



Morphology and multigene phylogeny reveal ten novel taxa in Ascomycota from terrestrial palm substrates (Arecaceae) in Thailand

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Abstract

Arecaceae is one of the important plant families in Thailand, and several of its representative genera are used for food, cosmetics, and energy sources. During the past eight years (2014 to 2022), more than 50 new taxa associated with terrestrial palm substrates have been reported in Thailand. The present study continued the survey of fungi associated with terrestrial palms in Thailand and based on both morphological characteristics and phylogenetic analyses of combined sequence data, fungal taxa belonging to the orders Amphisphaeriales, Diaporthales, Distoseptisporales, Glomerellales, Pleosporales, and Xylariales were identified. A new genus *Triseptatospora* and nine new species viz., *Acremoniisimulans cocois*, *Acrocalymma arengae*, *Bartalinia adonidiae*, *Cytospora calamicola*, *Distoseptispora licualae*, *Neopestalotiopsis elaeidis*, *Pteridiospora arengae*, *Triseptatospora calami*, and *Xenoanthostomella calami* are described and introduced here. Morphological illustrations, descriptions, and phylogenetic trees which indicate the placement of the new taxa are provided.

Keywords – 10 new taxa – Dothideomycetes – palm fungi – phylogeny – saprobe – Sordariomycetes – taxonomy – Thai fungi

Introduction

Palms (Arecaceae) are considered a major family of angiosperms (flowering plants) and are distributed worldwide, especially in tropical and sub-tropical regions. Palms are more diverse than other monocotyledonous families, perhaps more than any other family of seed plants (Dransfield et al. 2008). The palm family comprises more than 200 genera worldwide, and 32 of them are known to occur in Thailand (The Plant List 2013, Palms of Thailand 2021). The common and widespread

species are *Adonidia merrillii*, *Arenga pinnata*, *Calamus* sp., *Cocos nucifera*, *Elaeis guineensis*, and *Licuala glabra* (Arecaceae), which are particularly common in southern Thailand (Palms of Thailand 2021). The palm family members appeared in the fossil records of 110–120 Mya in the Middle Cretaceous (Friis et al. 2004). In Thailand, all palm genera had evolved during the Paleogene-Neogene period (Chandler 1957, 1962, Gregor & Hagns 1982, Kar 1985, Schrank 1994, Ergo 1996, Pan et al. 2006, Dransfield et al. 2008, Berggren 2020). *Arenga* is estimated to have evolved in the lower Eocene epoch (Kar 1985, Dransfield et al. 2008); *Calamus*, estimated at 63–73 Mya in the Cretaceous-Paleogene period (Chandler 1957, 1962, Schrank 1994, Pan et al. 2006); *Cocos*, estimated in the lower Oligocene epoch (Dransfield et al. 2008); *Elaeis*, estimated at 6 Mya in the late Miocene epoch (Ergo 1996, Pan et al. 2006); and *Licuala*, estimated at 62–65 Mya in the early Palaeocene epoch (Danian) (Gregor & Hagns 1982, Pan et al. 2006).

Microfungi associated with palms have been in the focus over a very long time, but the studies attracted more attention after the publication of Hyde (1988a). To date, more than 100 genera of palms have been studied for their associated microfungi (Farr & Rossman 2021). Several studies on palm-associated fungi have focused on saprobic life modes found in terrestrial, extreme (peat swamp), freshwater, and marine habitats (Hyde 1988a, Hyde et al. 2000, Pinnoi et al. 2006, 2009, Pilantanapak et al. 2005, Pinruan et al. 2007, Konta et al. 2016a, c, 2020a, 2021a, Mapook et al. 2020, Zhang et al. 2020, Tian et al. 2022). A few have focused on endophytic life modes involving living tissues (Taylor et al. 1999, Fröhlich et al. 2000, Lumyong et al. 2009, Pinruan et al. 2010a, Wikee et al. 2013, Saengket et al. 2021). In recent times, studies of plant-pathogen life modes associated with palms have been reported (Chou et al. 2019, El Meleigi et al. 2019, Marin-Felix et al. 2019, Kinge et al. 2019). The fungi on palms include numerous ascomycetes taxa, especially in *Cocos* and *Calamus* (Fröhlich & Hyde 2000, Pinnoi et al. 2009). Species in Amphisphaeriaceae, Oxydothidaceae (Amphisphaeriales), Hypocreaceae (Hypocreales), Meliolaceae (Meliolales), Mycosphaerellaceae (Mycosphaerellales), Phyllachoraceae (Phyllachorales), and Xylariaceae (Xylariales) are diverse on palms (Fröhlich & Hyde 2000).

In Thailand, studies on palm fungi have been carried out by outside researchers, e.g., Spaulding (1961), followed by Watson (1971), Giatgong (1980), and Hyde (1988b). Studies of microfungi associated with palms by Thai together with international mycologists have revealed quite a few novel and interesting taxa (Pinnoi et al. 2006, 2009, Pinruan et al. 2007, 2010a, Hidayat et al. 2006, To-anun et al. 2009, 2011, Liu et al. 2010, 2012, 2015, Phengsintham et al. 2013, Trakunyingcharoen et al. 2015, Konta et al. 2016b, 2017, 2020b, c, 2021b, Mapook et al. 2016, Tibpromma et al. 2018, Doilom et al. 2018, Wanasinghe et al. 2018, Phookamsak et al. 2019, Zhang et al. 2018). Pinruan et al. (2004a, b) used both morphology and DNA sequence data for the first time, to identify microfungi associated with palms in Thailand, whereas earlier studies used only morphological characteristics. The highest numbers of microfungi have been reported mainly on three palm species in Thailand viz., *Eleiodoxa conferta*, *Licuala longicalycata*, and *Nypa fruticans* (Giatgong 1980, Hyde & Sutton 1992, Pinruan et al. 2002, 2004a, b, 2007, 2008, 2010b, Pinnoi et al. 2003a, b, 2004, 2006, 2010, Pilantanapak et al. 2005, Liu et al. 2015, Hyde et al. 2017, 2019, Zhang et al. 2018, 2019, Jayasiri et al. 2019).

The substrates of Arecaceae have been reported to harbor a high fungal diversity in Thailand, especially those belonging to the Ascomycota. The members of the Sordariomycetes are the dominant group with 232 taxa, followed by Dothideomycetes (162 taxa), Ascomycetes genera *incertae sedis* (15 taxa), Leotiomycetes (17 taxa), Eurotiomycetes (seven taxa), Orbiliomycetes (six taxa), Lecanoromycetes (two taxa), and only a few taxa in the Basidiomycota (two taxa from Agaricomycetes and one taxon from the Exobasidiomycetes) (Pinruan et al. 2002, 2004a, b, c, d, 2007, 2008, 2010a, b, Pinnoi et al. 2003a, b, 2004, 2006, 2007, 2009, 2010, Liu et al. 2010, 2012, 2015, Phookamsak et al. 2015, 2019, Trakunyingcharoen et al. 2015, Hyde et al. 2016, 2017, 2019, 2020a, b, Konta et al. 2016a, b, c, 2017, 2020a, b, c, 2021a, b, Kumla et al. 2016, Zhang et al. 2018, 2019, 2020, Jayasiri et al. 2019, Chen et al. 2020, Saengket et al. 2021, Tian et al. 2022). In addition, Pleosporales and Xylariales are the best-represented orders in Dothideomycetes and Sordariomycetes, respectively.

In this study, we surveyed palm specimens from terrestrial ecosystems in the northern, western, and southern parts of Thailand. Based on both morphology and multigene phylogeny, we herein introduce one new genus, three new species in the Pleosporales; two new species in the Amphisphaeriales; one new species in each of the orders of Diaporthales, Distoseptisporales, Glomerellales, and Xylariales. Full descriptions, color images of macro- and micro-morphological characteristics, and phylogenetic trees to indicate the placement of new taxa are provided.

Materials & Methods

Isolates and morphology

Sample collection, morphological study, and isolation

Fresh materials were collected from several provinces in Thailand, during 2014–2018. Saprobe isolates included in this study were obtained from five parts of diverse hosts and identified as species of palm microfungi, based primarily on the presence of fruiting bodies (ascomata, conidiomata), asci, conidiophores, ascospores, and conidia. The method given in Senanayake et al. (2020) was used to carry out single spore isolation and to obtain pure cultures on MEA (malt extract agar). Isolations were established from a single ascospore or conidium; contents of the sectioned ascomata/conidiomata were transferred to a drop of sterile water on a flame-sterilized slide. Drops of the spore suspension were spread on a Petri-dish containing MEA and incubated at 25–28 °C overnight, or for more than 2 or 3 days, depending on the group of each fungus. Descriptions of culture characteristics were recorded after the transfer of germinated ascospores/conidia to new MEA media and incubated at 25–28 °C. All isolates were checked for sporulation on the culture medium after incubating for two months at 25–28 °C.

The fungal structures were examined with a Motic SMZ 168 series stereomicroscope and photographed with an Axio camera on a Zeiss Discover V8 stereomicroscope. Micromorphological structures were photographed by means of light microscopy, using a Canon 600D camera on a Nikon ECLIPSE 80i microscope. Distilled water, lactic acid, and/or lacto glycerol were used as mounting slide solutions, and Melzer's reagent was used to stain the apical ring structure in Sordariomycetes. Fungal structures were measured by Image Frame Work (IFW) version 0.9.7. Photo plates were made by Adobe Photoshop CS6. The herbarium specimens and living cultures were deposited in the Mae Fah Luang University herbarium (MFLU) and culture collection (MFLUCC), respectively. Faces of Fungi and Index Fungorum numbers were registered as outlined in Jayasiri et al. (2015) and Index Fungorum (2022).

DNA extraction, amplification, and sequencing

The genomic DNA of fungal mycelium was extracted using the Biospin Fungus Genomic DNA extraction Kit (BioFlux, P.R. China) and the genomic DNA of fungal fruiting bodies was extracted using the E.Z.N.A.® Forensic DNA kit (OMEGA Bio-Tek, P.R. China) following the manufacturer's protocols. The partial nuclear genes were subjected to PCR amplification and sequencing: 28S nrRNA gene (LSU), internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS) of the nrDNA operon, β -tubulin (TUB), translation elongation factor 1-alpha (TEF1), 18S ribosomal RNA (SSU), and RNA polymerase II second largest subunit (RPB2) using the primers listed in Table 1. The total volume of PCR mixtures for amplification was 25 μ l containing 8.5 μ l ddH₂O, 12.5 μ l 2 \times Easy Taq PCR Super Mix (mixture of Easy Taq™ DNA Polymerase, dNTPs and optimized buffer (Beijing Trans Gen Biotech Co., Chaoyang District, Beijing, PR China), 2 μ l of DNA template, and 1 μ l each of forward and reverse primers (10 pM). The quality of PCR products was checked on a 1% agarose gel electrophoresis stained with 4S green nucleic acid (Life Science Products & Services, Cat. No: A616694). Purification and sequencing of PCR products were carried out by Sangon Biotech Co., Shanghai, China. The resulting fragments were sequenced in both directions. The DNA sequences generated were analysed and consensus sequences were computed using SeqMan (DNASTAR).

Table 1 Partial gene regions and primers used in this study.

Gene/loci	PCR primers (forward/reverse)	References
LSU	LR0R/LR5	Vilgalys & Hester (1990)
ITS	ITS5/ITS4	White et al. (1990)
RPB2	fRPB2-5f/fRPB2-7cR	Liu et al. (1999)
SSU	NS1/NS4	White et al. (1990)
TEF1	983F/2218R	Rehner (2001)
TUB	T1/T22	O'Donnell & Cigelnik (1997)

Sequence alignment and phylogenetic analyses

The sequence data generated during this study were subjected to BLAST searches to identify closely related sequences available in the NCBI GenBank nucleotide database ([www http://blast.ncbi.nlm.nih.gov/](http://blast.ncbi.nlm.nih.gov/)). Sequence data were retrieved from GenBank based on recent publications. Raw forward and reverse sequences were assembled using SeqMan. Sequence alignments were carried out with MAFFT v.6.864b (Katoh & Standley 2013) and the alignments were manually improved where necessary. The sequence datasets were combined using Mega7 (Kumar et al. 2016). Maximum likelihood tree analyses with 1,000 bootstrap replicates were generated in the CIPRES Science Gateway platform (Miller et al. 2010) using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008, Stamatakis 2014) and GTR+I+G model of evolution. Bayesian analyses were conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronqvist 2001) to evaluate posterior probabilities (BYPP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). Two independent runs of six simultaneous Markov chains were run for 1M to 50M generations, depending on individual settings for the fungal group, and were sampled every 100th generation. The first 25% of the trees, representing the burn-in phase of the analyses, were discarded, while the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree (Cai et al. 2006). Convergence was determined when the average standard deviation of split frequencies reached less than 0.01. The phylogenetic trees were configured in FigureTree v1.4.0 (Rambaut 2012) and edited using Microsoft Office PowerPoint 2016 and Adobe Illustrator CS6 (Adobe Systems Inc., USA). The bootstrap support values for ML equal to or greater than 60% and BYPP equal to or greater than 0.95 are given above the nodes. All novel sequences generated in this study were deposited with the GenBank.

Results

The decisions to introduce new species follow the guidelines of Chethana et al. (2021), Pem et al. 2021, and Maharachchikumbura et al. (2021).

Dothideomycetes O.E. Erikss. & Winka 1997

Pleosporales Luttr. ex M.E. Barr 1987

Notes – Pleosporales is highly diverse and the largest order of Dothideomycetes. They contain numerous ecologically, and economically significant taxa, which are distributed worldwide (Hongsanan et al. 2020). Pleosporales is comprised of 91 families (Hongsanan et al. 2020, Wijayawardene et al. 2017, 2022).

Acrocalymmaceae Crous & Trakun. 2014

Notes – Alcorn & Irwin (1987) introduced *Acrocalymma* to accommodate a root pathogen, *A. medicaginis*, which had been previously identified as *Stagonospora meliloti*. In 2014, Trakunyingcharoen et al. (2014) confirmed the phylogenetic analysis of *Acrocalymma* species, viz., *A. aquatica*, *A. cycadis*, *A. fici*, *A. medicaginis*, and *A. vagum*. The results showed that the *Acrocalymma* clade represented an undefined lineage in Dothideomycetes (Trakunyingcharoen et al. 2014). Acrocalymmaceae was introduced with the single genus *Acrocalymma* as the type genus.

According to Liu et al. (2017), the divergence time was estimated for Acrocalymmmaceae with a stem age of 114 Mya (71–156 Mya) in the Cretaceous. The members of Acrocalymmmaceae have been reportedly associated with the two palm genera, *Eleiodoxa* and *Trachycarpus* (Taylor & Hyde 2003, Pinnoi et al. 2006).

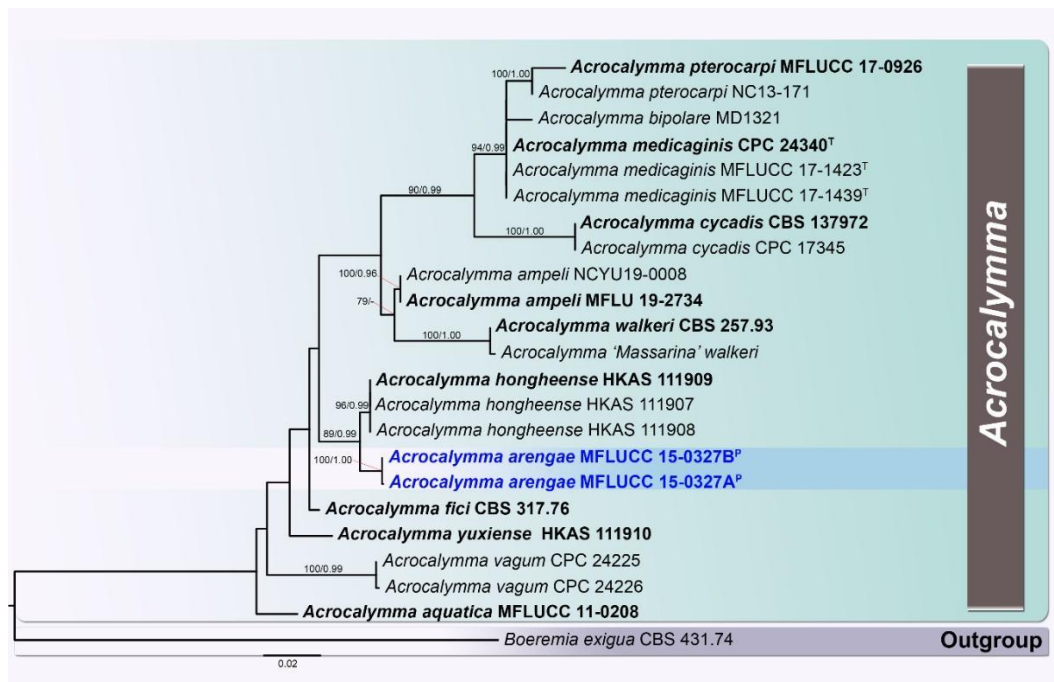


Figure 1 – The best scoring RAxML tree with a final likelihood value of -10419.141000 for a combined dataset of ITS, LSU, SSU, and RPB2 sequence data. The topology and clade stability of the combined gene analysis was compared with the single-gene analysis. The tree is rooted to *Boeremia exigua* (CBS 431.74). The matrix had 620 distinct alignment patterns with 52.51% undetermined characters and gaps. Estimated base frequencies were; A = 0.250925, C = 0.220570, G = 0.270536, T = 0.257969; substitution rates AC = 1.487807, AG = 2.762762, AT = 1.989307, CG = 0.698813, CT = 6.555054, GT = 1.000000; gamma distribution shape parameter α = 0.108948. Newly generated sequences are in blue. Ex-type/type strains are indicated in bold. Bootstrap support values for ML equal to or greater than 60% and BYPP equal to or greater than 0.95 are given above the nodes. ^T is the type species and ^P indicates palm microfungi from Thailand.

Acrocalymma Alcorn & J.A.G. Irwin 1987

Notes – *Acrocalymma* was introduced by Alcorn & Irwin (1987) to accommodate *Acrocalymma medicaginis*, which is the causative agent of a root and crown rot disease of lucerne (*Medicago sativa*) in Australia. Later, one sexual and four asexual morphs of *Acrocalymma* species were introduced to this genus (Zhang et al. 2012, Crous et al. 2014, Trakunyingcharoen et al. 2014). Members of *Acrocalymma* are pathogens and saprobes on leaf litter, seed pods, and wood in both terrestrial and freshwater habitats (Zhang et al. 2012, Crous et al. 2014, Trakunyingcharoen et al. 2014, Jayasiri et al. 2019, Mortimer et al. 2021, Tennakoon et al. 2021). The genus is characterized by immersed to semi-immersed conidiomata, globose to sub-globose pycnidia, hyaline conidiogenous cells with enteroblastic, cylindrical to fusiform conidia with a club-shaped mucilaginous appendage at each end (asexual morph). The sexual morph is characterized by having globose ascromata with unilocular, cylindrical, 8-spored asci of narrowly fusiform, hyaline to brown or pale reddish-brown, 1–3-septate ascospores, with a mucoid sheath (Alcorn & Irwin 1987, Zhang et al. 2012, Jayasiri et al. 2019, Li et al. 2020). Currently, 11 *Acrocalymma* epithets are listed in Species Fungorum (2022). *Acrocalymma medicaginis* has been reported to be associated with *Eleiodoxa conferta* in Thailand and *Trachycarpus fortunei* in China (Taylor & Hyde 2003, Pinnoi

et al. 2006). In this study, *Acrocalymma arengae* is reported from *Arenga pinnata* (Arecaceae) in Thailand based on morphological and phylogenetic evidence.

