neocosmospora rhizophorae* (MFLU 17-2588, holotype). a, b Ascomata superficial on the dead wood. c Section through an ascoma. d Peridium. e Paraphyses. f, g Asci. h–j Ascospores. k Culture on sea water PDA. Scale bars: b, c = 200 µm, d, e–g = 50 µm, h–j = 10 µm.



**Figure 62** – *Xenoacremonium brunneosporum* (MFLU 16-1204, holotype). a–c Appearance of stromata on host. d Vertical section through stromata. e Peridium. f Paraphyses. g–j Immature and mature asci. k–q Ascospores. Scale bars: a, b, d = 200  $\mu\text{m}$ , f–j = 50  $\mu\text{m}$ , e = 20  $\mu\text{m}$ , k–q = 10  $\mu\text{m}$ .

***Purpureocillium*** Luangsa-ard et al.

The genus *Purpureocillium* was introduced by Luangsa-ard et al. (2011) with *P. lilacinum* as the type species, and has been reported as an important human pathogen. Apart from the type species, *Purpureocillium* comprises three species viz *P. atypicola*, *P. lavendulum* and *P. takamizusanense* (Index Fungorum 2019). The type, *Paecilomyces lilacinus* is a ubiquitous, saprobic filamentous fungus often isolated from soil, decaying vegetation, insects, nematodes and laboratory air (as contaminant), and is a cause of infection in man and other vertebrates (Perdomo et al. 2013, Quandt et al. 2014). In addition, it also can colonize material such as catheters and plastic implants and can contaminate antiseptic creams and lotions (Castro et al. 1990, Orth et al. 1996, Itin et al. 1998).

***Purpureocillium lilacinum*** (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson, FEMS Microbiol. Lett. 321(2): 144 (2011) Fig. 64

Facesoffungi number: FoF 06185

*Saprobic* on decaying on submerged decaying wood of *Bruguiera* sp. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. *Colonies* scattered, black, upright, with abundant, mostly single, sometimes in pairs or multiple, erect synnemata, visible as aerial mycelium on host surface, with cylindrical, dark brown to black stipes, surrounded by hyaline, watery conidial masses. *Conidiophores* 650–800 µm in length, growing from the host surface, very long, consisting of a stipe, irregularly branched, hyaline to light brown and smooth-walled, generally verticillate, in multiple whorls, with swollen apex, with 3–4 phialides per branch, septate. Phialides up to 10–12 µm long, with a cylindrical basal portion tapering into a distinct neck. *Conidiogenous cells* in several whorls, cylindrical to subulate, straight or sometimes slightly curved phialidic, sometimes with percurrent proliferations. *Conidia* 4.5–6 × 1.5–2.8 µm ( $\bar{x}$  = 5 × 2.2 µm; n = 20), cylindrical to ovoid, with rounded apices and subtruncate to truncate bases, aseptate, hyaline, smooth-walled, in slimy heads.

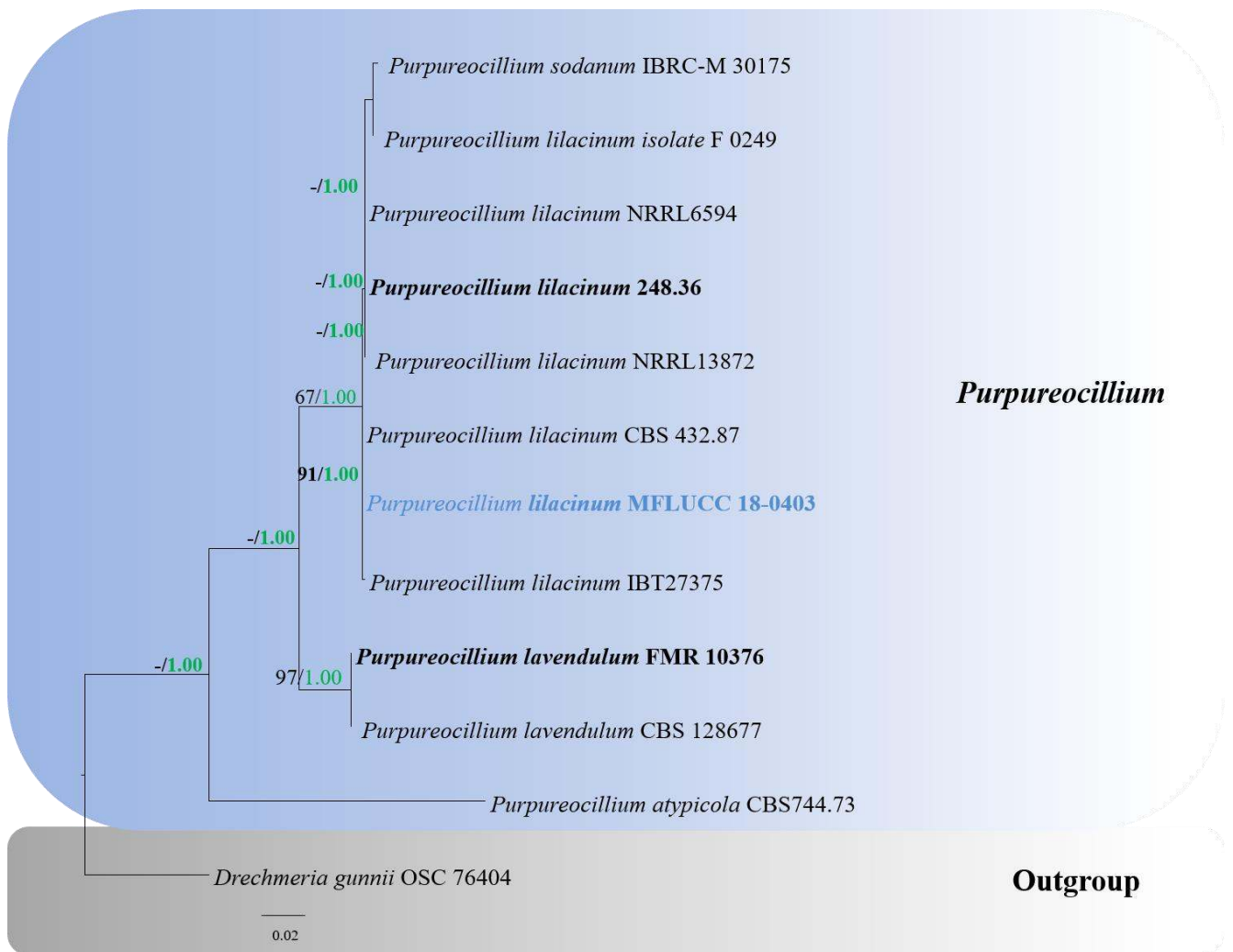
Culture characteristics – *Colonies* on seawater MEA fast growing, attaining a diameter of 25–35 mm after 7 days at 25 °C, consisting of a dense basal felt of numerous conidiophores giving a powdery texture, pale vinaceous, with sparse aerial mycelium, and producing a very faint yellow diffusible pigment. Reverse yellowish to vinaceous.

Material examined – THAILAND, Krabi Province, Mueang Krabi District, Khao Khram, on submerged decaying wood of *Bruguiera* sp. (Rhizophoraceae) wood, 30 January 2018, M.C. Dayaratne, MCD 152 (MFLU 17-2627); *ibid.*, (HKAS102058), living culture MFLUCC 18-0403.

GenBank numbers – ITS: MN047110; BTUB: MN077052

Additional GenBank number – LSU; MN017874

Notes – *Purpureocillium lilacinum* has been subjected to many studies because it has biotechnological properties and ability to cause human infections (Pastor & Guarro 2006, Fiedler & Sosnowska 2007, van Schooneveld et al. 2008, Wessolossky et al. 2008). This species produces vinaceous colonies, erect, thick and rough-walled conidiophores with verticillate branches, phialides with a swollen basal part tapering into a long, distinct neck, and ellipsoidal to fusiform, smooth to slightly roughened conidia (Samson 1974), and acremonium-like synanamorph (Okada et al. 1995). Our new strain (MFLUCC 18-0403) phylogenetically clustered together with existing *P. lilacinum* strains and also morphologically they are similar (Fig. 63). However, some studies have reported the intraspecific genetic variability of *P. lilacinum* from different habitats (Tigano-Milani et al. 1995, Inglis & Tigano 2006). Luangsa-ard et al. (2011) later compared a large data set of *P. lilacinum* isolates from different sources and showed there was no genetic differences detected among isolates. Perdomo et al. (2013) determined the phenotypic and genetic variability of a set of clinical and environmental isolates of *P. lilacinum* and showed that *P. lilacinum* formed a well-supported phylogenetic clade with low intraspecific variability that was supported by studies of Hyde et al. (2016). *Purpureocillium lilacinum* is listed in Jones et al. (2015) and [www.marinfungi.org](http://www.marinfungi.org) and our collection is an additional record of this species from marine habitats.



**Figure 63** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -16392.233800. The combined ITS, and BTUB sequence datasets comprised 11 strains of *Purpureocillium* and *Drechmeria gunnii* (OSC 76404) as the outgroup taxon. Tree topology of the ML analysis was similar to the BI. The matrix had 145 distinct alignment patterns, with 24.03% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.215906, C = 0.330368, G = 0.263083, T = 0.190643; substitution rates AC = 2.021294, AG = 3.185081, AT = 1.100483, CG = 1.557658, CT = 4.402658, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.332093. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.02 changes. The ex-type strains are in bold and new isolates in blue bold.

### **Subclass Sordariomycetidae** O.E. Erikss & Winka (= Meliolomycetidae P.M. Kirk & K.D. Hyde)

#### **Boliniales** P.F. Cannon

The order Boliniales, which was introduced by Cannon (Kirk et al. 2001), comprises saprobes within the subclass Sordariomycetidae. Boliniales is a monotypic order which includes a single family Boliniaceae, with nine genera (Maharachchikumbura et al. 2015, 2016, Wijayawardene et al. 2017a, 2018).

#### **Boliniaceae** Rick

The family Boliniaceae was established by Rick (1931) based on black ascostromata and cylindrical asci with smooth ascospores, and the type genus is *Camarops* (Karsten 1873). Initially, this family was placed in Xylariales based on morphological similarity on ascostromata and

ascospores (Barr 1990a, Romero & Samuels 1991). Andersson et al. (1995) showed that Boliniaceae was more closely related to Sordariales based on SSU sequence data. This family was subsequently placed in Boliniales by Kirk et al. (2001). Based on phylogenetic and morphological data nine genera were accepted in Boliniaceae (Untereiner et al. 2013, Wijayawardene et al. 2017a, 2018).

### ***Camarops* P. Karst.**

The genus *Camarops* is characterized by applanate or flat-pulvinate stromata, with separate ostioles at the surface and very long tubular perithecia with a palisade layer of cream or brownish coloured cells (Karsten 1873, Lumbsch & Huhndorf 2010). *Camarops hypoxyloides* is the type species of the genus (Karsten 1873). Currently, 30 species are listed in Index Fungorum (2019). *Camarops* species include members of wood-inhabiting ascomycetous fungi and represent an ecologically important fungal group with many members commonly occurring throughout temperate and tropical regions (Huhndorf & Miller 2008, Kim et al. 2014). *Camarops* species have not been reported from marine environments so far. Further collections and sequences are needed to confirm this identification.

***Camarops ustulinoides* (Henn.) Nannf., Svensk bot. Tidskr. 66(4): 370 (1972)**

Fig. 66

Facesoffungi number: FoF 06196

*Saprobic* on decaying wood of *Rhizophora* sp. Sexual morph: Stromata 8–20 mm × 4.5–8 mm, button-shaped, with abrupt angular to rounded margins, bark adhering, upper surface convex to nearly plane, with punctuate ostioles with raised circular rims, externally reddish brown to blackish, internally tan to black, external stromatal layer up to 1 mm thick, very hard, completely encasing fragile perithecia and entostroma, entostroma cheesy to woody. *Ascomata* 2.5–3 × 0.3–0.4 mm, perithecial cylindrical to ellipsoidal. *Asci* 25–40 μm × 3–6 μm ( $\bar{x}$  = 38.2 × 4.8 μm, n = 10), 8-spored, overlapping uniseriate, cylindrical, short- or long-stipitate. *Ascospores* 3.5–5.8 × 2.5–3 μm ( $\bar{x}$  = 4.2 × 2.6 μm, n = 20), grayish to brown to dark brown, ellipsoidal with one end somewhat acute, mostly with two guttules, with a germ pore at the more pointed end. Asexual morph: Unknown.

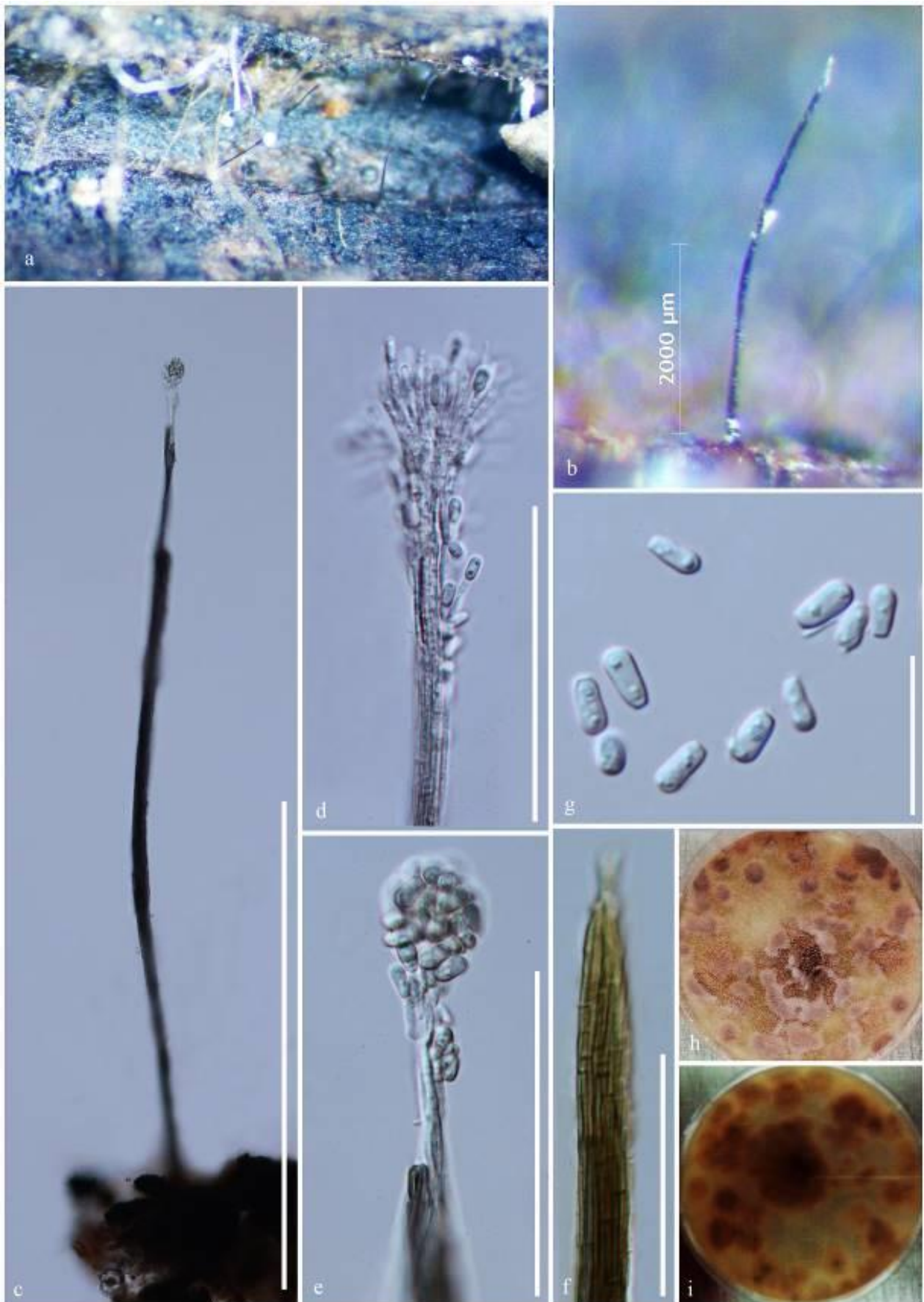
Material examined – THAILAND, Prachaub Kiri Khan Province, Pranburi, Sirinath Rajini center for mangrove forest studies on decaying wood of *Rhizophora* sp. (Rhizophoraceae), 02 December 2016, M. C. Dayarathne, MCD 088 (MFLU 18-0561); *ibid.*, (HKAS102093).

GenBank numbers – LSU: MN017889

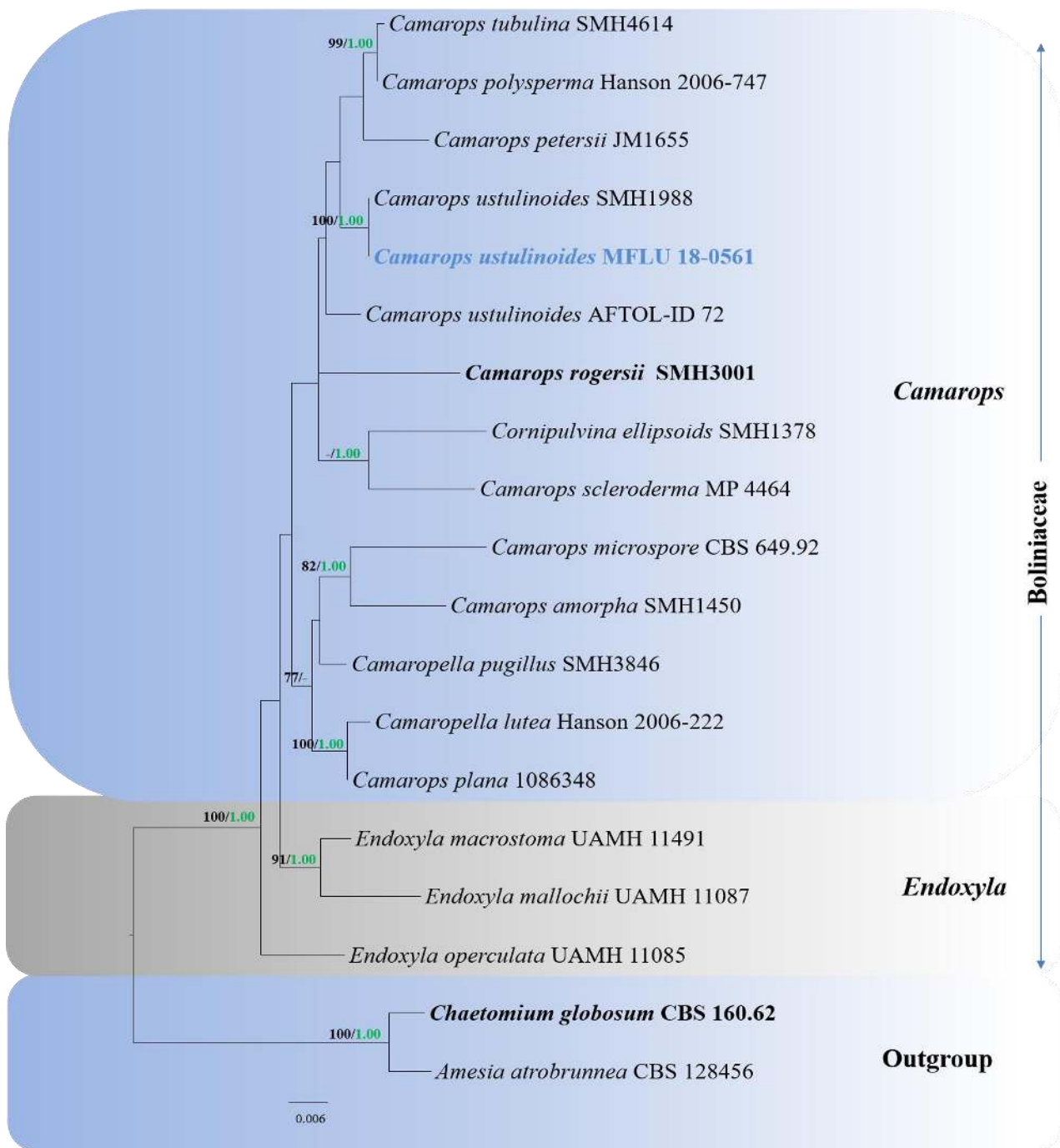
Notes – *Camarops ustulinoides* retains its uniqueness by having brown to blackish stromata adhering to bark, elongated perithecia in section and generally raised circular rims (Kim et al. 2014). Phylogenetic analyses with LSU data showed that our novel strain MFLU 18-0561 grouped together with *C. ustulinoides* (SMH1988) and their LSU sequence data are identical to each other (Fig. 65). Furthermore, they share similar morphologies as well. However, *C. ustulinoides* was previously found from trunk of a dead oak tree in Korea (Kim et al. 2014), from *Dacryodes excelsa* in West Indies (Minter et al. 2001), Costa Rica, French, Guiana, and Puerto Rico (Vasilyeva et al. 2007a, Huhndorf & Miller 2008) while our novel strain is from decaying wood of *Rhizophora* sp. in Thailand. This is a new geographical record for *C. ustulinoides* and the first report of *C. ustulinoides* from a marine based environment.

### **Chaetosphaeriales Huhndorf et al.**

Based on molecular analysis of LSU nrDNA sequence data Huhndorf et al. (2004) introduced the order Chaetosphaeriales. This order comprises four families: Chaetosphaeriaceae with 38 genera, Helminthosphaeriaceae with eight genera Leptosporiaceae with one genus and *Linocarpaceae* with two genera (Wijayawardene et al. 2017a, 2018).

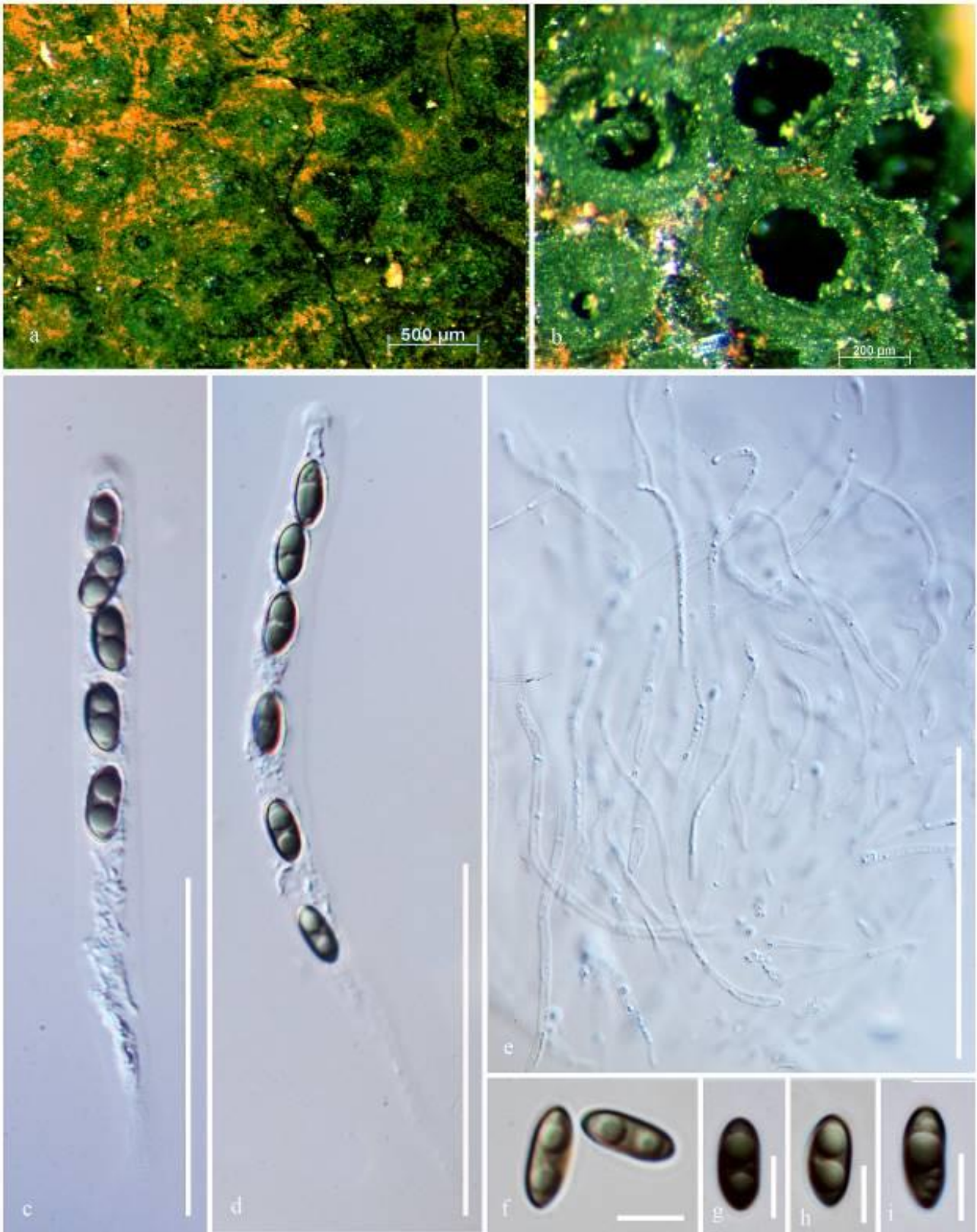


**Figure 64** – *Purpureocillium lilacinum* (MFLU 17-2627). a–c Conidiophores on host substrate. d, e Conidia attached to the phialides. f Erect synnematosse conidiophores. g Conidia. h, i Colonies on seawater MEA. (h-upper, i-lower). Scale bars: b, c = 200 μm, d–f = 100 μm, g = 10 μm.



**Figure 65** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -2336.436167. The combined LSU sequence datasets comprised 20 strains of *Boliniales* and related taxa with *Amesia atrobrunnea* (CBS 128456) and *Chaetomium globosum* (CBS160.62) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 134 distinct alignment patterns, with 4.39% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.252992, C = 0.241084, G = 0.312231, T = 0.193693; substitution rates AC = 1.427241, AG = 2.854867, AT = 1.357021, CG = 0.907692, CT = 16.364766, GT = 1.000000; gamma distribution shape parameter  $\alpha = 0.020000$ . Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.006 changes. The ex-type strains are in bold and new isolates in blue bold.





**Figure 66** – *Camarops ustulinoides* (MFLU 18-0561). a Stromata on host tissue. b Horizontal section of ascostromata. c, d Asci. e Paraphyses. f–i Ascospores. Scale bars: a = 500 µm, b = 200 µm, c–e = 20 µm, f–i = 2 µm.

***Chaetosphaeriaceae*** Réblová et al.

Réblová et al. (1999) established the family Chaetosphaeriaceae to accommodate *Chaetosphaeria* (Tulasne & Tulasne 1863). *Catenularia*, *Cylindrotrichum*, *Chalara*, *Chloridium*,

*Custingophora*, *Dictyochaeta*, *Menispora*, *Phialophora* and *Zanclospora* are reported as the asexual morphs of this family (Réblová et al. 1999, Ho et al. 2001, Huhndorf et al. 2001, Fernández & Huhndorf 2005, Fernández et al. 2006, Atkinson et al. 2007, Perera et al. 2016).

