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Article

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Additions to *Fissuroma* and *Neoastrosphaeriella* (Aigialaceae, Pleosporales) from palms

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Abstract

Recent examination of palm fungi led to the discovery of a group of fissuroma-like taxa, which share the following morphological features: slit-like ascomata, carbonaceous peridium, trabeculate pseudoparaphyses, cylindric-clavate or obclavate asci and fusiform, septate ascospores. Multi-gene phylogenetic analyses based on a combined ITS, LSU, SSU and TEF1-α sequence data support the establishment of *Fissuroma palmae* sp. nov. and *Neoastrosphaeriella phoenicis* sp. nov. *Fissuroma caryotae* and *N. aquatica* are also recovered as new host records from terrestrial palms. *Fissuroma palmae* is phylogenetically close to *F. caryotae* with strong support, but differs from *F. caryotae* in the appearance of pale brown mature ascospores, and the dimensions of asci and ascospores. *Neoastrosphaeriella phoenicis* is associated with submerged petioles of *Phoenix paludosa* in mangroves, providing an insight of a new habitat for *Neoastrosphaeriella* species. The hyaline to pale brown, fusiform, 1–3-septate, guttulate, verrucose ascospores of *N. phoenicis* distinguish it from other existing *Neoastrosphaeriella* species. Additional new taxa and their morphological features, ecological occurrence, as well as phylogenetic circumscription of genera in Aigialaceae are provided and discussed.

Key words – 2 new taxa – Arecaceae – Dothideomycetes – Phylogeny – Taxonomy

Introduction

The family Aigialaceae was introduced by Suetrong et al. (2009) to accommodate three marine genera *Aigialus* Kohlm. & S. Schatz, *Ascocratera* Kohlm. and *Rimora* Kohlm., Volkm.-Kohlm., Suetrong, Sakay. & E.B.G. Jones. Subsequently, two genera *Fissuroma* J.K. Liu, R. Phookamsak, E.B.G. Jones & K.D. Hyde and *Neoastrosphaeriella* J.K. Liu, E.B.G. Jones & K.D. Hyde from terrestrial habitats were included based on multi-gene phylogeny (Liu et al. 2011). Members of Aigialaceae are characterized by carbonaceous ascomata with slit-like or crater-like

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ostioles, trabeculate pseudoparaphyses, cylindric-clavate or obclavate asci and septate ascospores with a sheath or apical appendages (Suetrong et al. 2009, Liu et al. 2011, Hyde et al. 2013). The ascomata and ascospore morphologies are remarkable features to distinguish taxa in this family. *Aigialus*, the type genus of Aigialaceae, has immersed ascomata with apical slit-like ostioles and muriform brown ascospores with terminal appendages (Suetrong et al. 2009, Zhang et al. 2012). While the monotypic genus *Ascocratera* is distinct in having crater-like, erumpent to superficial ascomata, and ellipsoidal, 1-septate (3-septate when senescent) ascospores (Kohlmeyer 1986, Suetrong et al. 2009, Zhang et al. 2012). *Fissuroma*, *Neoastrosphaeriella* and *Rimora* share similar morphological features: slit-like, erumpent to superficial ascomata, and fusiform ascospores. However, *Rimora*, which is typified by *R. mangrovei* (≡ *Lophiostoma mangrovei*), can be distinguished by its broad oblong ascomata, thick peridium, cylindrical asci and 3-septate ascospores (Kohlmeyer & Vittal 1986, Suetrong et al. 2009, Liu et al. 2011). The phenotypic distinction between *Fissuroma* and *Neoastrosphaeriella* is not very clear but the two genera constitute distinct clades in the family Aigialaceae.

Liu et al. (2011) introduced *Fissuroma* to accommodate species with slit-like ascomata that were excluded from *Astrosphaeriella* Syd. & P. Syd., with *F. maculans* (Rehm) J.K. Liu, E.B.G. Jones & K.D. Hyde as the type species (≡ *Metasphaeria maculans* Rehm), and the combination *F. aggregata* (I. Hino & Katum.) R. Phookamsak., J.K. Liu, E.B.G. Jones & K.D. Hyde (≡ *Melanopsamma aggregate* I. Hino & Katum.). However, Phookamsak et al. (2015) named one of the represented *F. aggregate* strains as *F. neoaggregata* Phookamsak & K.D. Hyde, and introduced *F. bambusae* Phookamsak & K.D. Hyde, *F. fissuristoma* (J. Fröhl et al.) Phookamsak & K.D. Hyde and *F. thailandicum* Phookamsak & K.D. Hyde. Thereafter, Wanasinghe et al. (2018) discovered *F. calami* Wanas., E.B.G. Jones & K.D. Hyde and *F. caryotae* Wanas., E.B.G. Jones & K.D. Hyde from terrestrial palms, which are quite similar in morphology but phylogenetically distinct. Tennakoon et al. (2018) described *F. taiwanense* Tennakoon, C.H. Kuo & K.D. Hyde, which differs from the other species by broad cylindrical to obclavate asci and broad ascospores with distinctive long apical and basal appendages. Niranjan & Sarma (2018) reported *F. kavachabeejae* M. Niranjan & V.V. Sarma and *F. microsporum* M. Niranjan & V.V. Sarma in India but without molecular data. Eleven *Fissuroma* epithets are recorded in Index Fungorum (November, 2019), which are characterized by conical, hemisphaerical, carbonaceous ascomata, with flattened base and slit-like ostioles, trabeculate pseudoparaphyses, cylindric-clavate, short pedicellate asci, and 1-septate, fusiform ascospores (Liu et al. 2011, Phookamsak et al. 2015, Niranjan & Sarma 2018, Tennakoon et al. 2018, Wanasinghe et al. 2018).

During our investigation of palm fungal diversity, we collected five fissuroma-like taxa from palms in mangrove and terrestrial habitats in Thailand. Further morphological comparison and molecular phylogeny revealed that these collections belong to *Fissuroma* and *Neoastrosphaeriella*, respectively. In this study, a multi-gene phylogeny based on DNA sequence data and morphological comparisons are performed to identify the species. Two new species and two new host records are introduced to *Fissuroma* and *Neoastrosphaeriella*.