Acrocalymma arengae Konta & K.D. Hyde, sp. nov.

Fig. 2

Index Fungorum number: IF559674; FacesofFungi number: FoF 10819

Etymology – Refers to the host genus *Arenga*.

Holotype – MFLU 15-0302.

Saprobic on dead rachis and petioles of *Arenga pinnata*. Sexual morph: *Ascomata* 400–500 × 120–170 μm (\bar{x} = 455 × 150 μm, n = 10), dark brown sometimes covered with light grey, gnarled, warted hairs/setae, gregarious, immersed beneath host epidermis, not upright, laid on the substrate, visible as numerous, raised, dome-shaped areas on the host surface, obpyriform to ampulliform, uni-loculate, rough walls with setae, coriaceous, ostiolate. *Ostioles* 200–220 × 60–80 μm (\bar{x} = 215 × 70 μm, n = 10), centrally located, ostiolar canal filled with hyaline periphyses. *Peridium* 15–30 μm (\bar{x} = 23 μm, n = 10) wide, composed of dark red-brown to black cells, arranged in *textura globulosa* to *textura angularis*. *Pseudoparaphyses* 1.5–3 μm (\bar{x} = 2.1 μm, n = 10) wide, numerous, filamentous, branched, septate. *Asci* 60–190 × 15–30 μm (\bar{x} = 115 × 20 μm, n = 30), 1–8-spored, bitunicate, cylindrical to clavate, club-shaped, pedicellate, apically rounded with an ocular chamber. *Ascospores* 25–40 × 5–15 μm (\bar{x} = 30 × 10 μm, n = 30), overlapping 1–2-seriate, hyaline to pale brown, fusiform with acute ends, 1–3-septate, constricted at the septum, rough-walled, surrounded by a thick mucilaginous sheath. Asexual morph: Undetermined.

Culture characteristics – Ascospores germinating on MEA within 12 hr. Colonies on MEA from above, brown to greenish-brown at the centre, creamy white until margin; hyaline mycelia at the entire edge; dense, fluffy, and circular; colonies from below, dark-olivaceous brown at the centre, becoming yellowish-brown, and creamy white towards the edge.

Material examined – THAILAND, Phang-Nga Province, on dead rachis and petioles of *Arenga pinnata* (Arecaceae), 5 December 2014, Sirinapa Konta, PHR02a (MFLU 15-0302, holotype), ex-type living cultures, MFLUCC 15-0327A, B.

GenBank numbers – MFLUCC 15-0327A, B, LSU: ON650673, ON650674; ITS: ON650154, ON650155; SSU: ON650177, ON650178; RPB2: ON734014, ON734015; MFLUCC 15-0327A TUB: ON745966.

Notes – Phylogenetically, *Acrocalymma arengae* sp. nov. (MFLUCC 15-0327A and MFLUCC 15-0327B) forms a distinct lineage basal to *A. hongheense* (isolates HKAS 111907, HKAS 111908 and HKAS 111909) with 89% ML and 0.99 BYPP statistical support (Fig. 1). *Acrocalymma hongheense* differs from *A. arengae* in having a peridium with cells of *textura angularis*, 8-spored asci with a furcate to truncate pedicel and 1-septate ascospores (Mortimer et al. 2021), while our strains have peridium with cells of *textura globulosa* to *textura angularis*, 1–8-spored asci with a club-shaped pedicel, and 1–3-septate ascospores. Our new taxon shares similar morphology as in the sexual morph of *A. pterocarp*i and *A. walkeri* with 1–3-septate ascospores (Shoemaker et al. 1991, Jayasiri et al. 2019). Additionally, *A. arengae* differs from *A. pterocarp*i in having ostiolate ascomata with a long neck, and covered with light grey, gnarled, warted hairs, and setae; from *A. walkeri* with its larger asci (65–185 × 15–27 vs. 50–80 × 8–11 μm) and ascospores (33–36 × 10.5–7 vs. 19–22 × 4.5–5.5 μm) (Shoemaker et al. 1991, Jayasiri et al. 2019). However, we were unable to compare the morphology with *A. ampeli*, *A. aquaticum*, *A. bipolare*, *A. cycadis*, *A. fici*, *A. vagum*, and *A. yuxiense* as they reported only as asexual morphs (Alcorn & Irwin 1987, Zhang et al. 2012, Crous et al. 2014, Trakunyingcharoen et al. 2014, Dong et al. 2020, Mortimer et al. 2021, Tennakoon et al. 2021). In the BLASTn NCBI GenBank database search of LSU, ITS, SSU, RPB2 and TUB sequences, *Acrocalymma arengae* isolates (MFLUCC 15-0327A, B) are most similar; to *A. cycadis* (CBS 137972) with 97.78%, and 98.10% (LSU); to *A. aquatica* (MFLUCC 11-0208) with 91.46%, and 91.46% (ITS); to *Cryptocoryneum longicondensatum* (HHUF 30486) with 94.54%, and 94.40% (SSU); to *Alternaria burnsii* (GT037_010891) with 79.73%, and 79.82% (RPB2); and to *Neocucurbitaria salicis-albae* (CBS 144611) with 86.85% (TUB) similarity. Therefore, we introduce *A. arengae* as a new species, from *Arenga pinnata* (Arecaceae) in

Thailand, based on morphology and phylogeny. This is the first report of *Acrocalymma* species from *Arenga*. In addition, we noted that the asci could be 1-8-spored (ascospores) of this genus, which was reported as only 8-spores in asci, in the previous publications.

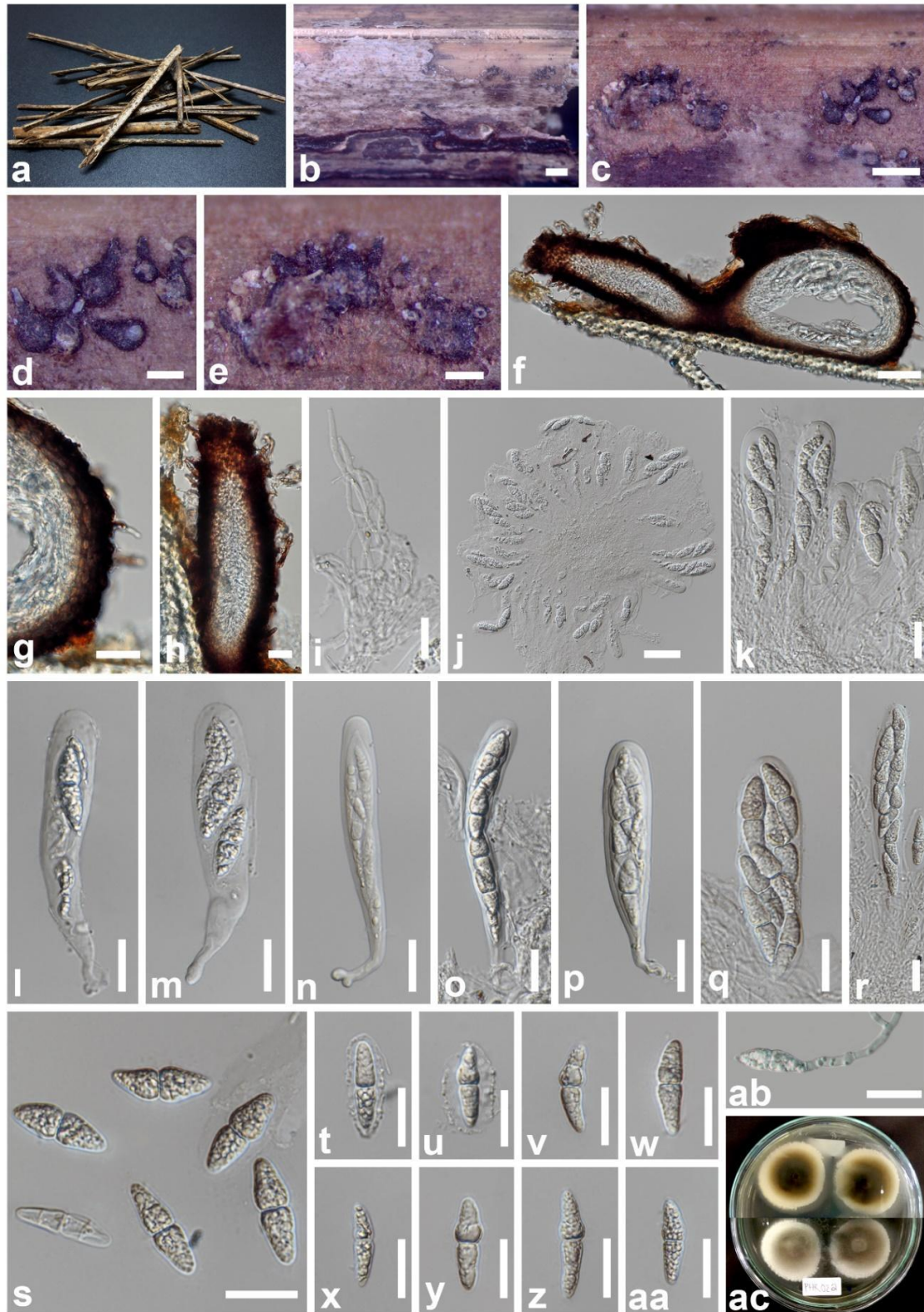


Figure 2 – *Acrocalymma arengae* (MFLU 15-0302, holotype). a Substrate. b Appearance of ascomata on host substrate with and without the outer layer of plant epidermis tissues. c–e Close up of ascomata. f Section of an ascoma. g Peridium. h Ostiole. i Pseudoparaphyses. j–r Asci. s–aa Ascospores. ab Ascospore with long tail. ac Petri dish with four cultures.

s–aa Ascospores. ab Germinated ascospore. ac Colonies on MEA (above reverse view, below upper view). Scale bars: b = 1 mm, c = 500 μm , d, e = 200 μm , f–i, k–ab = 20 μm , j = 50 μm .

Astrosphaeriellaceae Phook. & K.D. Hyde 2015

Notes – *Astrosphaeriellaceae* was introduced by Phookamsak et al. (2015) to accommodate two genera, viz., *Astrosphaeriella* and *Pteridiospora*. Ten genera are accepted in the *Astrosphaeriellaceae* viz., *Aquatospora*, *Astrosphaeriella*, *Astrosphaeriellopsis*, *Caryospora*, *Javaria*, *Mycopepon*, *Pithomyces*, *Pteridiospora*, *Quercicola*, and *Xenoastrosphaeriella* (Wijayawardene et al. 2022). *Astrosphaeriellaceae* is widespread in distribution and its members are usually saprobic or parasitic on bamboo, palms, or stout grasses (Phookamsak et al. 2015). The divergence time of this family is estimated with a stem age of 113 Mya (65–161 Mya) by Liu et al. (2017). The members of *Astrosphaeriellaceae* have been discovered in 33 palm genera (Farr & Rossman 2021). Herein, we introduce a new genus, *Triseptatospora*, and two new species namely *Pteridiospora arengae* and *Triseptatospora calami*, found on dead petioles of *Arenga pinnata* and *Calamus* sp. (Arecaceae) in Thailand.

Pteridiospora Penz. & Sacc 1897

Notes – *Pteridiospora* was introduced by Penzig & Saccardo (1897) to accommodate *P. javanica* as the type species. *Pteridiospora* is morphologically similar to *Astrosphaeriella* species but can be differentiated by asymmetrical ascospores with a large, mucilaginous sheath (Phookamsak et al. 2014). The sexual morph of this genus is characterized by carbonaceous superficial ascomata with ostioles, trabeculate pseudoparaphyses, 8-spored asci, broad-cylindrical to cylindrical-clavate asci with obclavate to ellipsoidal ascospores surrounded by a mucilaginous sheath. The asexual morph is characterized by semi-immersed to superficial conidiomata with globose to sub-globose, enteroblastic, phialidic, oblong to cylindrical or ampulliform conidiogenous cells, globose to sub-globose, aseptate, hyaline conidia forming chains (Phookamsak et al. 2014, 2015). The members of this genus can be found as saprobes, human and plant pathogens on a wide host range and are distributed in tropical and temperate regions (Phookamsak et al. 2015, Wijayawardene et al. 2017). One species of *Pteridiospora* (*P. munkii*) was found on *Phoenix sylvestris*, Arecaceae in India (Pande 2008). Currently, six *Pteridiospora* epithets are listed in Species Fungorum (2022).

Pteridiospora arengae Konta & K.D. Hyde, sp. nov.

Fig. 4

Index Fungorum number: IF559675; FacesofFungi number: FoF 10820

Etymology – Refers to the host genus *Arenga*.

Holotype – MFLU 15-0035.

Saprobic on dead petioles of *Arenga pinnata*. Sexual morph: *Ascostromata* 250–400 μm high, 550–850 μm diameter (\bar{x} = 325 \times 690 μm , n = 20), scattered, solitary, erumpent to superficial, mammiform to conical, visible as numerous, raised, black dome-shaped areas on the host surface, carbonaceous, ostiolate. *Ostiole* central, with pore-like opening. *Peridium* 40–70 μm (\bar{x} = 57 μm , n = 20) wide, of unequal thickness, poorly developed at the base, composed of dark brown to black, cells of *textura angularis*. *Pseudoparaphyses* 1–2 μm (\bar{x} = 1.34 μm , n = 20) wide, hyaline, numerous, filiform, trabeculate. *Asci* 80–150 \times 10–15 μm (\bar{x} = 115 \times 12 μm , n = 20), 8-spored, bitunicate, cylindrical, apically rounded, with a short club-shaped pedicel, apically rounded with a minute ocular chamber. *Ascospores* 25–40 \times 3–10 μm (\bar{x} = 32 \times 5.5 μm , n = 20), overlapping bi-seriate, ellipsoidal to sub-fusoid, 1(–3)-septate, hyaline, constricted at the septum, with rounded to acute ends, guttules, surrounded by a thick mucilaginous sheath. Asexual morph: Undetermined.

Culture characteristics – Ascospores germinating on MEA within 12 hr. Colonies on MEA from above, white-brown on the surface; wrinkled, irregular with undulate to lobate edge; colonies from below, yellow-brown, circulars, with undulate to lobate edge.

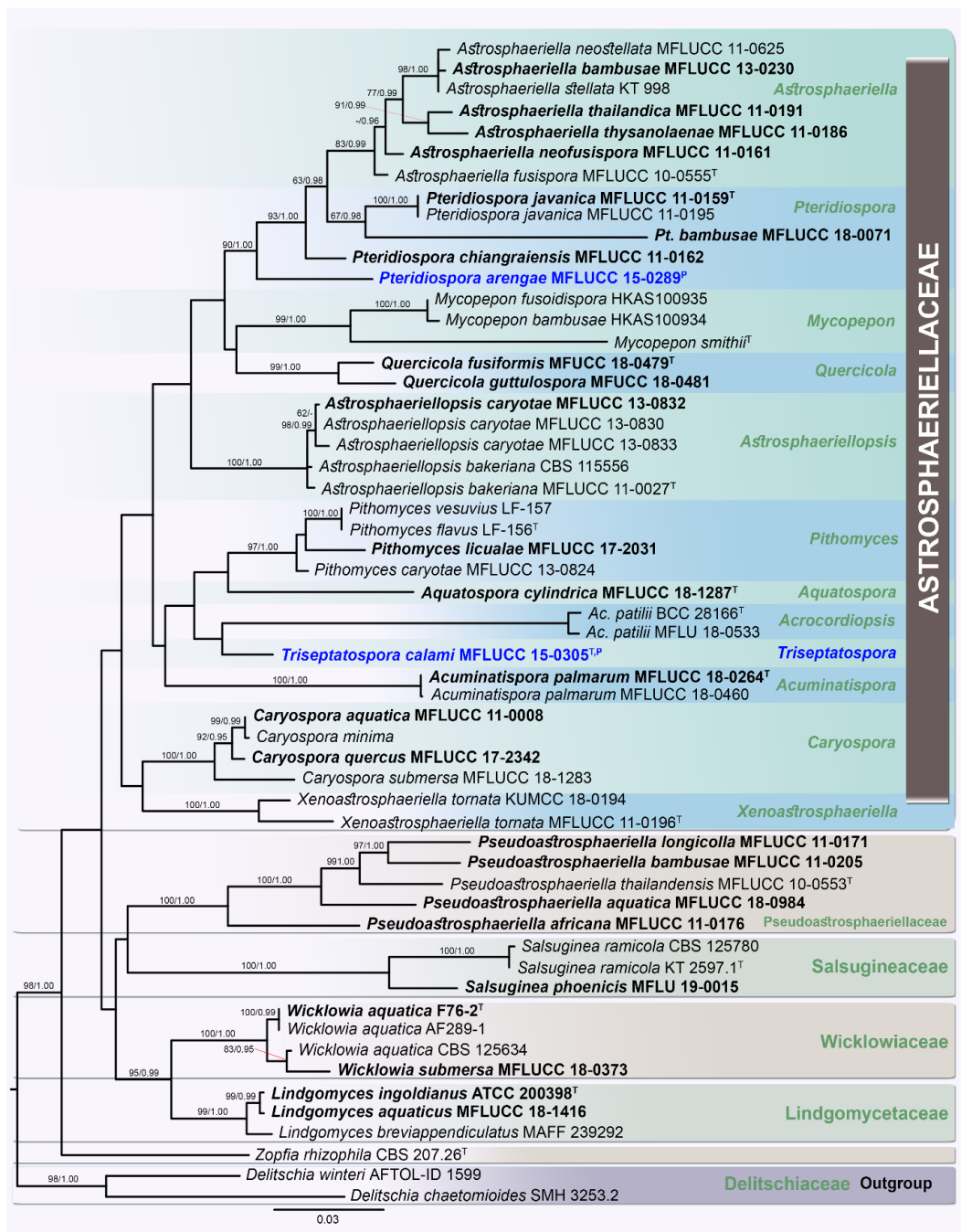


Figure 3 – The best scoring RAxML tree with a final likelihood value of -15298.294977 for a combined dataset of LSU, SSU, and TEF1 sequence data. The topology and clade stability of the combined gene analysis was compared with the single-gene analysis. The tree is rooted to *Delitschia chaetomioides* (SMH 3253.2), and *D. winteri* (AFTOL-ID 1599). The matrix had 1080 distinct alignment patterns with 32.74% undetermined characters and gaps. Estimated base frequencies were; A = 0.245730, C = 0.240721, G = 0.283167, T = 0.230382; substitution rates AC = 1.209403, AG = 3.473439, AT = 1.273268, CG = 1.220896, CT = 12.010203, GT = 1.000000; gamma distribution shape parameter α = 0.170362. Newly generated sequences are in blue. Ex-type/type strains are indicated in black bold. Bootstrap support values for ML equal to or greater than 60% and BYPP equal to or greater than 0.95 are given above the nodes. ^T is the type species and ^P indicates palm microfungi from Thailand.

Material examined – THAILAND, Phang-Nga Province, on dead petioles of *Arenga pinnata* (Arecaceae), 5 December 2014, Sirinapa Konta, PHR03a (MFLU 15-0035, holotype), ex-type living culture, MFLUC 15-0289.