### ***Chloridium* Link**

The hyphomycetous genus *Chloridium*, typified by *Chloridium viride*, is characterized by proliferating macronematous conidiophores (Link 1809). Gams & Holubová-Jechová (1976) treated *C. viride* as a synonym of *C. vermicularioides* (syn. *Melanopsammella vermicularioides*) which is congeneric with *M. inaequalis* (type of *Melanopsammella*) (Réblová & Winka 2000, Fernández et al. 2006). Réblová et al. (2016) considered *Chloridium* and *Melanopsammella* congeneric and recommended the use *Chloridium* over *Melanopsammella*. *Chloridium* species comprise macronematous or mononematous conidiophores, monophialidic or polyphialidic conidiogenous cells, with a distinct funnel-shaped collarete and hyaline to brown, guttulate or eguttulate conidia, with or without appendages (Seifert et al. 2011, Wei et al. 2018). Seventy-six names are currently listed under *Chloridium* in Index Fungorum (2019). *Chloridium* species have never been reported from marine habitats and herein we are introducing a novel *Chloridium* species from unidentified marine wood from the UK.

### ***Chloridium salinicola* Dayarathne & E.B.G. Jones, sp. nov.**

Fig. 68

Index Fungorum number: IF556603; Facesoffungi number: FoF 06197

Etymology – Epithet refers to the saline environment where the species found

Holotype – MFLU 19-1238

*Saprobic* on unidentified marine wood. Asexual morph: *Colonies* on natural substrate, effuse, thin hairy, brown. Mycelium partly superficial, partly immersed, consisting of branched, septate, smooth, thin-walled, subhyaline to pale hyphae. Stroma none. *Conidiophores* 100–300 × 5–8 µm wide, macronematous, mononematous, scattered, solitary, cylindrical, robust at the base, erect, straight or slightly flexuous, unbranched, brown, fading towards the apex, septate, smooth, percurrently proliferating. *Conidiogenous cells* monophialidic, integrated, terminal, cylindrical, with a collarete, subhyaline to pale brown. *Conidia* 18–30 × 4–6 µm ( $\bar{x}$  = 20 × 5.2 µm; n = 10), acrogenous, formed in slimy masses, ellipsoidal to cylindrical, hyaline, 1-septate, slightly or not constricted at the septa, smooth, thin-walled. Sexual morph: Undetermined.

Material examined – UK, Eastney, Hampshire, on unidentified marine wood, 23 January 2015, E.B Gareth Jones, GJ084 (MFLU 19-1238, holotype); ex-type living culture (MFLUCC 15-0382); *ibid.*, (BBH, isotype).

GenBank numbers – LSU: MN017890, MN420685; ITS: MN047125; BTUB: MN431494

Additional GenBank number – SSU: MN017944

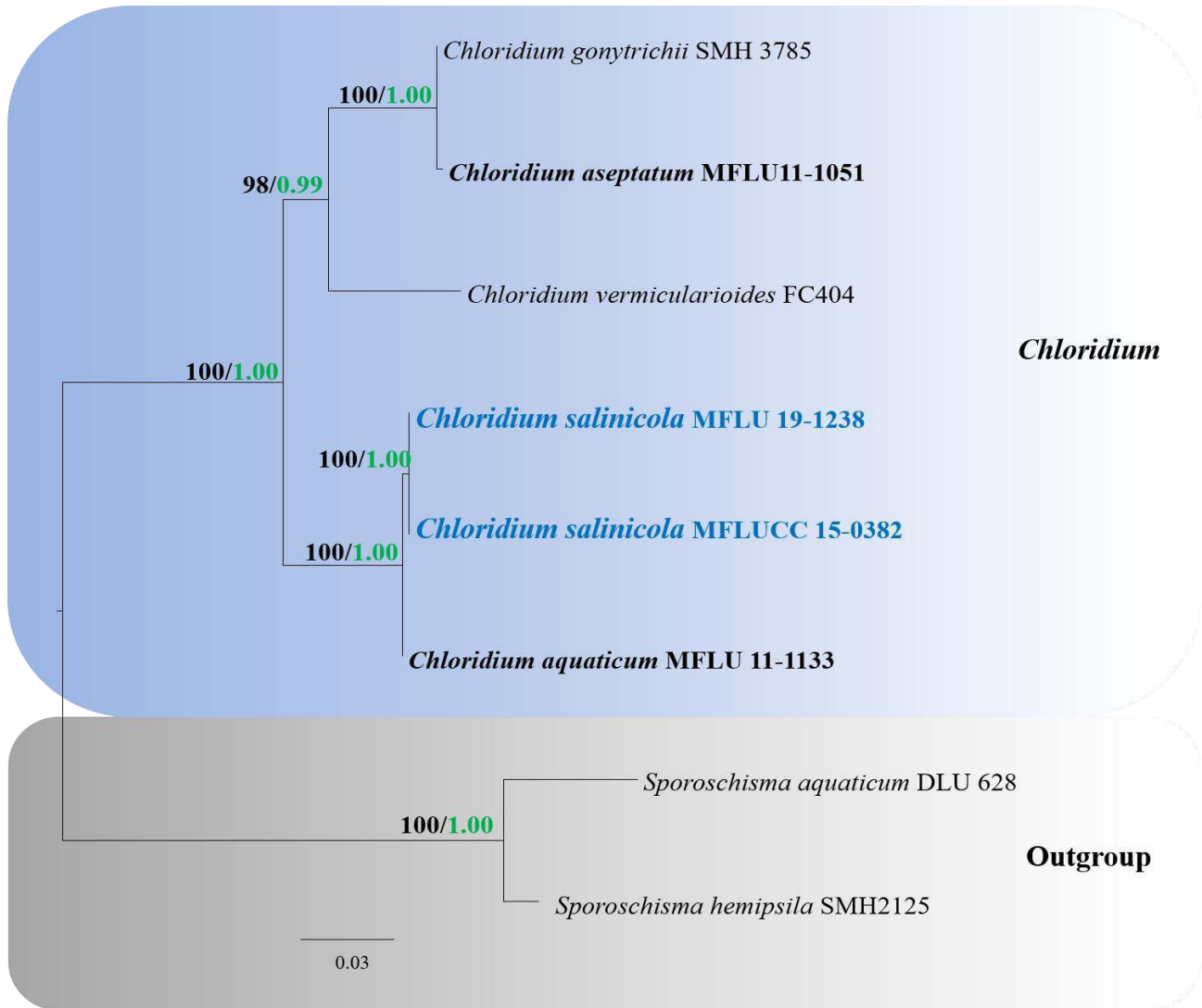
Notes – Phylogenetic analyses of concatenated ITS, LSU and BTUB sequences data showed that this novel strain grouped in a clade comprising *Chloridium* species with high statistical support (100% ML, 1.00 PP) (Fig. 67). *Chloridium salinicola* shares similar morphologies of the conidiophores, conidiogenous cells and mycelial characters of *Chloridium*. This novel strain phylogenetically closely resembles *C. aquaticum*, but can be clearly distinguish by conidial morphology. *Chloridium salinicola* has 1-septate, longer conidia (18–30 µm), whereas conidia of *C. aquaticum* are 1-celled and smaller (9–12 µm) (Wei et al. 2018). However, only one base pair different in ITS region out of 505 bp and there are no BTUB sequence data generated from *C. aquaticum* to compare the base pair differences.

### **Chaetosphaeriales genera *incertae sedis***

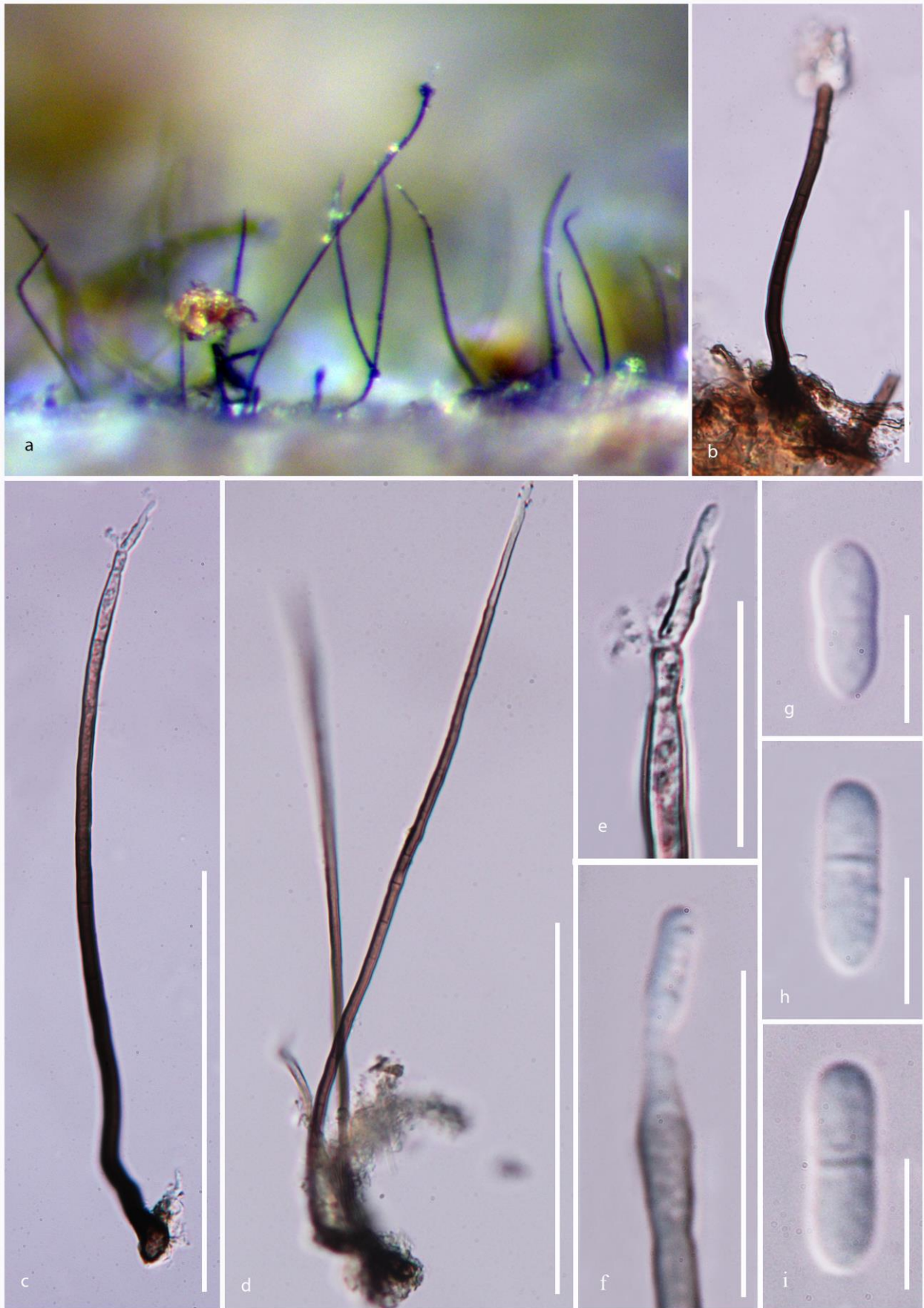
The genera, *Calvolachnella*, *Caudatispora*, *Erythromada*, *Lasiosphaeriella*, *Nawawia*, *Marvanova* and *Rimaconus* are currently place under Chaetosphaeriales genera *incertae sedis* (Wijayawardene et al. 2017a, 2018). An updated phylogentic tree to the order is provided in Fig. 69.

*Rimaconus* Huhndorf, F.A. Fernández, Joanne E. Taylor & K.D. Hyde

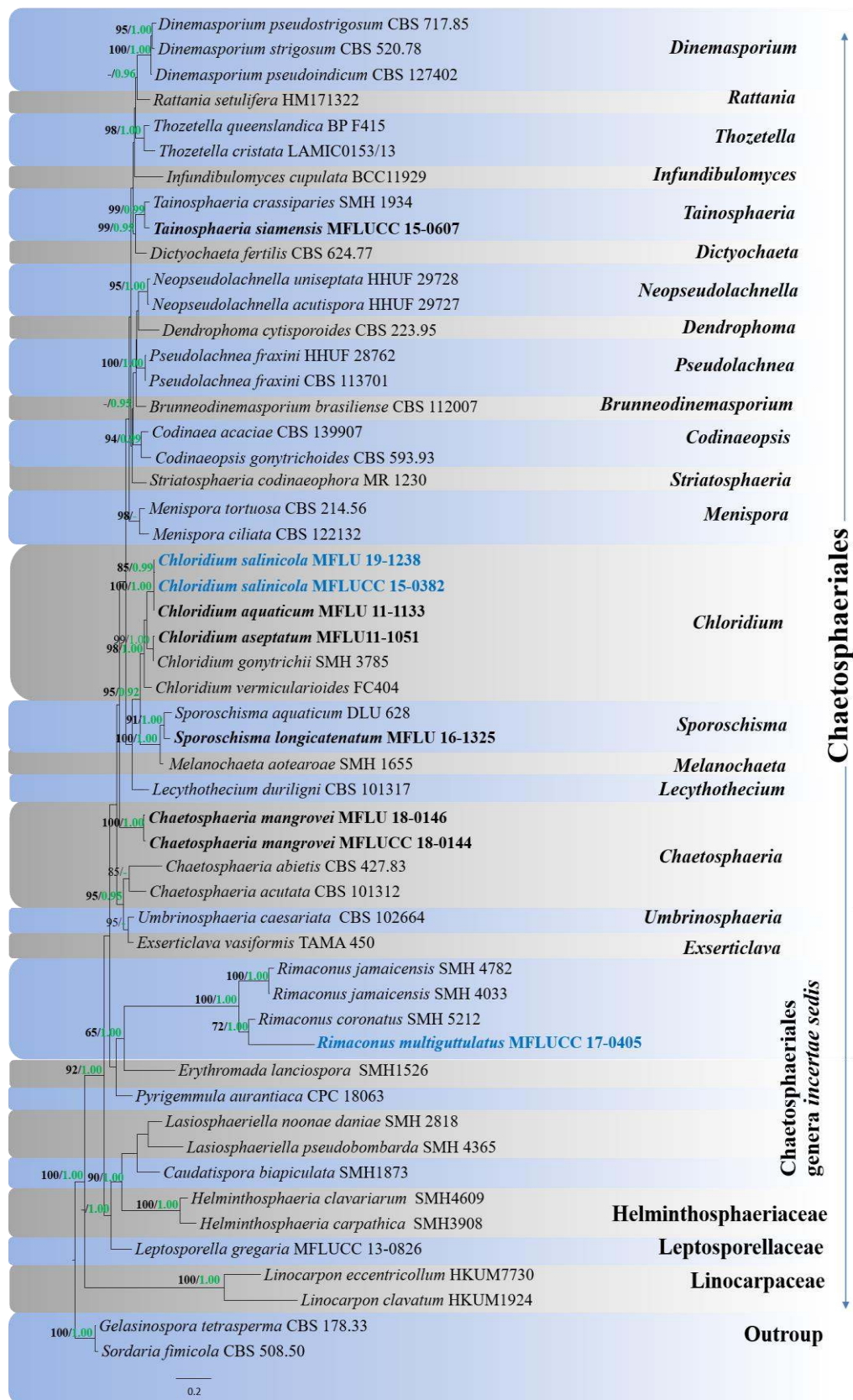
Huhndorf et al. (2001) introduced the genus *Rimaconus* to accommodate *Rimaconus jamaicensis*. Characteristics unique to this genus are immersed to erumpent, conical to hemispherical to applanate ascomata, unitunicate asci with a J-, apical ring, paraphyses with gelatinous coating and cylindrical, multiseptate ascospores (Huhndorf et al. 2001). There are two species *R. coronatus* and *R. jamaicensis* listed under this genus (Index Fungorum 2019) and we introduce a third species to this genus from a marine environment.



**Figure 67** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -4762.191078. The combined ITS, LSU and BTUB sequence datasets comprised 8 strains of *Chloridium* including *Sporoschisma aquaticum* (DLU 628) and *S. hemipsila* (SMH 2125) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 1104 distinct alignment patterns, with 60.52% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.217480, C = 0.280248, G = 0.293417, T = 0.208855; substitution rates AC = 0.978466, AG = 1.456047, AT = 1.060375, CG = 1.300170, CT = 7.572057, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.171569. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.03 changes. The ex-type strains are in bold and new isolates in blue bold.



**Figure 68** – *Chloridium salinicola* (MFLU 19-1238, holotype). a–b Colonies on substrate. c, d Conidiophore. e–f Proliferating conidiophores bearing conidia. g–i Conidia. Scale bars: b–d = 100  $\mu\text{m}$ , d, e, f = 20  $\mu\text{m}$ , g–i = 10  $\mu\text{m}$ .



**Figure 69** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -13756.917492. The combined ITS and LSU sequence datasets comprised 53 strains of *Chaetosphaeriales* including *Gelasinospora tetrasperma* (CBS 178.33) and *Sordaria fimicola* (CBS 508.50) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 1104 distinct alignment patterns, with 60.52% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.234344, C = 0.260336, G = 0.307692, T =

0.197628; substitution rates AC = 1.089279, AG = 1.587064, AT = 1.252250, CG = 0.834468, CT = 5.520909, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.296739. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.2 changes. The ex-type strains are in bold and new isolates in blue bold.

***Rimaconus multiguttulatus*** Dayarathne & K.D. Hyde, sp. nov.

Fig. 70

Index Fungorum number: IF556604; Facesoffungi number: FoF 06198

Etymology – Epithet refers to the multiguttulate ascospores

Holotype – MFLU 18-0535

*Saprobic* on submerged wood of *Bruguiera* sp. Sexual morph: *Ascomata* 700–850 × 300–400  $\mu\text{m}$ , conical to applanate when young, becoming conical with coronate projections around apical rim, solitary to gregarious in small groups, immersed becoming erumpent, with fragments of host cells adherent to the wall, surface roughened, dark brown appearing black ostiolate, papillate. *Peridium* 120–160  $\mu\text{m}$  wide, composed of several layers of dark brown cells, with coronate projections around periphery of apex. *Paraphyses* 2–4  $\mu\text{m}$  wide, abundant, persistent, narrow, branched, with guttules, tapering towards the apex. *Asci* 160–175 × 16–22  $\mu\text{m}$  ( $\bar{x}$  = 165 × 20.5  $\mu\text{m}$ ; n = 10), 8-spored, overlapping uni or bi seriate, cylindrical, unitunicate, apex tapered, with J- apical ring. *Ascospores* 25–42 × 10–14  $\mu\text{m}$ , ( $\bar{x}$  = 38 × 12.5  $\mu\text{m}$ , n = 20), broadly fusiform to cylindrical, broadly rounded at the ends, straight or curved, symmetrical, hyaline, 3-septate, without constrictions at septa, septa evenly distributed, with small, numerous guttules, without sheath or appendages. Asexual morph: undetermined.

Culture characteristics – Reaching 2 cm within 7 days on sea water PDA, when incubated at 25 °C, circular, flat, with diffuse margin, white, and becoming yellowish-white, to dull yellow with age.

Material examined – THAILAND, Ranong Province, Amphoe Maung, Mu 4 Tombol Ngao, Ranong Mangrove Research Center (GPS: 9°43' to 9°57'N; 98°29' to 98°39'E) on decaying wood of *Bruguiera* sp. (Rhizophoraceae), 06 December 2016, M.C. Dayarathne, MCD 048 (MFLU 18-0535, holotype), ex-type living culture, MFLUCC 17-0405.

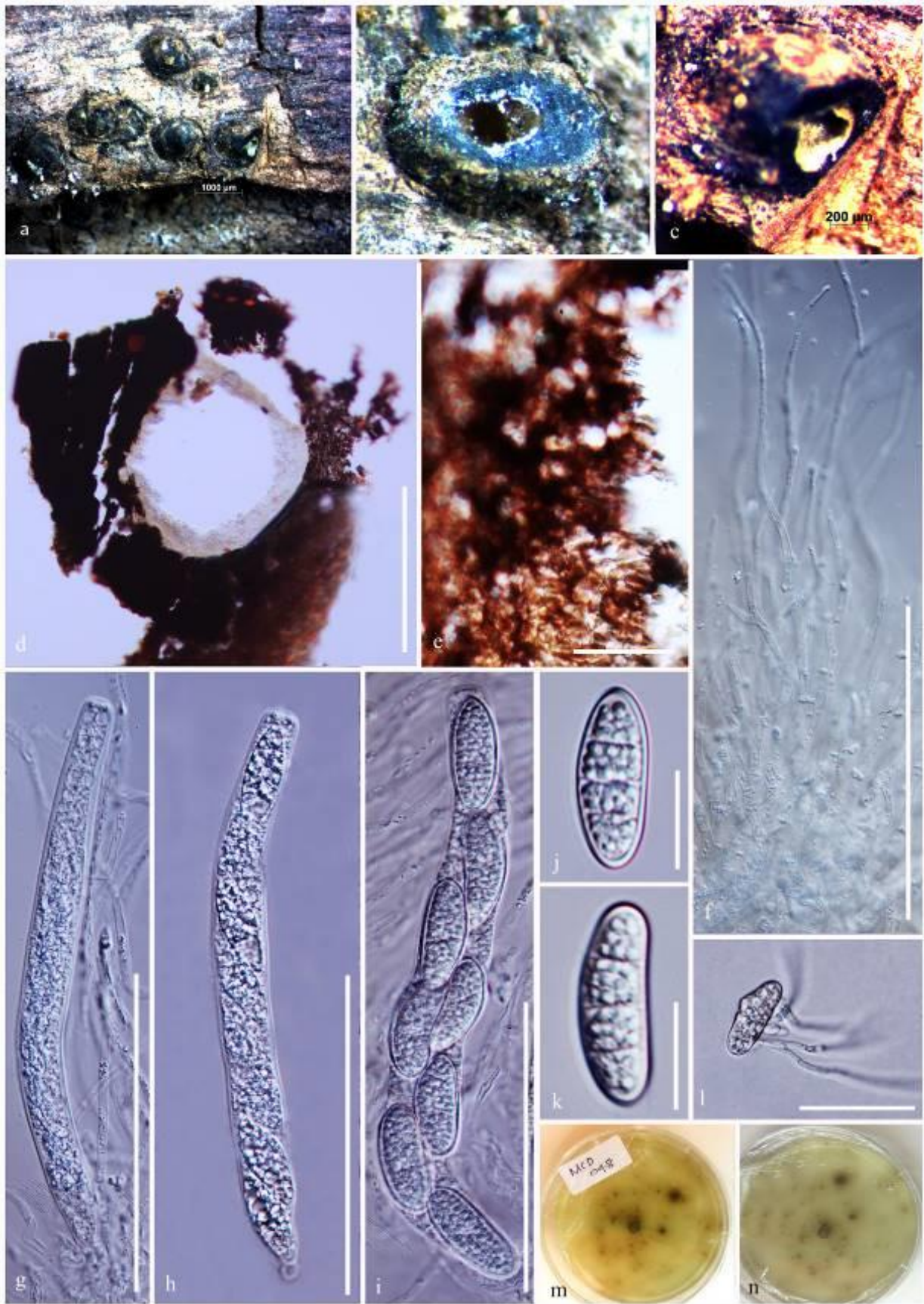
GenBank numbers – LSU: MN017891

Notes – *Rimaconus multiguttulatus* best fits the genus *Rimaconus* in having conical to applanate ascomata with coronate projections around the apical rim, persistent paraphyses, tapering towards the apex, cylindrical, unitunicate asci with tapered apex, with J- apical ring and broadly fusiform to cylindrical, 3-septate, ascospores that are broadly rounded at the ends with numerous guttules. The genus clusters with moderate support (72% ML, 1.00 PP) from *R. coronatus*. Ascospores of *Rimaconus multiguttulatus* can be easily distinguished from *R. coronatus* by its multiguttulate ascospores while in *R. coronatus* has 1–4 large guttules (Huhndorf & Miller 2011). Furthermore, ascospores of *R. coronatus* appear elongate and narrower than *R. multiguttulatus* (7.5–9  $\mu\text{m}$  vs 10–14  $\mu\text{m}$ ) (Huhndorf & Miller 2011). *Rimaconus multiguttulatus* is the first record of *Rimaconus* species from a marine based habitat. Their base pair differences in LSU loci (10.8%) also indicate they are two distinct taxa.

**Coniochaetales** Huhndorf et al. (= Cordanales M. Hern.-Rest. & Crous)

**Coniochaetaceae** Malloch & Cain

The family Coniochaetaceae comprises two genera, *Barrina* and *Coniochaeta*. Species belonging to Coniochaetaceae are cosmopolitan in distribution and exhibiting different modes of nutrition (Samarakoon et al. 2018, Jones et al. 2019). They can be saprobes on dung, plant litter or in soil, strongly acidic water with high heavy metal concentrations and food. As well, they can be pathogenic on plants or animals, and rarely in humans in terrestrial, freshwater and marine habitats (Ramaley 1997, Weber 2002, Weber et al. 2002, Huhndorf et al. 2004, García et al. 2006, Kirk et al. 2008, Damm et al. 2010, Maharachchikumbura et al. 2015, 2016, Wijayawardene et al. 2017a, Wanasinghe et al. 2018, Jones et al. 2019).



**Figure 70** – *Rimaconus multiguttulatus* (MFLU 18-0535, holotype). a Ascomata erumpent on decaying wood. b Section of ascoma. d Peridial wall layers. e Filamentous pseudoparaphyses. h–i Immature and mature asci. k–p Immature and mature ascospores. q Germinating ascospore. Scale bars: b = 100 µm, c–q = 10 µm.

***Coniochaeta*** (Sacc.) Cooke

The genus *Coniochaeta*, typified by *Coniochaeta ligniaria*, is characterized by dark brown to black, solitary or aggregated, typically setose, pyriform to globose ascomata, with or without ostioles. The peridium is membranaceous to pseudoparenchymatous, or rarely coriaceous (Damm et al. 2010, Samarakoon et al. 2018, Jones et al. 2019). A paraphysate hamathecium is present or absent. Asci are unitunicate and thin-walled, with a small non-amyloid, apical ring and ascospores are one-celled, usually dark brown and often laterally compressed, with a germ slit (Weber 2002, Asgari et al. 2007). In this study, we introduce two novel taxa to this genus from a marine habitat.