Materials & Methods

Specimens collection, examination and single spore isolation

Decayed rachides/petioles of palms were collected from mangrove and terrestrial habitats in Thailand (detailed information of collection sites and hosts are listed in the taxonomy section). Specimens were sorted and placed into plastic bags in the field along with the environment, geographic location and host information. Fungal fruiting bodies were observed using Motic SMZ 168 stereo microscope and free hand sections of fruiting bodies were made into slides mounted in water by using a syringe needle. Morphological characters were observed using a Carl Zeiss stereo microscope fitted with an AxioCam ERC 5S camera and photographed by a Nikon ECLIPSE 80i compound microscope fitted with a Canon EOS 600D digital camera. Measurements were taken by Tarosoft Image Frame Work program v. 0.9.7 (Liu et al. 2010) and images used for figures processed with Adobe Photoshop CS6 Extended v. 13.0 software. Isolations were obtained from single spore as described in Chomnunti et al. (2014). The strains isolated in this study were deposited in Mae Fah Luang University Culture Collection (MFLUCC). Herbarium specimens were deposited at the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (KUN-HKAS), Kunming, China. New taxa are established based on recommendations as outlined by Jeewon & Hyde (2016). The scientific names of the new taxa were registered in Index Fungorum and the FacesofFungi (Jayasiri et al. 2015).

DNA extraction, PCR amplification and sequencing

Fungal genomic DNA were extracted from fresh mycelia scraped from the margin of a colony on PDA that was incubated at 25–28ºC for one month, by following the manufacturer's instructions of the Ezup Column Fungi Genomic DNA Purification Kit (Sangon Biotech (Shanghai) Co., Ltd, China). In the case of failure to obtain pure cultures, the genomic DNA was extracted from fruiting bodies by using the E.Z.N.A. TM Forensic DNA Extraction Kit (OMEGA Bio-Tek, D3591-01, Norcross, GA, U.S.A.). Four gene regions were used for polymerase chain reaction (PCR) of the new collections: the internal transcribed spacers (ITS: ITS1-5.8S-ITS2), the large subunit of the nuclear ribosomal RNA genes (LSU), the small subunit of the nuclear ribosomal RNA (SSU), and the translation elongation factor-1 alpha (TEF1-α). The primers used were ITS5/ITS4 for ITS (White et al. 1990), LR0R and LR5 for LSU (Vilgalys & Hester 1990), NS1/NS4 for SSU (White et al. 1990) and EF1-983F/EF1-2218R for TEF1-α (Rehner 2001). The amplification reactions were performed in 25μL of PCR mixtures containing 9.5μL ddH_2O, 12.5μL 2× PCR MasterMix (TIANGEN Co., China), 1μL DNA template and 1μL of each primer. The PCR thermal cycle program for ITS, LSU, SSU and TEF1-α amplification were as follows: initial denaturing step of 94°C for 3 mins, followed by 40 cycles of denaturation at 94°C for 45 seconds, annealing at 56°C for 50 seconds, elongation at 72°C for 1 min, and final extension at 72°C for 10 mins. Purification and sequencing of PCR products were carried out with primers mentioned above at Sangon Biotech (Shanghai) Co., Ltd, China.

Sequence alignment and phylogeny analyses

A concatenated dataset of the ITS, LSU, SSU and TEF1-α sequences was used for phylogenetic analyses with the inclusion of reference taxa from GenBank (Table 1). Sequences were aligned using MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013) and then checked visually and manually optimized using BioEdit v.7.0.9 (Hall 1999). Taxa of Aigialaceae and representative families and genera in Pleosporales were included in the analyses. Maximum likelihood (ML), Maximum parsimony (MP) and Bayesian analyses were carried out and the detail procedures were followed Zhang et al. (2018, 2019). Phylogenetic tree was visualized by FigTree v.1.4.2 (Rambaut 2014). The phylogenetic tree and DNA sequence alignment were deposited in TreeBASE (study accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S25366).

Table 1 Taxa those were included in this study and their GenBank accession numbers. The type strains are indicated with superscript ^{T} and new generated strains are in bold.

Taxa	Strains/vouchers	GenBank accession numbers			
		ITS	**LSU**	**SSU**	**TEF1-α**
Acuminatispora palmarum	MFLUCC 18-0264 ^{T}	MN749105	MH390437	MH390401	MH399248
Acuminatispora palmarum	MFLUCC 18-0460	MN749106	MH390438	MH390402	MH399249
Aigialus grandis	BCC 18419	_	GU479774	GU479738	GU479838
Aigialus mangrovis	BCC 33563	_	GU479776	GU479741	GU479840
Aigialus parvus	BCC 18403	_	GU479778	GU479744	GU479842

Table 1 Continued.

Taxa	Strains/vouchers	GenBank accession numbers			
		ITS	**LSU**	**SSU**	**TEF1-α**
Aigialus rhizophorae	BCC 33572	_	GU479780	GU479745	GU479844
Ascocratera manglicola	BCC 09270	_	GU479782	GU479747	GU479846
Astrosphaeriella bambusae	MFLUCC 13-0230 T	_	KT955461	_	KT955424
Astrosphaeriella fusispora	MFLUCC 10-0555	JN846719	KT955462	KT955443	KT955425
Astrosphaeriella stellata	KT 998	_	AB524592	AB524451	_
Astrosphaeriellopsis bakeriana	CBS 115556	_	GU301801	_	GU349015
Astrosphaeriellopsis bakeriana	MFLUCC 11-0027 T	_	JN846730	JN846740	_
Astrosphaeriellopsis caryotae	MFLUCC 13-0832	_	MF588991	MF588981	MF588974
Astrosphaeriellopsis caryotae	MFLUCC 13-0833 T	_	MF588992	MF588982	_
Delitschia chaetomioides	SMH 3253.2	_	GU390656	_	_
Delitschia winteri	CBS 225.62	_	DQ678077	DQ678026	DQ677922
Fissuroma aggregata	KT 984	_	AB524591	AB524450	AB539105
Fissuroma aggregata	KT 767	_	AB524590	AB524449	_
Fissuroma bambusae	MFLUCC 11-0160 T	_	KT955468	KT955448	KT955430
Fissuroma calami	MFLUCC 13-0836 T	_	MF588993	MF588983	MF588975
Fissuroma caryotae	MFLU 17-1253 T	_	MF588996	MF588986	MF588979
Fissuroma caryotae	**MFLUCC 16-1383**	**MN735992**	**MN712335**	**MN699322**	**MN744228**
Fissuroma maculans	MFLUCC 10-0886 T	JN846710	JN846724	JN846734	_
Fissuroma maculans	MFLUCC 11-0023	JN846714	JN846728	JN846738	_
Fissuroma neoaggregata	MFLUCC 10-0554 T	JN846718	KT955470	KT955450	KT955432
Fissuroma palmae	**MFLU 19-0820**	_	**MN712336**	_	**MN744229**
Fissuroma taiwanense	FU30861 T	_	MG189605	MG189607	MG252072
Fissuroma thailandicum	MFLUCC 11-0206	_	KT955473	KT955453	KT955435
Massaria gigantispora	M26	HQ599397	HQ599397	HQ599447	HQ599337
Massaria inquinans	M19	HQ599402	HQ599402	HQ599444	HQ599342
Neoastrosphaeriella aquatica	MFLUCC 18-0209 T	MK138710	MK138829	MK138789	MK132866
Neoastrosphaeriella aquatica	**MFLU 19-0816**	**MN735993**	**MN712337**	**MN699323**	**MN744230**
Neoastrosphaeriella aquatica	**MFLUCC 18-1531**	**MN735994**	**MN712338**	_	**MN744231**
Neoastrosphaeriella phoenicis	**MFLUCC 18-1477 T**	**MN735995**	**MN712339**	**MN699324**	**MN744232**
Neoastrosphaeriella krabiensis	MFLUCC 11-0022	JN846711	JN846727	JN846735	_
Neoastrosphaeriella krabiensis	MFLUCC 11-0025 T	JN846715	JN846729	JN846739	_
Neoastrosphaeriella sp.	A70	_	GU205213	GU205233	_
Neoastrosphaeriella sribooniensis	MFLUCC 13-0834 T	_	MF588997	MF588987	MF588977
Rimora mangrovei	JK 5246A	_	GU301868	GU296193	_