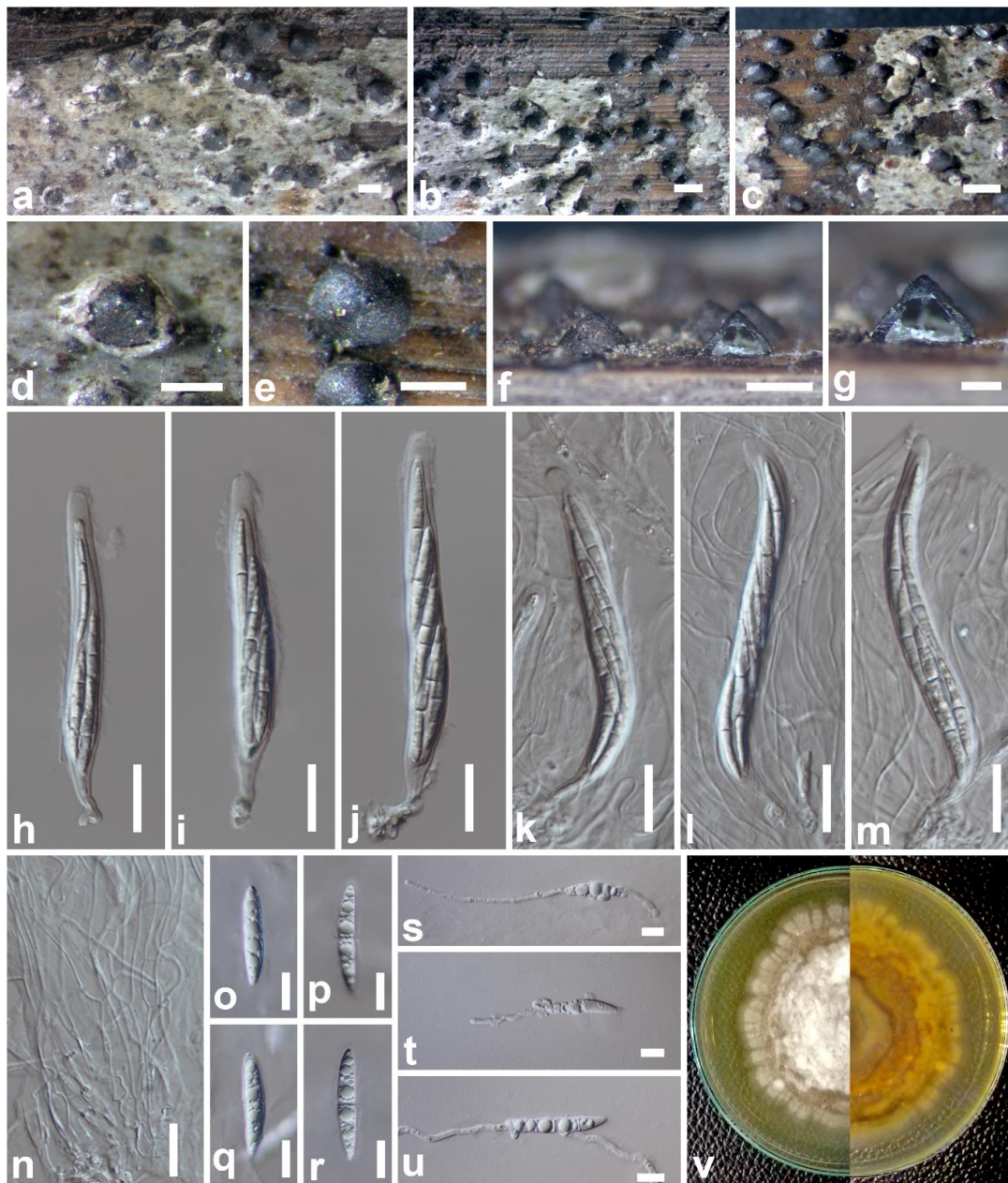


Figure 4 – *Pteridiospora arengae* (MFLU 15-0035, holotype). a–c Ascostromata on host. d, e Close-up of ascostroma. f, g Vertical section of ascostromata. h–m Asci. n Pseudoparaphyses. o–p Ascospores. s–u Germinated ascospores. v Colony on MEA. Scale bars: a = 1 mm, b–f = 500 μ m, g = 200 μ m, h–n = 20 μ m, o–u = 10 μ m.

GenBank numbers – LSU: ON650692; ITS: ON669255; SSU: ON650678; TEF1: ON734017.

Notes – *Pteridiospora arengae* morphologically fits into the generic concept of *Pteridiospora* (Penzig & Saccardo 1897). Based on the multi-gene sequence analyses, *Pteridiospora arengae* forms a distinct lineage basal to other *Pteridiospora* species with strong statistical support of 90% ML and 1.00 BYPP (Fig. 3). *Pteridiospora arengae* shares some similar characteristics with *Pt. bambusae*, *Pt. chiangraiensis*, and *Pt. javanica* in having erumpent to superficial, carbonaceous ascostromata, cylindrical asci with a short pedicels, and sub-fusoid ascospores with a thick mucilaginous sheath (Penzig & Saccardo 1897, Phookamsak et al. 2014, 2015, Hyde et al. 2018). However, *Pt. bambusae* and *Pt. chiangraiensis* differ from *Pt. arengae* with larger ascospores, which turn brown when mature (Phookamsak et al. 2014, 2015, Hyde et al. 2018). *Pteridiospora arengae* shares similarities to the type species, *Pt. javanica* in having hyaline ascospores at

maturity but differs from *Pt. javanica* in having short pedicels and 1-septate ascospores surrounded by a large appendage in the lower cell, with a wide mucilaginous sheath (Phookamsak et al. 2014, 2015, Hyde et al. 2018). In the BLASTn NCBI GenBank database search of LSU, ITS and SSU sequences, *Pteridiospora arengae* (MFLUCC 15-0289) is most similar; to *Lophiotrema mucilaginosus* (HMAS 255437) with 95.18% (LSU); to *Roussoella mediterranea* (MUT<ITA>:5369) with 86.26% (ITS); and *Astrosphaeriellopsis caryota* (MFLUCC 13-0832) with 99.24% (SSU) similarity. However, among the six species of this genus, only four have molecular data available in GenBank viz., *Pteridiospora bambusae*, *Pt. chiangraiensis*, *Pt. javanica*, and *Pt. spinosipora*. The sequence data of *Pt. spinosipora* strain CBS 478.69 (MH871121) from Vu et al. (2018) did not group with other species in the analyses of the combined sequence dataset of LSU, SSU, and TEF1, though it appeared to be a distant lineage from *Pteridiospora* and *Astrosphaeriellaceae* clades (the data not shown in this study). Thus, we excluded *Pt. spinosipora* (CBS 478.69) from our analyses. Hence, we introduce our collection as a new saprobic species from *Arenga pinnata* (Arecaceae) based on both morphology and phylogenetic evidences.

Triseptatospora Konta & K.D. Hyde, gen. nov.

Index Fungorum number: IF559676; FacesofFungi number: FoF 10821

Etymology – Refers to three septate ascospores of this genus.

Saprobic on dead or decaying petioles and wood in terrestrial habitats. Sexual morph: *Ascomata* scattered to gregarious, superficial, flat at the base, conspicuous on host surface, tightly attached to the host, numerous, raised, subglobose to lenticular or quadrilateral to dome-shaped, clustered, black, ostiolate. *Peridium* thick-walled, hyaline to dark brown composed of several layers of cells of *textura angularis* to *textura prismatica*. *Pseudoparaphyses* hyaline, numerous, filiform, trabeculate. *Asci* 6–8-spored, bitunicate, broadly clavate, apically rounded with an ocular chamber, short pedicels. *Ascospores* 2–3-seriate, fusiform, 3-septate, hyaline, constricted at the septum, enlarged at the second cell, guttulate, surrounded by a mucilaginous sheath. Asexual morph: Undetermined.

Type species – *Triseptatospora calami* Konta & K.D. Hyde

Notes – Based on phylogenetic analyses, our strain MFLUCC 15-0305 is well separated and forms an independent lineage between the two strains of *Acrocordiopsis patilii* (BCC 28166 and MFLUCC 18-0533), and the two strains of *Acuminatispora palmarum* (MFLUCC 18-0264 and 18-0460) with weak statistical support (Fig. 3). The weakly supported clades in the analyses are similar to those in Zhang et al. (2018), Dayarathne et al. (2020), and Dong et al. (2020). However, *Triseptatospora* can be distinguished from *Acrocordiopsis* and *Acuminatispora* by fusiform, 3-septate ascospores enlarged at the second cell and surrounded by a mucilaginous sheath. *Acuminatispora* have been found in the same host family in Thailand, but our new collection differs by having hyaline to brown, fusiform ascospores with acute or narrowly pointed ending cells, 1-septate (rarely 3) (Zhang et al. 2018), whereas *Acrocordiopsis* discovered in Thailand from mangrove wood is characterized by having ovoidal or ellipsoidal ascospores in cylindrical asci (Suetrong et al. 2008, Dayarathne et al. 2020). In the BLASTn NCBI GenBank database search of LSU, ITS, SSU and TEF1 sequences, *Triseptatospora calami* (MFLUCC 15-0305) is most similar; to *Lindgomyces pseudomadisonensis* (KT 2742) with 96.05% (LSU); to *Preussia procaviicola* (CBS 146981) with 83.80% (ITS); to *Lindgomyces angustiascus* (ILL A640-1a) with 99.43% (SSU); and *Occultibambusa jonesii* (GZCC 16-0117) with 93.13% (TEF1) similarity. *Triseptatospora calami* gen. et. sp. nov. is introduced here as a monospecific genus among the ten genera in *Astrosphaeriellaceae*. Additionally, our new taxon shares similar morphology to the generic concept of *Astrosphaeriella sensu lato* in having coriaceous ascostromata and distinct necks (Phookamsak et al. 2015). However, the current phylogenetic results place our new genus within *Astrosphaeriellaceae* until it is revised with further collections. In addition, *Triseptatospora* can be distinguished from other genera in *Astrosphaeriellaceae* and *Lindgomycetaceae*, based on the unique morphological traits of the sexual morph. Therefore, we introduce the new genus,

Triseptatospora with *T. calami* as the type species, from dead petioles of *Calamus* sp. (Arecaceae) in Thailand.

Triseptatospora calami Konta & K.D. Hyde, sp. nov.

Index Fungorum number: IF559677; FacesofFungi number: FoF 10822

Etymology – Refers to the host genus *Calamus*.

Holotype – MFLU 15-0280.

Fig. 5

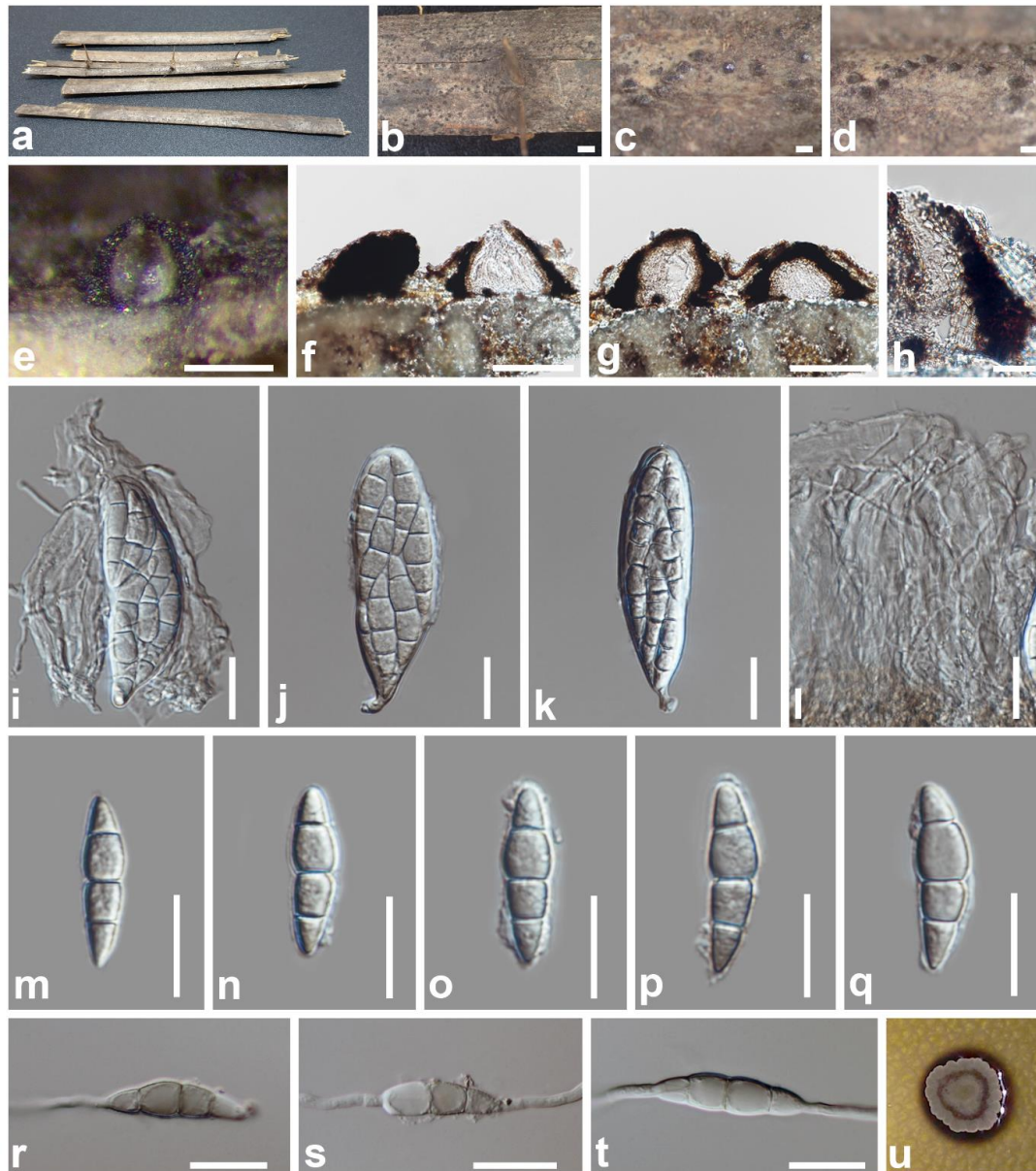


Figure 5 – *Triseptatospora calami* (MFLU 15-0280, holotype). a Substrate. b Appearance of ascostroma on host substrate. c Close-up of ascostroma (top view). d Close-up of ascostroma (side view). e Vertical section of ascostroma. f, g Section of ascostroma. h Peridium. i–k Asci. l Pseudoparaphyses. m–q Ascospores. r–t Germinated ascospores. u Colony on MEA. Scale bars: b = 1 mm, c, d = 200 μ m, e–g = 100 μ m, h–t = 20 μ m.

Saprobic on dead petioles of *Calamus* sp. Sexual morph: *Ascostromata* superficial, visible as numerous, raised, black dome-shaped on host surface, superficial, solitary to gregarious, coriaceous. *Ascomata* (55–)80–165(–225) \times 110–195(–340) μ m (\bar{x} = 97 \times 165 μ m, n = 20), sub-globose to lenticular or quadrilateral to dome-shaped, clustered, black, ostiolate. *Peridium* 20–90

μm ($\bar{x} = 36 \mu\text{m}$, $n = 20$) wide, thick-walled, hyaline to dark brown, composed of several layers of cells of *textura angularis* to *textura prismatica*. *Pseudoparaphyses* 1.5–4.5 μm ($\bar{x} = 2.3 \mu\text{m}$, $n = 20$) wide, hyaline, numerous, filiform, trabeculate. *Asci* 60–105 \times 15–30 μm ($\bar{x} = 84 \times 24 \mu\text{m}$, $n = 30$), 6–8-spored, bitunicate, broadly clavate, apically rounded with an ocular chamber, with a short pedicels. *Ascospores* 30–40 \times 5–15 μm ($\bar{x} = 35 \times 10 \mu\text{m}$, $n = 30$), 2–3-seriate, fusiform, 3-septate, hyaline, constricted at the septum, enlarged at the second cell, surrounded by a thin mucilaginous sheath. Asexual morph: Undetermined.

Culture characteristics – Colony on MEA. Colonies from above; olivaceous with mycelium medium dense, circular, fluffy at the middle, turning red-brown, towards grey-brown, wrinkled at the margin with lobate edge. Colonies from below; dark brown with an entire edge, producing red-brown to dark-brown pigmentation of medium.

Material examined – THAILAND, Phang-Nga Province, on dead petioles of *Calamus* sp. (Arecaceae), 6 December 2014, Sirinapa Konta, DNH02f (MFLU 15-0280, holotype), ex-type living culture, MFLUCC 15-0305.

GenBank numbers – LSU: ON650710; ITS: ON669256; SSU: ON650705; TEF1: ON734018.

Notes – The phylogenetic analyses showed that our strain forms a lineage sister to *Acrocordiopsis patilii* with an unsupported clade (Fig. 3). Our new species, *Triseptatospora calami*, shares similar characteristics with *Aquatospora cylindrica* in having superficial ascomata, clavate to narrowly ellipsoidal asci, with a short pedicels, and 2–4-seriate, hyaline ascospores (Dong et al. 2020). Moreover, *Triseptatospora calami* differs from *Aquatospora cylindrica* by its coriaceous ascomata with 3-septate fusiform ascospores surrounded by a thin mucilaginous sheath. In addition, the two middle cells of ascospores become pale brown when germinate. We introduce *Triseptatospora calami* as a new species based on both morphological comparison and phylogenetic analyses.

Sordariomycetes O.E. Erikss. & Winka 1997

Amphisphaeriales D. Hawksw. & O.E. Erikss. 1986

Notes – Amphisphaeriales was recently accepted with 21 families, including several genera in Amphisphaeriales genera *incertae sedis* (Wijayawardene et al. 2017, 2022, Hyde et al. 2020b, Samarakoon et al. 2022). The divergence time of this order has been estimated at 133 Mya (Hyde et al. 2020b).

Sporocadaceae Corda 1842

Notes – Sporocadaceae was introduced by Corda (1842) to accommodate *Sporocadus* as the type genus. Members of this family have been reported as saprobes, endophytes and pathogens on several hosts and are known as parasitic on humans and animals (Nag Raj 1993, Jaklitsch et al. 2016, Maharachchikumbura et al. 2016a, b, Liu et al. 2019, Hyde et al. 2020b, Ma et al. 2021). Currently, 35 genera are accepted into this family, with the divergence time of Sporocadaceae estimated at 52.22 Mya (Wijayawardene et al. 2017, 2022, Hyde et al. 2020b). The members of this family have been reported to be associated with 71 palm species (Farr & Rossman 2021). In this study, we introduce two new species, *Bartalinia adonidiae* and *Neopestalotiopsis elaeidis*, collected from the leaves of *Adonidia merrillii* and *Elaeis guineensis* (Arecaceae) in Thailand. Both of these new species were able to produce appressoria structures on media.

Bartalinia Tassi 1900

Notes – *Bartalinia* was introduced by Tassi (1900) to accommodate *B. robillarDoides* as the type species. *Bartalinia* species have been recorded on a wide range of hosts (Nag Raj 1993, Gangadevi & Muthumary 2008, Phookamsak et al. 2019, Tibpromma et al. 2020). The main asexual morphological features of *Bartalinia* are pycnidial conidiomata and the production of fusiform conidia, 3–4-septate, appendage-bearing with an acute or blunt apex (Senanayake et al. 2015). The members of this genus have a worldwide distribution, occurring as saprobes on various

host trees (some have been reported in freshwater) and are often associated with leaf spots (Wong et al. 2003, Maharachchikumbura et al. 2016b, Nguyen et al. 2019). There are 21 *Bartalinia* species listed in Species Fungorum (2022). While the member of *Bartalinia* has been associated with the palm genus *Borassus* (Mathur 1979). In this study, we introduce a new species of *Bartalinia adonidiae* collected from the leaves of *Adonidia merrillii* (Arecaceae) from Thailand.