***Coniochaeta arenariae*** Dayarathne & E.B.G Jones, sp. nov.

Fig. 72

Index Fungorum number: IF556606; Facesoffungi number: FoF 06200

Etymology – Epithet derived from the host epithet “*arenaria*”

Holotype – MFLU 19-1239

*Saprobic* on driftwood retrieved from the sea. Sexual morph: *Ascomata* 200–260 × 154–182 μm ( $\bar{x}$  = 252 × 174 μm, n = 5), perithecial, solitary to gregarious, non-stromatic, immersed to semi-immersed, globose, ostiolate, without setae. *Ostiole* central, papillate, with circular outline, lacking periphyses, filled with hyaline cells. *Peridium* 26–42 μm wide, brittle when dry, soft when rehydrated, two-layered. Inner layer consisting of hyaline to subhyaline, strongly compressed cells, outer layer consisting of densely packed, moderately thick-walled, brown cells of *textura prismatica*, darker towards outside. *Hamathecium* comprising numerous, 2–3 μm wide, filamentous, septate, paraphyses. *Asci* 130–148 × 12–16 μm ( $\bar{x}$  = 143 × 15.2 μm, n = 20), 8-spored, with short stipe, with a J- small apical apparatus. *Ascospores* 18–24 × 9.2–10.8 μm ( $\bar{x}$  = 22 × 10 μm, n = 30), overlapping uniseriate, ellipsoid, slightly laterally compressed, hyaline at first, becoming olivaceous-brown and finally dark brown and opaque, darker in 3% KOH, aseptate, with a conspicuous, straight germ slit across the entire length, guttulate, without sheath or appendages. Asexual morph: Undetermined.

Material examined – UK, Carmarthenshire, Wales, Cefn Sidan, Marram grass (*Ammophila arenaria*) in sand dune, 15 October 2017, E.B. Gareth Jones, GJ 445b (MFLU 19-1239, holotype); *ibid.*, (BBH, isotype), ex-type living culture MFLUCC 18-0405. SWEDEN, Bohuslän. Skaftö par., Fiskebäckskil, Rödbergsviken, 58°15′08″; 11°27′58″, on a large piece of driftwood retrieved from the sea, Leif Tibell, June 2017, GJ 396 (MFLU 19-1240).

GenBank numbers – LSU: MN017893, MN017896; ITS: MN047126

Notes – *Coniochaeta arenariae* is characteristic of *Coniochaeta* in its dark brown to black, pyriform ascomata with an ostiole, paraphysate, hamathecium, unitunicate, thin-walled asci with a small non-amyloid apical ring and one-celled, dark brown ascospores with a germ slit (Weber 2002, Asgari et al. 2007, Samarakoon et al. 2018). It grouped in a clade with *C. tetraspora* (Fig. 71). However, they are morphologically well-distinguished by ascomatal, asci and ascospore characteristics (Cain 1961). *Ascomata* of *C. tetraspora* are 135–220 × 80–190 μm, superficial, pyriform, ostiolate at the apex, but without a distinct neck region, and with a hairy upper part, while *ascomata* of *C. arenariae* are 200–260 × 154–182 μm, immersed to semi-immersed, globose, ostiolate, without setae at the upper part (Cain 1961). *Asci* (130–148 × 12–16 μm vs 50–55 × 8–11 μm) and *ascospores* (10–15 × 6.5–8 μm vs 18–24 × 9.2–10.8 μm) of *C. arenariae* are larger than those of *C. tetraspora* (Cain 1961). Furthermore, *asci* of *C. tetraspora* are initially eight spored and become four spored when mature (Cain 1961) and we could not observe four spored mature *asci* of *C. arenariae*. However, these species only have LSU data and only 2 base pairs different out of 456 bp (0.4%). Depending on considerable morphological differences, we herein introduce this species as a novel taxon within the genus *Coniochaeta*.

***Coniochaeta krabiensis*** Dayarathne & E.B.G Jones, sp. nov.

Fig. 73

Index Fungorum number: IF556605; Facesoffungi number: FoF 06199

Etymology – Epithet derived from the location Krabi, Thailand, where the species found.

Holotype – MFLU 16-1230



*Saprobic* on decaying submerged wood. Sexual morph: *Ascomata* 270–300 × 228–250 μm ( $\bar{x}$  = 250 × 230 μm, n = 5), perithecial, solitary to gregarious, non-stromatic, semi-immersed to superficial, pyriform, ostiolate, without setae. *Ostiole* central, papillate, filled with hyaline cells. *Peridium* 25–35 μm wide, brittle when dry, soft when rehydrated, two-layered. Inner layer consisting of hyaline to subhyaline, strongly compressed cells, outer layer consisting of moderately thick-walled, brown cells of *textura angularis*, darker towards outside. *Hamathecium* comprising numerous, 2–3 μm wide, filamentous, aseptate, paraphyses. *Asci* 108–122 × 2.8–4.7 μm ( $\bar{x}$  = 110 × 4 μm, n = 30), 8-spored, with short stipe, with a J- small apical apparatus. *Ascospores* 4.4–6.2 × 2.5–3.6 μm ( $\bar{x}$  = 6 × 3.1 μm, n = 30), uniseriate, ellipsoid, slightly laterally compressed, hyaline at first, becoming olivaceous-brown and finally dark brown and opaque, darker in 3% KOH, aseptate, with a conspicuous, straight germ slit across the entire length, guttulate. Asexual morph: Undetermined.

Material examined – THAILAND, Phang-nga Province, Mueang Phang-nga District, Tham Nam Phut, 8°25'52" N, 98°31'42" E, 0 m asl., on submerged marine wood, 7 December 2014, M. C Dayarathne, KRB006 (MFLU 16-1230, holotype); *ibid.*, (BBH, isotype).

GenBank numbers – LSU: MN017892

Additional GenBank number – SSU: MN017945

Notes – The novel taxon *Coniochaeta krabiensis* (MFLU 16-1230) grouped as a basal lineage in a clade comprising *C. tetraspora* (FMR 8167) and *C. arenariae* strains (MFLU 19-1239 and MFLU 19-1240) (Fig. 71). *Coniochaeta krabiensis* best fits the genus *Coniochaeta* by having dark brown to black, solitary or aggregated, pyriform ascomata with an ostiole, paraphysate, hamathecium, unitunicate, thin-walled asci with a small non-amyloid apical ring and one-celled, dark brown ascospores (Weber 2002, Asgari et al. 2007, Samarakoon et al. 2018). *Coniochaeta krabiensis* shows close phylogenetic affinities to *C. tetraspora* (Fig. 71). However, *C. tetraspora* can be clearly distinguished from *C. krabiensis* mainly by having 4-spored asci when immature and ascomata lacking setae. Furthermore, asci of *C. krabiensis* are longer and narrower than those of *C. tetraspora* (108–122 × 2.8–4.7 μm vs 50–55 × 8–11 μm) and ascospores of *C. krabiensis* are smaller than those of *C. tetraspora* (4.4–6.2 × 2.5–3.6 μm vs 10–15 × 6.5–8.0 μm) (Cain 1961). Both concatenated ITS and LSU gene analyses along with morphological characteristics, we establish this taxon as a novel species within the genus *Coniochaeta*.

## **Subclass Xylariomycetidae** O.E. Erikss & Winka

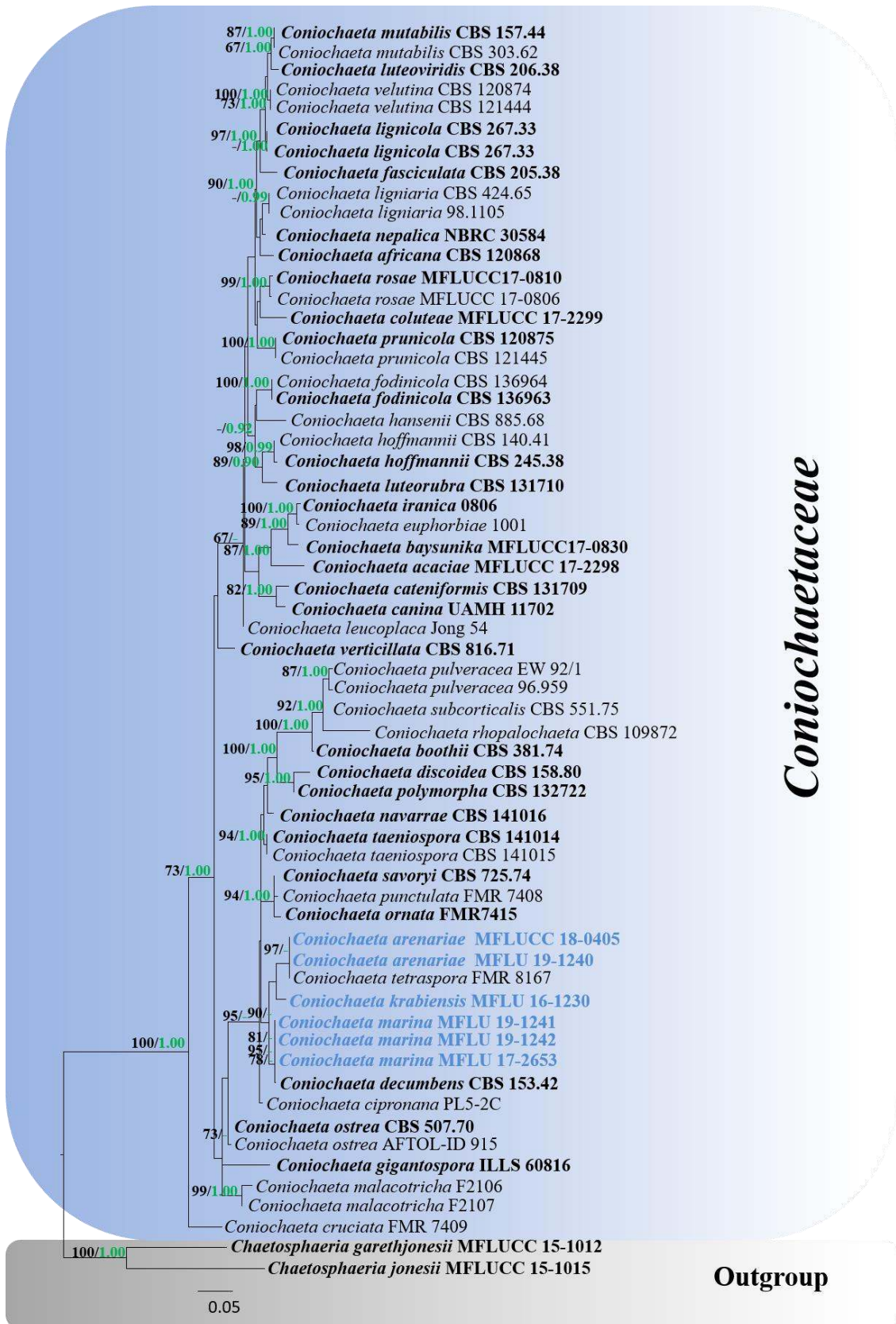
### **Xylariales** Nannf.

Xylariales was introduced by Nannfelt (1932) and is the largest order of perithecial ascomycetes. Familial relationships in this order have been investigated (Tang et al. 2009) and currently 34 families are accepted (Wendt et al. 2018, Daranagama et al. 2018, Voglmayer et al. 2019).

### **Diatrypaceae** Nitschke

Taxa in Diatrypaceae are characterized by perithecial ascomata, usually embedded in a black stroma, cylindrical-clavate to clavate, long pedicellate asci and allantoid ascospores (Glawe & Rogers 1984, Rappaz 1987, Mehrabi et al. 2015, De Almeida et al. 2016, Dayarathne et al. 2016, 2019c). Both coelomycetes (*Cytosporina*, *Libertella*) and hyphomycetes (*Phaeoisaria*) have been documented as asexual morphs of Diatrypaceae (Glawe & Rogers 1984, Wijayawardene et al. 2012, 2017a, b, De Almeida et al. 2016, Mehrabi et al. 2016). Kirk et al. (2008) listed 13 genera and 229 species in Diatrypaceae, and two marine saprotrophic genera, *Diatrypasimilis* and *Pedumispora* were added to the family by Klaysuban et al. (2014) and Abdel-Wahab et al. (2014), respectively. Later, *Monosporascus* and *Phaeoisaria*, were referred to the family by Maharachchikumbura et al. (2015). However, recently *Phaeoisaria* has been linked to the family Pleurotheciaceae (Luo et al. 2018, Wijayawardene et al. 2018, Hyde et al. 2018). Subsequently,

*Allocryptovalsa*, *Halodiatrype*, and *Halocryptovalsa* were added to the family by Senwana et al. (2017) and Dayarathne et al. (2016, 2019c).



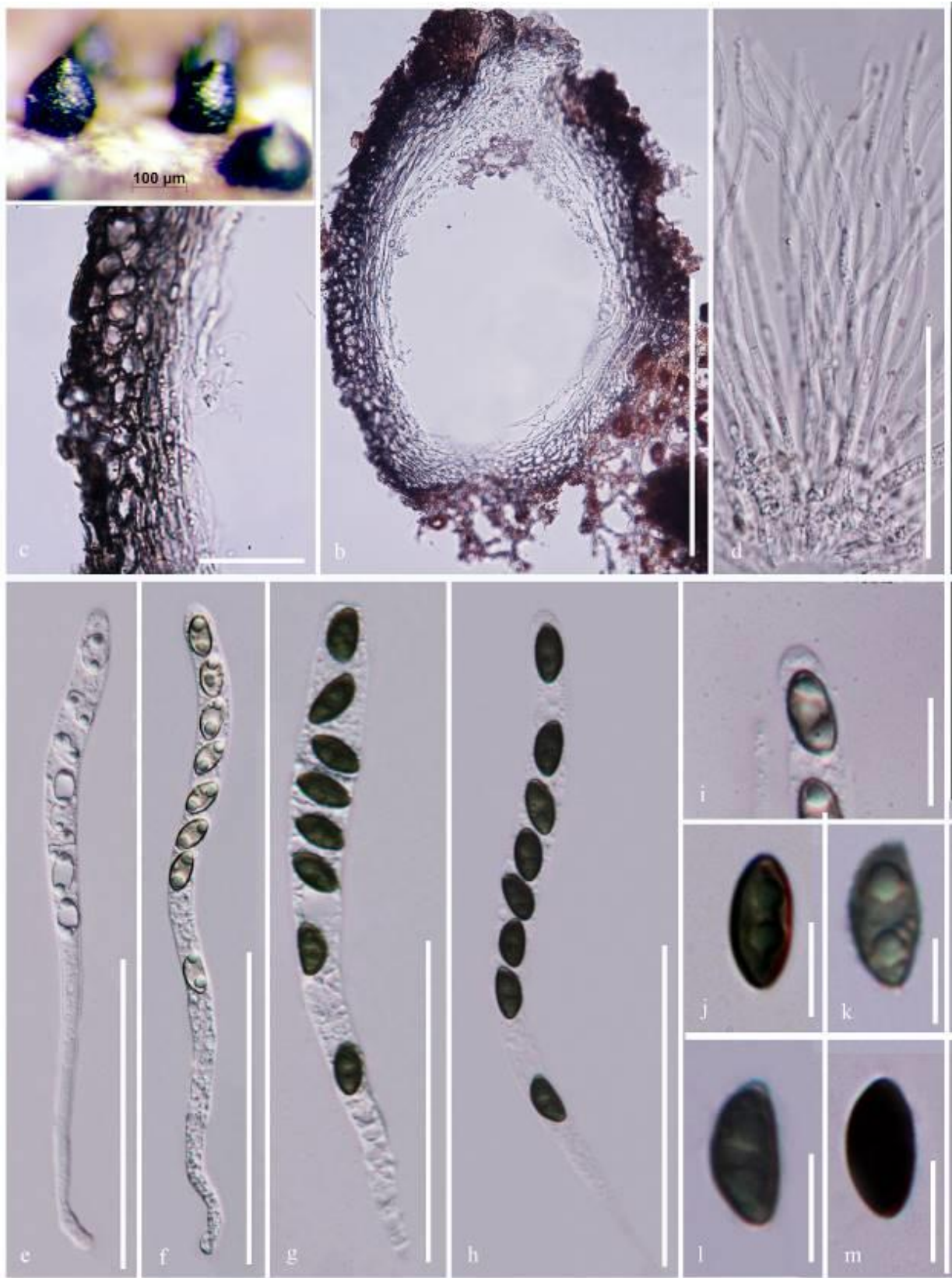
**Figure 71** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -6457.852038. The combined ITS and LSU sequence datasets comprised 59 strains of Coniochaetaceae and *Chaetosphaeria jonesii* (MFLUCC 15-1015) and *C. garethjonesii*

(MFLUCC 15-1012) (Chaetosphaeriaceae) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 498 distinct alignment patterns, with 32.21% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.251290, C = 0.238956, G = 0.283749, T = 0.226005; substitution rates AC = 1.232913, AG = 1.735029, AT = 1.048188, CG = 1.456387, CT = 5.779545, GT = 1.000000; gamma distribution shape parameter  $\alpha = 0.261744$ . Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.05 changes. The ex-type strains are in bold and new isolates in blue bold.

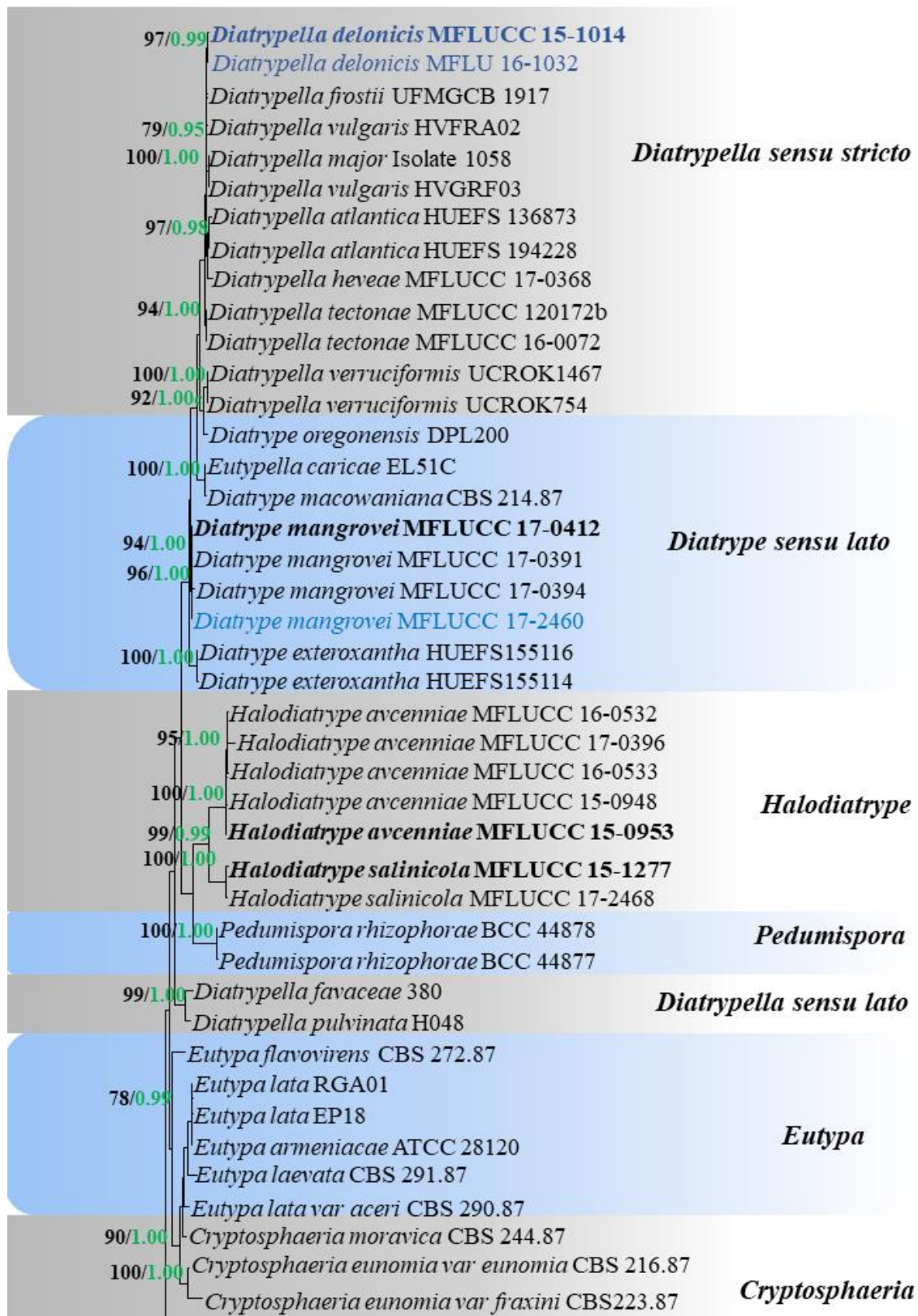


**Figure 72** – *Coniochaeta arenariae* (MFLU 19-1239, holotype). a, b Appearance of ascomata on host. c Section through ascoma. d Section through neck region. e Peridium. f Asci. g Paraphyses. h

Ascospores. i, j Cultures on PDA (i upper, j lower). Scale bars: a = 500  $\mu\text{m}$ , c, f, = 100  $\mu\text{m}$ , g = 50  $\mu\text{m}$ , h = 20  $\mu\text{m}$ , d, e = 10  $\mu\text{m}$ .



**Figure 73** – *Coniochaeta krabiensis* (MFLU 17-2653, holotype). a Appearance of ascomata on host. b Section through ascoma. c Peridium. d Paraphyses. e–h Asci. i Small apical apparatus. j–m Ascospores. Scale bars: a, d–h = 100  $\mu\text{m}$ , b = 200  $\mu\text{m}$ , c = 20  $\mu\text{m}$ , i–m = 10  $\mu\text{m}$ .



**Figure 74** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -16084.142687. The combined ITS and BTUB sequence datasets comprised 103 strains of Diatrypaceae and *Xylaria hypoxylon* (CBS 122620) and *Kretzschmaria deusta* (CBS 826.72) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 702 distinct alignment patterns, with 24.50% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.224322, C = 0.266497, G = 0.234450, T = 0.274732;

substitution rates AC = 1.069126, AG = 2.788495, AT = 1.253466, CG = 0.904409, CT = 3.268061, GT = 1.000000; gamma distribution shape parameter  $\alpha = 0.488277$ . Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.08 changes. The ex-type strains are in bold, new isolates in blue bold and new genus is in red.

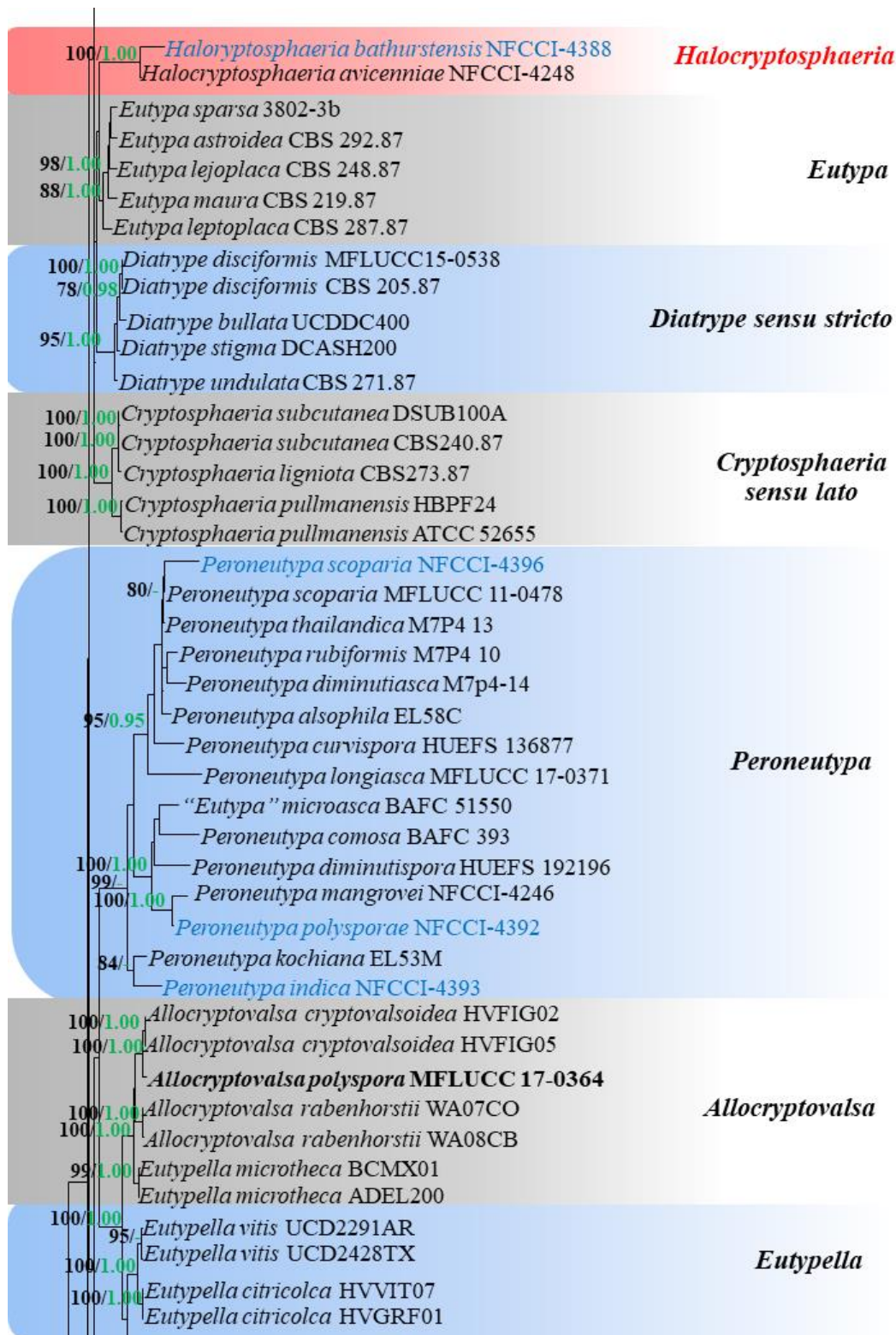


Figure 74 – Continued.

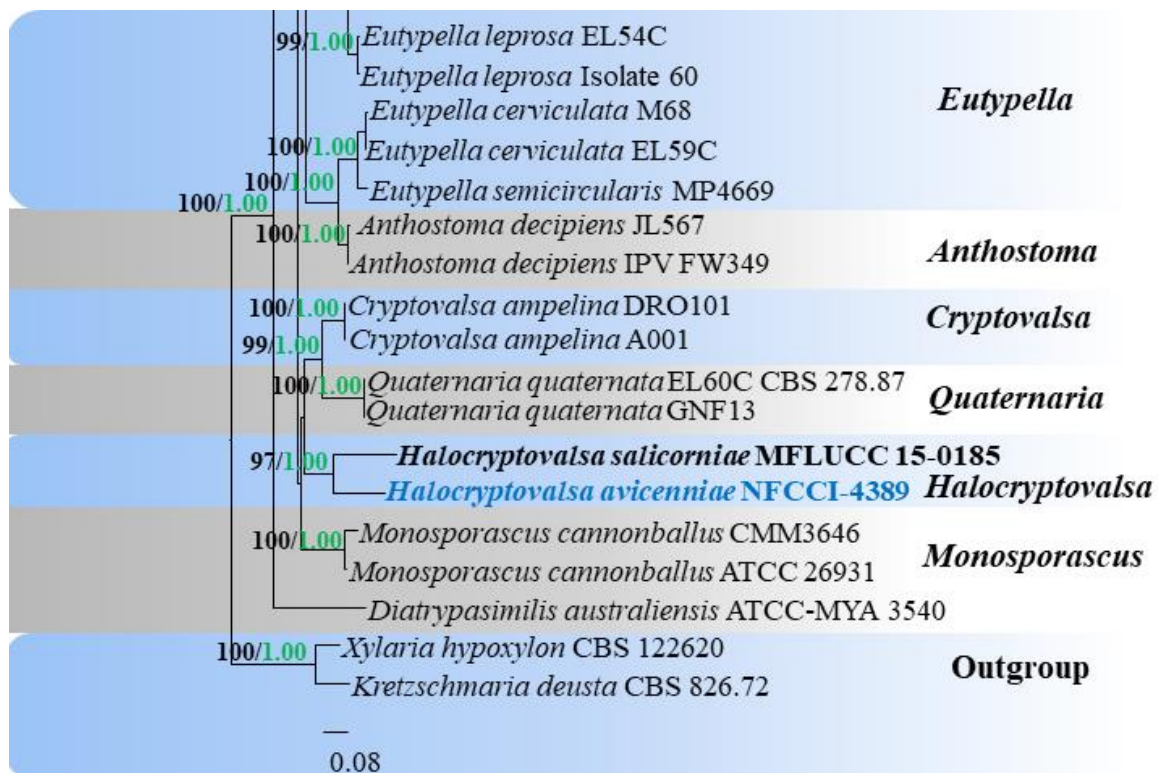


Figure 74 – Continued.