Abbreviations: **BCC** BIOTEC Culture Collection, Bangkok, Thailand; **CBS** Centraal Bureau voor Schimmel cultures, Utrecht, The Netherlands; **MFLU** Mae Fah Luang University Herbarium Collection; **MFLUCC** Mae

Fah Luang University Culture Collection, Chiang Rai, Thailand; **JK** J. Kohlmeyer; **KT** K. Tanaka; **SMH** S.M. Huhndorf.

Results

Phylogenetic analyses

The multi-gene dataset comprised 39 taxa and 3548 characters (ITS: 822 bp; LSU: 870 bp; SSU: 982 bp; TEF1-α: 874 bp) after alignment including gaps. RAxML, MP and Bayesian analyses were conducted and resulted in generally congruent topologies, and the familial and generic assignments are similar to previous studies (Liu et al. 2011, Phookamsak et al. 2015, Wanasinghe et al. 2018, Zhang et al. 2018, 2019). RAxML analysis based on ITS, LSU, SSU and TEF1-α yielded the best scoring tree (Fig. 1) with a final optimization likelihood value of –17277.762165. The matrix had 1195 distinct alignment patterns, with 30.16% undetermined characters or gaps. Estimated base frequencies were: A = 0.243435, C = 0.249933, G = 0.281605, T = 0.225027; substitution rates AC = 1.136319, AG = 3.245371, AT = 1.300447, CG = 1.299865, CT = 8.692916, GT = 1.000000; gamma distribution shape parameter α = 0.706134. Maximum parsimony analyses indicated that 2387 characters were constant, 351 variable characters parsimony-uninformative and 810 characters are parsimony-informative. A heuristic search yield one equally most parsimonious trees (TL = 2680, CI = 0.633, RI = 0.723, RC = 0.458, HI = 0.367).

The newly generated strains in this study are nested in the genera *Fissuroma* and *Neoastrosphaeriella*. Two taxa belonging to *Fissuroma* are closely related and can be recognized as two species, *F. caryotae* and a new species *F. palmae*. Two of the other three taxa clustered with *Neoastrosphaeriella aquatica*, and one formed a distinct lineage in *Neoastrosphaeriella*, which can be recognized as a new species *N. phoenicis*.

Taxonomy

Fissuroma caryotae Wanas., E.B.G. Jones & K.D. Hyde, Mycological Progress 17(5): 579 (2018)
Fig. 2

Facesoffungi number: FoF03608

Saprobic on rachides of palms. Sexual morph: *Ascomata* 600–630 µm long, 420–575 µm diam. on host surface, in vertical section 220–270 µm high, 425–560 µm diam., black, gregarious, coriaceous, conical, hemisphaerical, unilocular, semi-immersed to erumpent, beneath the host surface, base applanate and immersed. *Ostioles* central, periphysate, with carbonaceous slit-like opening. *Peridium* up to 60 µm wide, black, thick at sides towards the apex, poorly developed at the base, base and corners comprising a mixture of host tissue and brown to hyaline fungal cells, arranged in a *textura angularis* to *textura prismatica*. *Hamathecium* up to 1.5 µm wide, hyaline, filamentous, trabeculate pseudoparaphyses, anastomosing, embedded in a gelatinous matrix. *Asci* 110–160 × 14–21 µm ($\bar{x}$ = 139 × 17.5 µm, n = 22), 8-spored, bitunicate, cylindric-clavate or obclavate, with short furcate to truncate pedicel, apex narrow and rounded, with an ocular chamber. *Ascospores* 40–54 × 6–9.5 µm ($\bar{x}$ = 47 × 8 µm, n = 40), overlapping 1–3-seriate at the base, 1-seriate at the apex, hyaline, fusiform with acute ends, 1-septate, constricted at the septum, smooth-walled, surrounded by a distinct thin sheath. Asexual morph: Undetermined.

Culture characteristics – Colonies growing well on PDA media and attaining a diameter about 2 cm after 21 days at 25 °C, circular, medium dense, tufted colony center elevated, white grayish mycelium, becoming obverse olive to gray-green, reverse dark green.

Material examined – THAILAND, Chiang Mai Province, Mae Taeng District, Pa Pae, Mushroom Research Center, on the petiole of *Calamus* sp. (Arecaceae), 24 August 2016, S.N. Zhang, SNT12 (MFLU 19-2280, living culture MFLUCC 16-1383).