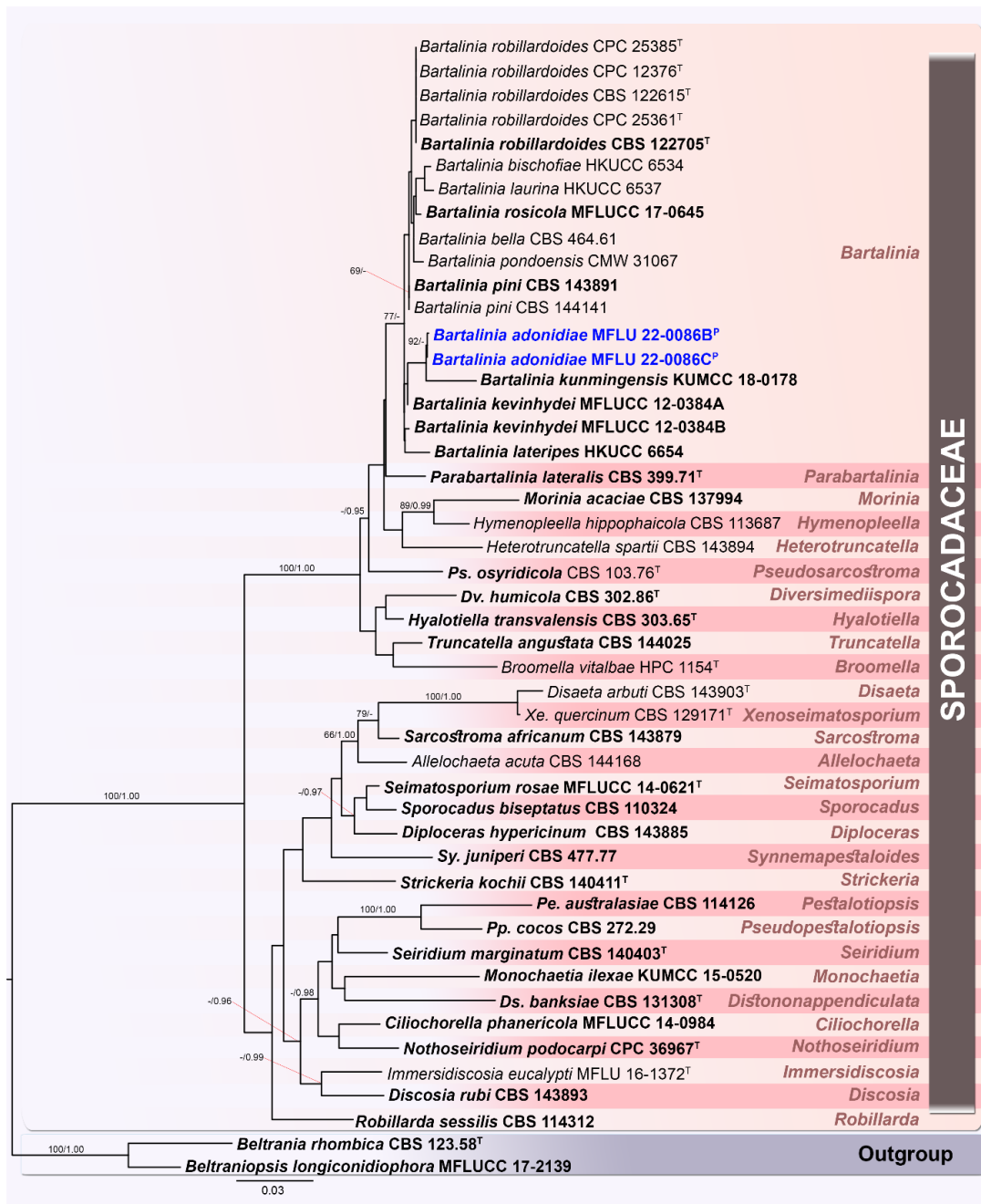


Figure 6 – The best scoring RAxML tree with a final likelihood value of -7577.599260 for a combined dataset of ITS and LSU sequence data. The topology and clade stability of the combined gene analysis was compared with the single-gene analysis. The tree is rooted to *Beltrania rhombica* (CBS 123.58), and *Beltraniopsis longiconidiophora* (MFLUCC 17-2139). The matrix had 550 distinct alignment patterns with 17.36% undetermined characters and gaps. Estimated base frequencies were; A = 0.261739, C = 0.208302, G = 0.259364, T = 0.270595; substitution rates AC = 1.116426, AG = 3.285061, AT = 1.724720, CG = 1.083075, CT = 4.707212, GT = 1.000000; gamma distribution shape parameter α = 0.162618. Newly generated sequences are in blue. Ex-type/type strains are indicated in black bold. Bootstrap support values for ML equal to or greater

than 60% and BYPP equal to or greater than 0.95 are given above the nodes. ^T is the type species and ^P indicates palm microfungi from Thailand.

Bartalinia adonidiae Konta & K.D. Hyde, sp. nov.

Fig. 7

Index Fungorum number – IF559714; FacesofFungi number: FoF 10823

Etymology – Refers to the host genus *Adonidia*.

Holotype – MFLU 22-0086.

Saprobic on the dead part of the living leaves of *Adonidia merrilli*. Sexual morph: Undetermined. Asexual morph: Coelomycetous. *Conidiomata* 30–60 × 50–100 μm (\bar{x} = 55 × 70 μm, n = 10), subglobose to lenticular, visible as dark grey to black areas, immersed, slightly raised, solitary to gregarious, uniloculate. *Conidiomata walls* 10–15 μm (\bar{x} = 12 μm, n = 10) wide, slightly thick-walled, of equal thickness, comprising several cell layers of light brown to brown, pseudoparenchymatous cells of *textura angularis*, paler towards the inner layers. *Conidiophores* arising from the inner cavity reduced to conidiogenous cells. *Conidiogenous cells* 3–7 × 1.8–3.8 μm (\bar{x} = 5 × 3 μm), enteroblastic, annellidic, integrated, hyaline, ampulliform to subcylindrical, or obclavate, aseptate, smooth-walled. *Conidia* 14–21.8 × 3.6–6.2 μm (\bar{x} = 17.2 × 4.8 μm, n = 100), cylindrical to inequilateral, straight to slightly curved, 2–4-septate, constricted at the septa, with the longest cell at the second from the base, bearing appendages; basal cell 2–3.2 μm (\bar{x} = 2.5 μm) long, obconic, truncate at base, hyaline, thin and smooth-walled; second cell from the base 3.3–4.6 μm (\bar{x} = 4.1 μm) long, pale yellowish; third cell 2.5–3.3 μm (\bar{x} = 2.7 μm) long, pale yellowish; fourth cell 2.4–3.2 μm (\bar{x} = 2.8 μm) long, pale yellowish; apical cell 2.4–3.2 μm (\bar{x} = 2.6 μm) long; apical appendages conical, hyaline and smooth-walled, forming three-branched tubular, flexuous, 6–18 μm long (\bar{x} = 10 μm); basal appendages 1.3–3 μm (\bar{x} = 1.9 μm) long, single, absent at immature state, tubular, unbranched, centric. *Appressoria* irregular, hyaline, rough-walled, 5–16.8 × 7–20 μm (\bar{x} = 11 × 11.2 μm, n = 50).

Culture characteristics – Colonies on MEA. Colonies from above; white fluffy at the beginning, dense, circular, turning yellow-brown from the middle, becoming red-brown to green-brown, and creamy brown towards edge, entire margin at maturity. Colonies from below; olivaceous-green, white at the entire edge with circular layers.

Material examined – THAILAND, Kanchanaburi Province, on dead parts of living leaves of *Adonidia merrillii* (Arecaceae), 8 September 2018, Machima Saengket, KCB03 (MFLU 22-0086, holotype).

GenBank numbers – MFLU 22-0086B, C, LSU: ON650681, ON650682; ITS: ON650703, ON650704.

Notes – The taxonomic identification of the fungal genus *Bartalinia* reveals that it is a monophyletic clade of saprophytes associated with leaf spots on the leaves of various hosts (Matsushima 1987, Pinggen et al. 2000, Li et al. 2002, Andrianova et al. 2007, Liu et al. 2019, Phookamsak et al. 2019, Tibpromma et al. 2020). In the BLASTn NCBI GenBank database search of LSU, and ITS sequences, *Bartalinia adonidiae* (strains MFLU 22-0086B, C) are most similar; to *B. robillardoides* (CBS 122705) (strains B), and *B. pondoensis* (CBS 125525) (strains C) with 99.46%, and 99.76% (LSU); and to *B. pondoensis* (CMW 31067) (strains B, C) with 99.33%, 99.17% (ITS) similarity. Based on the multi-gene analyses (Fig. 6), our isolates showed close affinities to *B. kunmingensis* but were different in shape, septation, and size of conidia (Phookamsak et al. 2019). *Bartalinia adonidiae* differs from *B. kunmingensis* in sub-globose to lenticular conidiomata, cylindrical to inequilateral conidia, 2–4-septate, constricted at the septa, shorter basal cells, the second cell from the base, the third cells, the fourth cells, and the basal appendages are; 2.0–3.2 μm vs. 2.5–4 μm, 3.3–4.6 μm vs. 6.5–8 μm, 2.5–3.3 vs. 4–5.5 μm, 2.4–3.2 vs. 4–5.5(–6) μm, 1.3–3 vs. 5–6 μm, respectively, and overlapping in size of the apical appendages (6–18 vs. 10–20 μm), while, *B. kunmingensis* has globose to sub-globose conidiomata, cylindrical to sub-cylindrical, conidia 4-septate, not constricted at the septa (Phookamsak et al. 2019). Only *B. robillardoides* has been recorded, associated with *Borassus flabellifer* (Arecaceae) in India (Mathur 1979). Thus, we introduce our collection as a new species, *Bartalinia adonidiae*, from the dead part

of the living leaves of *Adonidia merrillii* (Arecaceae) in Thailand. This is the first report of an appressorium structure for this genus.

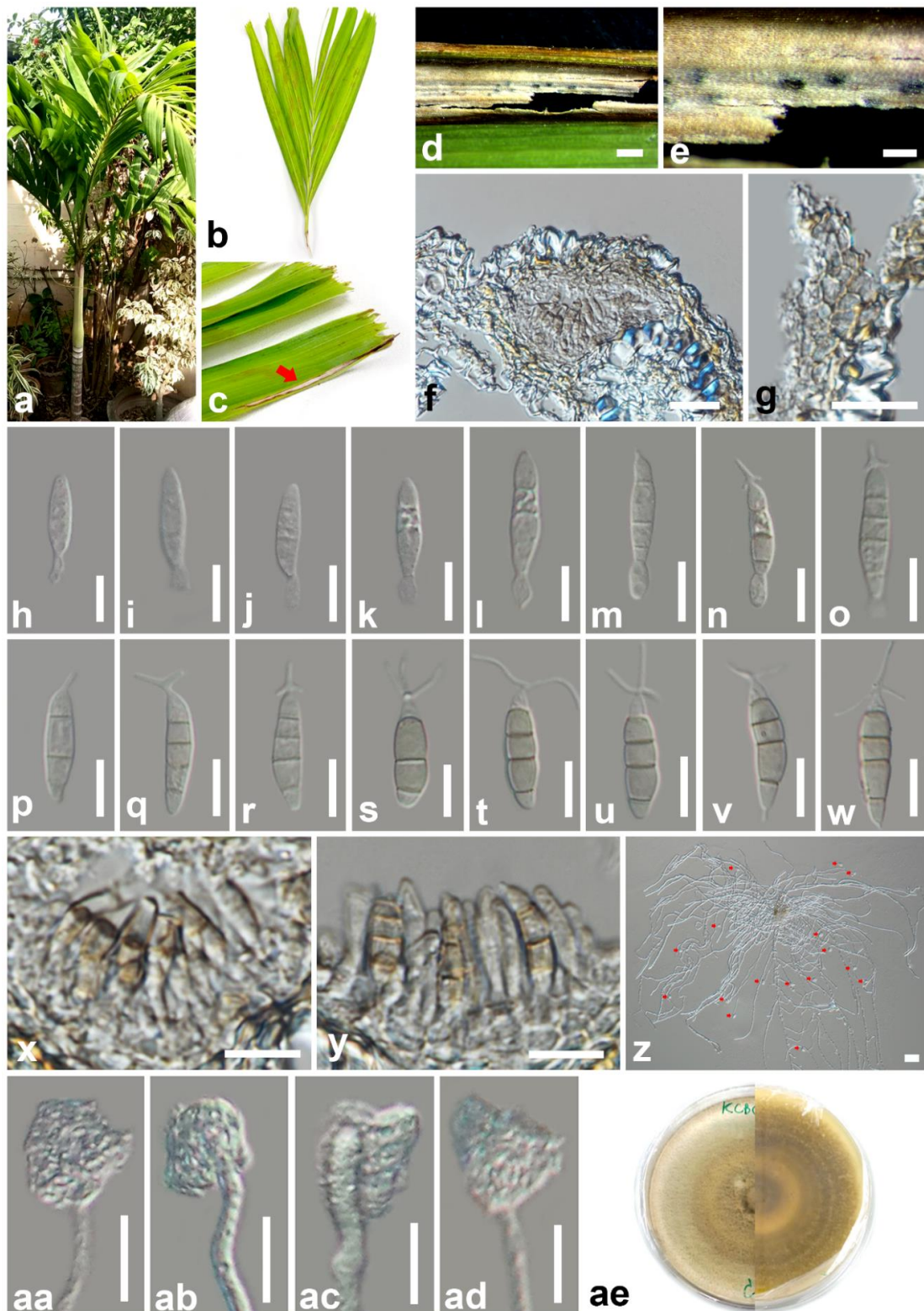


Figure 7 – *Bartalinia adonidiae* (MFLU 22-0086, holotype). a *Adonidia merrillii* tree. b, c Substrate. d, e Conidiomata on host substrate. f Vertical section of conidioma. g Conidioma wall. h–o, x, y Conidiogenous cells with conidia. p–w Conidia. z Germinated conidium with appressoria (pointed with red arrows). aa–ad Appressoria. ae Colony on MEA. Scale bars d = 500 μ m, e = 200 μ m, f, g, z = 20 μ m, h–y, aa–ad = 10 μ m.

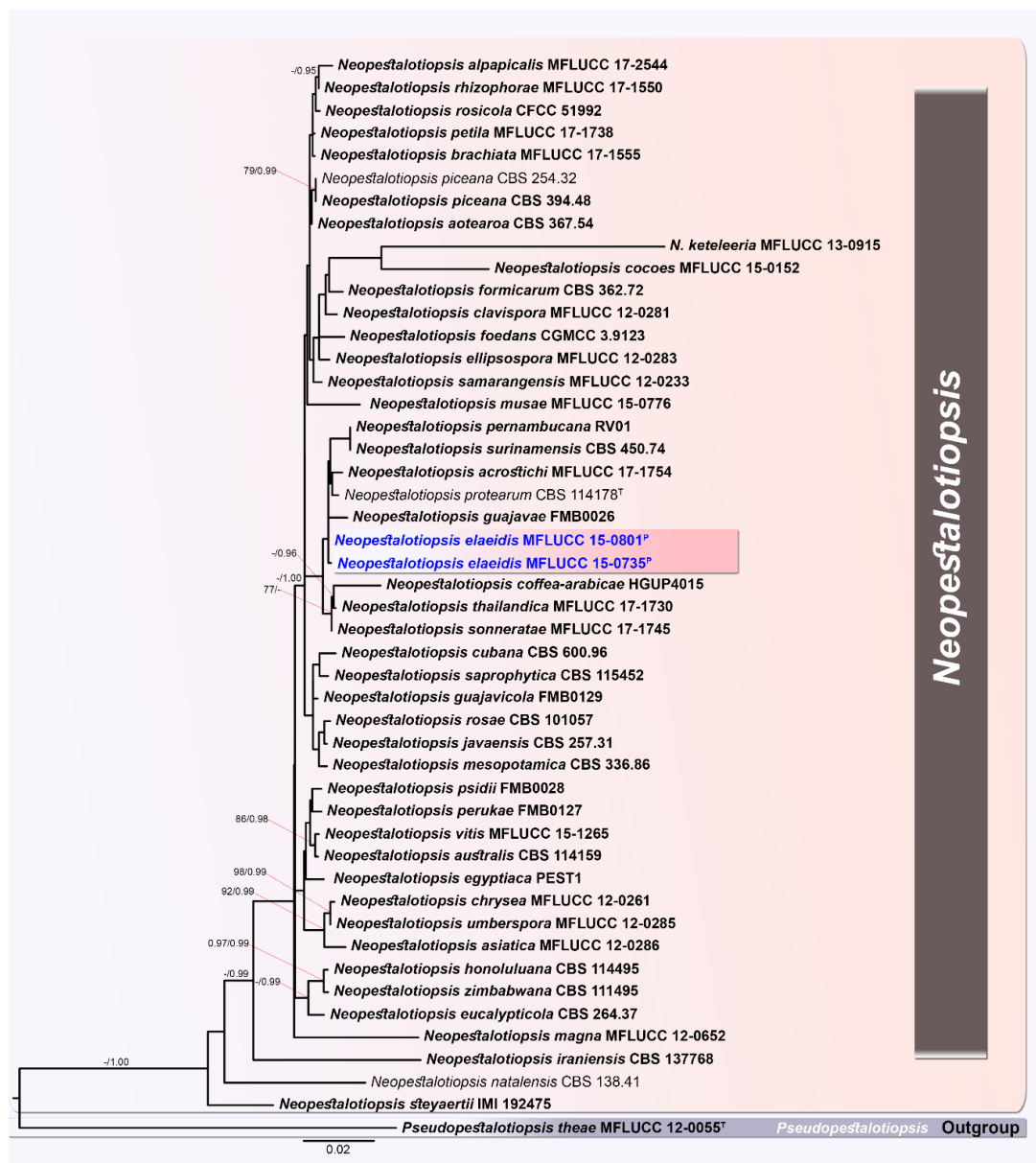


Figure 8 – The best scoring RAxML tree with a final likelihood value of -7856.565209 for the combined dataset of ITS, TUB, and TEF1 sequence data. The topology and clade stability of the combined gene analysis was compared with the single-gene analysis. The tree is rooted to *Pseudopestalotiopsis theae* (MFLUCC 12-0055). The matrix had 699 distinct alignment patterns with 29.48% undetermined characters and gaps. Estimated base frequencies were; A = 0.237201, C = 0.273328, G = 0.216876, T = 0.272595; substitution rates AC = 0.651524, AG = 3.114610, AT = 1.558132, CG = 0.696324, CT = 4.053130, GT = 1.000000; gamma distribution shape parameter $\alpha = 0.167791$. Newly generated sequences are in blue. Ex-type/type strains are indicated in black bold. Bootstrap support values for ML equal to or greater than 60% and BYPP equal to or greater than 0.95 are given above the nodes. ^T is the type species and ^P indicates palm microfungi from Thailand.