*Diatrype* Fr., Summa veg. Scand.

*Diatrype* was established by Fries (1849) with *Diatrype disciformis* as the type species with taxa often found on decaying wood (Dayarathne et al. 2019c). Several species are reported as canker forming pathogens on forest trees (Senanayake et al. 2015). The asexual morph of *Diatrype* is reported as libertella-like and dumortieria-like (Kirk et al. 2008, Wijayawardene et al. 2012, 2017b, Maharachchikumbura et al. 2015, 2016, Senanayake et al. 2015, Dayarathne et al. 2019c). Dayarathne et al. (2019c) introduced the first marine *Diatrype* species *D. mangrovei* and we provide a new host record with its asexual morph.

*Diatrype mangrovei* Dayarathne & K.D. Hyde, Cryptog. Mycol. (2019)

Fig. 75

Facesoffungi number: FoF 03949

*Saprobic* on submerged decaying wood of *Bruguiera cylindrica*. Sexual morph: *Ascostromata* 0.9–2 × 0.8–1 cm, scattered or aggregated on host, erumpent, arising through cracks on the bark, edges of ascostromata remaining as pointed, angular parts, carbonaceous, with numerous perithecia immersed in one stroma. *Ostirole* opening through host bark and appearing as black spots, composed of an outer layer of dark brown, small, tightly packed, thin parenchymatous cells and an inner layer of yellowish white, large, loosely packed, parenchymatous cells. *Ascomata* 450–580 × 310–336 μm ( $\bar{x}$  = 490 × 325 μm, n = 5), perithecia immersed in stromatic tissue, aggregated, brown, globose to sub-globose, narrowing towards the apex and very narrow at the base of ostiolar canal, thin-walled, ostiolate; ostiolar canal, periphysate, ostiolar opening covered with carbonaceous, black cells; periphyses hyaline, filamentous. *Peridium* 40–42 μm wide, comprising an outer layer of yellow-brown, thick-walled cells of *textura angularis* and a thin, inner stratum of yellow, thick-walled cells of *textura angularis*. *Asci* 85–120 × 6–8 μm ( $\bar{x}$  = 100 × 7.2 μm, n = 30), unitunicate, 8-spored, with long, narrow, thin-walled stalk, with cylindrical, thick-walled, swollen upper portion, apex flat, with J-, cylindrical, conspicuous apical ring. *Ascospores* 5–8 × 1.5–2.5 μm ( $\bar{x}$  = 7.5 × 2 μm, n = 30), seriate, hyaline becoming yellowish at maturity, allantoid, unicellular, thin-walled, with small fat globules at each end, smooth-walled. Asexual morph: *Conidiomata* erumpent, sub-globose to globose, 200–450 × 150–240 ( $\bar{x}$  = 230 × 180 μm, n

= 6), solitary to aggregated, deeply immersed in a stroma with the ascomata of the sexual stage, pale yellow to light brown. *Peridium* 15–25 thick, comprising brown, thick-walled *textura angularis* and pseudoparenchymatous cells merged with the host tissue. *Conidiophores* 25–45 × 1–2.5 ( $\bar{x}$  = 30.5 × 2 μm, n = 10), aseptate, straight or curved, hyaline, rarely branched with one conidiogenous cell. *Conidiogenous cells* 10–25 × 1–2.5 ( $\bar{x}$  = 18 × 1.4 μm, n = 10), cylindrical, mostly straight, discrete or integrated, arising from pseudoparenchymatous cells, hyaline, unicellular, with wide base producing conidia at the apex. *Conidia* 20–40 × 0.5–1.8 ( $\bar{x}$  = 30.2 × 1 μm, n = 30), hyaline, numerous, filiform, straight, curved or hook like, with blunt ends.

Culture characteristics – Colonies on PDA reaching 5 cm diam. after 2 weeks at 20–25 °C, medium dense, irregular, flat, surface smooth with undulate edge, colony from above yellow at the margin, greenish yellow at the center; from below yellowish white at the margin, greenish yellow at the center; mycelium greenish yellow, not produced pigmentation.

Material examined – THAILAND, Krabi Province, Phang Nga, on mangrove sp., 31 August 2017, M.C. Dayarathne, MCD 101 (MFLU 17-2585); *ibid.*, (HKAS102029), living culture (MFLUCC 17-2460).

GenBank numbers – ITS: MN047112

Notes – *Diatrype mangrovei* was introduced by Dayarathne et al. (2019c) from intertidal attached wood of *Bruguiera cylindrica* from Ranong, Thailand. Our new isolate (MFLUCC 17-2460) grouped in a clade comprising *D. mangrovei* (MFLUCC 17-0412, MFLUCC 17-0391, MFLUCC 17-0394) with high statistical support (96% ML, 1.00 PP) (Fig. 74) Furthermore, the new isolate and their ITS sequences are identical to *D. mangrovei*. Hence, we confirm the strain MFLUCC 17-2460 as *D. mangrovei* and this is the first record of this species from *Rhizophora* wood and collected from Krabi which expands its occurrence on a new host. Furthermore, we present the first report of the asexual morph (libertella-like) of this species.

***Halocryptosphaeria*** Dayarathne, Devadatha, V.V. Sarma & K.D. Hyde, gen. nov.

Index Fungorum number: IF556800; Facesoffungi number: FoF 06495

*Saprobic* on decaying wood of *Avicennia marina*. Sexual morph: *Stromata* immersed, blackening the wood surface. *Entostroma* poorly developed, dorsally limited by a black zone binding the stromatic area. *Ascomata* spherical to sub spherical, regularly spaced, submerged in the wood, occasionally deeply buried, long necked, raised, and blackening the wood surface. *Ostiole* poorly developed or conical, not sulcate. *Peridium* wide, comprising thin white line under the black hymenium, composed of three layers; a subhymenial layer of hyaline hyphae of *textura globosa-angularis* mixed with ascogenous elements, a middle layer comprising guttulate hyaline cells, forming a *textura globosa*, an outer most layer composed of melanized cells interdispersed with wood elements, wider near the ostiolar canal. *Hamathecium* lining the whole cavity. *Paraphyses* numerous, hyaline, aseptate, persistent. *Asci* clavate, J-, long pedicellate. *Ascospores* light-brown to olive-brown, aseptate, allantoid, containing oil droplets and limited by a thin epispore, lacking sheath or appendages. Asexual morph *Phomopsis* like. *Conidiomata* immersed, sub-globose to globose, solitary to aggregated, deeply immersed in a stroma with the ascomata of the sexual stage, pale yellow to light brown, occurring on the host. *Conidiomatal wall* thick, comprising brown, thick-walled *textura angularis* and pseudoparenchymatous cells merged with the host tissue. *Conidiophores* lacking septa, straight or curved, hyaline, rarely branched, with one conidiogenous cell. *Conidiogenous cells* cylindrical, mostly straight, discrete or integrated, arising from pseudoparenchymatous cells, hyaline, unicellular, with wide base producing conidia at the apex. *Conidia* hyaline, numerous, filiform, straight or curved or hook-like, with blunt ends.

Type – *Halocryptosphaeria bathurstensis* (K.D. Hyde & Rappaz) Dayarathne & K.D. Hyde

Notes – *Halocryptosphaeria* is characterized in having poorly developed entostroma, dorsally limited by a black zone binding the stromatic area, submerged or occasionally deeply buried long necked ascomata and olive-brown, aseptate ascospores. The genus *Halocryptosphaeria* resembles *Cryptosphaeria*. Morphologically *Halocryptosphaeria* is distinct from *Cryptosphaeria ligniota* and *Cryptosphaeria pulmanensis* in having light-brown ascospores containing oil droplets versus pale



yellow ascospores in the latter two, and also by occurring in a marine habitat (Rappaz 1987).



**Figure 75** – *Diatrype mangrovei* (MFLUCC 17-2460). a Appearance of ascostromata on host. b Section through ascostromata. c Section through ascocysts. d Peridium. e, f Asci. g Ascospores. h Conidiogenous cells and conidiophores. i–k Conidia. l Germinating ascospore. m, n Culture on

PDA (m-upper, n-lower). Scale bars: b = 500  $\mu\text{m}$ , c = 200  $\mu\text{m}$ , e, f = 100  $\mu\text{m}$ , d = 50  $\mu\text{m}$ , i–k = 20  $\mu\text{m}$ , g = 5  $\mu\text{m}$ .

The species *Cryptosphaeria ligniota*, *Cryptosphaeria pullmanensis* and *Cryptosphaeria subcutanea* are highly specific to host plants in Salicaceae (*Populus* and *Salix* spp.), and reported from terrestrial habitats (Rappaz 1987). *Cryptosphaeria pullmanensis* has oblong to reniform, occasionally septate, brown ascospores, *Cryptosphaeria subcutanea* has allantoid to cylindrical, brown ascospores, whereas *Cryptosphaeria ligniota* has allantoid pale yellow ascospores. While *Cryptosphaeria ligniota* and *Cryptosphaeria subcutanea* have J<sup>+</sup> asci, *Halocryptosphaeria* species discussed in this paper have J<sup>-</sup> asci (Clement & Shear 1931). In our phylogenetic analyses *Halocryptosphaeria* species formed a well-supported (100% ML, 1.00 pp) clade (Fig. 74). This species also resembles *Halodiatrype* species in lacking stromatic tissues and nature of ascospores. However, ascomata of *Halodiatrype* species are deeply immersed in a darkened pseudostroma while stromata of *Halocryptosphaeria* are immersed but blackening the wood surface with a poorly developed entostroma, which is dorsally limited by a black zone binding the stromatic area. Therefore, by considering distinct morphological characteristics and phylogenetic placement we introduce a novel genus *Halocryptosphaeria* to accommodate *H. bathurstensis* and *H. avicenniae* (= *Cryptosphaeria avicenniae*).

***Halocryptosphaeria bathurstensis*** (Dayarathne & K.D. Hyde) Dayarathne, Devadatha, V.V. Sarma & K.D. Hyde, comb. nov. Fig. 76

≡ *Eutypa bathurstensis* K.D. Hyde & Rappaz

Index Fungorum number: IF556801; Facesoffungi number: FoF 04597

*Saprobic* on decaying wood of *Avicennia marina*. Sexual morph: *Stromata* extend up to 0.5–2 cm long, immersed, blackening the wood surface. *Entostroma* poorly developed, dorsally limited by a black zone binding the stromatic area. *Ascomata* 250–600 × 200–450  $\mu\text{m}$ , ( $\bar{x}$  = 402 × 347  $\mu\text{m}$ , n = 5), spherical to sub spherical, regularly spaced, submerged in the wood, occasionally deeply buried, long necked, raised, and blackening the wood surface. *Ostiole* up to 60–200 × 60–100  $\mu\text{m}$  ( $\bar{x}$  = 142 × 80  $\mu\text{m}$ , n = 5), poorly developed or conical, not sulcate. *Peridium* 10–30  $\mu\text{m}$  ( $\bar{x}$  = 19  $\mu\text{m}$ , n = 5), wide, comprising thin white line under the black hymenium, composed of three layers; a subhymenial layer of hyaline hyphae of *textura globosa-angularis* mixed with ascogenous elements, a middle layer comprising guttulate hyaline cells, forming a *textura globosa*, an outer most layer composed of melanized cells interdispersed with wood elements, wider near the ostiolar canal. *Hamathecium* lining the whole cavity. *Paraphyses* numerous, hyaline, aseptate, persistent. *Asci* 42–50 × 5–8  $\mu\text{m}$  ( $\bar{x}$  = 46 × 6  $\mu\text{m}$ , n = 10), 8-spored, clavate, J<sup>-</sup>, long pedicelate. *Ascospores* 5–10 × 1–2.5  $\mu\text{m}$  ( $\bar{x}$  = 8 × 1.5  $\mu\text{m}$ , n = 30), olive-brown, aseptate, allantoid, guttulate. Asexual morph *Phomopsis* like

Culture characteristics – Ascospores germinating on seawater agar within 24 hours, germ tubes arising from both sides of the ascospores. Colonies on MEA with moderate growth, reaching 30–50 mm diameter after 15 days of incubation at room temperature, white to cream, reverse pale brown, cottony, surface undulate, irregular. Culture isolated from the single ascospore remained non-sporulating even after incubation at room temperature for one month.

Material examined – INDIA, Tamil Nadu, Parangipettai mangroves (11.59°N 79.5°E), on decaying wood of *Avicennia marina* (Acanthaceae), 23 April 2016, B. Devadatha (AMH-9952, reference specimen designated here), living culture (NFCCI-4248).

GenBank numbers – ITS: MN061366; BTUB: MN431496

Notes – *Halocryptosphaeria bathurstensis* (≡ *Eutypa bathurstensis*) collected from Parangipettai mangroves is morphologically similar to *Halocryptosphaeria bathurstensis* (≡ *Eutypa bathurstensis*) reported by Hyde & Rappaz (1993). With strong morphological support we designate this specimen (AMH-9952) as a reference specimen for *H. bathurstensis* (*sensu* Ariyawansa et al. 2014) with the objective of providing the availability of a reliable herbarium material in good condition and corresponding molecular data for future studies. *Eutypella naqsii*

resembles *Halocryptosphaeria bathurstensis* but clearly distinct in having small groups of ascomata and ascospores that are hyaline to pale yellow. Hence, a new combination *Halocryptosphaeria bathurstensis* has been introduced for *Eutypa bathurstensis* based on both morphological characteristics and its closer relationship in phylogenetic analysis with *Cryptosphaeria* by Dayarathne et al. (2019c). *Halocryptosphaeria bathurstensis* and *H. avicenniae* share identical morphological characteristics and overlapping dimensions of the ascomata and ascospores. However, *H. bathurstensis* is distinct from *H. avicenniae* in having longer necks, shorter asci bearing spore part and olive-brown ascospores. Furthermore, pairwise alignment comparison of ITS gene regions of *H. bathurstensis* and *H. avicenniae* resulted in a difference of 2.89% (out of 461 bp).

### ***Halocryptovalsa* Dayarathne & K.D. Hyde**

*Halocryptovalsa* was introduced by Dayarathne et al. (2019c), which includes cryptovalsa-like marine based diatrypaceous fungi with poorly developed stromata that lack horizontal or vertical furrows within the ascomatal necks (Dayarathne et al. 2019c). Two species are recognized: *Halocryptovalsa avicenniae* and *Halocryptovalsa salicorniae* under this genus. ITS and BTUB sequence data has been generated for *Halocryptovalsa avicenniae* for the first time in this study. Our phylogenetic analyses with combined ITS and BTUB confirmed the previous inclusion of *Halocryptovalsa avicenniae* based only on LSU and SSU data within the genus *Halocryptovalsa* (Dayarathne et al. 2019c).

***Halocryptovalsa avicenniae* (Abdel-Wahab, Bahkali & E.B.G. Jones) Dayarathne & K.D. Hyde, Cryptog. Mycol. (2019)** Fig. 77

Facesoffungi number: FoF 03947

Etymology – Epithet refers to the host, *Avicennia* sp., on which the species was found.

Holotype – MFLU 17-2588

*Saprobic* on decaying wood of *Avicennia marina*. Sexual morph *Ascomata* 120–327 µm high, 150–350 µm diam. ( $\bar{x}$  = 237 × 225 µm, n = 5), deeply immersed in a single layer of a wide-spreading stroma, flask-shaped, single or aggregated and sometimes confluent. Necks 100–140 µm long, 50–70 µm diam. ( $\bar{x}$  = 113 × 60 µm, n = 5), ostiolate, periphysate with a small papilla protruding above the substrate and lacking furrows. *Peridium* 18–30 µm diam., ( $\bar{x}$  = 23 µm, n = 5), forming *textura angularis*; outer layer of small, thick-walled, melanized cells, brown to dark-brown, interspersed with cells of the substrate; and an inner layer of hyaline, elongate cells of *textura angularis*. *Paraphyses* hyaline, septate, deliquescent. *Asci* 100–140 × 10–16 µm ( $\bar{x}$  = 113 × 12 µm, n = 15), polysporous (128–256 ascospores per ascus), cylindrical fusoid, tapering to the pedicel, tapering to the upper part to a truncate, thick ended apex, with a faint, subapical, non-amyloid ring at the base of an invagination of the ascus apex. *Ascospores* 4–8 × 1–2 µm ( $\bar{x}$  = 6 × 1.5 µm, n = 25), allantoid, unicellular, yellow-brown to brown. Asexual morph Undetermined.

Material examined – INDIA, Tamil Nadu, Parangipettai mangroves (11.59°N 79.5°E), on decaying wood of *Avicennia marina* (Acanthaceae), 23 April 2018, B. Devadatha, AMH-10006, living culture, NFCCI-4389.

GenBank numbers – ITS: MN062160, BTUB: MN520613

Notes – *Halocryptovalsa avicenniae* ( $\equiv$  *Cryptovalsa avicenniae*) and its asexual morph were reported by Abdel-Wahab et al. (2017) from decayed intertidal wood of *Avicennia marina* from Tarut mangrove, Arabian Gulf, Saudi Arabia. Recently Dayarathne et al. (2019c) introduced *Halocryptovalsa* based on the combined SSU and LSU phylogenetic analyses. The *H. avicenniae* strains and *H. salicorniae* grouped together in a monophyletic clade and were distantly related to *Cryptovalsa* species (Dayarathne et al. 2019c). *Halocryptovalsa avicenniae* can be distinguished from *Cryptovalsa* species and other members of Diatrypaceae in having larger ascomata that lack papillial furrows and a greater number of ascospores per ascus (Abdel-Wahab et al. 2017). Our collection of *Halocryptovalsa avicenniae* (AMH-10006) from Parangipettai mangroves shares similar characters with *H. avicenniae* (MAW-2017a) from Tarut mangrove (Abdel-Wahab et al.

2017). However, the former is distinct in having smaller ascomata and we did not observe the asexual morph on the host, but both share characters of overlapping asci and ascospore dimensions and occur on *Avicennia marina* (Abdel-Wahab et al. 2017). Furthermore, phylogenetic analysis based on LSU rDNA revealed that *Halocryptovalsa avicenniae* (NFCCI-4389) clustered together with *H. avicenniae* strains (MAW-2017a) in a monophyletic clade with significant support from ML 100%. Mutli-loci phylogeny with ITS and BTUB genes showed that *Halocryptovalsa avicenniae* (NFCCI-4389) nested between *H. salicorniae* and *Cryptovalsa ampelina* with low bootstrap support. *Halocryptovalsa avicenniae* strains (MAW-2017a) lack ITS and BTUB sequences for comparison with our strain. Based on morphological and molecular data we confirm our collection of *Halocryptovalsa avicenniae* (NFCCI-4389) as similar to *H. avicenniae* strains (MAW-2017a) as reported by Abdel-Wahab et al. (2017).

### ***Halodiatrype* Dayarathne & K.D. Hyde**

Dayarathne et al. (2016) introduced the genus *Halodiatrype* as it differs from other genera in this family by having ascomata lacking stromatal tissues, comparatively large ascospores with septa and a libertella-like asexual morph. Generally, Diatrypaceae species have well-developed ascostromata with aseptate ascospores (Maharachchikumbura et al. 2015, Dayarathne et al. 2019c). This genus currently comprises *H. avicenniae*, *H. mangrovei* and *H. salinicola* while Dayarathne et al. (2019c) amended the generic description to include asexual morph characteristics of *H. avicenniae*. Our collection from Krabi yielded *H. salinicola* from *Rhizophora* sp. while the previous record was from unidentified mangrove wood (Dayarathne et al. 2016).

### ***Halodiatrype salinicola* Dayarathne & K.D. Hyde, Mycosphere 7(5): 617 (2016)**

Fig. 78

Facesoffungi number: FoF02021

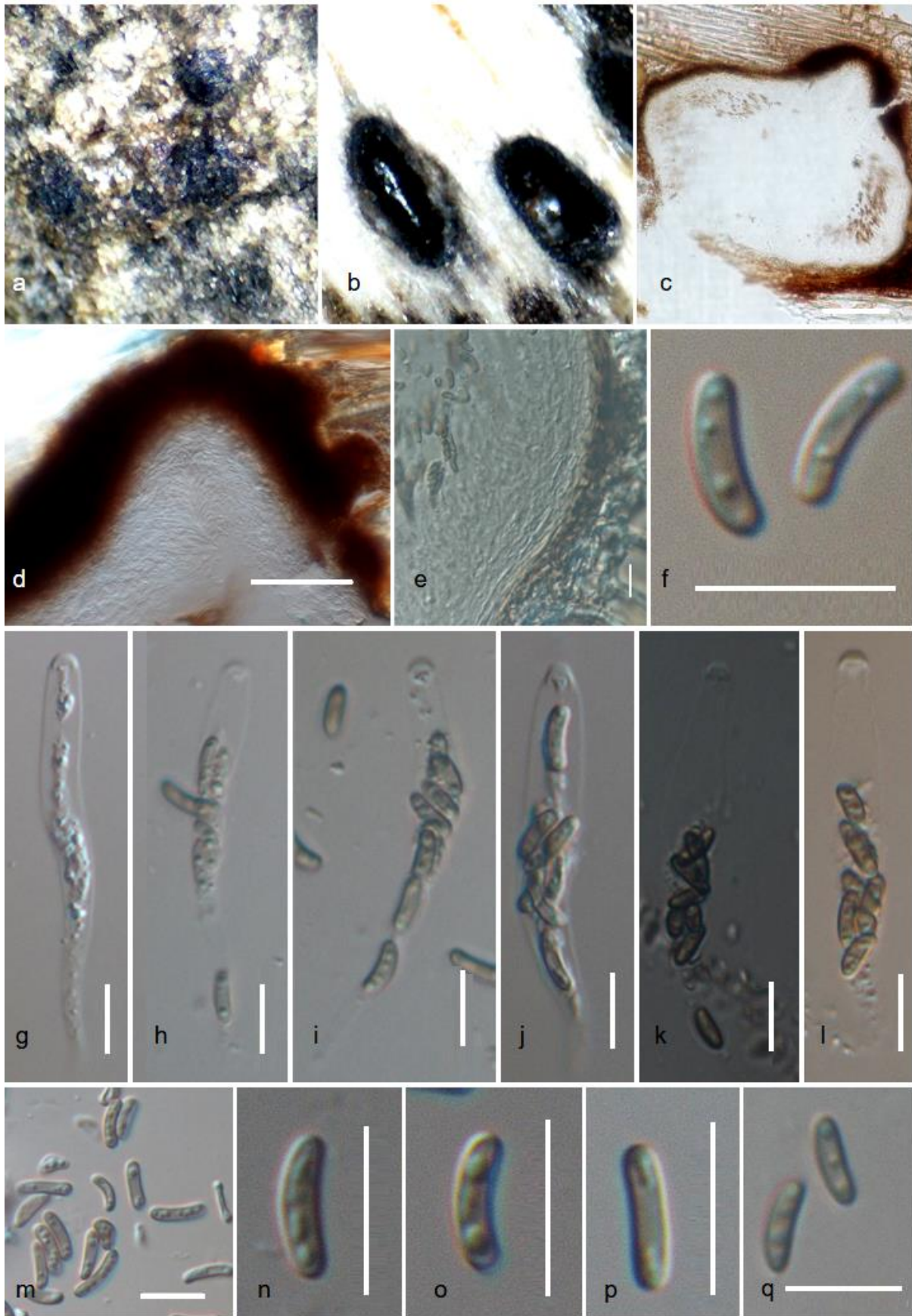
*Saprobic* on submerged decayed wood of *Rhizophora* sp. Sexual morph *Ascomata* 120–135 µm high, 100–120 µm diam., solitary to scattered, lacking stromatic tissues, deeply immersed, dark brown to black, globose to subglobose, uni-loculate, ostiolate, papillate. *Ostioles* central, apex composed of brown outer amorphous layer and inner yellow cells of *textura porrecta* and ostiolar canal filled with periphyses. *Peridium* up to 2.5 µm wide, thick, a single layer of amorphous fungal tissue fused with the host cells, incorporating occasionally in the host tissues. *Paraphyses* 2–3.5 µm wide, arising from the base of perithecia, composed of long, wide, filamentous, thin-walled cells, guttulate, unbranched, septate, slightly constricted at septum, narrow towards the apex, with apex blunt. *Asci* 45–50 × 6–10 µm ( $\bar{x}$  = 48 × 7.2 µm, n = 20), 8-spored, unitunicate, clavate, long pedicellate, apically rounded, with J- subapical ring, initially deliquescing. *Ascospores* 6–8.5 × 2–3 µm ( $\bar{x}$  = 7.2 × 2.4 µm, n = 30), overlapping bi- to tetra-seriate, allantoid, light brown, aseptate, 1-septate at maturity, not constricted at the septum, smooth-walled with one to few small guttules, slightly curved. Asexual morph Undetermined.

Culture characteristics – Colonies on PDA, reaching 10 cm diam. after 2 weeks at 20–25 °C, medium dense, irregular, slightly raised, surface slightly rough, with uneven edge, undulate margin, cottony, colony from above: white to grey; from below: yellowish white at the margin, yellow to brown at the centre; mycelium greenish-grey, not produced pigmentation on PDA media.