Notes – Phylogenetic analyses results showed that one of the fissuroma-like strain cluster with *Fissuroma caryotae* with high statistical support (Fig. 1). The new collection is identified as *Fissuroma caryotae* found from *Calamus* sp., and its ascomatal morphology on host surface is similar to *F. calami* in Wanasinghe et al. (2018). However, the molecular data of the new isolate (MFLUCC 16-1383) is identical to the ex-type strain of *F. caryotae* (MFLUCC 17-1253), with only one nucleotide difference in the LSU rDNA and TEF1-α sequence data, respectively. Morphologically, the two *F. caryotae* specimens are also comparable in the average dimensions of asci (139 × 17.5 µm vs. 137 × 15.5 µm) and ascospores (47 × 8 µm vs. 44 × 7.5 µm). A new host record of *F. caryotae* is reported and its living culture is provided herein.

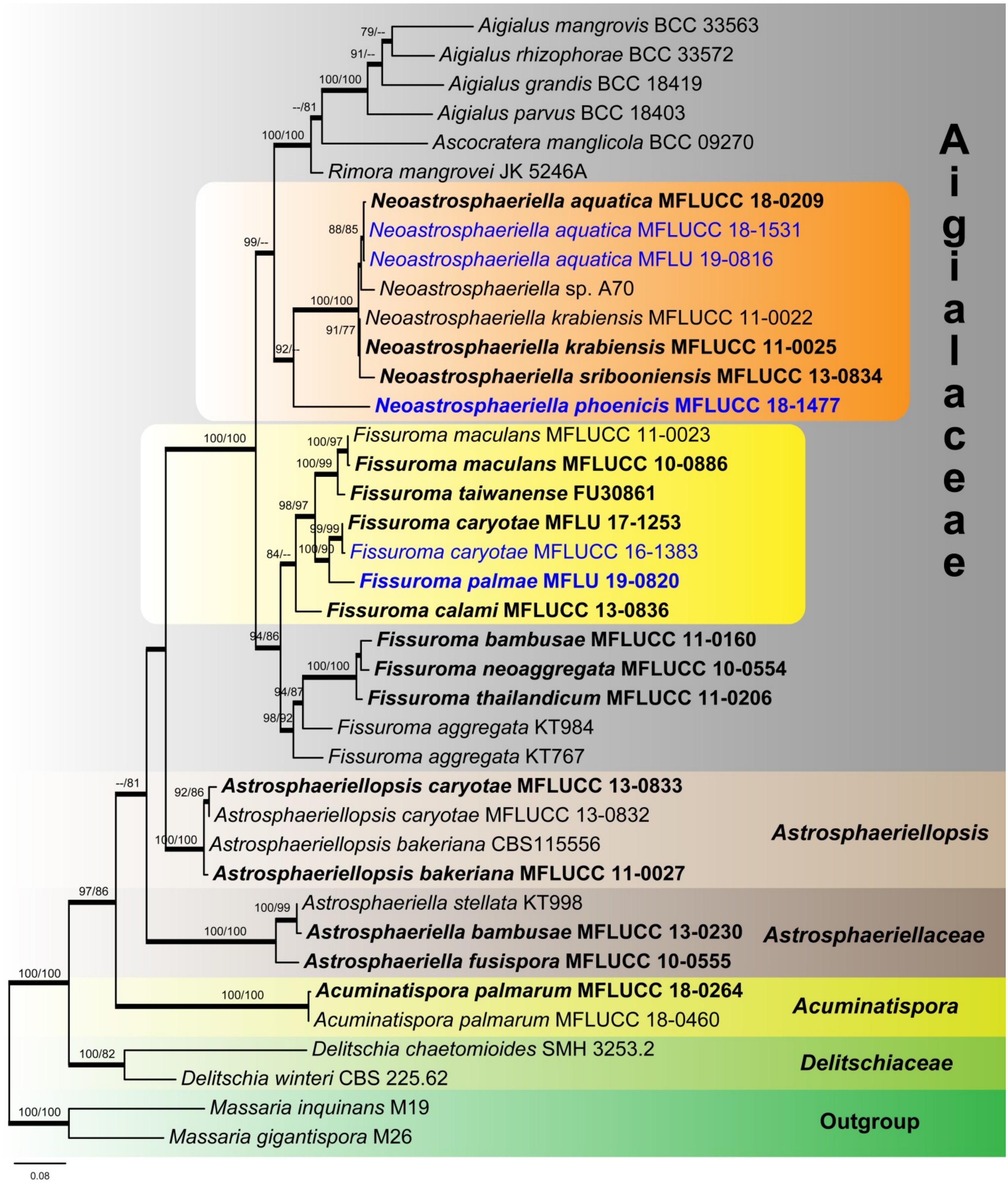

Figure 1 – RAxML tree of Aigialaceae and selected representative families in Pleosporales, based on analysis of ITS, LSU, SSU and TEF1-α gene region sequences data. Bootstrap values for ML

and MP equal to or greater than 75% are placed (ML/MP) above the branches respectively. Branches with Bayesian posterior probabilities (PP) from MCMC analysis equal or greater than 0.95 PP are in bold. The ex-type strains are in bold, and the new generated sequences are indicated in bold blue. The tree is rooted with *Massaria gigantispora* (M26) and *M. inquinans* (M19).