***Neopestalotiopsis* Maharachch., K.D. Hyde & Crous 2014**

Notes – *Neopestalotiopsis* was introduced by Maharachchikumbura et al. (2014) to accommodate *N. protearum* as the type species. *Neopestalotiopsis* species have been found to cause post-harvest diseases, leaf spots, fruit rots, fruit cankers, flower diseases, and root rot on a variety of hosts (Darapanit et al. 2021, Gualberto et al. 2021, Prasannath et al. 2021, Sun et al. 2021, Ul Haq et al. 2021). *Pestalotiopsis* and *Pseudopestalotiopsis* are very similar to *Neopestalotiopsis* in

morphology but can be distinguished based on conidial pigmentation and molecular phylogeny (Maharachchikumbura et al. 2014). Currently, 48 records are listed in Species Fungorum (2022).

Neopestalotiopsis elaeidis Konta & K.D. Hyde, sp. nov.

Fig. 9

Index Fungorum number: IF559678; FacesofFungi number: FoF 10824

Etymology – Refers to the host genus *Elaeis*.

Holotype – MFLU 15-1466.

Saprobic on dead leaves of *Elaeis guineensis*. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 100–140 × 180–250 μm (\bar{x} = 120 × 215 μm, n = 10), acervuli, sub-globose to lenticular, visible as black spots, or black dome-shape, raised, solitary to gregarious, uniloculate. *Conidiomata* walls 5–10 μm (\bar{x} = 9 μm, n = 20) wide, thick-walled, comprising several cell layers of dark brown, pseudoparenchymatous cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, discrete, simple, short, filiform. *Conidia* 10–20 × 3–7 μm (\bar{x} = 16 × 5.5 μm, n = 20), fusiform to ellipsoid, straight to slightly curved, 4-septate; basal cell conic, hyaline, thin and smooth-walled, 2.4–4.2 μm (\bar{x} = 3.3 μm) long, brown, verruculose; second cell from base brown, 2.5–4.4 μm (\bar{x} = 3.5 μm) long; third cell brown, 2.8–4.2 μm (\bar{x} = 3.5 μm) long; fourth cell darker brown, 2.4–5 μm (\bar{x} = 3.8 μm) long; apical cell 2.3–4.2 μm (\bar{x} = 3.2 μm) long, hyaline, cylindrical to subcylindrical; apical appendages 10–20 μm long (\bar{x} = 12 μm), (mostly 3); basal appendage present (1 or rarely absent), filiform 2–6 μm (\bar{x} = 3.5 μm). *Appressoria* irregular, hyaline, rough-walled, 3.4–4.6 × 3.4–4.6 μm (\bar{x} = 3.9 × 4.1 μm).

Culture characteristics – Colonies on MEA. Colonies from above; white, circular, mycelia medium dense, flat or raised, with filiform margin, fluffy, with black spot of fruiting bodies. Colonies from below, same as above.

Material examined – THAILAND, Songkhla Province, on dead leaves of *Elaeis guineensis* (Arecaceae), 16 June 2015, Benjarong Thongbai, SK01d (MFLU 15-1466, holotype), ex-type living culture, MFLUCC 15-0735; Chiang Mai Province, on dead leaves of unidentified palm (Arecaceae), 19 July 2015, Putarak Chomnunti, Fg01c (MFLU 15-2334), living culture, MFLUCC 15-0801.

GenBank numbers – MFLUCC 15-0735, LSU: ON650696; ITS: ON650690; SSU: ON650698; RPB2: ON734016; TEF1: ON734012.

MFLUCC 15-0801, LSU: ON650695; ITS: ON650689; SSU: ON650697; TEF1: ON734011.

Notes – In the phylogenetic analyses, *Neopestalotiopsis* isolates (MFLUCC 15-0801, MFLUCC 15-0735) clustered basal to *N. pernambucana* RV01, *N. surinamensis* CBS450.74, *N. acrostichi* MFLUCC 17-1754, *N. protearum* CBS 114178 and *N. guajavae* FMB0026 (Fig. 8). Taxonomic identification of *Neopestalotiopsis* is difficult because of the cryptic evolutionary history of the genus. Therefore, we were unable to compare the morphological characteristics of closely related taxa in the phylogenetic tree (Fig. 8) as they are very similar. In the BLASTn NCBI GenBank database search of LSU, ITS, SSU, RPB2 and TUB sequences, *Neopestalotiopsis elaeidis* isolates (MFLUCC 15-0735, and MFLUCC 15-0801) are most similar; to *N. piceana* (CBS 394.48) with 100% (LSU); to *N. eucalypticola* (CBS 264.37) with 99.82% (ITS); to *Pseudopestalotiopsis thailandica* (MFLUCC 17-1724) (isolate MFLUCC 15-0735) and to *N. petila* (MFLUCC 17-1737) (isolate MFLUCC 15-0801) with 100%, and 100% (SSU); to *N. protearum* (CBS 114178) with 99.45%, and 99.82% (RPB2); and to *Seiridium marginatum* (SEI) (isolate MFLUCC 15-0801) and to *Pestalotiopsis ellipsospora* (MFLUCC12-0283) (isolate MFLUCC 15-0801) with 93.79%, and 99.47% (TUB) similarity. Based on our investigation, the phylogenetic data revealed our collection as a novel species of *Neopestalotiopsis*. Thus, we introduce our collection as a new species from dead leaves of *Elaeis guineensis* and leaves of unidentified palm (Arecaceae). Appressoria in *Neopestalotiopsis elaeidis* were also observed and presented. *Neopestalotiopsis elaeidis* sp. nov. could be used for further studies in the investigation of why saprobe life mode on the dead leaves of palms is able to produce appressoria (specialized cells of many fungal plant pathogens) as oil palm is an economically important plant, worldwide.

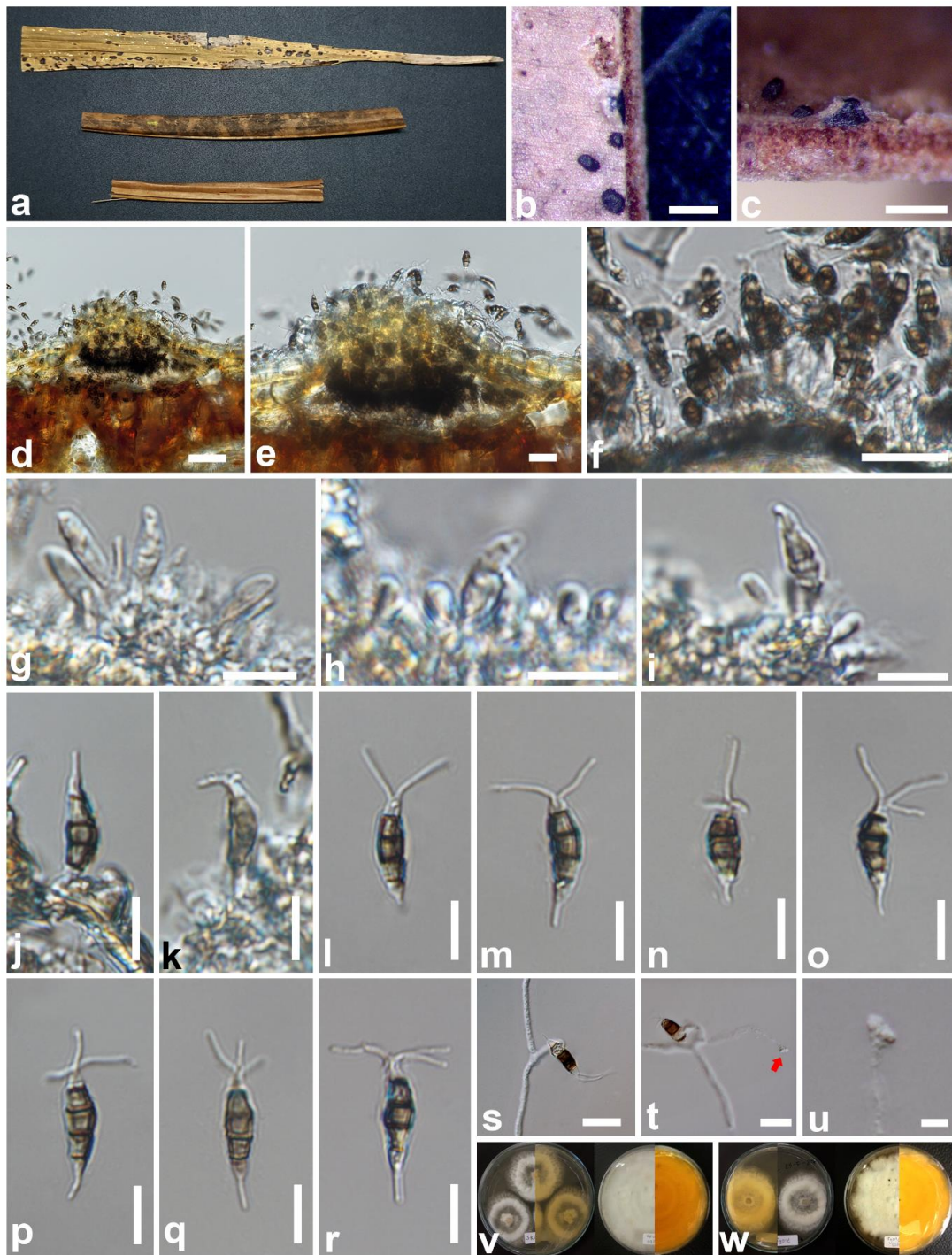


Figure 9 – *Neopestalotiopsis elaeidis* (MFLU 15-1466, holotype, MFLU 15-2334, isotype). a Host substrate. b Close-up of conidioma (top view). c Close-up of conidioma (side view). d, e Vertical section of conidioma. f–k Conidiogenous cells with conidia. l–r Conidia. s Germinated conidium of MFLUCC 15-0735. t Germinated conidium with appressorium (pointed with red arrow) of MFLUCC 15-0801. u Appressorium. v Colonies on MEA of MFLUCC 15-0735. w Colonies on MEA of MFLUCC 15-0801. Scale bars b, c = 200 μ m, d = 50 μ m, e, f, s, t = 20 μ m, g–r = 10 μ m, u = 3 μ m.

Diaporthales Nannf. 1932

Notes – Diaporthales consists of 32 families, while several genera are included in Diaporthales genera *incertae sedis* (Hyde et al. 2020b). The members of this order are generally endophytes, pathogens, saprobes, and parasites (Rossman et al. 2007, Alvarez et al. 2016).



Figure 10 – The best scoring RAxML tree with a final likelihood value of -34685.287080 for a combined dataset of ITS, LSU, TEF1, and TUB sequence data. The topology and clade stability of the combined gene analysis was compared with the single-gene analysis. The tree is rooted to *Diaporthe vaccinii* (CBS 160.32). The matrix had 1951 distinct alignment patterns with 61.30%

undetermined characters and gaps. Estimated base frequencies were; A = 0.241028, C = 0.275774, G = 0.254780, T = 0.228418; substitution rates AC = 1.673651, AG = 2.995992, AT = 1.634918, CG = 1.091344, CT = 6.017604, GT = 1.000000; gamma distribution shape parameter α = 0.281526. Newly generated sequences are in blue. Ex-type/type strains are indicated in bold. Bootstrap support values for ML equal to or greater than 60% and BYPP equal to or greater than 0.95 are given above the nodes. ^T is the type species and ^P indicates palm microfungi from Thailand.

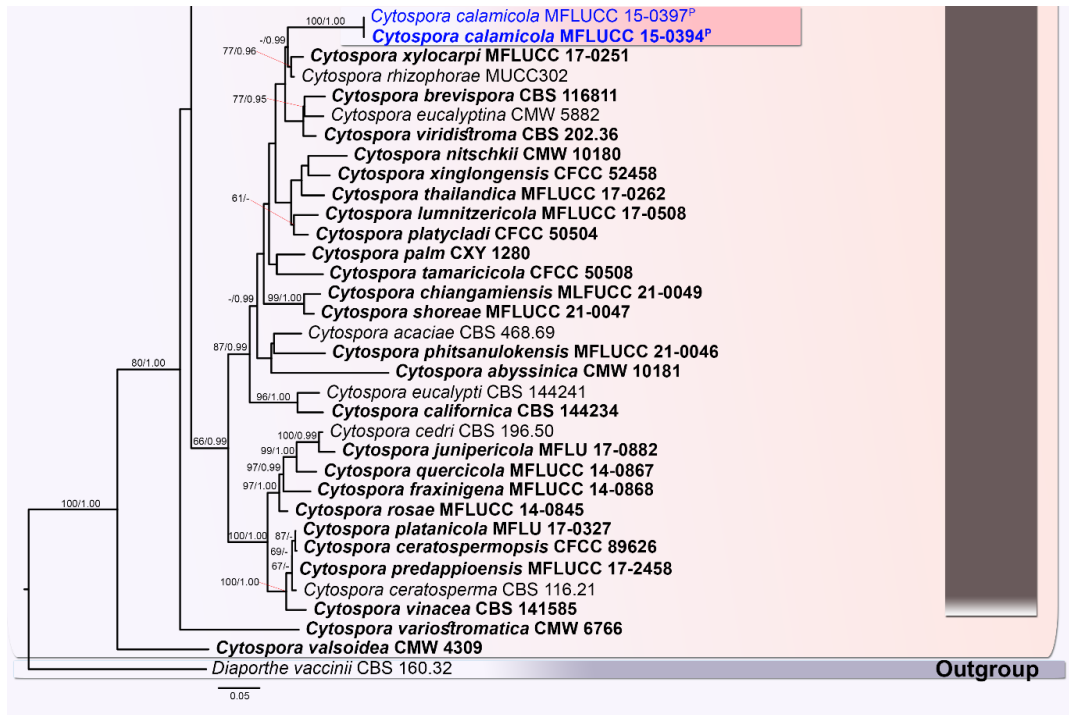


Figure 10 – Continued.

Cytosporaceae Fr. 1825

Notes – Cytosporaceae was introduced by Fries (1825). Six genera are accepted into this family, viz., *Cryptascoma*, *Cytospora*, *Pachytrype*, *Paravalsa*, *Waydora*, and *Xenotypa*, with the divergence time for the family estimated at 58.4 Mya (Hyde et al. 2020b).

Cytospora Ehrenb. 1818

Notes – *Cytospora* was introduced by Ehrenberg (1818). The members of this genus comprise important pathogens causing canker and dieback diseases in a wide range of plants (Lawrence et al. 2017, Norphanphoun et al. 2018, Fan et al. 2020, Jiang et al. 2020). The main characteristics of *Cytospora* are that they have coelomycetous asexual morphs producing single or a labyrinthine of loculate stromata, filamentous conidiophores, enteroblastic, phialidic conidiogenous cells, and aseptate, hyaline, allantoid conidia (Fan et al. 2020, Shang et al. 2020), while the sexual morph is characterized by immersed to erumpent ascostromata, embedded in ectostromatic disc, a J- apical ring, ellipsoid to clavate asci, and ellipsoid to allantoid, hyaline, aseptate ascospores (Norphanphoun et al. 2018, Fan et al. 2020). Recently, three new *Cytospora* species have been introduced to accommodate the diverse taxa of this genus; *C. Chiangmaiensis*, *C. phitsanulokensis* and *C. shoreae*, while screening biocontrol agents against pathogenic fungi (Monkai et al. 2021). *Cytospora* have been found on palm hosts worldwide, such as *Cytospora palmarum* on the palms (Arecaceae) in Florida, U. S. A. (Alfieri et al. 1984, Anonymous 1960), *Cytospora* sp. on leaf spot of palm (Arecaceae) in Florida, U. S. A. (Alfieri et al. 1984), *C. angularis* on *Arenga pinnata* in the Philippines (Teodoro 1937), *C. calami* on *Calamus* sp. in the Philippines (Reinking 1919, Teodoro 1937), *C. chrysosperma* on *Caryota urens* in India (Mathur

1979), *Cytospora* sp. on *Caryota urens* in Myanmar (Thaung 2008), *C. palmarum* on leaf blight of *Cocos nucifera* in Florida, U. S. A. (Alfieri et al. 1984), *C. palmicola* on *Cocos nucifera* in Mexico (McGuire & Crandall 1967), Philippines (Reinking 1918, 1919, Teodoro 1937), Puerto Rico, American Virgin Islands (Stevenson 1975), *Cytospora* sp. on *Cocos nucifera* in the Malay Peninsula (Thompson & Johnston 1953), and *C. yatay* on *Cocos yatay* in Argentina (Farr 1973). Currently, 318 *Cytospora* records are listed in Species Fungorum (2022). In this study, we introduce a new species of *Cytospora calamicola* collected from dead petioles of *Calamus* sp. (Arecaceae) from Thailand.

Cytospora calamicola Konta & K.D. Hyde, sp. nov.

Figs 11, 12

Index Fungorum number: IF559679; FacesofFungi number: FoF 10825

Etymology – Refers to both morphs being found on the host genus *Calamus*. The Latin words “colo” or “-cola” refer to inhabit.

Holotype – MFLU 15-0262.

Saprobic on dead petiole of *Calamus* sp. Sexual morph: *Ascomata* 260–380 × 185–290 µm, scattered, immersed, eventually with erumpent neck arising through cracks in bark epidermis, uniloculate, globose to sub-globose or irregular, whitish-yellow on the host surface, with long neck. *Neck* up to 120–250 µm long, central, lined with periphyses. *Peridium* 13–16 µm wide, comprising an outer layer of yellow to pale-brown, thick-walled cells of *textura prismatica* and inner layer, hyaline, thin-walled layer of cells. *Paraphyses* absent. *Asci* 15–20 × 3–5 µm, (\bar{x} = 16.5 × 5 µm, n = 50), 8-spored, unitunicate, cylindrical to clavate, with thin-walled pedicel, apex flat. *Ascospores* 3–5 × 1–2 µm, (\bar{x} = 4.3 × 1.7 µm, n = 100), biseriate or crowded, hyaline, fusiform or allantoid, oblong, unicellular, aseptate, smooth-walled (Fig. 11). Asexual morph: Coelomycetous. *Conidiomata* 250–300 × 110–175 µm, appearing as brownish-yellow areas, pycnidia semi-immersed, solitary or aggregated, globose to sub-globose, with long neck. *Peridium* 10–13 µm wide, comprising pale-brown, thick-walled, cells of *textura epidermoidea*. *Conidiophores* erect, hyaline. *Conidiogenous cells* enteroblastic, proliferating percurrently to produce small, hyaline, ellipsoidal conidia. *Conidia* 1–3 × 1–2 µm, (\bar{x} = 1.8 × 1.3 µm, n = 20), oblong to fusiform, small, unicellular, hyaline and smooth-walled (Fig. 12).

Culture characteristics – Colonies on MEA after ascospores and conidia germinated. Colonies from above; white to creamy, mycelia medium dense, circular, flat, entire margin. Colonies from below; yellow-white, circular with entire edge.

Material examined – THAILAND, Phang-Nga Province, on dead petiole of *Calamus* sp. (Arecaceae), 6 December 2014, Sirinapa Konta, DNH04g (MFLU 15-0262, holotype), ex-type living culture, MFLUCC 15-0397; *Ibid.*, DNH04c (MFLU 15-0259, HKAS95033, HKAS95034, isotypes), living culture MFLUCC 15-0394.

GenBank numbers – MFLUCC 15-0397 (Asexual morph), LSU: ON650679; ITS: ON650702; SSU: ON650693; TEF1: ON734013.

MFLUCC 15-0394 (Sexual morph), LSU: ON650680; SSU: ON650694.