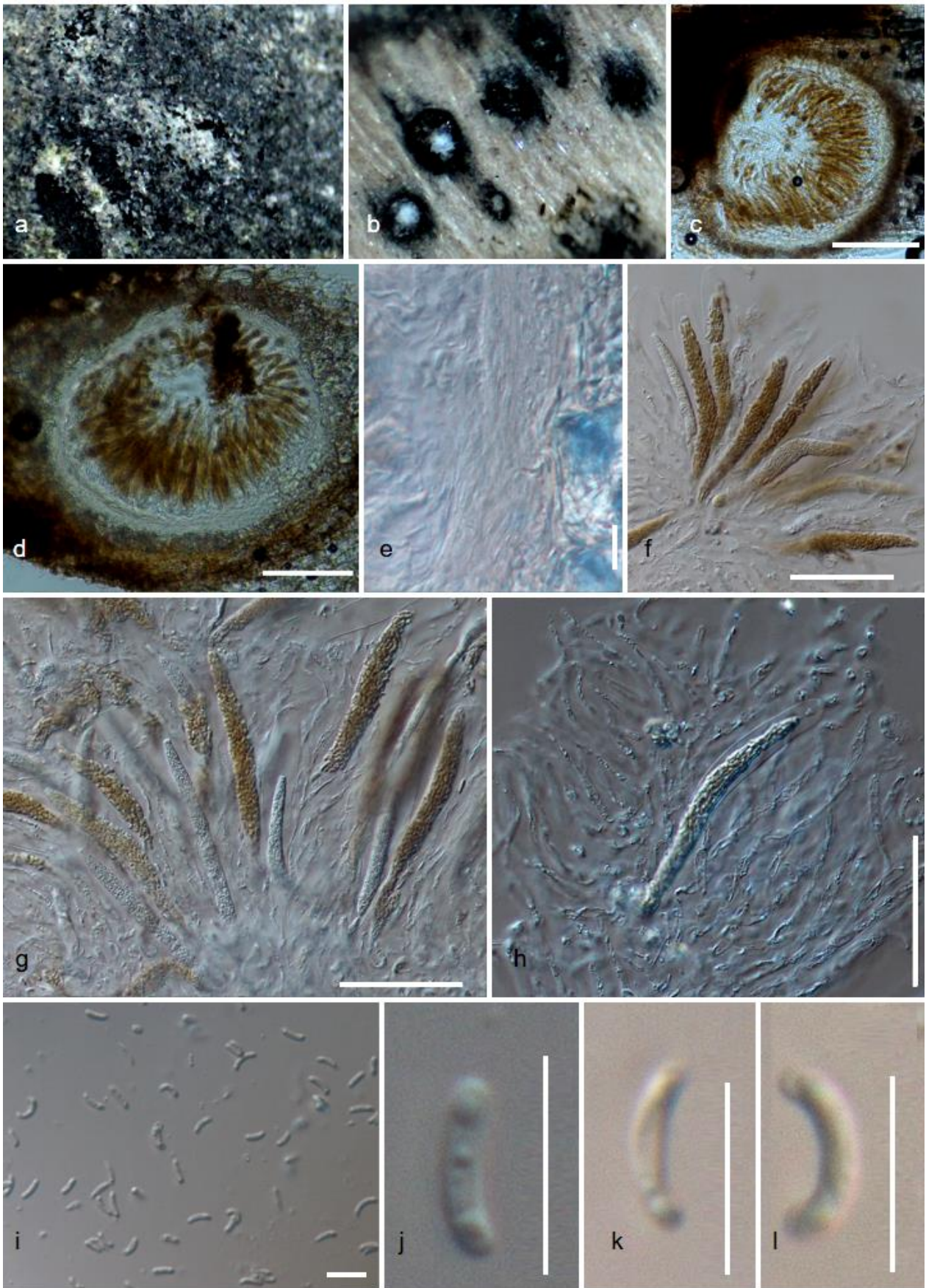
Material examined – THAILAND, Krabi Province, Phang Nga, on mangrove wood, 31 August 2017, M.C. Dayarathne MCD 113 (MFLU 17-2595); *ibid* (HKAS102038), living culture (MFLUCC 17-2468).

GenBank numbers – ITS: MN047113

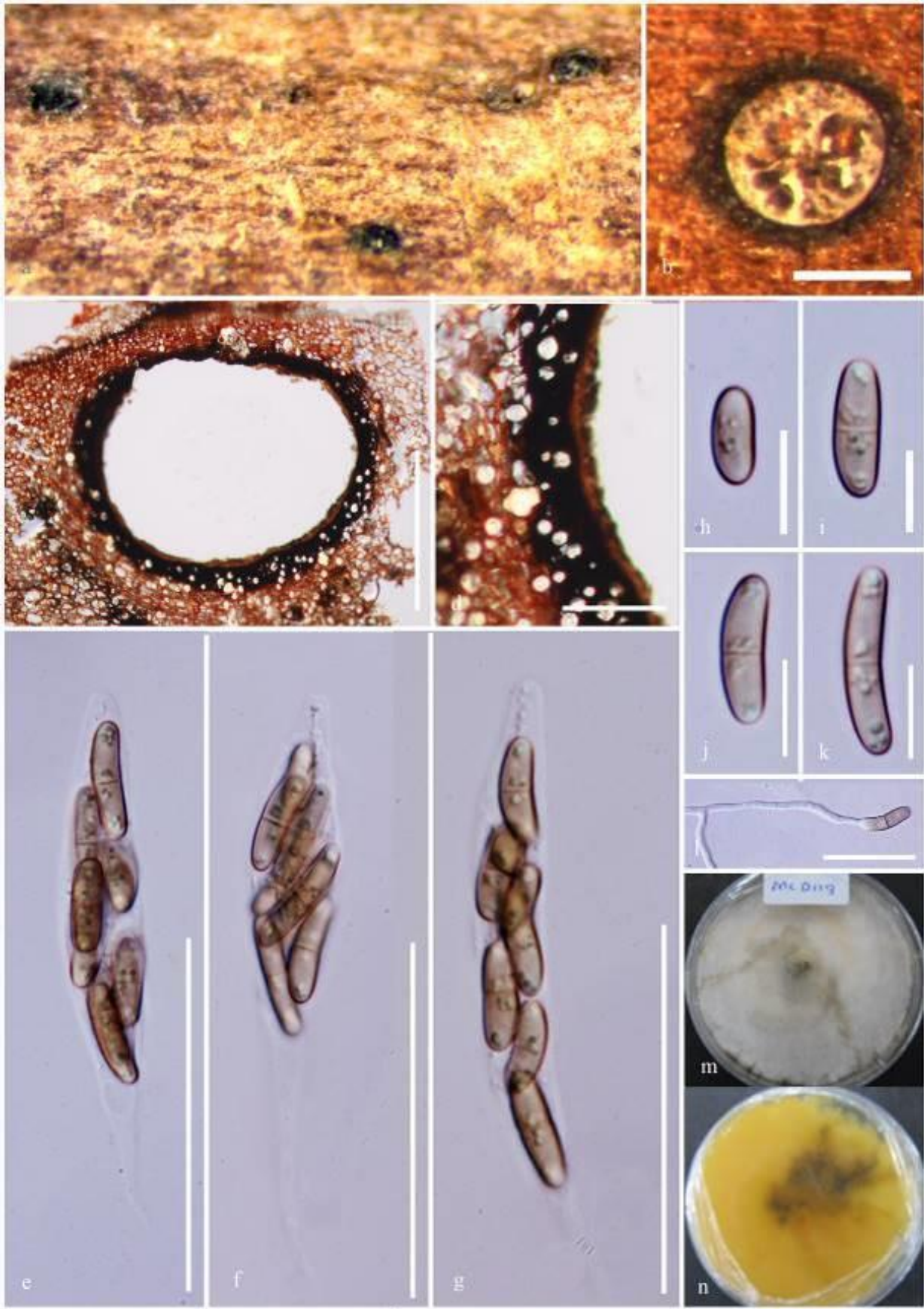
Notes – *Halodiatrype salinicola* introduced by Dayarathne et al. (2016) is characterized by immersed ascomata with a cylindrical or subconical, periphysate ostiole, apically rounded, clavate asci and allantoid, 0–1-septate, and light brown ascospores. Our collection (MFLU 17-2595) is morphologically similar to *Halodiatrype salinicola*. Comparison of ITS nucleotides between *Halodiatrype avicenniae* and *H. salinicola* resulted in 9.9% (out of 532 bp), which reveals that both are different species.



**Figure 76** – *Halocryptosphaeria bathurstensis* (AMH-9952). a Ascomata erumpent, immersed in decaying wood of *Avicennia marina*. b Horizontal sections of ascoma. c Vertical section of ascoma. d Ostiole. e Peridium. g-l Immature and mature asci. f, m-q Ascospores. Scale bars: c = 100  $\mu\text{m}$ , d = 50  $\mu\text{m}$ , e-q = 5  $\mu\text{m}$ .



**Figure 77** – *Halocryptovalsa avicenniae* (AMH-10006). a, b Ascomata immersed in the decaying wood. c, d Longitudinal sections of ascomata. e Section showing peridium. h Paraphyses. f–g Immature and mature asci. i–l Ascospores. Scale bars: c–d = 100  $\mu$ m, f–h = 50  $\mu$ m, i–l = 10  $\mu$ m.



**Figure 78** – *Halodiatrype salinicola* (MFLUCC 17-2468). a Appearance of ascomata on host. b Transverse section of ascoma on the host. c Vertical section through ascoma. d Peridium. e–g Asci. h–k Ascospores. l Germinating spore. m, n Culture on MEA (m = from above, n = from below). Scale bars: b, c = 100  $\mu$ m, e–g = 20  $\mu$ m, d, h–k = 5  $\mu$ m.

***Peroneutypa* Berl.**

Berlese (1902) introduced the genus *Peroneutypa* to include *P. bellula*, *P. corniculata* and *P. heteracantha* but without designating a type species. Rappaz (1987) proposed *P. bellula* as the type species and the genus was considered to be a synonym of *Eutypella*. However, based on morphological characteristics and phylogenetic data (Acero et al. 2004), *Peroneutypa* was reinstated by Carmarán et al. (2006). The species of *Peroneutypa* are saprobes or pathogens and are widely distributed in terrestrial habitats and are also found in marine habitats (Lumbsch & Huhndorf 2010, Maharachchikumbura et al. 2015, Shang et al. 2017, Hyde et al. 2019). *Peroneutypa* species formed a well-supported (99 % ML) distinct clade in our phylogram (Fig. 74).

***Peroneutypa scoparia* (Schwein.) Carmarán & A.I. Romero, in Carmarán, Romero & Giussani, Fungal Diversity 23: 84 (2006)** Fig. 79

Facesoffungi number: FoF 01998

*Saprobic* on decaying wood of *Avicennia marina*. Sexual morph *Stromata* 1–2 cm wide, poorly developed between perithecial necks, perithecia gregarious, dark brown to black, semi-immersed to raised, erumpent through the host tissue with superficial necks. *Ascomata* 200–300 µm high, 150–300 µm diam. ( $\bar{x}$  = 257 × 185 µm, n = 10), immersed to erumpent, globose to sub globose, gregarious to solitary, ostiolate, with short-beaks, periphysate, brown to black. *Ostiolar canals* 60–100 µm ( $\bar{x}$  = 75 µm, n = 5) wide, with moderate neck length 30–60 µm ( $\bar{x}$  = 45 µm, n = 5), cylindrical, straight, dark-brown to black. *Periphyses* filamentous, short, 0.5–2 µm ( $\bar{x}$  = 1.5 µm, n = 10). *Peridium* 15–35 µm ( $\bar{x}$  = 22 µm, n = 10) wide, comprising two layers, inner stratum with many layers of hyaline cells of *textura angularis* and outer stratum with 2–3 layers of light brown to black cells of *textura angularis*. *Hamathecium* composed of paraphyses that are 1–2 µm ( $\bar{x}$  = 1 µm, n = 20) wide, filamentous, longer than asci, septate, embedded in a gelatinous matrix. *Asci* 19–24 × 3–5 µm ( $\bar{x}$  = 20 × 4 µm, n = 20), 8-spored, unitunicate, cylindrical to clavate, apically rounded to truncate with a J- apical ring, short pedicellate. *Ascospores* 3–5 × 1–1.5 µm ( $\bar{x}$  = 4 × 1 µm, n = 30), uniseriate to biserially arranged, overlapping, hyaline to pale yellow, oblong to allantoid or slightly curved, aseptate, smooth-walled, with small guttules. Asexual morph Undetermined.

Culture characteristics – Colonies on MEA fast growing, reaching 50–80 mm diameter after 15 days of incubation at room temperature, white initially, becoming light grey at maturity, reverse light yellow, cottony, punctiform, flat, circular, entire.

Material examined – INDIA, Puducherry, Karaikal mangroves (11.5°N 79.5°E), on submerged decaying wood of *Avicennia marina* (Acanthaceae), 14 April 2016, B. Devadatha (AMH-10007), living culture, NFCCI-4396

GenBank numbers – ITS: MN061369, BTUB: MN431499

Notes – Our collection (AMH-10007) shares similar morphological characters with the type of *Peroneutypa scoparia* such as ascomata, overlapping asci and ascospore dimensions (Carmarán et al. 2006, Shang et al. 2018). Hence, we consider our collection as *P. scoparia*, which forms the first report of this fungus from a marine habitat in India (Jones et al. 2015), as it was earlier reported from a marine habitat in Thailand (Haye et al. 2019). *Peroneutypa scoparia* is reported as a severe pathogen of grapevine and also produces “nasty compounds” (Helaly et al. 2018).

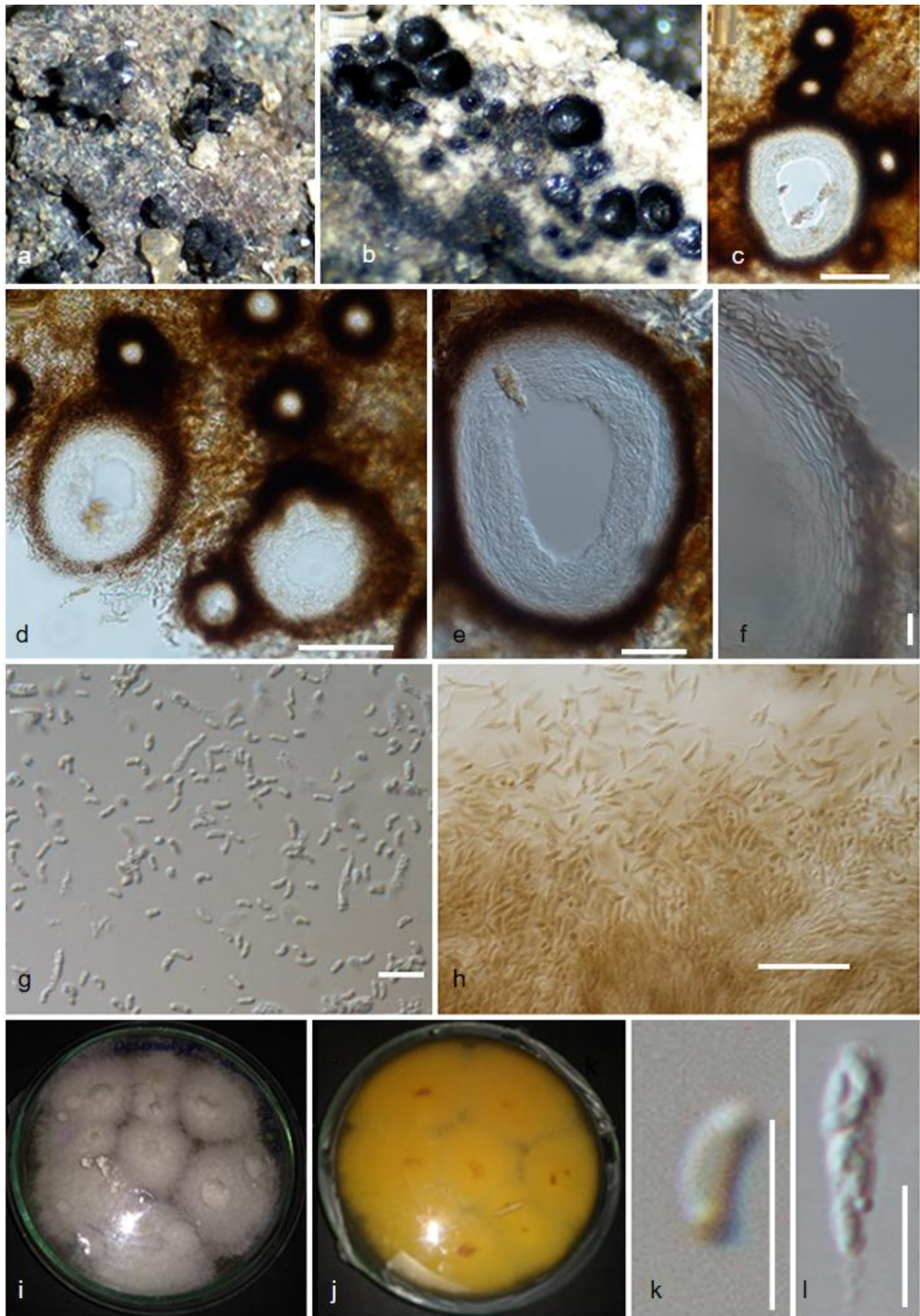
***Peroneutypa indica* Devadatha, V.V. Sarma & E.B.G Jones, sp. nov.** Fig. 80

Index Fungorum number: IF556226; Facesoffungi number: FoF 06187

Etymology – Specific epithet in reference to the country India.

*Saprobic* on decaying wood of *Suaeda monoica*. Sexual morph: *Stromata* well-developed between perithecial necks, perithecia gregarious, dark brown to black, semi-immersed to raised, erumpent through the host tissue with superficial necks. *Ascomata* 350–600 µm high, 130–300 µm diam. ( $\bar{x}$  = 375 × 202 µm, n = 10), erumpent to immersed, globose to sub globose, gregarious to solitary, brown to black, ostiolate, periphysate. *Ostiolar canal* 100–250 µm ( $\bar{x}$  = 175 µm, n = 5) wide, with moderate neck length 100–350 µm ( $\bar{x}$  = 231 µm, n = 5), cylindrical, straight, dark-brown to black.





**Figure 79** – *Peroneutypa scoparia* (AMH-10007). a, b Ascomata erumpent and semi-immersed in the decaying wood of *Avicennia marina*. c–e Longitudinal sections of ascomata. f Section showing peridium. h, l Immature and mature asci. g, k Ascospores. i–j Front and reverse view of culture. Scale bars: c–e = 100  $\mu$ m, h = 50  $\mu$ m, g, k–l = 10  $\mu$ m.

*Peridium* 15–35  $\mu\text{m}$  ( $\bar{x}$  = 22  $\mu\text{m}$ ,  $n$  = 10) wide, comprising two layers, inner stratum with many layers of hyaline cells of *textura angularis* and outer stratum with 2–3 layers of light brown to black cells of *textura angularis*. *Hamathecium* composed of paraphyses that are 1–2  $\mu\text{m}$  ( $\bar{x}$  = 1  $\mu\text{m}$ ,  $n$  = 10) wide, filamentous, embedded in a gelatinous matrix. *Asci* 35–47  $\times$  3–4  $\mu\text{m}$  ( $\bar{x}$  = 42  $\times$  3.5  $\mu\text{m}$ ,  $n$  = 20), 8-spored, unitunicate, cylindrical to clavate, apically rounded to truncate with a J-apical ring, short pedicellate. *Ascospores* 4–8  $\times$  1–2  $\mu\text{m}$  ( $\bar{x}$  = 5.5  $\times$  1.3  $\mu\text{m}$ ,  $n$  = 30), uniseriate to biserially arranged, overlapping, hyaline, straight to allantoid, aseptate, smooth-walled, lacking guttules, light brown in mass. Asexual morph: Undetermined.

Culture characteristics – Colonies on malt extract agar fast growing, reaching 50–80 mm diam. after 15 days of incubation at room temperature, white initially, becoming light grey at maturity, reverse light yellow, cottony, punctiform, flat, circular, entire.

Material examined – INDIA, Tamil Nadu, Tiruvarur, Muthupet mangroves (10.4°N 79.5°E), on decaying wood of *Suaeda monoica* (Amaranthaceae), 28 November 2015, B. Devadatha, (AMH-10002, holotype), ex-type living culture, NFFCCI-4393.

GenBank numbers – ITS: MN061368, BTUB: MN431498

Notes – *Peroneutypa indica* shares similar morphological characters with *P. kochiana* in having hyaline allantoid ascospores and overlapping ascospore dimensions (Carmaran et al. 2006). However, *Peroneutypa indica* is distinct from *P. kochiana* in having longer asci (35–47 vs 18–28  $\mu\text{m}$  long) with J-ve apical ring and occurs in a marine habitat. Whereas *Peroneutypa kochiana* has shorter asci with J+ apical rings and reported from terrestrial habitats Carmaran et al. 2006). *Peroneutypa* species such as *P. cosmosa*, *P. mangrovei*, *P. mackenziei*, *P. rubiformis* and *P. scoparia* have shorter asci in contrast to *P. Indica* (Senwanna et al. 2017, Shang et al. 2017, 2018). Phylogenetic results also revealed that *Peroneutypa indica* clusters with *P. kochiana* in a monophyletic clade with a significant support from ML 82%. Furthermore, a comparison of ITS nucleotides between our taxon and *P. kochiana* (GenBank: AJ302462) resulted in 7% difference (out of 528 bp). Based on the significant support from morpho-molecular data we introduce *Peroneutypa indica* as a new species in *Peroneutypa*.

***Peroneutypa polysporae*** Devadatha, V.V. Sarma & E.B.G Jones, sp. nov.

Fig. 81

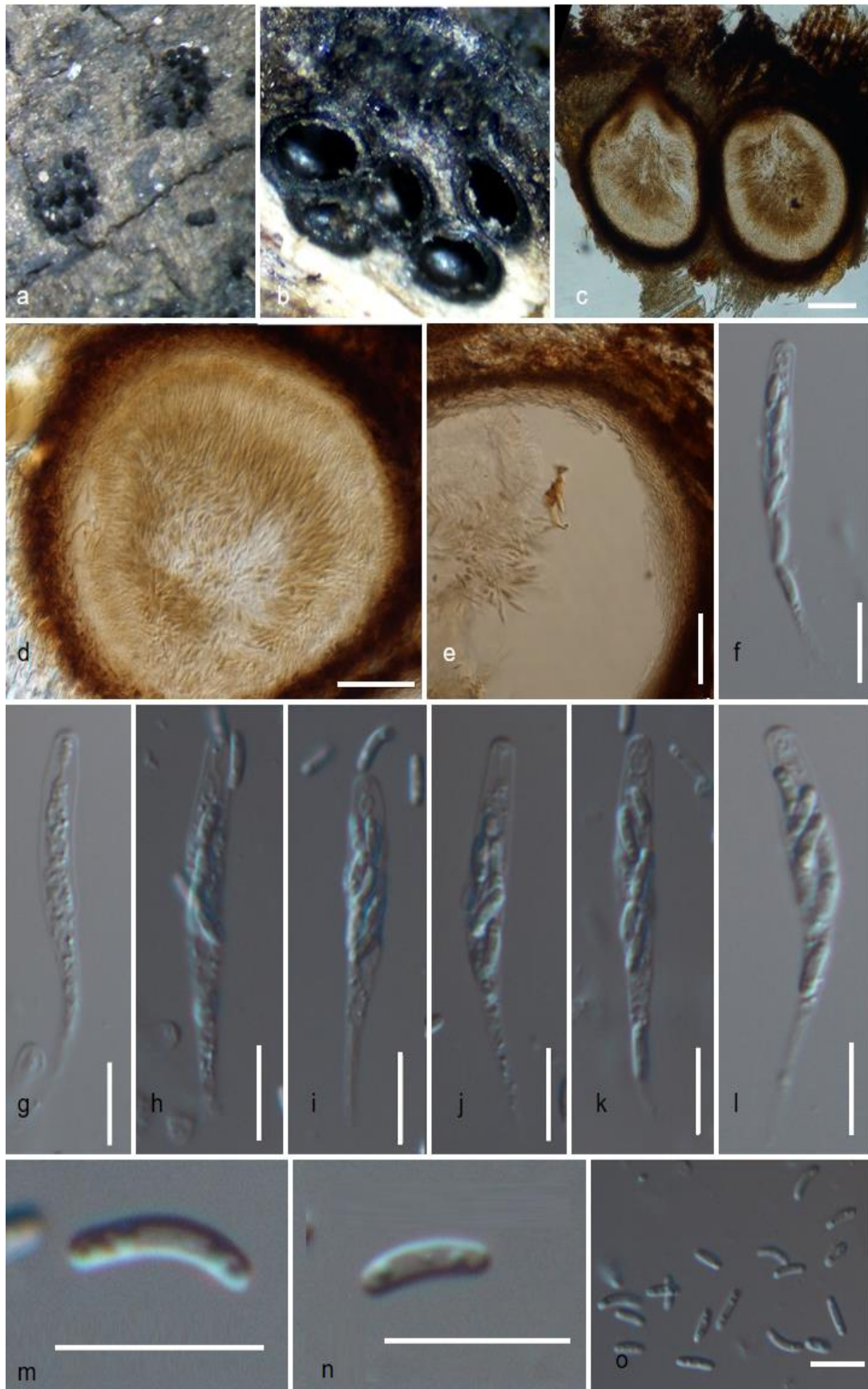
Index Fungorum number: IF556227; Facesoffungi number: FoF 06188

Etymology – Specific epithet in reference to polysporous asci.