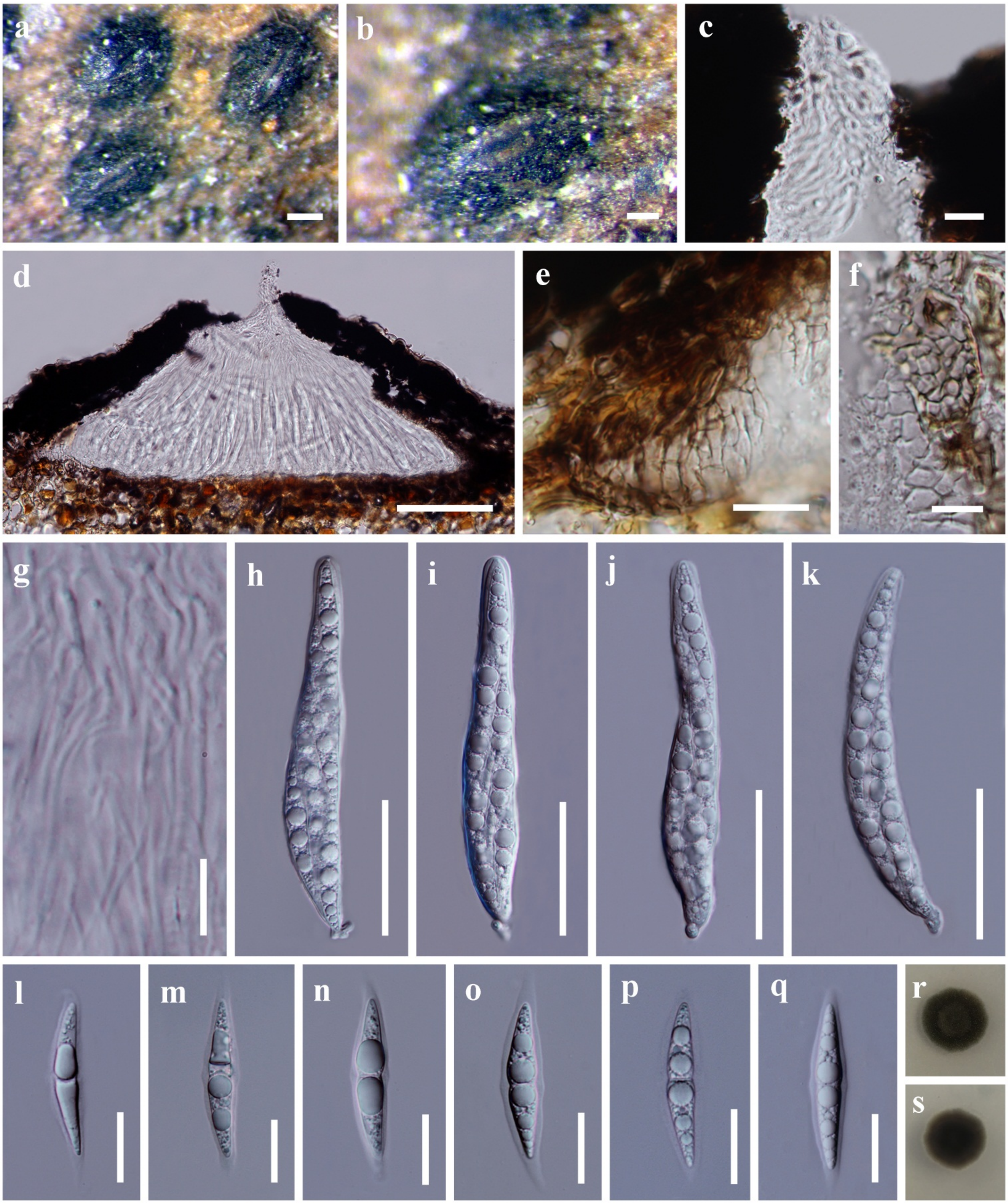

Figure 2 – *Fissuroma caryotae* (MFLU 19-2280). a, b Appearance of ascomata on host surface. c Ostiole with periphyses. d Vertical section of ascoma. e, f Structure of peridium. g Trabeculate

pseudoparaphyses. h–k Asci. l–q Ascospores. r, s Colony on PDA. Scale bars: a = 200 µm, b, d = 100 µm, h–k = 50 µm, e, l–q = 20 µm, c, f, g = 10 µm.

Fissuroma palmae S.N. Zhang, K.D. Hyde & J.K. Liu, sp. nov. Fig. 3

Index Fungorum number: IF556950; Facesoffungi number: FoF 06862

Etymology – The epithet refers to the general name of the host plant.

Holotype – MFLU 19-0820

Saprobic on rachides of palms. Sexual morph: *Ascomata* 410–760 µm long, 225–490 µm diam. on host surface, in vertical section 150–280 µm high, 380–480 µm diam., black, scattered, rarely clustered, coriaceous, conical, hemisphaerical, unilocular, semi-immersed to erumpent or superficial, form beneath the host surface, base applanate and immersed. *Ostioles* central, periphysate, with carbonaceous slit-like opening. *Peridium* 25–60 µm wide, black, thick at sides and thinner at the base, base and corners comprising hyaline to brown fungal cells, which arranged in a *textura angularis*. *Hamathecium* up to 1 µm wide, hyaline, filamentous, trabeculate pseudoparaphyses, anastomosing, embedded in a gelatinous matrix. *Asci* 97–157 × 14–24 µm ($\bar{x}$ = 121.5 × 19.5 µm, n = 30), 8-spored, bitunicate, cylindric-clavate, short pedicellate, apically rounded with an ocular chamber. *Ascospores* 40–52 × 6–10 µm ($\bar{x}$ = 45 × 8.5 µm, n = 30), overlapping, 2-seriate, hyaline, becoming brown when mature or senescent, fusiform with acute ends, 1-septate, slightly constricted at the septum, smooth-walled, surrounded by a mucilaginous sheath. *Sheath* drawn out 3–6.5 µm long, 3.5–6.5 µm wide at both ends, up to 5.5 µm at the middle. Asexual morph: Undetermined.

Material examined – THAILAND, Phang-nga Province, Thap Put District, Bo Sean, Tao Thong Waterfall, on the rachides of *Arenga pinnata* (Wurmb) Merr. (Arecaceae), 30 August 2017, S.N. Zhang, SNT253A (MFLU 19-0820, holotype; HKAS 105486, isotype).

Notes – *Fissuroma palmae* is different from other *Fissuroma* species in the dimensions of the asci and ascospores (Table 2). Phylogenetically, *Fissuroma palmae* clustered together with strains of *F. caryotae* (100% ML, 90% MP and 1.00 PP) but represented as a distinct lineage. In addition, the comparison of sequence data between the two species showed that there are 7 nucleotide differences across the 829 nucleotides (0.84%) and 20 nucleotide differences across the 646 nucleotides (3.1%) of LSU and TEF1-α respectively. *Fissuroma palmae* differs from *F. caryotae* in having cylindric-clavate asci, which is also shorter than that of *F. caryotae* in average dimension (121.5 × 19.5 µm vs. 137 × 15.5 µm and 139 × 17.5 µm) (Table 2).

Neoastrosphaeriella aquatica D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, Phytotaxa 391: 201 (2019) Fig. 4

Facesoffungi number: FoF04910

Saprobic on palms or decaying submerged wood in freshwater habitat. Sexual morph: *Ascomata* 280–450 µm long, 280–365 µm diam., in vertical section 210–250 µm high × 350–390 µm long, black, coriaceous, slightly carbonaceous, unilocular, hemispherical, scattered, erumpent, semi-immersed to immersed, with a flattened base, beneath the host surface, with a central slit-like ostiole over almost the entire length. *Ostioles* slit-like, central. *Peridium* 25–65 µm wide, black, composed of dark brown thick-walled cells of *textura angularis*. *Hamathecium* 1–1.5 µm wide, trabeculate pseudoparaphyses, hyaline, anastomosing, embedded in a gelatinous matrix. *Asci* 90–120 × 10–20 µm ($\bar{x}$ = 111 × 16 µm, n= 20), 8-spored, bitunicate, fissitunicate, cylindric-clavate or obclavate, short pedicellate, apex narrow and rounded, with a small ocular chamber. *Ascospores* 30–40 × 5–8 µm ($\bar{x}$ = 35.5 × 6.5 µm, n= 40), overlapping, 1–2-seriate, fusiform, hyaline when young, becoming brown and verrucose when mature, tapering to pointed apices, 1-septate, slightly constricted at the septum, surrounded by a mucilaginous sheath. Asexual morph: Undetermined.