Notes – The morphology of the new species, *Cytospora calamicola*, fits with the generic concept of *Cytospora*. Our strains were found in both sexual and asexual morphs, and they form a distinct lineage but are placed among *Cytospora* species in the phylogeny results with 0.99 BYPP statistical support (Fig. 10). In the BLASTn NCBI GenBank database search of LSU, ITS and TEF1 sequences, *Cytospora calamicola* isolates MFLUCC 15-0394 and MFLUCC 15-0397 are most similar; to *C. xylocarpi* (MFLU 17-0708) and *C. tibouchinae* (CBS 141324) with 97.94% and 97.12% (LSU); to *C. xinjiangensis* (CFCC 53183) (isolate MFLUCC 15-0397) with 89.47% (ITS); and to *Phialemonium obovatum* (CBS 279.76) (isolate MFLUCC 15-0397) with 92.81% (TEF1) similarity. Based on morphology and phylogeny, we introduce *Cytospora calamicola* as a new species from Thailand. In addition, we noted that several *Cytospora* species have been associated with palm hosts and most of them still lack molecular sequence data, thus, fresh material has to be recollected for the use of epitypification.

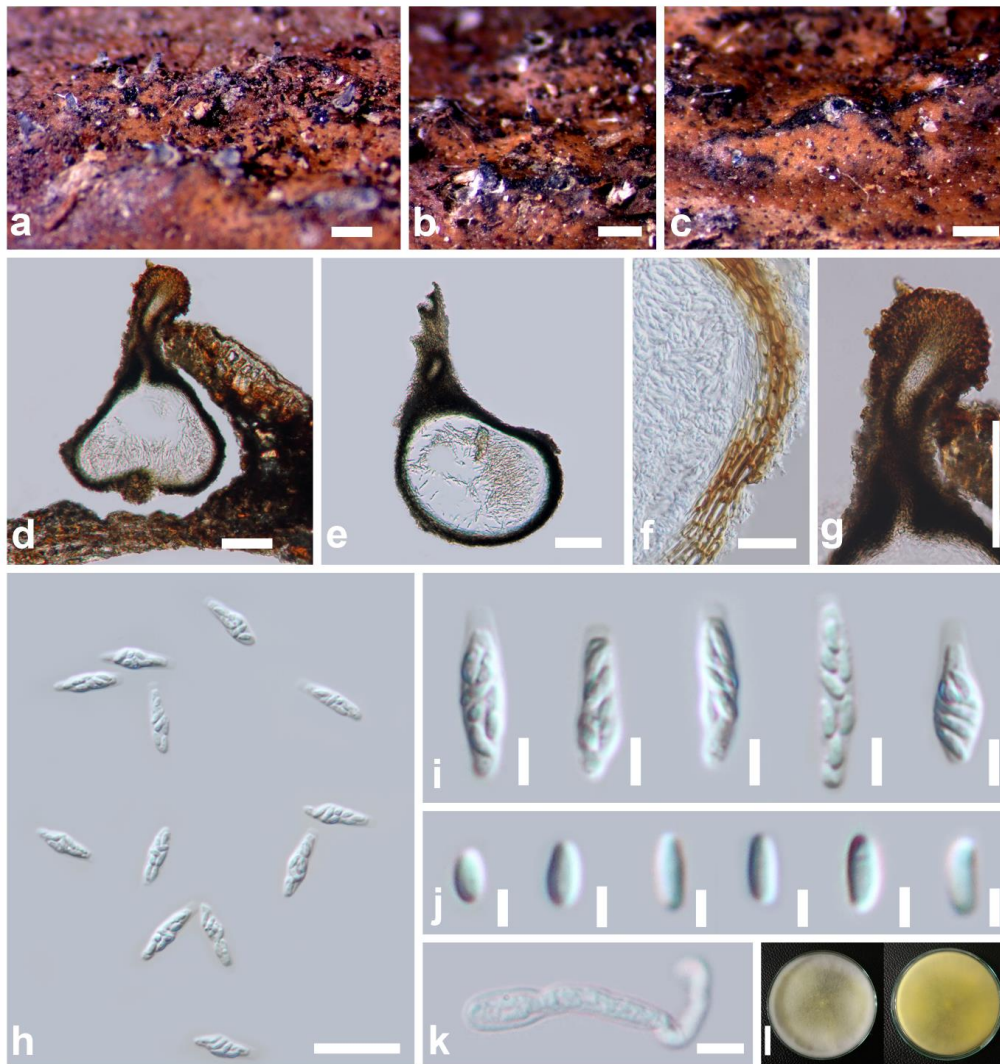


Figure 11 – *Cytospora calamicola* (MFLU 15-0259). a–c Appearance of ascomata on host substrate. d, e Section of an ascoma. f Peridium. g Ostiolar neck. h, i Asci. j Ascospores. k Geminated ascospore. l Colony on MEA. Scale bars: a–c= 500 μ m, d, e, g =100 μ m, f = 20 μ m, i = 5 μ m, j, k = 2 μ m.

Distoseptisporales Z.L. Luo, K.D. Hyde & H.Y. Su 2019

Notes – Distoseptisporales was introduced by Hyde et al. (2020b) to accommodate Distoseptisporaceae and *Sporidesmium*-like taxa. Based on morphology and phylogeny, Distoseptisporales clusters together in a distinct clade, within Diaporthomycetidae with strong statistical support (Hyde et al. 2020b).

Distoseptisporaceae K.D. Hyde & McKenzie 2016

Notes – Distoseptisporaceae was introduced by Su et al. (2016) to accommodate a single genus, *Distoseptispora*. Most of the fungi in this family are known from freshwater habitats. More freshwater fungal species of *Distoseptispora* have been reported recently by Dong et al. (2021), Yang et al. (2021), Phukhamsakda et al. (2022), and Zhai et al. (2022). The divergence time for Distoseptisporaceae was estimated at 190.57 Mya (Hyde et al. 2020b).

Distoseptispora Hyde & E. McKenzie & S. Maharachchikumbura 2016

Notes – *Distoseptispora* was introduced by Su et al. (2016) to accommodate *D. fluminicola* as the type species. *Distoseptispora* species have been published with their full descriptions of asexual and sexual morphs, molecular data, and geographical distributions (Su et al. 2016, Tibpromma et

al. 2018, Luo et al. 2018, 2019, Sun et al. 2020, Dong et al. 2021, Li et al. 2021, Yang et al. 2021). The asexual morph of *Distoseptispora* is a hyphomycetous form and is characterized by macronematous, mononematous conidiophores with septate, unbranched, olivaceous to brown, cylindrical monoblastic conidiogenous cells sub-hyaline or pigmented, euseptate or distoseptate conidia (Su et al. 2016). The sexual morph is characterized by having immersed to semi-immersed ascomata, sub-globose to ellipsoidal, ostiolate, with a short neck, peridium elongated cells of *textura angularis* or *textura prismatica*, cylindrical asci 8-spored, fusiform, hyaline, 0–3-septate ascospores with a mucilaginous sheath (Yang et al. 2021). Recently, Phukhamsakda et al. (2022) and Zhai et al. (2022) introduced more new species in the genus and 45 species are accepted in *Distoseptispora*. *Distoseptispora palmarum* has been reported as an asexual morph associated with *Cocos nucifera* (Arecaceae) in Thailand (Hyde et al. 2019). In this study, we introduce a new species, *Distoseptispora licualae*, collected from the dead leaves of *Licuala glabra* (Arecaceae) from Thailand.

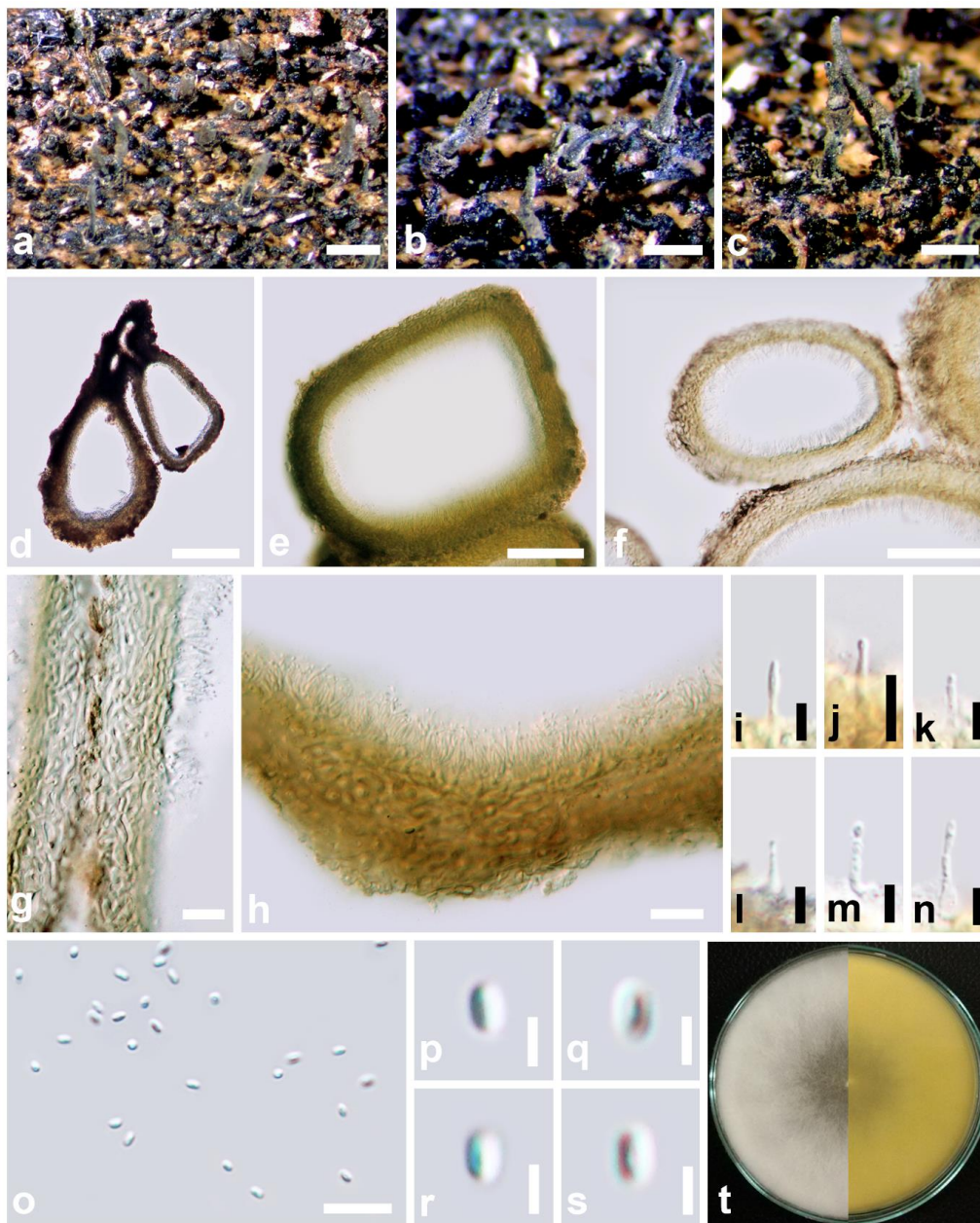


Figure 12 – *Cytospora calamicola* (MFLU 15-0262, holotype). a Appearance of conidiomata on host substrate. b, c Close up of conidiomata. d–f Sections of conidiomata. g Peridium. h–i Peridium. j–k Conidiophores. l–m Conidia. n–o Ascospores. p–q Asci. r–s Ascospores. t Petri dish with yellowish growth.

h–n Conidiogenous cells with conidia. o–s Conidia. t Colony on MEA. Scale bars: a = 1,000 μm, b, c = 500 μm, d = 100 μm, e, f = 50 μm, g–h = 10 μm, i–o = 5 μm, p–s = 2 μm.

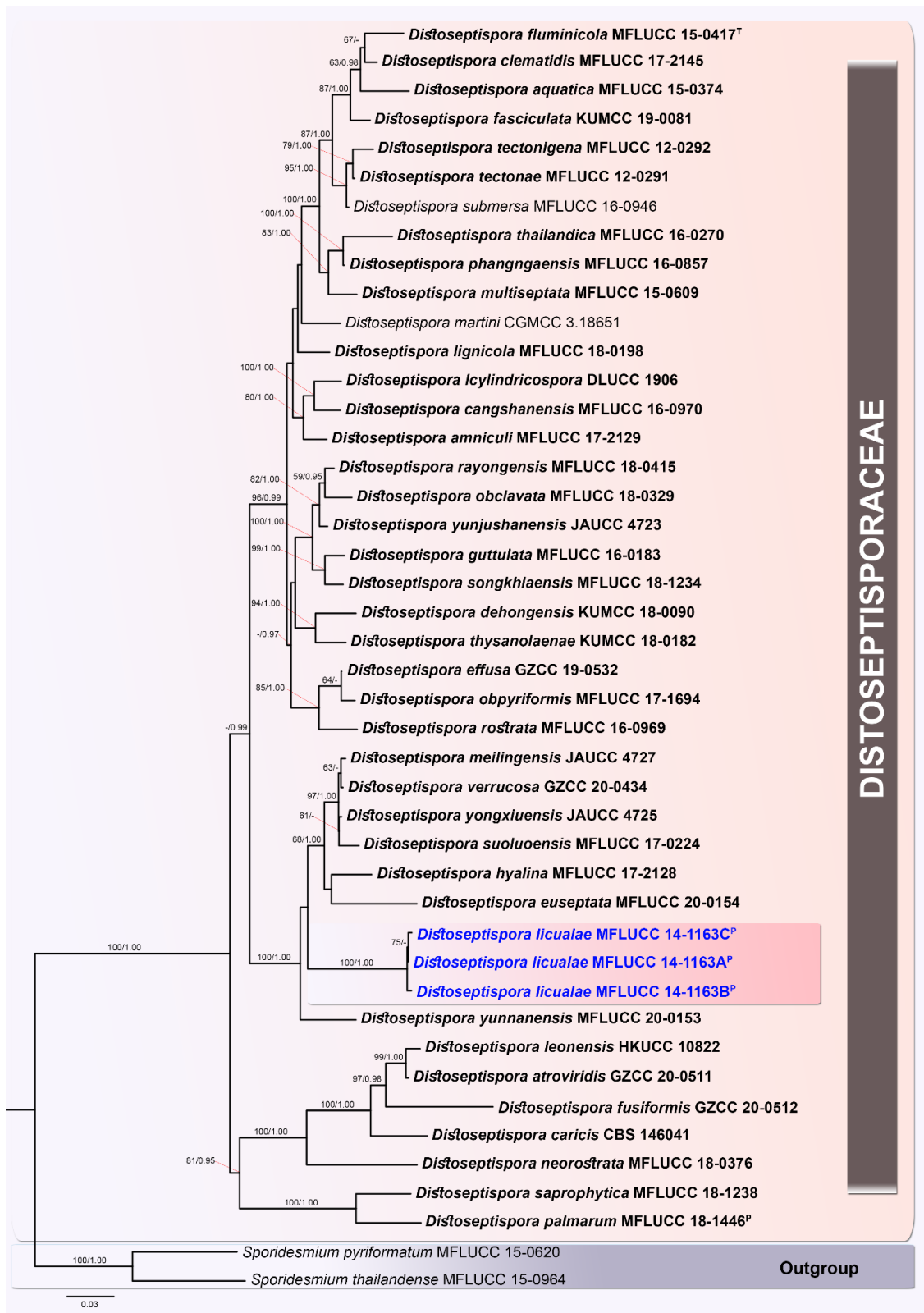


Figure 13 – The best scoring RAxML tree with a final likelihood value of -19782.043632 for a combined dataset of LSU, SSU, ITS, and TEF1 sequence data. The topology and clade stability of the combined gene analysis was compared with the single-gene analysis. The tree is rooted to *Sporidesmium pyriformatum* (MFLUCC 15-0620), and *S. thailandense* (MFLUCC 15-0964). The matrix had 1202 distinct alignment patterns with 52.22% undetermined characters and gaps.

Estimated base frequencies were; A = 0.241171, C = 0.259122, G = 0.280846, T = 0.218861; substitution rates AC = 1.098843, AG = 2.032031, AT = 1.359599, CG = 0.800555, CT = 5.800575, GT = 1.000000; gamma distribution shape parameter α = 0.180532. Newly generated sequences are in blue. Ex-type/type strains are indicated in bold. Bootstrap support values for ML equal to or greater than 60% and BYPP equal to or greater than 0.95 are given above the nodes. ^T is the type species and ^P indicates palm microfungi from Thailand.

Distoseptispora licualae Konta & K.D. Hyde, sp. nov.

Fig. 14

Index Fungorum number: IF559680; FacesofFungi number: FoF 10826

Etymology – Refers to the name of the host genus, *Licuala*.

Holotype – MFLU 15-0014.

Saprobic on dead leaves of *Licuala glabra*. Sexual morph: *Ascomata* 250–300 μm long, 120–140 μm diam., solitary or gregarious, semi-immersed to superficial, not upright, laid on the substrate, perithecial, sub-globose to ellipsoidal, dark brown, ostiolate, with a short neck through host surface. *Peridium* 15–30 μm (\bar{x} = 21 μm , n = 10) wide, thick coriaceous, comprising brown cells of *textura angularis*. *Paraphyses* 2–6 μm (\bar{x} = 4 μm , n = 30) wide, long, cylindrical, septate, hyaline, smooth-walled. *Asci* 120–210 \times 8–15 μm (\bar{x} = 158 \times 11 μm , n = 25), 6–8-spored, cylindrical, with a short pedicel, obtuse at the apex, apex with a J-, apical ring. *Ascospores* 20–30 \times 5–10 μm (\bar{x} = 24 \times 7 μm , n = 100), overlapping, uniseriate, inequilateral to fusiform, straight, rarely slightly curved, hyaline, aseptate, thin- and smooth-walled, guttulate, with a thick mucilaginous sheath. Asexual morph: Undetermined.

Culture characteristics – Ascospore germinated on PDA, one germ tube produced from one end. Colonies from above; light grey, dense, raised at the center, wrinkled, covered with white aerial mycelium, becoming olive-green with fimbriate at the margin. Colonies from below; light-grey with collapsed centre, mycelia turning olive-green at the edge.

Material examined – THAILAND, Narathiwat Province, on dead leaves of *Licuala glabra* (Arecaceae), 16 August 2014, Saithong Kaewchai NR06a (MFLU 15-0014, holotype), ex-type living culture, MFLUCC 14-1163A, B, C.

GenBank numbers – MFLUCC 14-1163A, B, C, LSU: ON650675, ON650676, ON650677; ITS: ON650686, ON650687, ON650688; SSU: ON650699, ON650700, ON650701; MFLUCC 14-1163A, B, TEF: ON734007, ON734008.

Notes – *Distoseptispora* species are commonly found as freshwater fungi, mainly with hyphomycetous morphs. Our collections share a similar morphology with *D. hyalina* in having sexual morphological characteristics (Yang et al. 2021). Both strains of *D. licualae* and *D. hyalina* shared an overlapping size range of ascospores with a thick mucilaginous sheath but can be different as our strains have aseptate ascospores and have been found as saprobes on dead leaves of *Licuala glabra* in a terrestrial habitat, while *D. hyalina* has 0–3-septate ascospores and has been reported from submerged wood in freshwater (Yang et al. 2021). In the multigene phylogeny, our strains (MFLUCC 14-1163A, B, C) clustered basal to *Distoseptispora euseptata*, *D. hyalina*, *D. suoluoensis*, *D. verrucosa*, and *D. yunnanensis*, well-separated from other species and with strong statistical support (100% ML, 1.00 BYPP, Fig. 13). In the BLASTn NCBI GenBank database search of LSU, ITS, SSU and TEF1 sequences, *Distoseptispora licualae* isolates (MFLUCC 14-1163A, B, C) are most similar; to *D. hyalina* (MFLUCC 17-2128) with 96.16%, 96.18%, and 96.14% (LSU); to *D. verrucosa* (GZCC 20-0434) (strains A, B), *D. bambusae* (MFLU 20-0261) (strain C) with 83.50%, 83.50%, 87.80% (ITS); to *D. hyalina* (MFLUCC 17-2128) (strains A, C) with 96.42%, 97.10% (SSU); and to *D. hyalina* (MFLU 21-0137) (strains A, B) with 95.11%, 95.16% (TEF1) similarity. *Distoseptispora palmarum* has been recorded as an asexual morph and associated with *Cocos nucifera* (Arecaceae) in Thailand (Hyde et al. 2019). Phylogenetic analyses indicate that our new species forms a distinct lineage (Fig. 13). Therefore, based on morphology and molecular data, our collection is introduced as a new terrestrial species (*Distoseptispora licualae*) that occurs on the dead leaves of *Licuala glabra* (Arecaceae). This is the second sexual morph report in this genus.