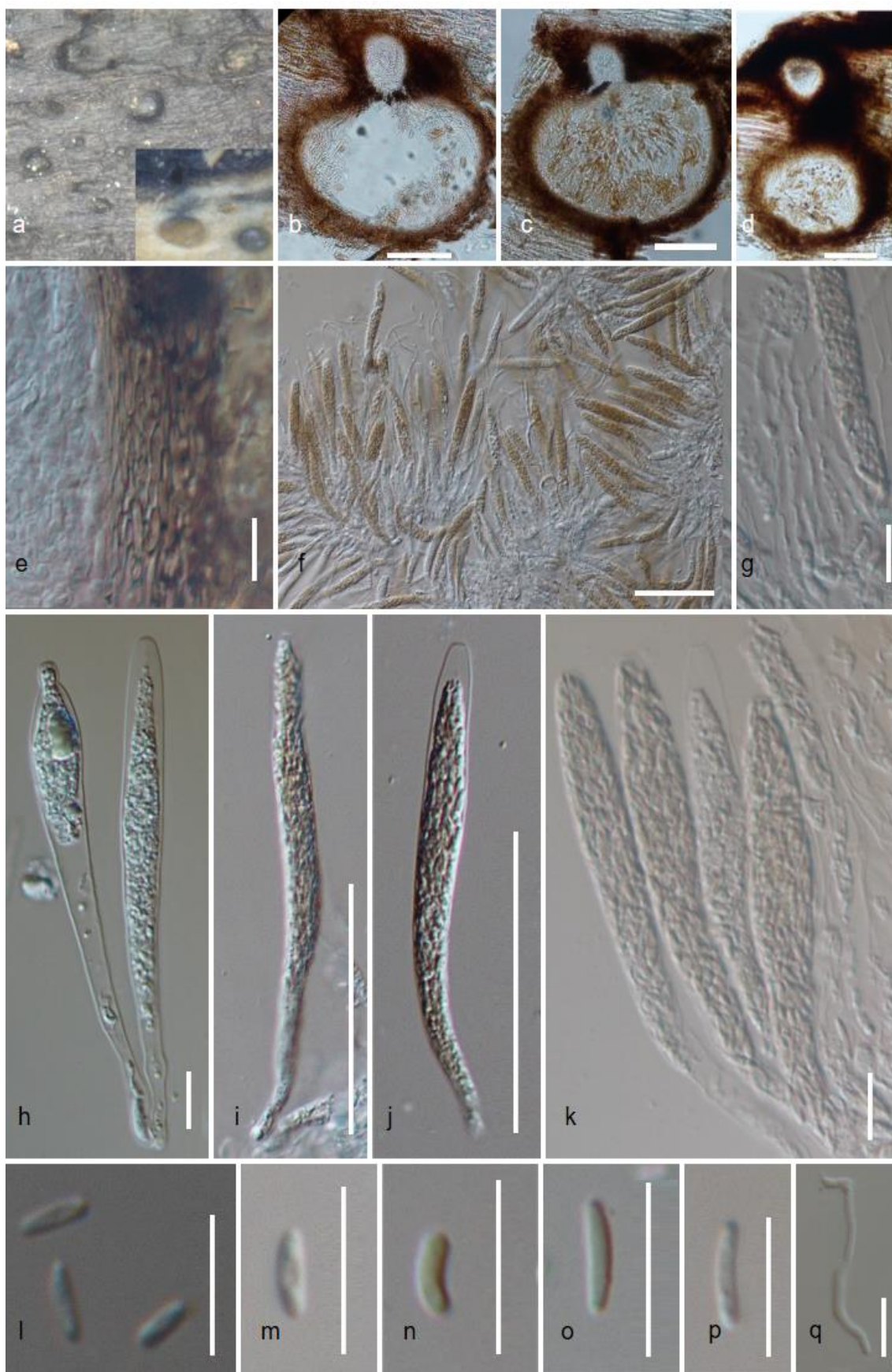
*Saprobic* on decaying wood of *Suaeda monoica*. Sexual morph: *Ascomata* 200–430  $\mu\text{m}$  high, 100–370  $\mu\text{m}$  diam. ( $\bar{x}$  = 286  $\times$  223  $\mu\text{m}$ ,  $n$  = 10), deeply immersed in a single layer of a wide-spreading stroma, flask-shaped, single or aggregated and sometimes confluent, ostiolate, periphysate, with a small papilla protruding above the substrate and having furrows. *Ostiolar canal* 130–250  $\mu\text{m}$  long, 60–100  $\mu\text{m}$  diam. ( $\bar{x}$  = 113  $\times$  60  $\mu\text{m}$ ,  $n$  = 5). *Peridium* 15–50  $\mu\text{m}$  diam. ( $\bar{x}$  = 23  $\mu\text{m}$ ,  $n$  = 5), forming *textura angularis*; outer layer of small, thick-walled, melanized cells, brown to dark-brown, interspersed with cells of the substrate; and an inner layer of hyaline, elongate cells of *textura angularis*. *Hamathecium*: *Paraphyses* hyaline, septate, deliquescent. *Asci* 110–155  $\times$  5–7.5  $\mu\text{m}$  ( $\bar{x}$  = 113  $\times$  12  $\mu\text{m}$ ,  $n$  = 15), polysporous, cylindrical fusoid, tapering towards the pedicel, tapering to the upper part to a truncate, thick ended apex, with a faint, subapical, non-amyloid ring at the base of an invagination of the ascus apex. *Ascospores* 5–15  $\times$  1–2.5  $\mu\text{m}$  ( $\bar{x}$  = 9  $\times$  1.8  $\mu\text{m}$ ,  $n$  = 25), multiseriate, allantoid, unicellular, hyaline to pale yellow. Asexual morph: not observed.

Material examined – INDIA: Tamil Nadu, Tiruvarur, Muthupet mangroves (10.4°N 79.5°E), on decaying wood of *Suaeda monoica* (Amaranthaceae) 28 November 2015, B. Devadatha (AMH-10016, holotype), ex-type living culture, NFFCCI-4392

GenBank numbers – ITS: MN061367; BTUB: MN431497



**Figure 80** – *Peroneutypa indica* (AMH-10002, holotype). a Ascomata erumpent and semi-immersed in the decaying wood of *Suaeda monoica*. b–d Longitudinal sections of ascomata. e Section showing peridium. f–l Immature and mature asci. m–o Ascospores. Scale bars: c–d = 100 μm, e–o = 10 μm.



**Figure 81** – *Peroneutypa polysporae* (AMH-10016, holotype). a Ascomata semi-immersed in the decaying wood of *Suaeda monoica*. b–d Longitudinal sections of ascomata. e Section showing peridium. g Paraphyses. f–k Immature and mature asci. l–p Ascospores. q Germinating ascospore. Scale bars: b–d = 100  $\mu$ m, f–j = 50  $\mu$ m, h, k, l–q = 10  $\mu$ m.

Notes – *Peroneutypa polysporae* shares similar morphological characters with *Cryptovalsa suaedicola* in having immersed ascomata, cylindric-fusoid, multi-spored asci and ascospores that are hyaline (Spooner 1981). However, *Peroneutypa polysporae* is different from *Cryptovalsa suaedicola* in having longer asci (110–155 vs 80–130 µm long) and ascospores (5–15 vs 4–6 µm long) (Spooner 1981). *Cryptovalsa mangrovei* and *Halocryptovalsa avicenniae* are distinguishable from *Peroneutypa polysporae* in having larger ascomata and yellow to brown ascospores (Inderbitzin et al. 1999). *Halocryptovalsa salicorniae* and *Cryptovalsa halosarceicola* differ from *Peroneutypa polysporae* in possessing smaller ascomata, asci and ascospores (Hyde 1993a). The phylogenetic analyses based on individual LSU gene data and combined ITS and BTUB genes placed our taxon under *Peroneutypa*. *Peroneutypa polysporae* clusters with *P. mangrovei* in a monophyletic clade with a significant support from ML 100% and 1.00 PP. Furthermore, a nucleotide difference of 4.8% (out of 517 bp with gaps) was found within ITS gene region of *Peroneutypa polysporae* and *P. mangrovei*. *Peroneutypa polysporae* is distinct from *P. mangrovei* and other species of *Peroneutypa* in having multispored asci. *Peroneutypa* species usually have 8-spored asci. Interestingly, however, our *Peroneutypa polysporae*, though possesses polysporous asci, clustered along with other species of *Peroneutypa* in our multigene phylogenetic analyses thus suggesting that *Peroneutypa* can also accommodate taxa with polysporous asci (Shang et al. 2017, 2018). Molecular phylogenetic results also suggest that *Cryptovalsa* and *Halocryptovalsa* are distantly related to *Peroneutypa polysporae*. Hence a new species, *Peroneutypa polysporae* is introduced in the genus *Peroneutypa* based on the significant support from morphology and phylogeny.

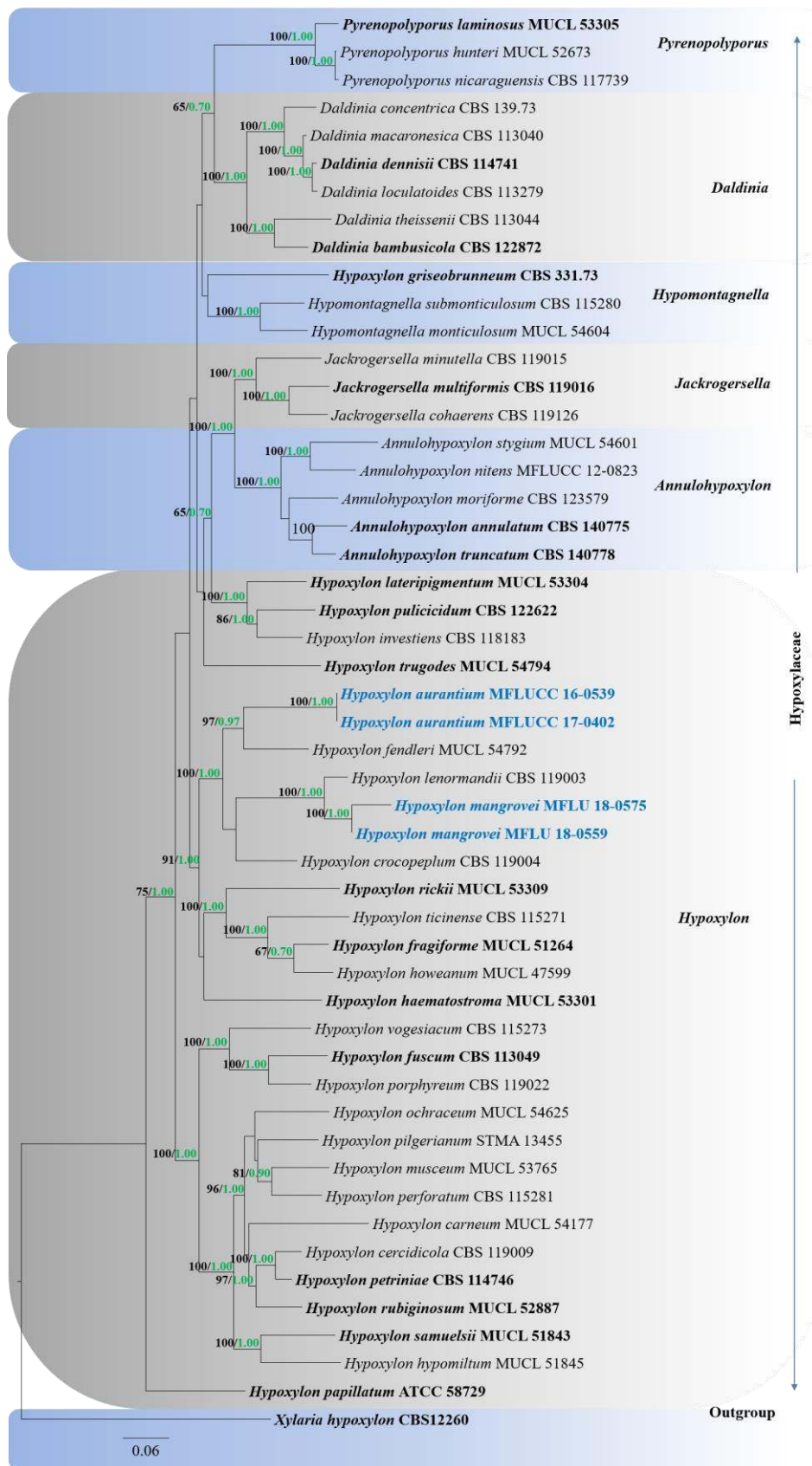
#### **Hypoxylaceae** DC., in Lamarck & de Candolle

Hypoxylaceae, is one of the most species-rich family of Ascomycota and has also been studied intensively for their wealth of biologically active secondary metabolites (Helaly et al. 2018). Polyphasic approaches by Daranagama et al. (2018) and Wendt et al. (2018), has resulted in a more reliable classification for Hypoxylaceae, however, some genera are still unresolved (Lambert et al. 2019). At present there are 20 genera in this family (Wijayawardene et al. 2018, De Long et al. 2019, Lambert et al. 2019).

#### **Hypoxylon** Bull.

The genus *Hypoxylon* is one of the largest genera within the family Hypoxylaceae with over 300 species (Wijayawardene et al. 2018). *Hypoxylon* species are cosmopolitan in distribution (Hyde et al. 2017). Most *Hypoxylon* species produce stromata and have been shown to produce high yields of secondary metabolites, which show characteristic colour reactions in potassium hydroxide solutions. This feature that is used to discriminate between species (Lambert et al. 2019). Analytical chromatographic methods (HPLC) to recognize the stromatal compounds and secondary metabolite profiles of *Hypoxylon* species provide more reliable evidence in species identification (Kuhnert et al. 2014). These chemical profiles are mostly species specific and important to validate the erection of new species (Cheng et al. 2015). This genus was revised by Ju & Rogers (1996), and later re-evaluate by Hsieh et al. (2005). This genus was last revised by Ju & Rogers (1996), and the first molecular phylogeny resulted in the segregation of the former sect. *Annulata* s. *Wendt* et al. (2018) accommodated this genus within Hypoxylaceae, which was resurrected and emended. *Wendt* et al. (2018) segregated *Jackrogersella* from *Annulohypoxylon* and also resurrected the genus *Pyrenopolyporus* for the species of *Hypoxylon* polyporus complex. *Hyponomagnella* was recently recognized as separate genus and contains species of the *Hypoxylon monticulosum* complex, but even the remainder of *Hypoxylon* appears rather heterogeneous and was divided into several clades in the recently published multi-locus genealogies. This suggests that *Hypoxylon* must eventually be subdivided furthermore, but this should only be undertaken once fresh material representing the numerous old species that had already been described in the 19<sup>th</sup> and early 20<sup>th</sup> century has been collected and sequenced. *Hypoxylon croceum* and *H. teeravasati* are the only

reports of *Hyoxylon* species from marine environment, which shows more species remain unexplored (Phookamsak et al. 2019).



**Figure 82** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -56278.895136. The combined LSU, ITS, RPB2 and BTUB sequence

datasets comprised 64 strains of *Hypoxylaceae* and *Xylaria hypoxylon* (CBS12260) as the outgroup taxa. Tree topology of the ML analysis was similar to the BI. The matrix had 2107 distinct alignment patterns, with 12.73% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.237999, C = 0.265221, G = 0.254759, T = 0.242022, substitution rates AC = 1.436086, AG = 4.571825, AT = 1.596379, CG = 1.014392, CT = 6.218983, GT = 1.000000; gamma distribution shape parameter  $\alpha$  = 0.268346. Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.06 changes. The ex-type strains are in bold and new isolates in blue bold.

***Hypoxylon aurantium*** Dayarathne, E.B. G. Jones & K.D. Hyde, sp. nov.

Fig. 83

Index Fungorum number: IF556597; Facesoffungi number: FoF 06189

Etymology – Epithet signifies the bright orange Ascostromata

*Saprobic* on wood. Sexual morph: *Ascostromata* 1.5–2 × 0.08–1 × 0.06–0.12 cm ( $\bar{x}$  = 1.8 × 0.085 × 0.07 cm, n = 5), effuse-pulvinate, with conspicuous perithecial mounds, surface bright orange; orange red granules immediately beneath surface and between ascostromata, with KOH extractable pigments orange, the tissue below the perithecial layer inconspicuous. *Ascostromata* 130–150 × 7–9.5  $\mu$ m, ( $\bar{x}$  = 145 × 8.2  $\mu$ m, n = 15), globose to sub globose, ostiolate. *Paraphyses* 2.5–3.8  $\mu$ m wide, hyaline, aseptate, unbranched. *Asci* 110–125 × 5.8–9  $\mu$ m, ( $\bar{x}$  = 118 × 6.2  $\mu$ m, n = 15), 8-spored, unitunicate, cylindrical, pedicellate, with an inamyloid ascal apical apparatus. *Ascospores* 12–14 × 7–9.5  $\mu$ m ( $\bar{x}$  = 12.4 × 6.5  $\mu$ m, n = 25), uniseriate, one-celled, ellipsoid, with narrowly rounded ends, initially light brown, becoming brown to dark brown, with a straight, germ slit spore-length, smooth or with inconspicuous coil-like ornamentation, epispore smooth. Asexual morph: Undetermined.

Culture characteristics – Colonies on MEA at 25–28 °C covering 15 cm diam. within 14 days, at first white, becoming greenish yellow, with diffuse margins, usually with vinaceous-buff to isabelline pigments diffusing beyond colonies; reverse amber to olivaceous.

Material examined – THAILAND, Krabi Province, Tha Pom Khlong Song Nam (N 08°12'50.4", E 98°46'42.7"), on submerged decaying mangrove wood, 16 December 2015, M. C. Dayarathne KLA009 (MFLU 16-1202, holotype); *ibid.*, (HKAS101986, isotype), ex-type living cultures MFLUCC 16-0539. THAILAND, Ranong Province, Amphoe Maung, Mu 4 Tombol Ngao, Ranong Mangrove Research Center (GPS: 9°43' to 9°57'N; 98°29' to 98°39'E) on submerged decaying wood of *Rhizophora apiculata* (Rhizophoraceae), 06 December 2016, M.C. Dayarathne, MCD 044 (MFLU 18-0531), living culture MFLUCC 17-0402

GenBank numbers – LSU: MN017878, MN017879; ITS: MN047114, MN047115; RPB2: MN077081

Additional GenBank numbers – SSU MN017936, MN017937

Notes – *Hypoxylon aurantium* is a distinctive *Hypoxylon* species in possessing a bright orange ascostromatal surface which is somewhat similar to *H. fendleri* (Vasilyeva et al. 2007b). Furthermore, *H. aurantium* formed a sister lineage to *H. fendleri* with high statistical support (100% ML, 1.00 PP) (Fig. 82). Nevertheless, *H. aurantium* has an inamyloid ascal apical apparatus while *H. fendleri* has amyloid ascal apical apparatus (Vasilyeva et al. 2007b). We also compared the base pair for ITS and BTUB gene loci accounts 52 out of 604 (84%) and 163 bp out of 1028 bp (15%) respectively. Furthermore, the occurrence of *H. aurantium* is on mangrove wood in Thailand while *H. fendleri* is a neotropical species. Hence, *H. aurantium* is warranted as a novel species within *Hypoxylon*.

***Hypoxylon mangrovei*** Dayarathne, E.B.G. Jones & K.D. Hyde, sp. nov.

Fig. 84

Index Fungorum number: IF556598; Facesoffungi number: FoF 06190

Etymology – Epithet refers to the mangrove host

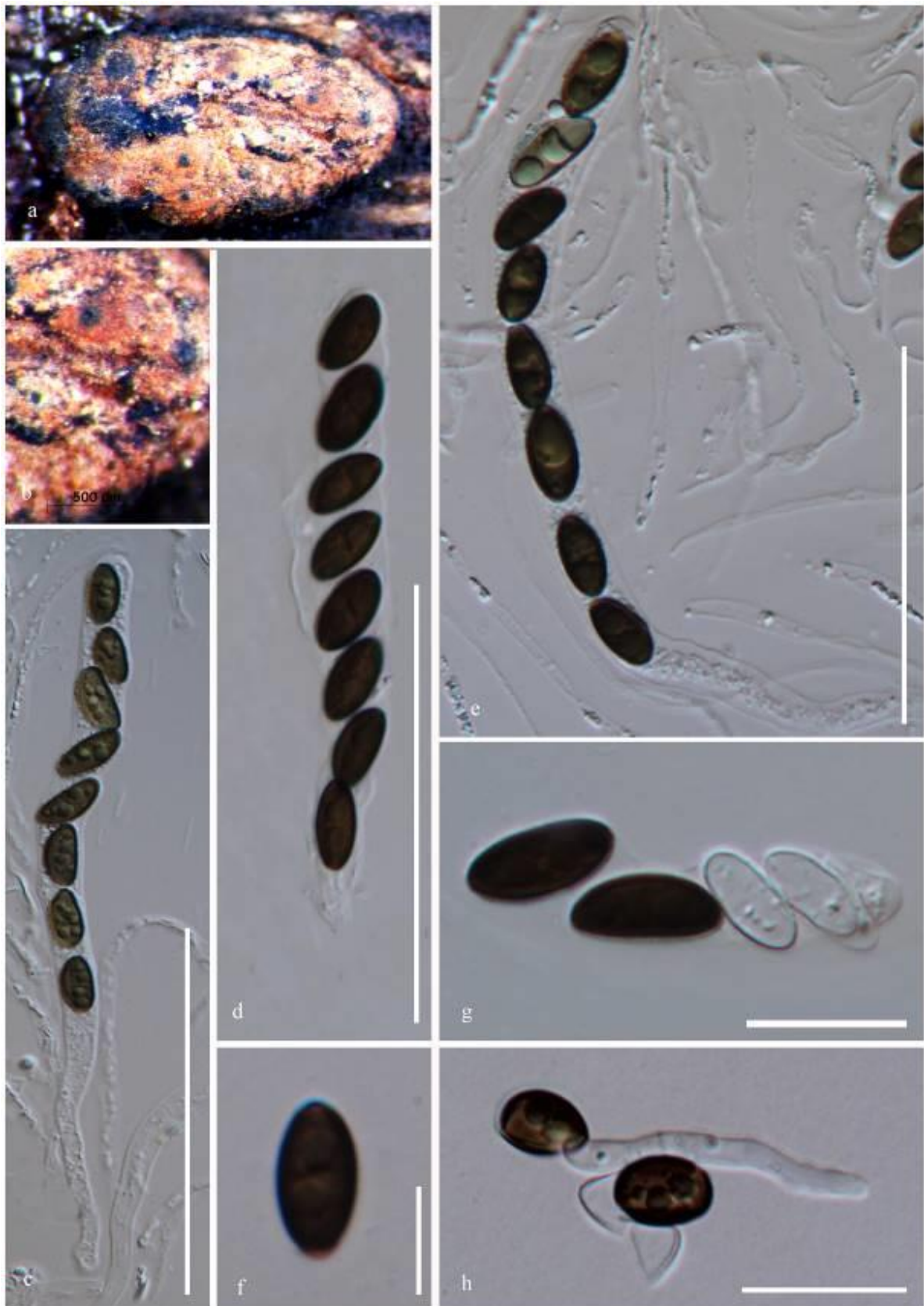
*Saprobic* on submerged mangrove wood. Sexual morph: *Ascostromata* 2–3.5 × 1.5–2.8 × 0.4–1 mm ( $\bar{x}$  = 3 × 2 × 0.8 mm, n = 15), glomerate, with inconspicuous perithecial mounds, surface dark vinaceous, dark brick or orange red granules immediately beneath surface and between

perithecia, with KOH extractable pigments orange, the tissue below the ascomata layer inconspicuous.



**Figure 83** – *Hypoxylon aurantium* (MFLU 16-1202, holotype). a, b Ascostromata in wood. c, d Horizontal section through ascostromata. e Paraphyses. f–i Asci. j–m Ascospores. n Germinating ascospore. o, p Cultures on MEA (o upper, p lower). Scale bars: a, b = 1 mm, c = 200  $\mu$ m, e–i = 50  $\mu$ m, j–m = 10  $\mu$ m.





**Figure 84** – *Hypoxylon mangrovei* (MFLU 18-0559 holotype). a Stromata in wood. b Surface of stromata with ostioles. c–e Asci and paraphyses. f, g Ascospores. h Mature ascospore with dehiscent perispore in KOH. Scale bars: b = 500  $\mu$ m, d = 100  $\mu$ m, c, e = 50  $\mu$ m, f–h = 10  $\mu$ m.

*Ascomata* 0.3–0.6 mm in diameter, perithecial, globose, ostiolate. *Paraphyses* 3–4.5  $\mu$ m, hyaline, aseptate, unbranched. *Asci* 130–150  $\times$  7–9.5  $\mu$ m, ( $\bar{x}$  = 145  $\times$  8.2  $\mu$ m, n = 15), 8-spored, unitunicate,

cylindrical, pedicellate, with a J- apical ring. *Ascospores* 12–14.5 × 5–6.5 μm, ( $\bar{x}$  = 12.8 × 5.8 μm, n = 30), uniseriate, one-celled, ellipsoid-inequilateral, with narrowly rounded ends, brown to dark brown, with sigmoid germ slit spore-length, smooth or with inconspicuous coil-like ornamentation, episporium smooth. Asexual morph: Undetermined.

Material examined – THAILAND, Prachau Kiri Khan Province, Pranburi, Sirinath Rajini center for mangrove forest studies on decaying wood of *Rhizophora* sp. (Rhizophoraceae), 02 December 2016, M.C. Dayarathne, MCD 085 (MFLU 18-0559 holotype); *ibid.*, (HKAS102092 isotype). THAILAND, Phetchaburi Province, Cha-am, on decaying submerged mangrove wood, 18 January 2018, M.C. Dayarathne, MCD 190 (MFLU 18-0575).

GenBank numbers – LSU: MN017880; ITS: MN047116, MN047117; BTUB: MN077053, MN077054

Additional GenBank number – SSU: MN017938

Notes – Strains of *H. mangrovei* formed a well-separated (100% ML, 1.00 PP) lineage which phylogenetically closely resembles *H. lenormandii* (Fig. 82). *Hypoxylon lenormandii* is different from *H. mangrovei* by the size of stromata (2–3.5 × 1.5–2.8 × 0.4–1 mm vs 13–85 × 6–27 × 0.5–1 mm) (Kuhnert et al. 2015). Considering the base pair differences of *H. mangrovei* and *H. lenormandii* for ITS, 27 bp out of 470 bp (5.7%) and BTUB 73 out of 594 (12.3%) and RPB2 data which warrants two clearly distinct taxa. *Hypoxylon mangrovei*, *H. fendleri*, *H. trugodes* and *H. crocopleum* share similar stromatal characters (Liu et al. 2015). However, *H. fendleri*, *H. trugodes* and *H. crocopleum* are distantly placed from *H. mangrovei* in our phylogram.

### **Xylariaceae** Tul. & C. Tul.

Tulasne & Tulasne (1963) introduced the family Xylariaceae with the term “Xylariei” and by then the family concept had not even been established (Stadler et al. 2013). The exact number of accepted genera of this family is doubtful while the recent outline of Ascomycota (Wijayawardene et al. 2018) listed 44 genera among the 38 genera are established by molecular data. The segregation of Xylariaceae into the subfamilies Hypoxyloideae and Xylarioideae has been supported by molecular data and several genera of Xylariaceae have transferred to various families i. e. Graphostromataceae and Lopadostomataceae (Tang et al. 2007, Stadler et al. 2013, Daranagama et al. 2015, Maharachchikumbura et al. 2015, 2016).