Culture characteristics – Colonies growing well on PDA media and attaining a diameter about 3.5 cm after 21 days at 25°C, medium dense, flattened colony, slightly radiating with concentric ring of cottony mycelium at edge of colony, obverse olive to gray-green, reverse dark green.

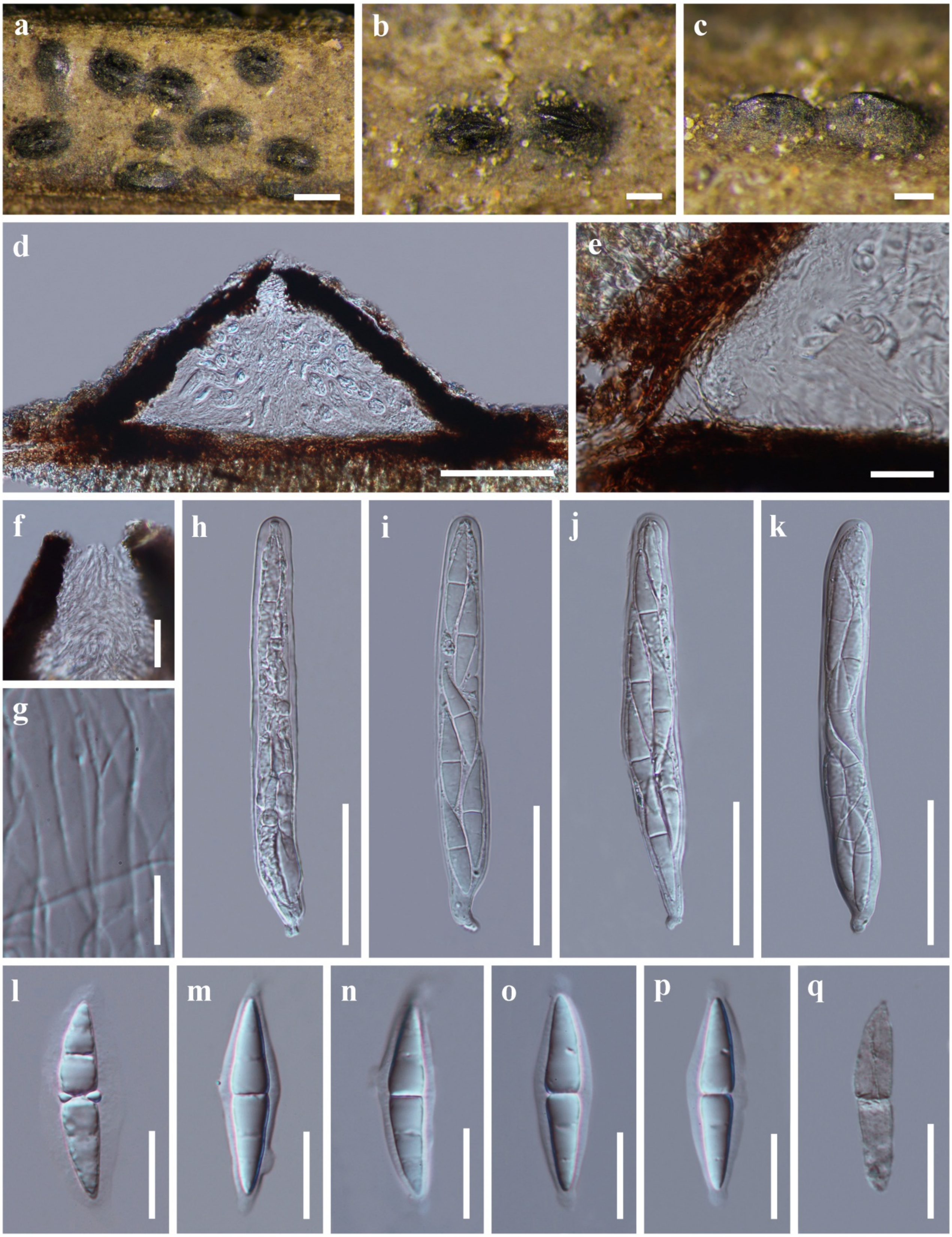

Figure 3 – *Fissuroma palmae* (MFLU 19-0820, holotype). a–c Appearance of ascomata on host surface. d Vertical section of ascoma. e Structure of peridium. f Ostiole with periphyses. g Trabeculate pseudoparaphyses. h–k Asci. l–q Ascospores. Scale bars: a = 500 µm, b, c = 200 µm, d = 100 µm, h–k = 50 µm, e, f, l–q = 20 µm, g = 10 µm.

Table 2 The asci and ascospores morphology, habitats and host records of *Fissuroma* species.