Figure 14 – *Distoseptispora licualae* (MFLU 15-0014, holotype). a Substrate. b, c Appearance of ascomata on host substrate. d Close up of an ascoma. e Section of an ascoma. f Asci. g Asci stain in Melzer's reagent. h–l Ascospores. m J- apical ring. n Paraphyses (red arrow pointing the separation and white arrow pointing the long stalk). o Peridium. p Germinated ascospore. q Colony on MEA. Scale bars: b = 500 μ m, c = 200 μ m, d = 100 μ m, e, h–p = 20 μ m, f, g = 50 μ m.

Glomerellales Chadef. ex Réblová, W. Gams & Seifert 2011

Notes – Five families, Australiascaceae, Glomerellaceae, Malaysiascaceae, Plectosphaerellaceae and Reticulascaceae, are accepted (Hyde et al. 2020b). The divergence time for Glomerellales was estimated at 215.97 Mya (Hyde et al. 2020b).

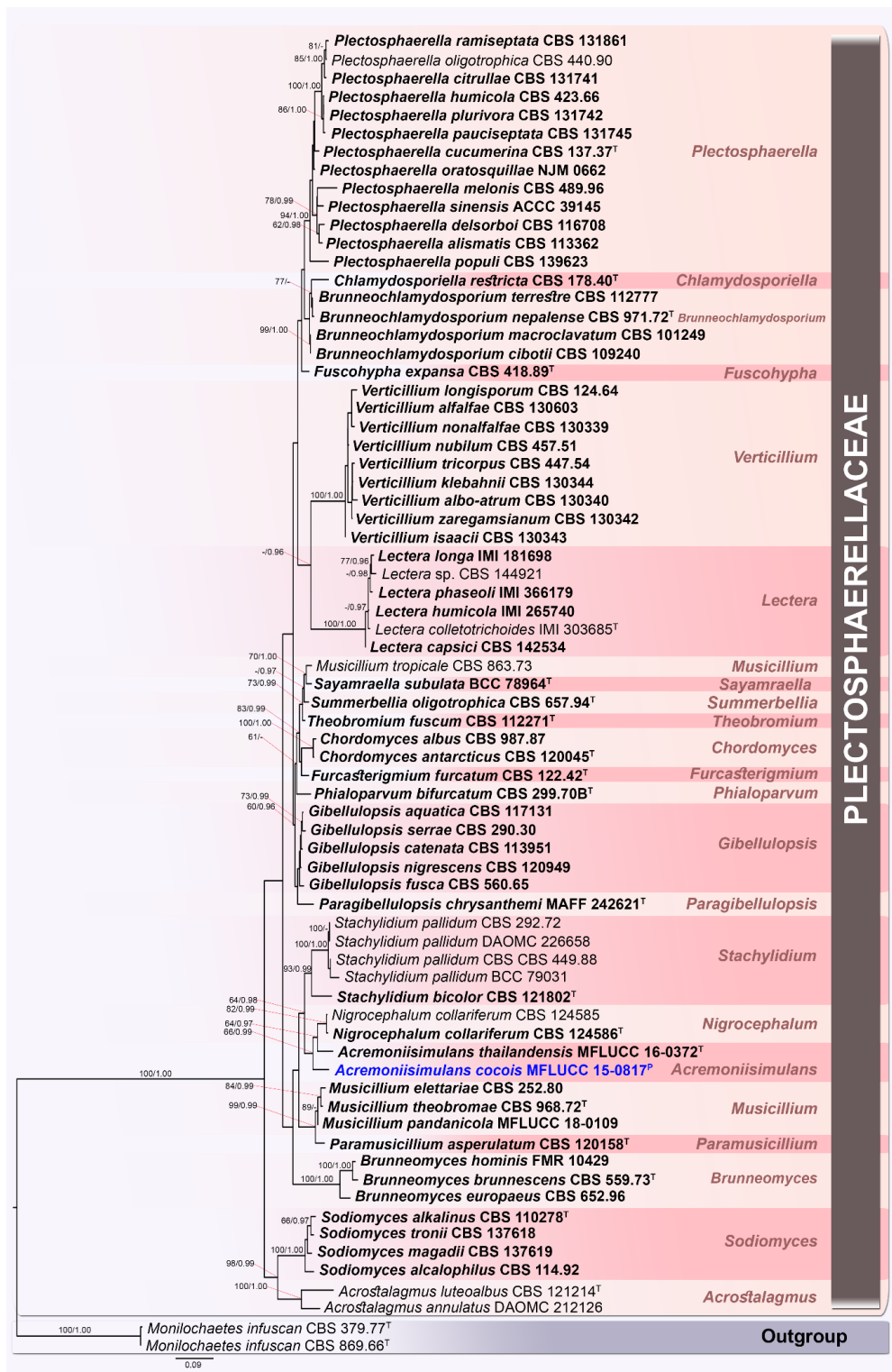


Figure 15 – The best scoring RAxML tree with a final likelihood value of -10269.766509 for a combined dataset of LSU and ITS sequence data. The topology and clade stability of the combined gene analysis was compared with the single-gene analysis. The tree is rooted to *Monilochaetes infuscan* (CBS 379.77, CBS 869.66). The matrix had 585 distinct alignment patterns with 14.04% undetermined characters and gaps. Estimated base frequencies were; A = 0.232604, C = 0.263827, G = 0.295820, T = 0.207749; substitution rates AC = 1.084283, AG = 1.945391, AT = 2.097202, CG = 0.597644, CT = 5.991097, GT = 1.000000; gamma distribution shape parameter α = 0.200792. Newly generated sequences are in blue. Ex-type/type strains are indicated in black bold. Bootstrap support values for ML equal to or greater than 60% and BYPP equal to or greater than

0.95 are given above the nodes. ^T is the type species and ^P indicates palm microfungi from Thailand.

Plectosphaerellaceae W. Gams, Summerb. & Zare 2007

Notes – Plectosphaerellaceae was erected by Zare et al. (2007) with *Plectosphaerella* as the type genus. The first complete study of the phylogenetic relationships of Plectosphaerellaceae was carried out by Zare et al. (2007). Later, Giraldo & Crous (2019) updated and resolved this family with 22 genera within Plectosphaerellaceae. Moreover, 15 new species and ten new combinations were introduced to accommodate the family (Graldo & Crous 2019). Subsequently, a few publications related to Plectosphaerellaceae have been published (Monkai et al. 2020, Crous et al. 2021). Currently, 24 genera are accepted in this family (Hyde et al. 2020b). The divergence time for Plectosphaerellaceae was estimated at 122.16 Mya (Hyde et al. 2020b). The family Plectosphaerellaceae has been reported as associated with 14 palm genera (Lu et al. 2000, Zhuang 2001, Taylor & Hyde 2003, Capdet & Romero 2010).

Acremoniisimulans Tibpromma & K.D. Hyde 2018

Notes – *Acremoniisimulans* was introduced by Tibpromma et al. (2018) to accommodate *A. thailandensis* as the type species. This monospecific genus was introduced to accommodate an asexual morph having hyphomycetous characteristics of pale brown conidiophores and conidia (Tibpromma et al. 2018). Currently, only *Acremoniisimulans thailandensis* is listed in Species Fungorum (2022) under this genus.

Acremoniisimulans cocois Konta & K.D. Hyde, sp. nov.

Fig. 16

Index Fungorum number: IF559681; FacesofFungi number: FoF 10827

Etymology – Refers to the host genus *Cocos*.

Holotype – MFLU 15-2350.

Saprobic on the dead petiole of *Cocos nucifera*. Sexual morph: *Ascomata* 170–230 × 110–200 μm (\bar{x} = 200 × 140 μm, n = 10), solitary, immersed to semi-immersed, oval to globose, or irregular, with central raised, dark, papillae, ostiole with periphyses. *Peridium* 10–25 μm (\bar{x} = 20 μm, n = 20) wide, outer cells merging with the host epidermal cells, composed of dark brown to black cells of *textura angularis*. *Paraphyses* 2–5 μm (\bar{x} = 3.2 μm, n = 50) wide, hyaline, branched, septate, longer than asci. *Asci* 30–65 × 6–13 μm (\bar{x} = 44 × 9 μm, n = 50), 4–8-spored, unitunicate, oblong to clavate, pedicellate, apex rounded, a J- apical ring. *Ascospores* 9–14 × 3–6 μm (\bar{x} = 11.4 × 4.3 μm, n = 100), biseriate, oblong to broadly oblong, straight or curved, rough, or sometimes conical at both ends, hyaline, 1-celled, guttules. Asexual morph: Undetermined.

Culture characteristics – Colony on MEA, colonies from above; white, medium dense, flat, smooth surface, entire edge, fluffy to velvety with smooth aspects, cream to white at the margin, white in the center. Colonies from below; white at the margin, white to cream in the center.

Material examined – THAILAND, Prachuap Khiri Khan Province, on dead petioles of *Cocos nucifera* (Arecaceae), 30 July 2015, Sirinapa Konta, PJK041 (MFLU 15-2350, holotype), ex-type living culture, MFLUCC 15-0817.

GenBank numbers – LSU: ON650672; SSU: ON650683; ITS: ON650691.

Notes – Based on the combined LSU and ITS phylogeny, our isolate clustered basal to *Acremoniisimulans thailandensis* with 66% ML and 0.99 BYPP support (Fig. 15). *Acremoniisimulans thailandensis* was previously recorded as saprobic on *Pandanus* sp. (Pandanaeae) in Prachuap Khiri Khan Province, Thailand, and only an asexual morph was reported (Tibpromma et al. 2018). In the BLASTn NCBI GenBank database search of LSU, ITS and SSU sequences, *Acremoniisimulans cocois* isolate (MFLUCC 15-0305) is most similar to *Chlamydosporiella restricta* (CBS 178.40) with 98.18% (LSU); to *Acremonium collariferum* (CBS 124586) with 88.25% (ITS); and to *Chordomyces antarcticum* (CBS 120045) with 99.26%, and 94.40% (SSU) similarity.

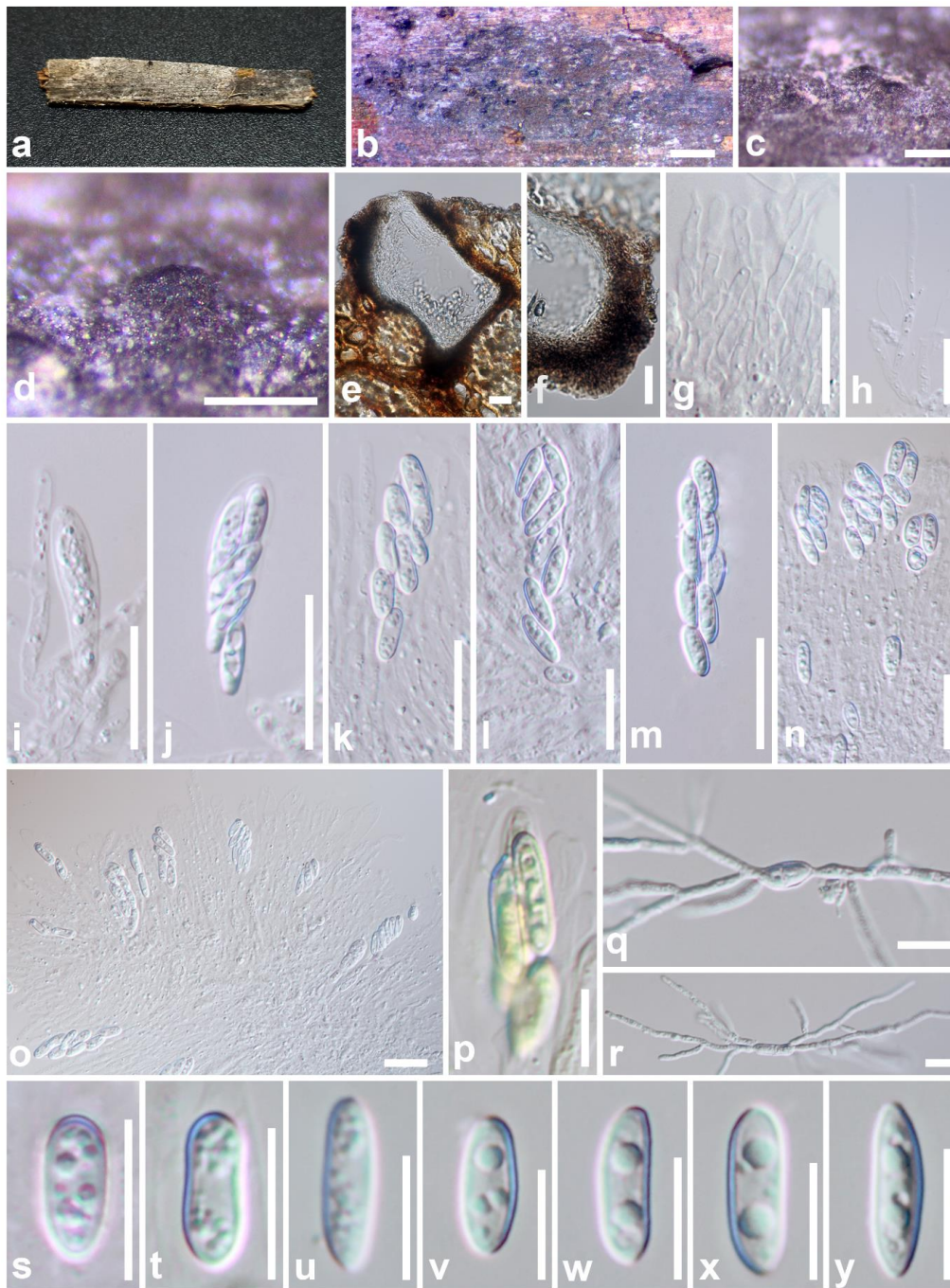


Figure 16 – *Acremoniisimulans cocois* (MFLU 15-2350, holotype). a Substrate. b Appearance of ascomata on host substrate. c, d Close-up of ascomata. e Section of an ascoma. f Peridium. g, h Paraphyses. i–o Asci. p J- apical ring. q, r Germinated ascospores. k–m Ascospores. Scale bars: b = 1 mm, c, d = 200 μ m, d = 100 μ m, e = 50 μ m, f–o = 20 μ m, p–y = 10 μ m.

We compared base pair differences of the LSU gene between our collection and the type species of *Acremoniisimulans* and the results showed that there is less than 1.5% nucleotide differences, while ITS has 3.8% differences (out of 551 bp, without gaps), which reveals that they belong to the same genus, but as a different species. Additionally, our new species shares the same locality, the same period of collecting, and same the monocotyledon host substrate as the type species of *Acremoniisimulans*. Unfortunately, we were unable to observe asexual characteristics in the culture and our strain was found as a sexual morph. Tibpromma et al. (2018) established *Acremoniisimulans* as a monotypic genus, but this genus has not been included in the phylogenetic tree of the family Plectosphaerellaceae in any study. Our phylogenetic tree (Fig. 15) indicated that

the genus *Acremoniisimulans* clusters with the genus *Nigrocephalum* with good statistical support of 64% ML and 0.97 BYPP; their morphology is very similar in having hyphomycetous asexual morph and without sexual morph, brown septate conidiophores with aseptate pale-brown conidia. However, *Nigrocephalum* differs from *Acremoniisimulans* as *Acremoniisimulans* was found in a plant (Pandanaeae), while *Nigrocephalum* was found in a human (Tibpromma et al. 2018, Graldo & Crous 2019). Based on the closest affinities, phylogenies, and the priorities of the studies published, *Acremoniisimulans* and *Nigrocephalum* have been introduced in 2018 and 2019, respectively (Tibpromma et al. 2018, Giraldo & Crous 2019). We introduce our taxon as a new saprobic species under *Acremoniisimulans* with the first sexual morph associated with dead petioles of *Cocos nucifera* (Arecaceae).

Xylariales genera incertae sedis

Notes – Recent studies have revealed that *Anthostomella* species and anthostomella-like taxa are species-rich genera (Daranagama et al. 2015, 2016, 2018, Dai et al. 2017, Tibpromma et al. 2018, Voglmayr et al. 2018, Hyde et al. 2020b, Konta et al. 2021b, Samarakoon et al. 2022). In the last few years, numerous anthostomella-like taxa have been explored and described as new genera, viz., *Alloanthostomella*, *Anthostomelloides*, *Haploanthostomella*, *Neoanthostomella*, *Nigropunctata*, *Pseudoanthostomella*, and *Xenoanthostomella* (Daranagama et al. 2015, 2018, Dai et al. 2017, Tibpromma et al. 2018, Hyde et al. 2020b, Konta et al. 2021b, Samarakoon et al. 2022). Based on recent phylogenetic studies, most of the *Anthostomella* and anthostomella-like taxa cluster apart from the Xylariaceae (Xylariales) and are treated and accepted as paraphyletic groups within the Xylariales genera *incertae sedis* (Fig. 17, Konta et al. 2021b, Samarakoon et al. 2022). We followed the current revision of this group by Samarakoon et al. (2022). In this study, we introduce a new taxon into the Xylariales genera *incertae sedis* with the available data; it is taxonomically prudent to name our collection as a new species, until it is proven not to be so, with more research knowledge.

***Xenoanthostomella* Mapook & K.D. Hyde 2020**

Notes – *Xenoanthostomella* was introduced by Hyde et al. (2020b) to accommodate *X. chromolaenae* as the type species, which has morphological affinities with *Anthostomella* but a distinct lineage. *Xenoanthostomella* has been accepted in Xylariales genera *incertae sedis*. Currently, only one species is reported from this genus (Species Fungorum 2022). The holotype species of *X. chromolaenae* has been recorded from Lampang Province, Thailand on the dead stem of *Chromolaena odorata* (Asteraceae) (Hyde et al. 2020b). The second report of *Xenoanthostomella chromolaenae* was found on a rachis of *Nephrolepis* sp. (Nephrolepidaceae) from Nan Province, Thailand (Samarakoon et al. 2022).

***Xenoanthostomella calami* Konta & K.D. Hyde, sp. nov.**

Fig. 18

Index Fungorum number: IF559682; FacesofFungi number: FoF 10828

Etymology – Refers to the host genus *Calamus*.

Holotype – MFLU 15-0003.