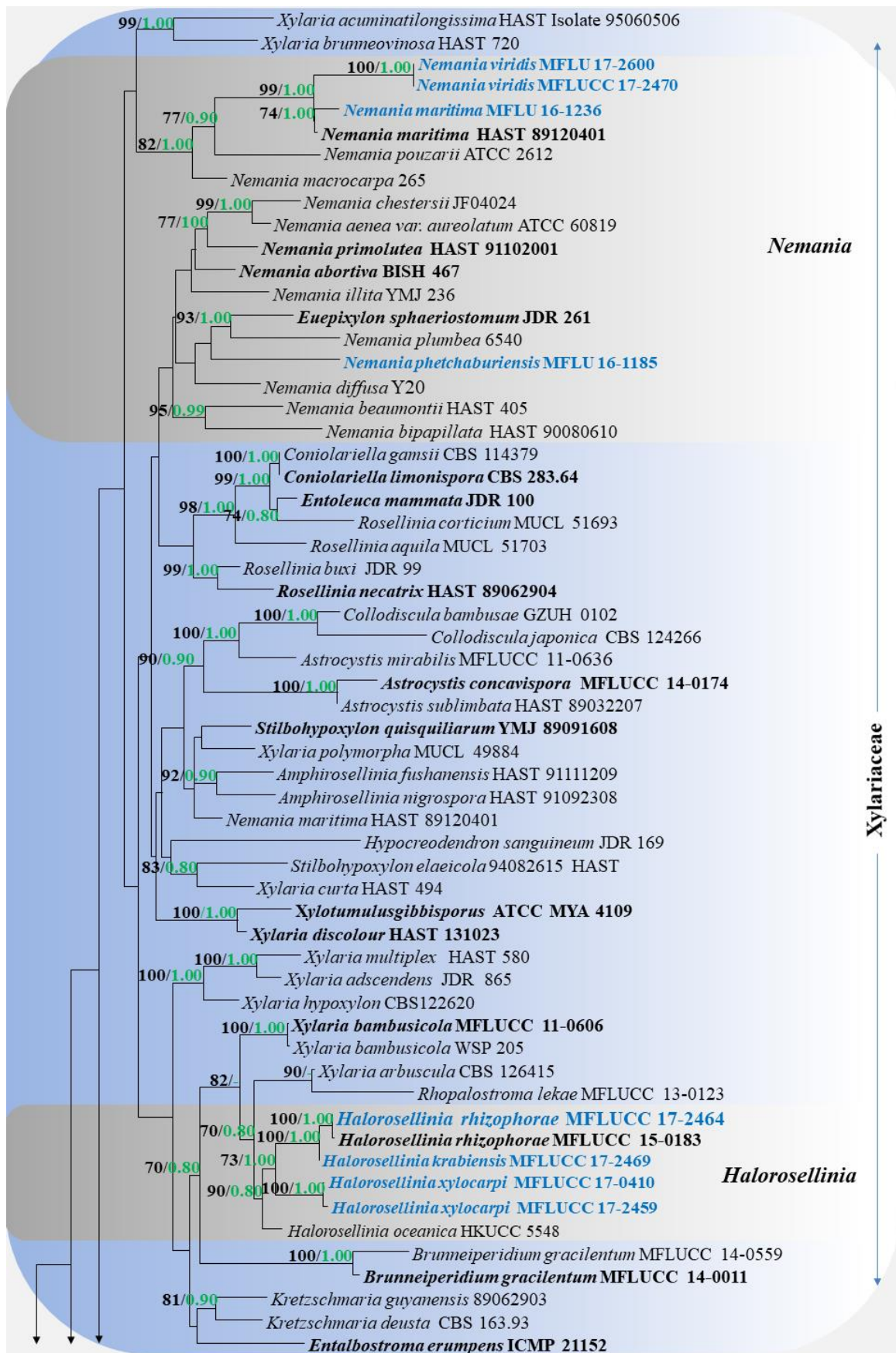
### **Halorosellinia** Whalley et al.

*Halorosellinia* was introduced by Whalley et al. (1999) as a monotypic genus to accommodate *Halorosellinia oceanica* (previously referred to as *Hypoxylon oceanicum*). The genus is characterized by uniperitheciate ascromata which are immersed in a pseudostroma (Hyde et al. 2016). *Halorosellinia* currently comprises three species (Hyde et al. 2016, Wijayawardene et al. 2018).

***Halorosellinia rhizophorae*** Dayar., E.B.G. Jones & K.D. Hyde, in Li et al., Fungal Diversity 78: 10.1007/s13225-016-0366-9, [117] (2016) Fig. 86

Facesoffungi number: FoF 01811

*Saprobic* on decaying wood of *Rhizophora* sp. attached to the plant. Sexual morph: *Pseudostromata* 1–2.5 × 0.9–1.5 mm ( $\bar{x}$  = 2 × 1.2 mm, n = 5), semi-immersed, pulvinate to hemispherical, in clusters of up to 20 uni peritheciate pseudostromata, surface black, carbonaceous, lacking ascromatal projections. In section pseudostromata comprises host cells, filled with amorphous black fungal material. *Ascromata* 345–360 × 90–100 μm ( $\bar{x}$  = 350 × 100 μm, n = 10), immersed in pseudostroma, subglobose to hemispherical, black, ostioles papillate. *Peridium* 25–38 μm wide, two-layered, outer layer of cells of *textura angularis*, black, fusing at the outside with the pseudostromata, inner layer of elongate cells, dark brown to black. Paraphyses 1– 3 μm wide, hyaline, abundant, persistent, aseptate. *Asci* 160–270 × 12–18 μm ( $\bar{x}$  = 220 × 15 μm, n= 20), overlapping, 8-spored, cylindrical, long pedicellate, unitunicate, with J+, rectangular apical ring.



**Figure 85** – Maximum likelihood analysis with 1000 bootstrap replicates yielded a best tree with the likelihood value of -56278.895136. The combined LSU, ITS BTUB and RPB2 sequence datasets comprised 114 strains of Xylariales and *Achaetomium macrosporum* (CBS 532.94), *Chaetomium elatum* (CBS374.66) and *Sordaria fimicola* (CBS 723.96) as the outgroup taxa. Tree

topology of the ML analysis was similar to the BI. The matrix had 2107 distinct alignment patterns, with 37.32% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.237999, C = 0.265221, G = 0.260159, T = 0.242022; substitution rates AC = 1.436086, AG = 4.571825, AT = 1.596379, CG = 0.254759, CT = 6.218983, GT = 1.000000; gamma distribution shape parameter  $\alpha = 0.268346$ . Maximum likelihood bootstrap (ML, black) values > 65% and Bayesian posterior probabilities (PP, green) > 0.90% are given above the nodes. The scale bar indicates 0.1 changes. The ex-type strains are in bold and new isolates in blue bold.

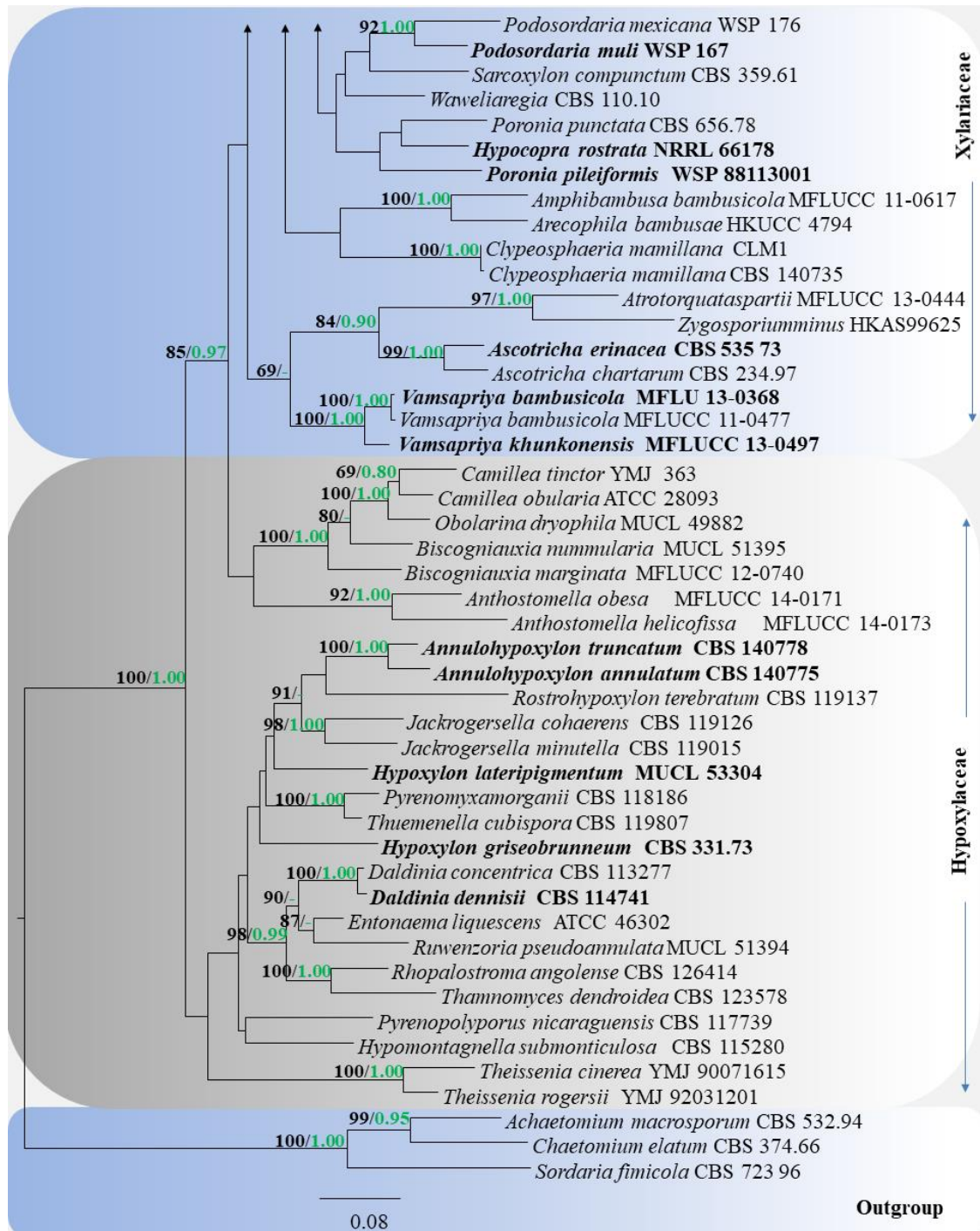
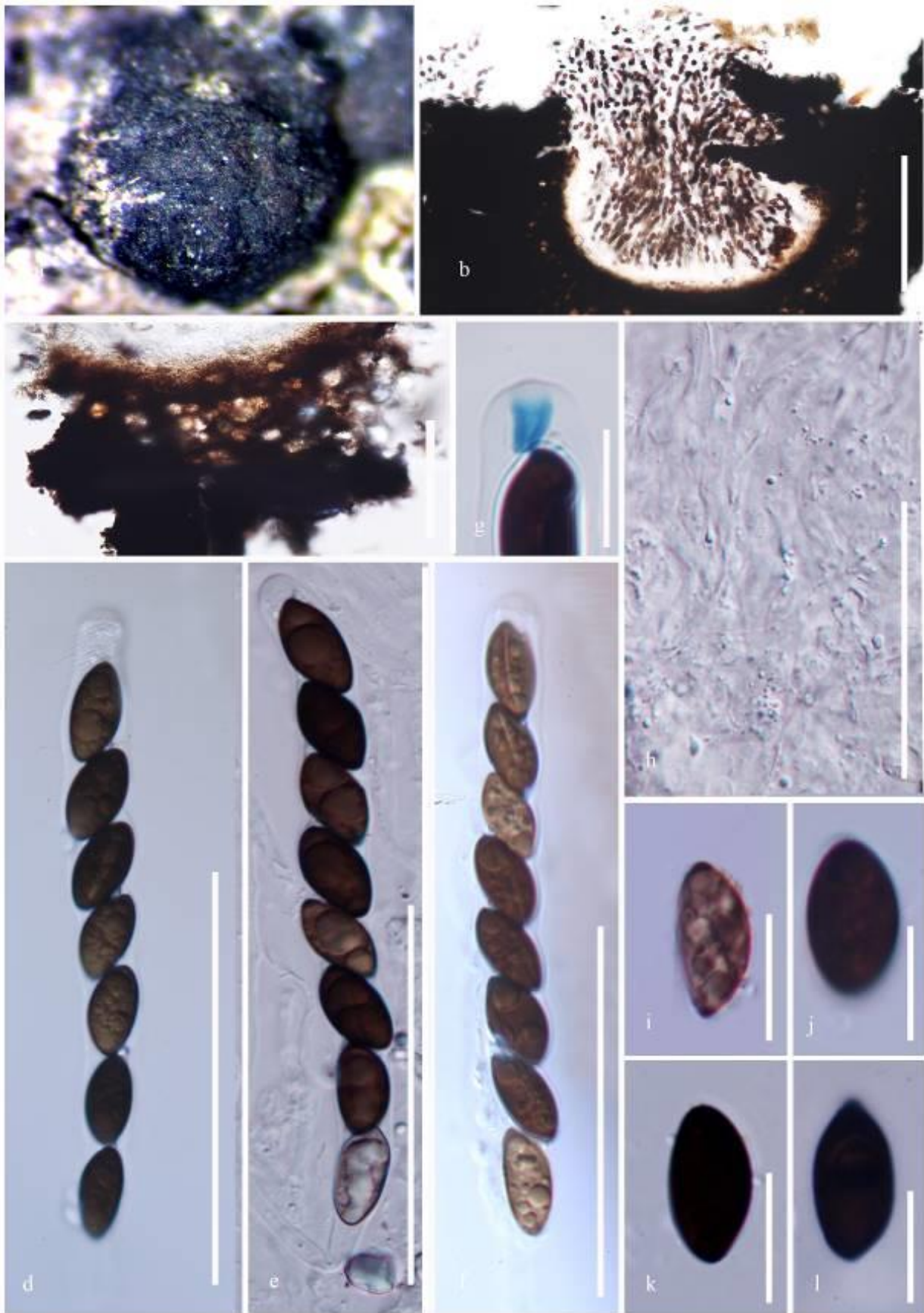


Figure 85 – Continued.



**Figure 86** – *Halorosellinia rhizophorae* (MFLU 17-2591). a Appearance of pseudostromata on host. b Vertical section through pseudostroma. c Peridium. d-f Asci. g Apical apparatus stained blue with Melzer's reagent. h Paraphyses. i-l Ascospores. Scale bars: b = 200  $\mu$ m, d-f = 100  $\mu$ m, c, d, h = 50  $\mu$ m, i-l = 20  $\mu$ m, g = 10  $\mu$ m.

*Ascospores* 20–35 × 10–15 µm ( $\bar{x}$  = 30 × 12 µm, n = 30) overlapping uniseriate, light brown when immature, dark to opaque brown when mature, more or less equilaterally ellipsoid, ventral side varying in degree of convex curvature, upper end broadly rounded, lower end slightly pointed, 1-celled, 1–2-guttulate, without appendages, germ slit on the ventral side, straight,  $\frac{3}{4}$  total length of spore. Asexual morph: Undetermined.

Culture characteristics – *Colonies* on PDA at 25–28 °C reaching 10 cm in 14 days, whitish colonies, zonate with diffuse margins, reverse at first whitish and turning light brown after 3–4 days.

Material examined – THAILAND, Krabi Province, Phang Nga, on mangrove sp. (Rhizophoraceae) wood, 31 August 2017, M.C. Dayarathne MCD 108 (MFLU 17-2591); *ibid.*, HKAS102036. Living culture MFLUCC 17-2464.

GenBank numbers – LSU: MN017882; ITS: MN047118; BTUB: MN431492

Additional GenBank number – SSU: MN017939

Notes – Our new strain (MFLU 17-2591) is morphologically similar to *Halorosellinia rhizophorae* which formed a sister group (Fig. 80). *Halorosellinia rhizophorae* differs from the type *H. oceanica* in lacking ascomatal projections (Whalley et al. 1999). This is the second collection of this species from Krabi, Thailand. Our earlier collection of this species was from submerged decaying wood while this collection is from an above ground decaying wood which was not inundated in marine water. BTUB sequence data for this species is provided for the first time.

***Halorosellinia krabiensis*** Dayarathne, & K.D. Hyde, sp. nov.

Fig. 87

Index Fungorum number: IF556599; Facesoffungi number: FoF 06191

Etymology – Name referring to the location, Krabi, Thailand where the species found.

Holotype – MFLU 17-2596

*Saprobic* on decaying mangrove wood submerged in marine habitats. Sexual morph: *Pseudostromata* 1.5–2 × 0.8–1.2 mm ( $\bar{x}$  = 1.8 × 1 mm, n = 5), semi-immersed, pulvinate to hemispherical to conical, in clusters of uni-peritheciate pseudostromata, surface black, carbonaceous, lacking ascomatal projections. In section pseudostromata comprises host cells, filled with amorphous black fungal material. *Ascomata* immersed in pseudostroma, subglobose to hemispherical, black, ostioles papillate. *Paraphyses* 1–2 µm wide, hyaline, abundant, persistent, aseptate. *Asci* 118–128 × 15–18.5 µm ( $\bar{x}$  = 126 × 16 µm, n = 20), 8-spored, cylindrical, long pedicellate, unitunicate, with J+, rectangular apical ring. *Ascospores* 14–18 × 8–10.5 µm ( $\bar{x}$  = 16 × 9.5 µm, n = 30) overlapping biseriate, light brown when immature, becoming dark brown when mature, more or less equilaterally ellipsoid, ventral side varying in degree of convex curvature, ends broadly pointed, lower end sometimes slightly rounded, 1-celled, 1–2-guttulate, without appendages, germ slit on the ventral side, straight. Asexual morph: Undetermined.

Culture characteristics – Colonies on PDA at 25–28 °C reaching 3 cm in 7 days, whitish, zonate with diffuse margins, reverse at first whitish and turning light brown after 3–4 days.

Material examined – THAILAND, Krabi Province, Phang Nga, on mangrove wood, 31 August 2017, M.C. Dayarathne MCD 114 (MFLU 17-2596, holotype); *ibid.*, (HKAS102035 isotype), ex-type living culture MFLUCC 17-2469.

GenBank numbers – LSU: MN017883; ITS: MN047119; BTUB: MN431493

Notes – *Halorosellinia krabiensis* shares distinctive features of *Halorosellinia* including a poorly developed pseudostromata which lack extractable pigments in KOH, asci with a relatively large apical apparatus that becomes dark blue in Melzer's reagent and ascospores with a prominent, straight germ slit on the ventral side (Whalley et al. 1999). *Halorosellinia krabiensis* is similar to *H. oceanica* and *H. rhizophorae* in ascospore morphology being one-celled, brown ascospores at maturity, more or less equilaterally ellipsoid with a germ slit at the ventral side (Whalley et al. 1999). The new species, is clearly different from the type *Halorosellinia oceanica* in lacking ascomatal projections and asci and ascospore measurements (Table 7). *Halorosellinia oceanica* was collected from *Rhizophora* in Florida, USA but it is a widely occurring mangrove species (Pang et al. 2011). *Halorosellinia krabiensis* has ascospores with pointed ends, and the lower end sometimes

slightly rounded while the ascospores of *H. rhizophorae* have broadly rounded upper ends, slightly pointed lower ends (Liu et al. 2015). Ascospore arrangement within the asci is the most distinguishable character of these two species with *H. krabiensis* having overlapping biseriate and *H. rhizophorae* in uniseriate ascospores. Phylogenetic analyses of combined ITS, LSU, BTUB, RPB2 data confirmed the placement of *H. krabiensis* within the family Xylariaceae and formed a sister clade to *H. rhizophorae* with high statistical support of 100% ML, 1.00 pp (Fig. 85). Base pair differences for ITS and BTUB of *H. krabiensis* and *H. rhizophorae* are 2 bp out of 597 bp (0.3%) and 16 bp out of 920 bp (1.7%) respectively.

***Halorosellinia xylocarpi*** Dayarathne & K.D. Hyde, sp. nov.

Fig. 88

Index Fungorum number: IF556600; Facesoffungi number: FoF 06192

Etymology – Name referring to the host genus *Xylocarpus*.

Holotype – MFLU 18-0545

*Saprobic* on submerged mangrove wood in marine habitats. Sexual morph: *Pseudostromata* 0.6–1.0 × 0.5–0.8 mm ( $\bar{x}$  = 0.8 × 0.6 mm, n = 5), semi-immersed, pulvinate to hemispherical, in clusters of uni-peritheciate pseudostromata, surface black, carbonaceous, lacking ascomatal projections. In section pseudostromata comprises host cells, filled with amorphous black fungal material. *Ascomata* 300–340 × 340–352  $\mu$ m ( $\bar{x}$  = 335 × 348  $\mu$ m, n = 5), immersed in pseudostroma, subglobose to hemispherical, black, ostioles papillate. *Peridium* 25–38  $\mu$ m wide, two-layered, outer layer of cells of *textura angularis*, black, fusing at the outside with the pseudostromata, inner layer of elongate cells, dark brown to black. *Paraphyses* 2–3  $\mu$ m wide, hyaline, abundant, persistent, aseptate. *Asci* 126–135 × 20–28  $\mu$ m ( $\bar{x}$  = 130 × 25  $\mu$ m, n = 20), 8-spored, cylindrical, long pedicellate, unitunicate, with J+, cylindrical apical ring. *Ascospores* 20–26 × 10–14  $\mu$ m ( $\bar{x}$  = 24 × 12  $\mu$ m, n = 30) overlapping bi-seriate, hyaline and becoming opaque green and dark brown when mature, more or less equilaterally ellipsoid, straight or ventral side varying in degree of convex curvature, both ends often pointed, sometimes lower end broadly rounded, 1-celled, 1–2-guttulate, without appendages, germ slit on the ventral side, straight, total length of spore. Asexual morph: Undetermined.

Culture characteristics – Colonies on PDA at 25–28 °C reaching 6 cm in 7 days, whitish, zonate with diffuse margins, reverse at first whitish and turning light brown after 3–4 days.

Material examined – THAILAND, Ranong Province, Ranong, on decaying, submerged *Xylocarpus* sp. (Meliaceae) wood, 07 December 2016, M.C. Dayarathne, MCD 62 (MFLU 18-0545, holotype); *ibid.*, (HKAS102084, isotype), ex-type living cultures (MFLUCC 17-0410). THAILAND, Krabi Province, Phang Nga, on decaying submerged wood of *Rhizophora* sp. (Rhizophoraceae), 31 August 2017, M.C. Dayarathne, MCD 100 (MFLU 17-2584); *ibid.*, (HKAS102028), living culture (MFLUCC 17-2459).

GenBank numbers – LSU: MN017884, MN017885; ITS: MN047120, MN047121; BTUB: MN077076

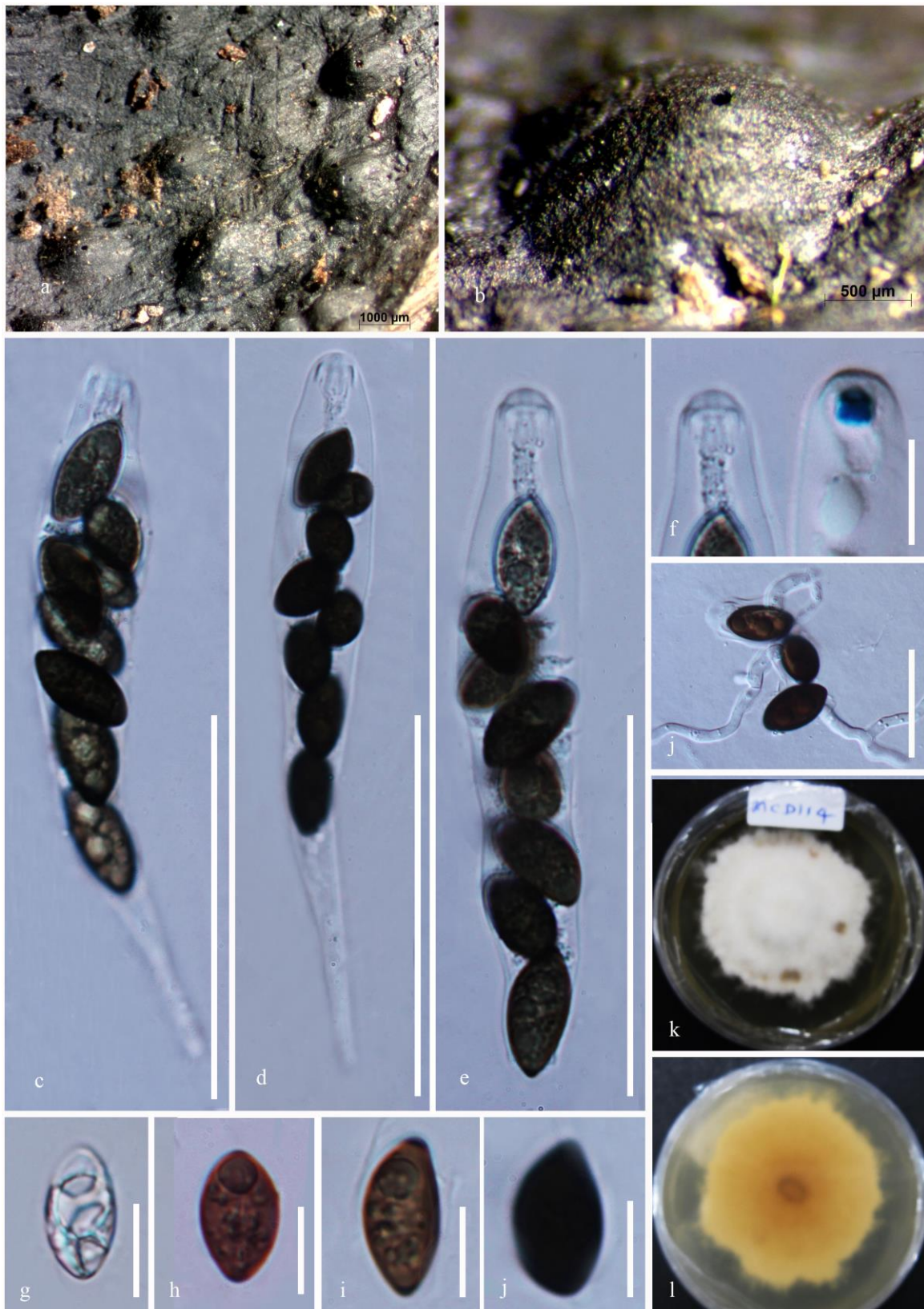
Additional GenBank numbers – SSU MN017940, MN017941

Notes – *Halorosellinia xylocarpi* is distinguishable for all the other *Halorosellinia* species by the unique opaque green colouration of the ascospores. *Halorosellinia xylocarpi* formed a well-supported lineage (100% ML, 1.00 PP) basal to a clade comprising strains *H. rhizophorae* and *H. krabiensis* (Fig. 85). Base pair differences of *H. xylocarpi* and *H. krabiensis* for ITS and BTUB are 68 bp out of 597 bp (11%) and 38 bp out of 938 bp (4%), respectively. In ITS locus 60 bp out of 593 bp (10%) are different among *H. xylocarpi* and *H. oceanica*. Pseudostromata, asci, ascospores and paraphyses measurements of all the *Halorosellinia* species are presented in Table 7.