Taxa	Asci		Ascospores		Habitats & host records	References
	Morphology	Size (µm)	Morphology	Size (µm)		
Fissuroma aggregata (YAM 20365, holotype)	Obclavate	143–185 × 15–19.5 ($\bar{x}$ = 164.5 × 17.3)	Hyaline, becoming brown at maturity, fusiform, 1-septate, smooth-walled, guttulate, thin sheath	46–61(–64) × 7–9.5 ($\bar{x}$ = 55.9 × 8.2)	Terrestrial/ *Phyllostachys bambusoides* (Poaceae)	Phookamsak et al. 2015
F. bambusae (MFLU 11-0196, holotype)	Cylindric-clavate or obclavate	(150–)170–187(–194) × (15–)17–19(–22) ($\bar{x}$ = 178.1 × 18.5)	Hyaline, fusiform, 1-septate, smooth-walled, thick sheath	(40–)45–47(–52) × 6–8 (–9) ($\bar{x}$ = 46.2×7.1)	Terrestrial/ Bamboo	Phookamsak et al. 2015
F. calami (MFLU 17-1251, holotype)	Cylindric-clavate or obclavate	100–130 × 19–22 ($\bar{x}$ = 120.3 × 20.3)	Hyaline, fusiform, 1-septate, smooth-walled, guttulate, thin sheath	40–45 × 7–9 ($\bar{x}$ = 43.5 × 8.2)	Terrestrial/ *Calamus rotang* (Arecaceae)	Wanasinghe et al. 2018
F. caryotae (MFLU 17-1253, holotype)	Cylindric-clavate or obclavate	120–150 × 14–18 ($\bar{x}$ = 136.8 × 15.4)	Hyaline, 1-septate, fusiform with acute ends, smooth-walled, thin sheath	40–50 × 7–9 ($\bar{x}$ = 44.2 × 7.5)	Terrestrial/ *Caryota urens* (Arecaceae)	Wanasinghe et al. 2018
F. caryotae (MFLU 19-2280, reference specimen)	Cylindric-clavate or obclavate	110–160 × 14–21 ($\bar{x}$ = 139 × 17.5)	Hyaline, 1-septate, fusiform with acute ends, smooth-walled, thin sheath	40–54 × 6–9.5 ($\bar{x}$ = 47 × 8)	Terrestrial/ *Calamus* sp. (Arecaceae)	This study
F. fissuristoma (IFRD 294-002, holotype)	Obclavate	(124–)130–150(–166) × (16–)18–19(–26) ($\bar{x}$ = 144.1 × 19.3)	Hyaline, fusiform, 1-septate, becoming brown with 3-septa at maturity, thin sheath	(43–)45–50(–55) × 7–9 ($\bar{x}$ = 48.2 × 8.4)	Terrestrial/ *Calamus conirostris* (Arecaceae)	Phookamsak et al. 2015
F. kavachabeejae (AMH-9963, holotype)	Cylindrical	142–167 × 15–20 ($\bar{x}$ = 153.3 × 16.5)	Hyaline, fusiform, 1-septate, smooth-walled, guttulate, with sheath	37.3–47.4 × 4.7–6.7(–8.1) ($\bar{x}$ = 42.1 × 6.2)	Terrestrial/ *Calamus andamanicus* (Arecaceae)	Niranjan & Sarma 2018
F. maculans (MFLU 11-1143, type species)	Cylindric-clavate or obclavate	65–125 × 10–17 ($\bar{x}$ = 85 × 13)	Hyaline, fusiform, 1-septate, smooth-walled, with sheath	29–38 × 4–8 ($\bar{x}$ = 30 × 6.5)	Terrestrial/ *Arenga westerhoutii* (Arecaceae), *Metroxylon sagu* (Arecaceae)	Liu et al. 2011
F. microsporum (AMH-9962, holotype)	Cylindrical, Cylindric-oblong	(75.9–)80.3–103.6 × 7.4–8.7 ($\bar{x}$ = 94 × 8.4)	Hyaline, fusiform with 1-septate, 2–3 pseudo-septa, with thick sheath and polar appendages	14.6–21.8 × 3.5–4 ($\bar{x}$ = 18.7 × 3.2)	Terrestrial/ *Borassus flabellifer* (Arecaceae)	Niranjan & Sarma 2018

Table 2 Continued.

Taxa	Asci		Ascospores		Habitats & host records	References
	Morphology	Size (µm)	Morphology	Size (µm)		
F. neoaggregata (MFLU 11-0146, holotype)	Cylindric-clavate or obclavate	155–197 × 15–18.5 ($\bar{x}$ = 177 × 16.5)	Hyaline, elongate-fusiform, 1-septate, smooth-walled, guttulate, with sheath	38.5–54 × 7–10.5 ($\bar{x}$ = 47.5 × 8.5)	Terrestrial/ Bamboo	Liu et al. 2011, Phookamsak et al. 2015
F. palmae (MFLU 19-0820, holotype)	Cylindric-clavate	97–157 × 14–24 ($\bar{x}$ = 121.5 × 19.5)	Hyaline, becoming brown when mature or senescent, fusiform, 1-septate, smooth-walled, thin sheath	40–52 × 6–10 ($\bar{x}$ = 45 × 8.5)	Terrestrial/ *Arenga pinnata* (Arecaceae)	This study
F. taiwanense (F31005, holotype)	Broadly cylindrical to obclavate	(97–)100–120(–125) × (23.5–)24–29(–30) ($\bar{x}$ = 110 × 26.5)	Hyaline, fusiform, 1-septate, smooth-walled, thick sheath, with club-shaped appendages	52–56 × 8.5–10 ($\bar{x}$ = 53.5 × 9.4)	Terrestrial/ *Hedychium coronarium* (Zingiberaceae)	Tennakoon et al. 2018
F. thailandicum (MFLU 11-0156, holotype)	Cylindrical to cylindric-clavate	(150–)170–190(–204) × 15–18(–19.5) ($\bar{x}$ = 176.9 × 17)	Hyaline, fusiform, 1-septate, smooth-walled, thick sheath	(40–)43–46(–52) × 6–7(–9) ($\bar{x}$ = 45.4 × 7.1)	Terrestrial/ Bamboo	Phookamsak et al. 2015

Material examined – THAILAND, Phang-nga Province, Kapong District, Mo, on the petiole of *Metroxylon sagu* Rottb. (Arecaceae), 29 August 2017, S.N. Zhang, SNT190 (MFLU 19-0816 = HKAS 105481); *ibid.*, on the rachides of palm species, 29 August 2017, S.N. Zhang, SNT240 (MFLU 19-0819, HKAS 105485), living culture MFLUCC 18-1531.

Notes – *Neoastrosphaeriella aquatica* was introduced by Bao et al. (2019). In this study, our two newly collected isolates clustered together with *N. aquatica* (Fig. 1). The comparison of sequence data of these three strains showed that there is only one nucleotide difference in the ITS regions and one gap in the TEF1-α sequence data. We identify them as *Neoastrosphaeriella aquatica* based on the morphology (Table 3) and phylogeny, and report the new habitats and host record for *N. aquatica.*

Neoastrosphaeriella phoenicis S.N. Zhang, E.B.G. Jones & J.K. Liu, sp. nov. Fig.5

Index Fungorum number: IF556951; Facesoffungi number: FoF06863

Etymology – The epithet refers to the host plant, of which the fungus was collected.