Saprobic on dead rachis of *Calamus* sp. Sexual morph: *Ascomata* 100–150 × 60–150 μm (\bar{x} = 125 × 115 μm, n = 20), solitary, immersed, visible as black spots, raised, thick carbonaceous cap, in cross-section ampulliform to ovoid or oppyriiform to irregular. *Ostioles* 45–60 × 60–85 μm (\bar{x} = 52 × 73 μm, n = 20), central, shiny black, conspicuous, ostiolar canal periphysate. *Peridium* 5–60 μm (\bar{x} = 27.5 μm, n = 40) wide, comprising hyaline to brown cells of *textura angularis*. *Paraphyses* 1–6 μm (\bar{x} = 3.6 μm, n = 70) wide, long, cylindrical, septate with small guttules, hyaline, smooth-walled. *Asci* 50–90 × 10–15 μm (\bar{x} = 67.5 × 13 μm, n = 30), 6–8-spored, unitunicate, clavate, constricted near the apex, discoid, J+ apical ring in Melzer's reagent, apex rounded, with a short pedicels. *Ascospores* 10–16 × 4–7 μm (\bar{x} = 12.4 × 5.3 μm, n = 40), uni- to bi-seriate, hyaline to pale brown when immature, dark olivaceous-brown at maturity, 1–3-large guttules when immature, multi-granule at maturity, oblong to narrowly ellipsoid, inequilateral to broadly fusiform, slightly curved,

limoniform, aseptate, thick mucilaginous sheath, with a straight germ slit along the entire spore length. Asexual morph: Undetermined.

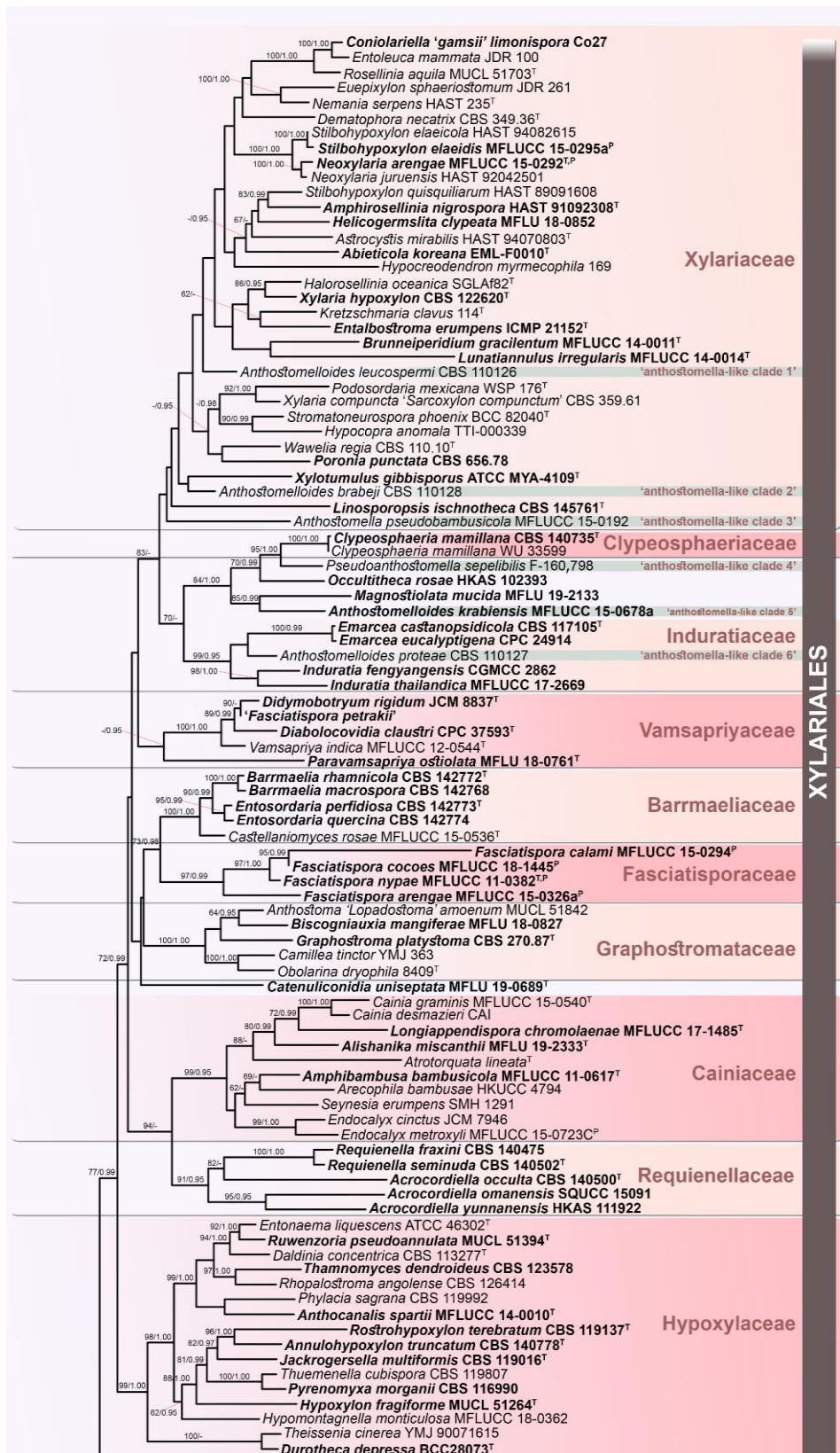


Figure 17 – The best scoring RAxML tree with a final likelihood value of -136659.837263 for a combined dataset of ITS, LSU, TUB, and RPB2 sequence data. The topology and clade stability of the combined gene analysis was compared with the single-gene analysis. The tree is rooted to Delonicolales. The matrix had 4588 distinct alignment patterns with 63.22% undetermined

characters and gaps. Estimated base frequencies were; A = 0.243582, C = 0.258911, G = 0.258955, T = 0.238552; substitution rates AC = 1.250500, AG = 3.143800, AT = 1.446898, CG = 1.054424, CT = 5.644779, GT = 1.000000; gamma distribution shape parameter α = 0.452172. Newly generated sequences are in blue. Ex-type/type strains are indicated in black bold. Bootstrap support values for ML equal to or greater than 60% and BYPP equal to or greater than 0.95 are given above the nodes. ^T is the type species and ^P indicates palm microfungi from Thailand.

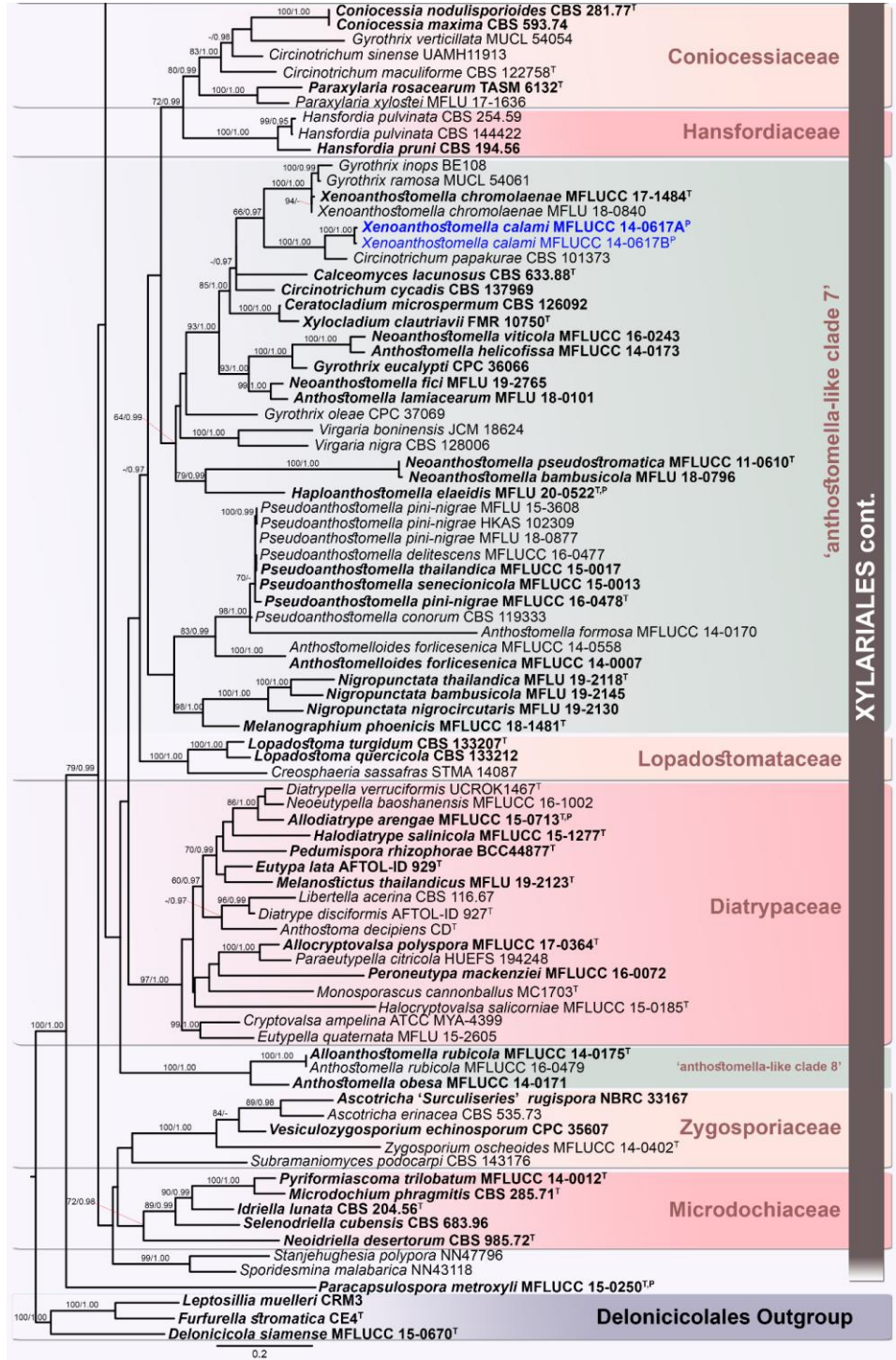


Figure 17 – Continued.

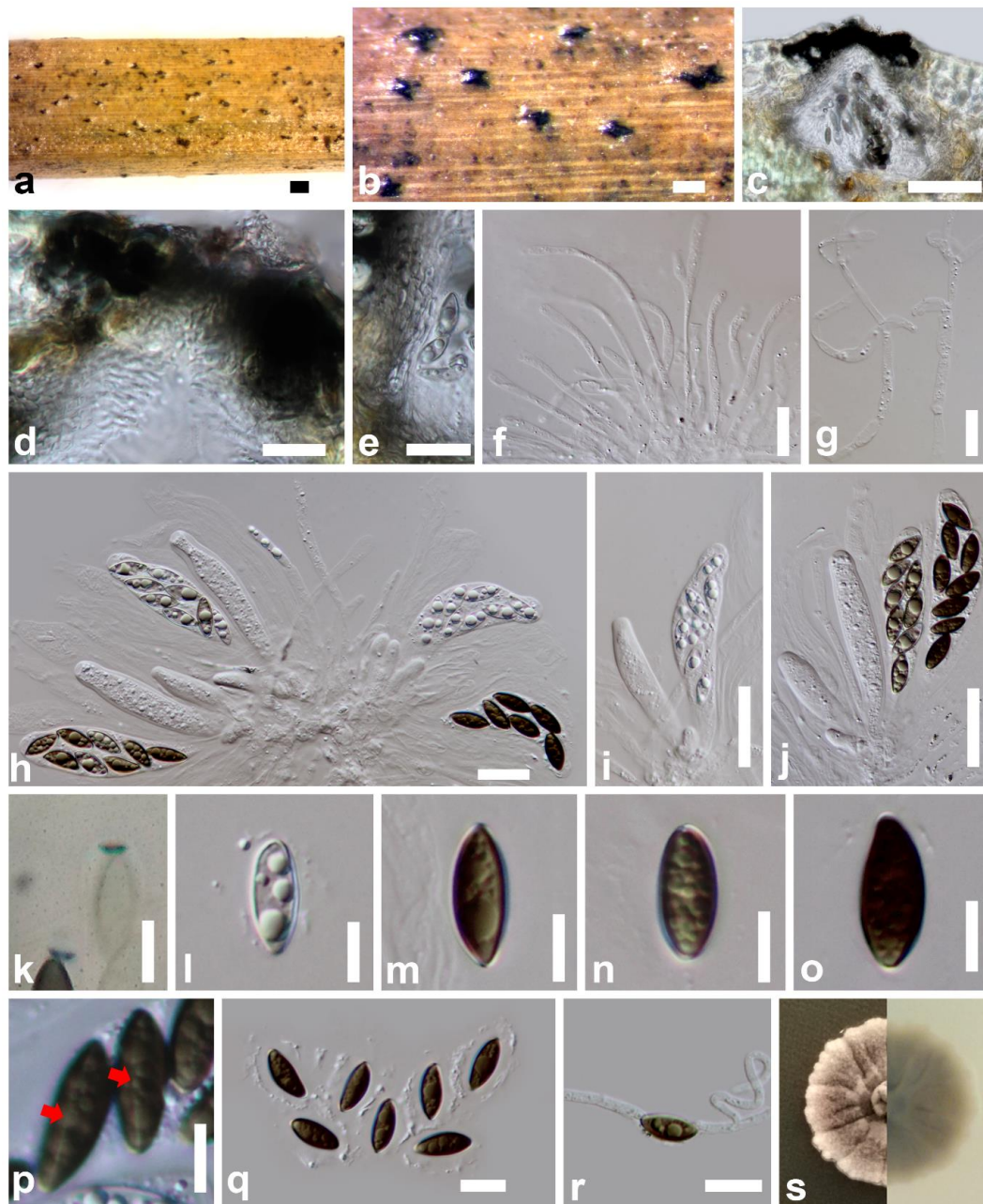


Figure 18 – *Xenoanthostomella calami* (MFLU 15-0003, holotype). a, b Appearance of ascomata on the substrate. c Section of an ascoma. d Periphyses. e Peridium. f, g Paraphyses. h–j Asci. k J+ apical ring. l–o Ascospores. p Germ slit. q Mature ascospores with mucilaginous sheaths. r Germinated ascospore. s Colony on MEA. Scale bars: a = 500 μm , b = 200 μm , c = 50 μm , d–j = 20 μm , k–p = 5 μm , q, r = 10 μm .

Culture characteristics – Colonies on MEA after ascospore germinated. Cultures from above; brown, dense, circular, edge undulate or erose or lobate, wrinkled, folded, brown, next circular dark olivaceous-brown, white-creamy at edge. Colonies from below; dark olivaceous-brown at the center, wrinkled, folded, white-creamy at the edge.

Material examined – THAILAND, Chiang Mai Province, on a dead rachis of *Calamus* sp. (Arecaceae), 11 August 2014, Sirinapa Konta, P07b (MFLU 15-0003, holotype), ex-type living culture, MFLUCC 14-0617A, B.

GenBank numbers – MFLUCC 14-0617A, B, LSU: ON650706, ON650707; ITS: ON650684, ON650685; SSU: ON650708, ON650709; TEF1: ON734009, ON734010; TUB: ON745964, ON745965.

Notes – Based on the multi-gene phylogenetic analyses (Fig. 17), *Xenoanthostomella calami* clustered among six genera, *Calceomyces*, *Ceratocladium*, *Circinotrichum*, *Gyrothrix*, *Xenoanthostomella*, and *Xylocladium* in anthostomella-like clade 7 (Fig. 17), and the placement is similar to the studies of Hyde et al. (2020b) and Samarakoon et al. (2022). Herein, we treat our collection as a new species under the genus *Xenoanthostomella* based on a sister branch in the multi-gene phylogeny, and the morphology is more closely related to *Xenoanthostomella* than to *Calceomyces*, *Ceratocladium*, *Circinotrichum*, *Gyrothrix*, or *Xylocladium*. *Xenoanthostomella calami* is similar to *X. chromolaenae* (holotype) in having immersed ascomata with 6–8-spored asci, dark olivaceous-brown ascospores. Moreover, our new species is different from the two strains of the type species in having ascomata with a thick carbonaceous cap, irregular ascomata in cross-section, preformed clavate asci, and bi-seriate ascospores with a thick mucilaginous sheath and straight germ slit. Samarakoon et al. (2022) have mentioned that the presence of a spiral germ slit ascospore is a key characteristic among the species/genera clustered between *Xenoanthostomella*. In the BLASTn NCBI GenBank database search of LSU, ITS, SSU, TEF1 and TUB sequences, *Xenoanthostomella calami* isolates (MFLUCC 14-0617A, B) are most similar; to *X. chromolaenae* (MFLUCC 17-1484) with 98.98%, and 99% (LSU); to *Circinotrichum cycadis* (CBS 137969) with 86.28%, and 86.16% (ITS); to *X. chromolaenae* (MFLUCC 17-1484) with 99.23%, and 99.42% (SSU); to *Allodiatrype arengae* (MFLUCC 15-0713) with 91.04%, and 90.86% (TEF1); and to *Calceomyces lacunosus* (CBS 633.88) with 90.73%, and 90.63% (TUB) similarity. In this study, we introduced a new species *Xenoanthostomella calami* as the second species for this genus, and this is the first report of a *Xenoanthostomella* species on a *Calamus* host (Arecaceae).

Discussion

In this study, saprobic fungi associated with the dead rachis, petioles, and leaves of the Arecaceae viz., *Adonidia merrillii*, *Arenga pinnata*, *Calamus* sp., *Cocos nucifera*, *Elaeis guineensis*, and *Licuala glabra* are reported from terrestrial habitats in Thailand. Six palm genera were sampled from six Provinces in Thailand viz., Chiang Mai, Kanchanaburi, Narathiwat, Phang-Nga, Prachuap Khiri Khan, and Songkhla. A new genus and nine new species associated with palm substrates in Thailand are described above, based on multi-gene analyses in combination with morphological data.

In this study, we found that most of the new taxa associated with *Calamus* sp. and *Cocos nucifera* referring to the information by Fröhlich & Hyde (2000) and Pinnoi et al. (2009). Fröhlich & Hyde (2000) mentioned that the members of the Ascomycetes are the primary group of fungi that are found on palm substrates, and *Cocos* and *Calamus* (Arecaceae), were the richest sources of palm ascomycetes.

In addition, two new saprobic species, *Bartalinia adonidiae* and *Neopestalotiopsis elaeidis*, in the family Sporocadaceae, occurred on the dead parts of palm substrates. They produced appressoria (present in many fungal plant pathogens as used to infect host plants) from germ tubes after conidia germinated on the media. This is evidence that a species can change its lifestyle from saprobic to pathogenic or endophytic to pathogenic. For example, when the host is stressed, some endophytic fungi may become pathogenic, and when the host is dead, some endophytic/pathogenic fungi may become saprobic. Many known saprobe species obtained in this study produced appressoria on culture media, while members of several genera, viz., *Leptosporella*, *Neolinocarpon*, *Neoxylaria*, and *Oxydothis* are reported as saprobes (Konta et al. 2016c, 2020a, Hyde et al. 2020b). This lifestyle change of palm fungi needs future in-depth studies.

Conclusion

The present study investigated the microfungi associated with palms in several Provinces in Thailand. Although most fungal families coincided with the expected origin of the host family (Arecaceae from worldwide), all new species identified in this study are probably not specific to palm hosts. The dominant group of our new species is Sordariomycetes rather than

Dothideomycetes, which is consistent with the previous studies on palm fungi. This confirms that palms are rich in Sordariomycetes, which could be associated with a younger divergent time of Sordariomycetes than Dothideomycetes, and which is still evolving. This study adds a new genus and nine new species to Thailand's assemblage of palm-associated microfungi based on morphology and phylogeny. Further, studies with more collections are necessary to better understand the fungal diversity associated with palms in Thailand.

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