### ***Nemania*** Gray

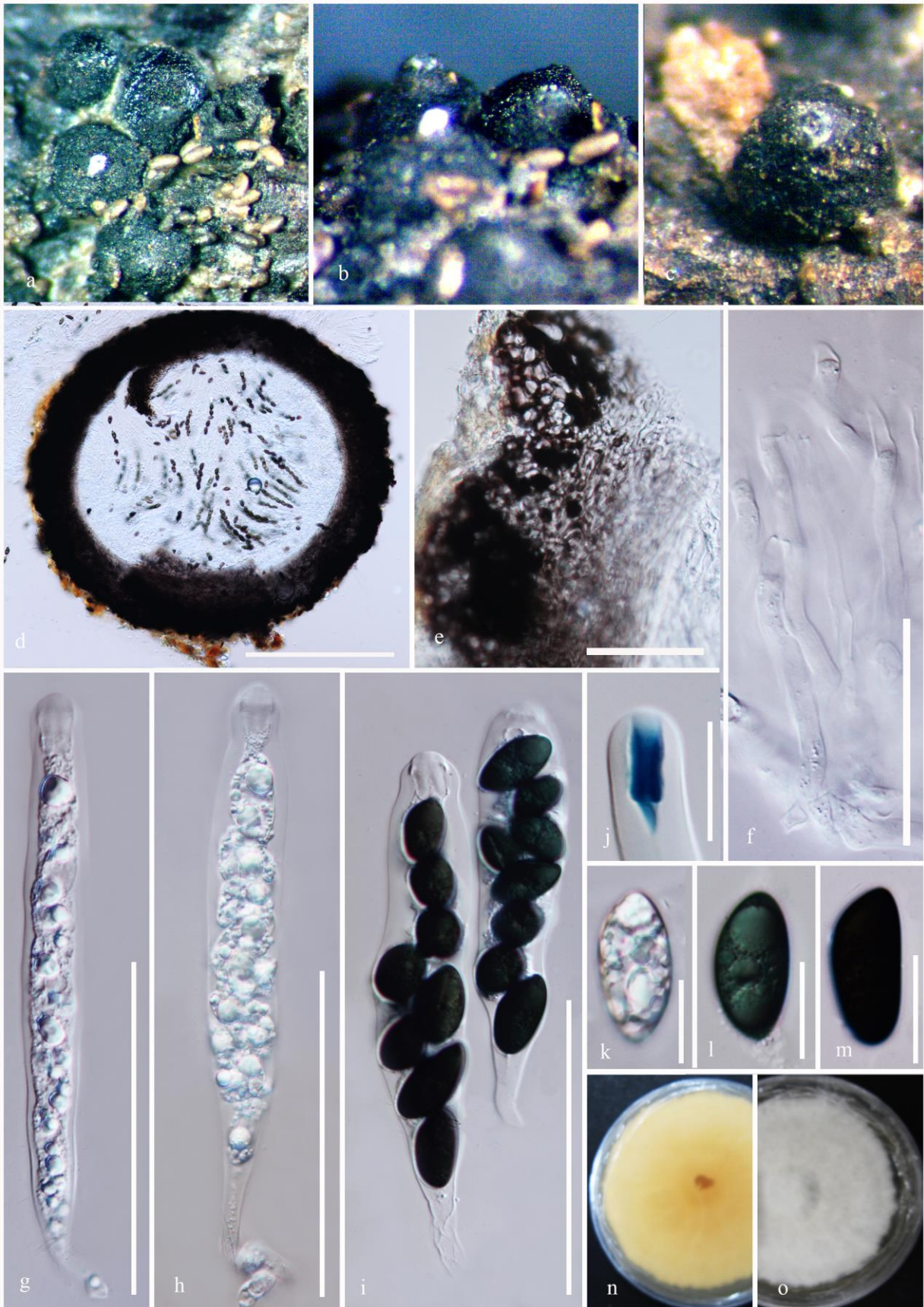
*Nemania* was erected for a heterogeneous assemblage of taxa by Gray (1821). Subsequently, *Nemania* was emended and reinstated by Pouzar (1985) to accommodate the *Hypoxylon selpens*-complex of *Hypoxylon* subsection *Prinocinerea* of Miller (1961). As emended by Pouzar (1985) *Nemania* species develop superficial stromata, lacking both a subiculum and bright pigments that

are soluble in KOH, and also possess a J+ or J-, rectangular apical apparatus that is higher than it is broad. The apical ring is more similar to those of *Kretzschmaria* and *Xylaria* than those of *Hypoxylon* (Pouzar 1985, Smith & Hyde 2001).



**Figure 87** – *Halorosellinia krabiensis* (MFLU 17-2596 holotype). a, b Appearance of pseudostromata on host. f Apical apparatus before and after staining blue with Melzer's reagent. c-e Asci. g-j Ascospores. Scale bars: b = 200 µm, c = 100 µm, d, e = 20 µm, f-i = 50 µm, j-m = 20 µm.





**Figure 88** – *Halorosellinia xylocarpi* (MFLU 18-0545, holotype). a, b Appearance of pseudostromata on host. b Horizontal section through pseudostroma. c Section through stromata. d Peridium. e Apical apparatus stained blue with Melzer's reagent. f-h Asci. I Paraphyses. j-m Ascospores. Scale bars: b = 200  $\mu$ m, c = 100  $\mu$ m, d, e = 20  $\mu$ m, f-i = 50  $\mu$ m, j-m = 20  $\mu$ m.

**Table 7** Morphological comparison of *Halorosellinia* species.

Characters	<i>H. oceanica</i>	<i>H. krabiensis</i>	<i>H. xylocarpi</i>	<i>H. rhizophorae</i>
<b>Pseudostromata (mm)</b>	0±4–0±8 in diam.	1.5–2 × 0.8–1.2	0.6–1.0 × 0.5–0.8	1.5–2.5 × 0.9–1
<b>Asci (µm)</b>	177–219	118–128 × 15–18.5	126–135 × 20–28	165–270
<b>Ascospores (µm)</b>	(17±9–)18±7–26(–28)–7±5–13(–13±5)	14–18 × 8–10.5	20–26 × 10–14	24–36 × 10–15
<b>Paraphyses (µm)</b>	2–2±5 wide	1–2 wide	2–3 wide	1–3 wide

*Nemania maritima* Y.M. Ju & J.D. Rogers, Nova Hedwigia 74(1-2): 102 (2002)

Fig. 89

Facesoffungi number: FoF 06193

*Saprobic* on submerged root of *Rhizophora* sp. Sexual morph: *Stromata* 0.4–0.6 mm diam., scattered, subglobose to globose, enclosing one to several perithecia, erumpent, with lower to middle part embedded in the substrate, surface dark brown, smooth; carbonaceous immediately beneath the surface and between the perithecia, tissue beneath the perithecial layer inconspicuous, ostioles obtusely conical, black. *Asci* 65–90 × 4–7 µm ( $\bar{x}$  = 70 × 6 µm, n = 20), short-stipitate, with apical ring bluing in Melzer's iodine reagent, cuneate. *Ascospores* 6.5–10 × 3–6 µm ( $\bar{x}$  = 7.8 × 4 µm, n = 30), uni-seriate, light brown to brown, unicellular, ellipsoidal, with broadly rounded ends, guttulate, with straight germ slit much less than spore-length. Asexual morph: Undetermined.

Material examined – THAILAND, Krabi Province, Krabi, 8°25'52" N, 98°31'42" E, 0 m asl., on submerged root of *Rhizophora* sp., 7 December 2014, M.C. Dayarathne, KRB 016 (MFLU 16-1236); *ibid.*, HKAS102022.

GenBank numbers – LSU: MN017886; ITS: MN047122

Notes – Our new strain (MFLU 16-1236) is morphologically similar to *N. maritima* with subglobose to globose stromata which enclose one to several perithecia, asci with a J- apical ring bluing in Melzer's iodine reagent and with light brown to brown, unicellular, ellipsoidal, guttulate ascospores with broadly rounded ends and straight germ slit shorter than its spore-length (Ju & Rogers 2002). Furthermore, strain (MFLU 16-1236) grouped together with *Nemania maritima* (JF 03075) in a well-supported clade (67% ML, 1.00 PP) (Fig. 85). This was reported from *Kandelia candel* while our collection is from *Rhizophora* sp.

*Nemania viridis* Dayarathne & K.D. Hyde, sp. nov.

Fig. 90

Index Fungorum number: IF556601; Facesoffungi number: FoF 06194

Etymology – Epithet derived from the Latin word for the characteristic opaque green ascospores.

Holotype – MFLU 17-2600

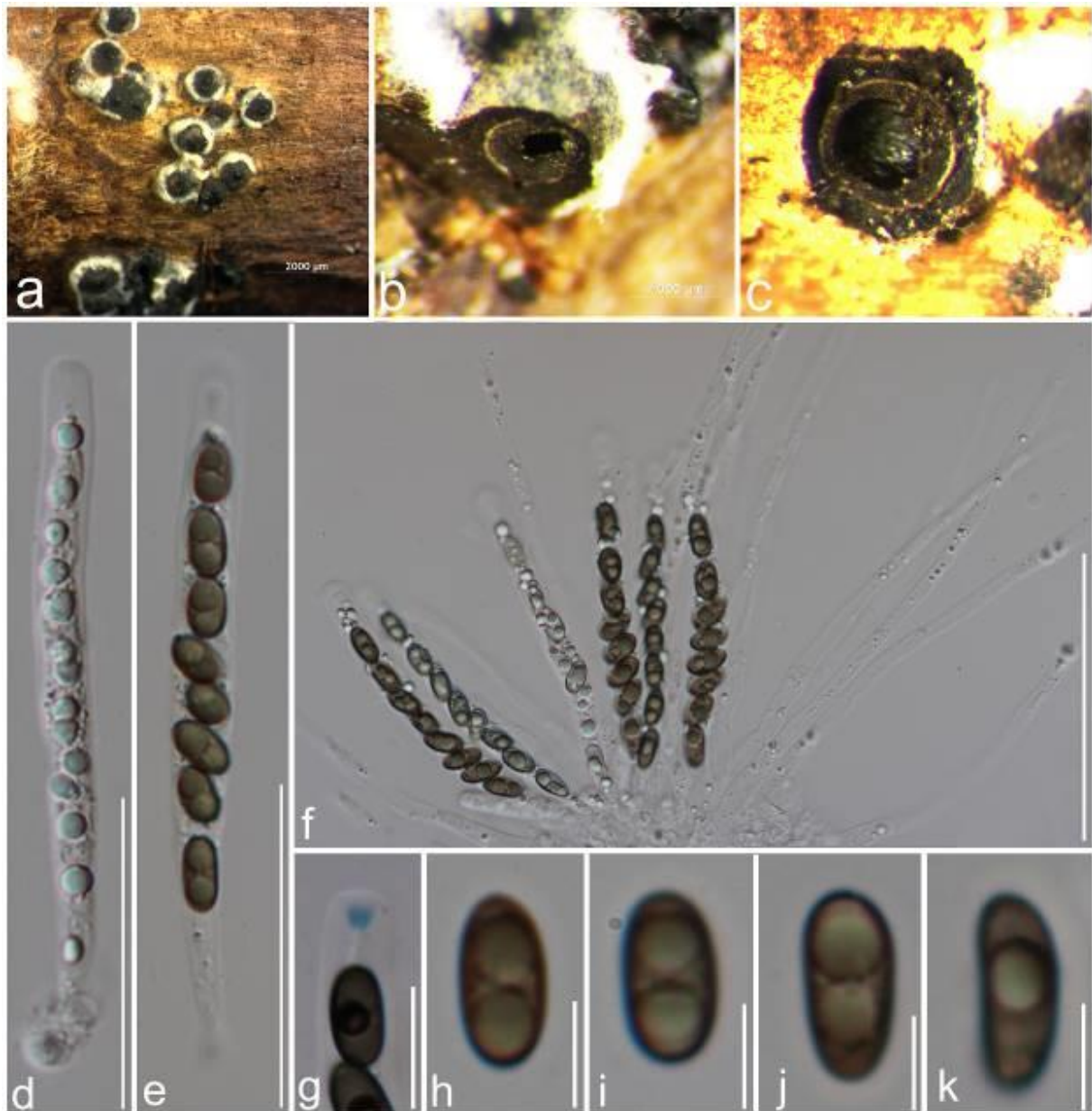
*Saprobic* on submerged wood of *Rhizophora* sp. (Rhizophoraceae). Sexual morph: *Stromata* 0.5–0.8 mm diam, pulvinate to effused-pulvinate, superficial, surface black, plane or with inconspicuous perithecial mounds, carbonaceous tissue immediately beneath the surface and between the perithecia; tissue beneath the perithecial layer inconspicuous to conspicuous, blackish brown. *Ascomata* spherical to globose, ostioles higher than the stromatal surface, with openings papillate to coarsely papillate, with surrounding area slightly flattened. *Peridium* 25–38 µm wide, two-layered, outer layer of cells of *textura angularis*, black, fusing at the outside with the pseudostromata, inner layer of elongate cells, dark brown to black. *Asci* 136–150 × 8–12 µm ( $\bar{x}$  = 147 × 10 µm, n = 20), broad, 8-spores, cylindrical, short pedicellate, unitunicate, with a J- apical ring. *Ascospores* 15–17 × 6–8 µm ( $\bar{x}$  = 16.8 × 6.8 µm, n = 30), uni-seriate, initially hyaline, becoming opaque green, brown when mature, unicellular, ellipsoid, with narrowly rounded ends, guttulate, with straight, conspicuous germ slit. Asexual morph: Undetermined.

Culture characteristics – Colonies on sea water PDA at 25–28 °C reaching 15 cm in 14 days, medium dense, circular, pulvinate, smooth, with entire margins, from about white, reverse at first whitish and turning yellow to light brown when mature.

Material examined – THAILAND, Phetchaburi Province, Cha-am, on submerged, decaying *Rhizophora* sp. (Rhizophoraceae) wood, 30 August 2017, M.C. Dayarathne, MCD 119 (MFLU 17-2600, holotype); *ibid.*, (HKAS102033, isotype), living culture MFLUCC 17-2470.

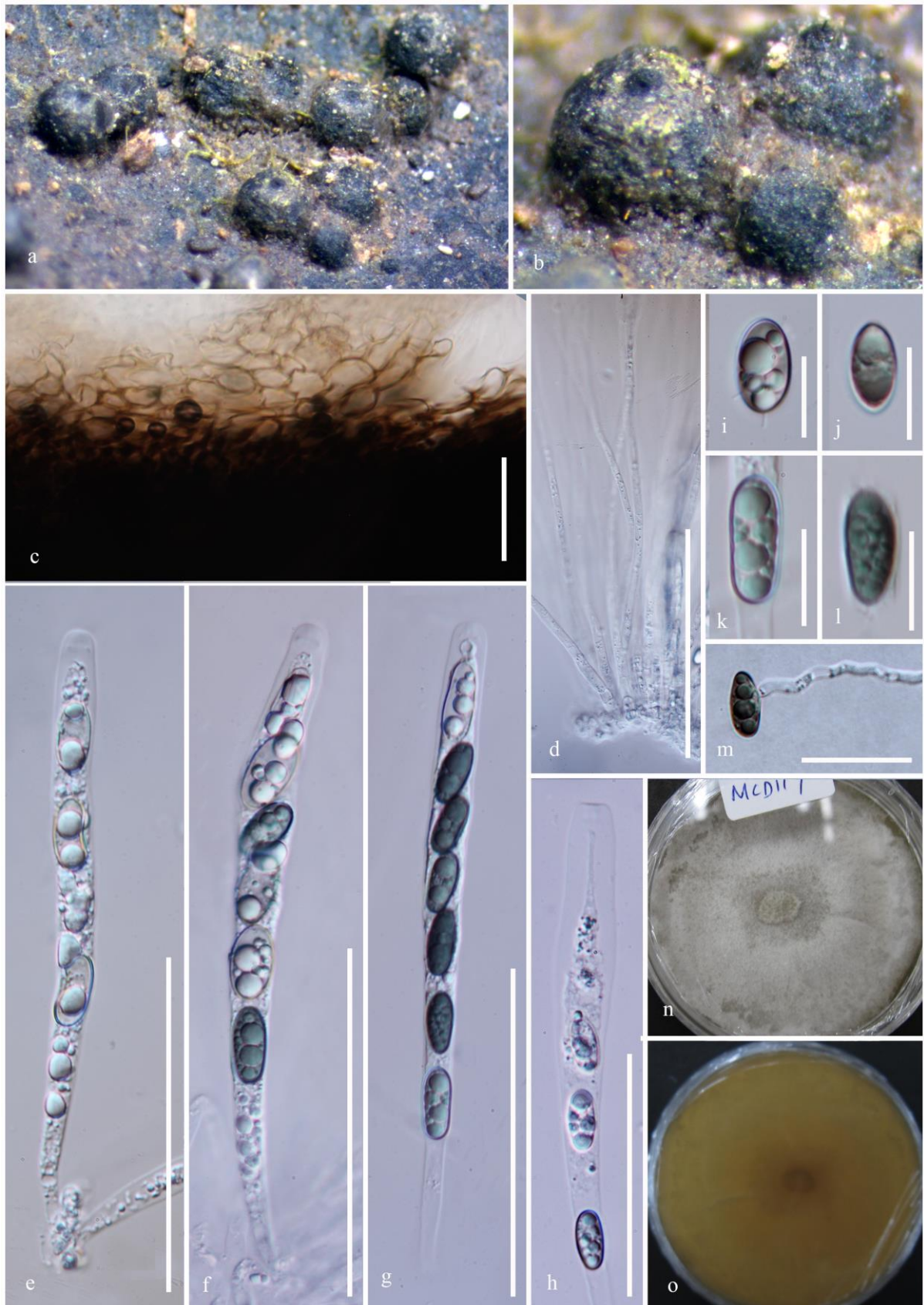
GenBank numbers – LSU: MN017887, ITS: MN047123

Notes – *Nemania viridis* shares morphological similarities with *N. maritima*. *Nemania viridis* formed a well separated distinct lineage from *N. maritima* with high statistical support (74% ML, 1.00 PP) (Fig. 85). Ascospores of both species are unicellular, ellipsoid, with narrowly rounded ends, guttulate, with a straight, conspicuous germ slit (Ju & Rogers 2002), however, *N. viridis* has characteristic opaque brown ascospores while *N. maritima* has brown ascospores. Additionally, ascospores of *N. viridis* are larger ( $136\text{--}150 \times 8\text{--}12 \mu\text{m}$  vs  $65\text{--}90 \times 4\text{--}7 \mu\text{m}$ ) and asci are longer than ( $15\text{--}17 \times 6\text{--}8 \mu\text{m}$  vs  $6.5\text{--}10 \times 3\text{--}6 \mu\text{m}$ ) that of *N. maritima*. We also compared the base pair differences of ITS gene regions of *N. viridis* and *N. maritima* (non-type) are accounted 48 bp out of 561 bp (8%).



**Figure 89** – *Nemania maritima* (MFLU 16-1236). a Stromata on host. b Vertically section of

ascostromata. c. Horizontally section of ascostromata. d–f Asci and paraphyses. g. Apical ring stained dark blue with Melzer’s reagent. h–k Ascospores. Scale bars: f = 100  $\mu$ m, d, e = 50  $\mu$ m, g = 10  $\mu$ m h–k = 5  $\mu$ m.



**Figure 90** – *Nemanium viridis* (MFLU 17-2600, holotype). a, b Appearance of pseudostromata on

host. c Peridium. d Paraphyses. e–h Asci i–l Ascospores. m Germinating ascospore. n, o Culture on sea water PDA (n upper, o lower). Scale bars: e–g = 100  $\mu\text{m}$ , d, h, m = 50  $\mu\text{m}$ , f–i = 50  $\mu\text{m}$ , c, i–l = 20  $\mu\text{m}$ .

*Nemania phetchaburiensis* Dayarathne, E.B.G. Jones. & K.D. Hyde, sp. nov.

Fig. 91

Index Fungorum number: IF556602; Facesoffungi number: FoF 06195

Etymology – name referring to the host genus *Rhizophora*.

Holotype – MFLU 16-1185

*Saprobic* on root pieces of *Rhizophora* sp. submerged in marine water. Sexual morph: *Ascstromata* 800–1600  $\times$  1300–2100  $\mu\text{m}$  ( $\bar{x}$  = 1200  $\times$  1700  $\mu\text{m}$ , n=5), semi immersed to superficial with base immersed pulvinate to hemispherical, in clusters of up to 20 uniperitheciate stromata, shiny, surface black, carbonaceous. In section, stromata comprising host cells filled with amorphous black fungal material. *Ascomata* immersed in stroma, subglobose to hemispherical, black, ostioles papillate. Peridium 80–135  $\mu\text{m}$  wide two-layered, outer layer of cells of *textura angularis* fusing at the outside with the pseudostromata, inner layer of lighter elongate cells. *Paraphyses* 2–3  $\mu\text{m}$  wide, filamentous, hyaline, septate, flexuous, branched, numerous and embedded in a gelatinous matrix. *Asci* 90–150  $\times$  12–13  $\mu\text{m}$  ( $\bar{x}$  = 120  $\times$  12.5  $\mu\text{m}$ , n=20), 6–8-spored, cylindrical, overlapping, short pedicellate, unitunicate, apical ring apically truncate with a 5  $\times$  2.3  $\mu\text{m}$  wedge-shaped, subapical ring which becomes dark blue in Melzer's reagent. *Ascospores* 14–16  $\times$  6–8  $\mu\text{m}$  ( $\bar{x}$  = 15  $\times$  6.5  $\mu\text{m}$ , n=20) overlapping uniseriate, light brown at immature stage, dark to greenish brown when mature, more or less inequilaterally ellipsoid, ventral side varying in degree of convex curvature, upper and lower ends slightly pointed, one-celled, 1–2 guttules, germination slit usually clearly seen on the ventral side, straight, conspicuous. Asexual morph: Undetermined.

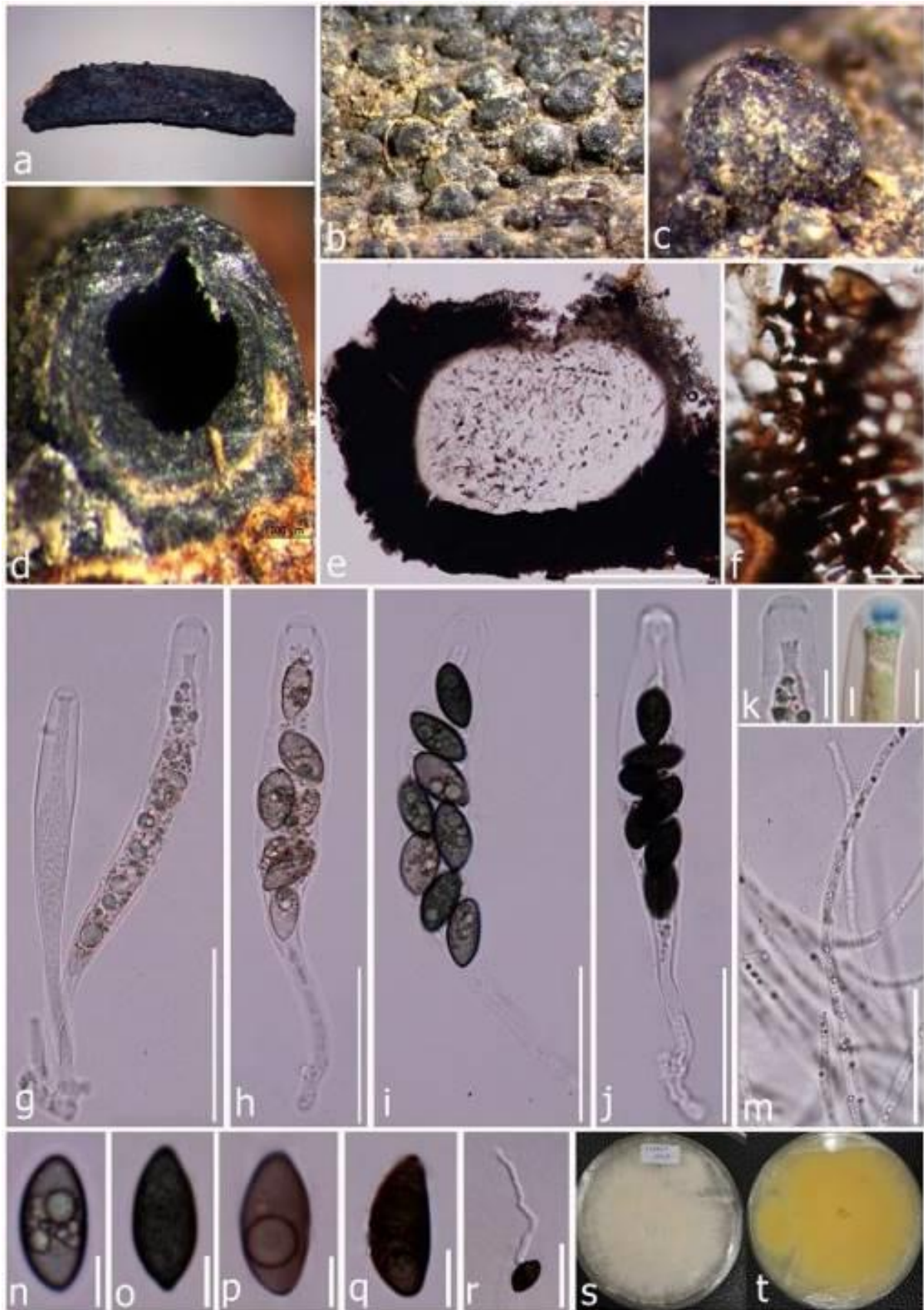
Culture characteristics – Colonies on sea water PDA at 25–28  $^{\circ}\text{C}$  reaching 5 cm in 7 days, whitish colonies, zonate with diffuse margins, reverse at first whitish and turning yellow to light brown after 3–4 days.

Material examined – THAILAND, Phetchaburi Province, Hat Chao Samran, 47 $^{\circ}$  72506' E, 40 $^{\circ}$  25038' N, 0 m asl., on intertidal decayed wood of *Rhizophora* sp. at a mangrove stand, 28 August 2015, M.C. Dayarathne, CHAM007 (MFLU 16-1185, holotype); *ibid.*, (HKAS 97420, isotype), ex-type living culture MFLUCC 15-0953.

GenBank numbers – LSU: MF615402; ITS: MN047124

Additional GenBank number – SSU: MN017943

Notes – *Nemania phetchaburiensis* is characterized by having pulvinate to hemispherical stromata in clusters of up to 20 uniperitheciate stroma and 1–2 guttulate, inequilaterally ellipsoid ascospores with the ventral side varying in degree of convex curvature and with a clearly seen germination slit usually on the ventral side. *Nemania phetchaburiensis* grouped closely to *N. diffusa* (isolate NC1333) and *N. plumbea* (JF TH 04-01), but with weak statistical support (Fig. 89). Morphologically *N. phetchaburiensis* and *N. diffusa* have effused-pulvinate ascostromata with spherical to obovoid perithecia (Ju & Rogers 2002). They can be distinguished by ascospore size (14–16  $\times$  6–8  $\mu\text{m}$  vs 9.5–13.5  $\times$  5–6  $\mu\text{m}$ ) (Ju & Rogers 2002). Furthermore, 98 bp of ITS locus are different out of 645 bp (15%) between *N. phetchaburiensis* and *N. diffusa*. *Nemania phetchaburiensis* and *N. plumbea* also have effused-pulvinate ascostromata like in *N. phetchaburiensis*, but, ascomata of *N. plumbea* are spherical to ellipsoid, while *N. phetchaburiensis* has subglobose to hemispherical ascomata (Tang et al. 2007). There are 102 base pair difference of ITS locus out of 642 bp (15.2%) of these two species. However, further collections of this novel taxon are required as no DNA sequence data from protein coding genes are available for this species.



**Figure 91** – *Nemaniam phetchaburiensis* (MFLU 16-1185, holotype). a, b, c Stromata on host. d, e Section through stroma. f Peridium. g–j Asci. k Apical ring without staining. l. Apical ring stained dark blue with Melzer’s reagent. m Paraphyses. n–q Ascospores. r Germinating ascospore. s, t Cultures on PDA (s from above, t from below). Scale bars: b, d = 1000  $\mu\text{m}$ , e = 500  $\mu\text{m}$ , h–j = 50  $\mu\text{m}$ , g, m, r = 20  $\mu\text{m}$ , f = 10  $\mu\text{m}$ , n–q = 5  $\mu\text{m}$ .

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