Holotype – MFLU 19-0807

Saprobic on palm in mangrove habitat. Sexual morph: *Ascomata* 535–730 µm long, 350–505 µm diam., in vertical section 260–295 µm high, 360–415 µm long, black, coriaceous, slightly carbonaceous, unilocular, hemispherical, scattered, erumpent, semi-immersed to immersed, with a flattened base, beneath the host surface, with a central slit-like ostiole over almost the entire length, base applanate and immersed. *Ostioles* central, slit-like opening. *Peridium* 40–80 µm wide, the outside layer black, with host cells and fungal tissue, and the inner layer thin, comprising fungal cells of *textura angularis*, thinner at the base. *Hamathecium* 1–1.5 µm wide, trabeculate pseudoparaphyses, hyaline, anastomosing, embedded in a gelatinous matrix. *Asci* 110–130 × 15–22 µm ($\bar{x}$ = 121 × 19.5 µm, n = 15), 8-spored, fissitunicate, obclavate, short pedicellate, apex narrow and rounded, with a small ocular chamber. *Ascospores* 40–60 × 6–11 µm ($\bar{x}$ = 49 × 8 µm, n = 20), 1–2 seriate, fusiform, hyaline when young, becoming brown to dark brown and verrucose when mature or senescent, tapering to pointed apices, 1–3-septate, constricted at the septum, surrounded by a mucilaginous sheath 1–2 µm wide, which is slightly drawn out at the apices. Asexual morph: Undetermined.

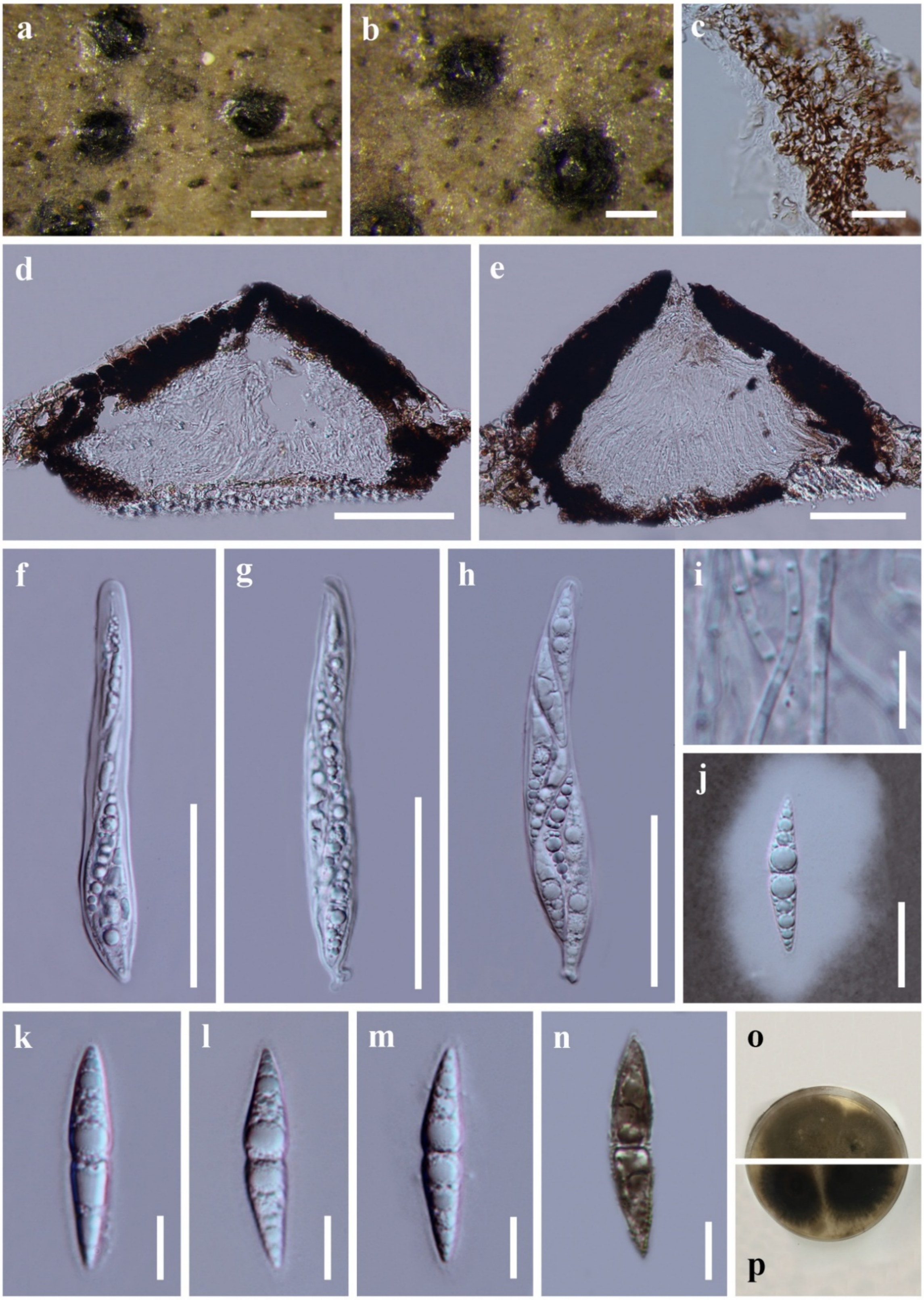

Figure 4 – *Neoastrosphaeriella aquatica* (MFLU 19-0816, MFLU19-0819). a, b Appearance of ascomata on host surface. c Structure of peridium. d, e Vertical section of ascoma. f–h Asci. i Pseudoparaphyses. j–n Ascospores; j ascospore in India ink showing sheath. o, p Colonies on PDA. Scale bars: a = 500 µm, b = 200 µm, d, e = 100 µm, f–h = 50 µm, c, j = 20 µm, i, k–n = 10 µm.

Table 3 The asci and ascospores morphology, habitats and host records of *Neoastrosphaeriella* species.

Taxa	Asci		Ascospores		Habitats & host records	References
	Morphology	**Size (µm)**	**Morphology**	**Size (µm)**		
N. alankrithabeejae (AMH-9961, holotype)	Cylindrical, obclavate	132.2–154.5 × (19.2–)21.1–32.5 ($\bar{x}$ = 143.5 × 25.4)	Hyaline to pale-brown at maturity, broad-fusiform, 1-septate with two pseudo-septa, verrucose	40.2–46.7 × 8.5–9.3(–10) ($\bar{x}$ = 44.2 × 9)	Terrestrial / *Calamus andamanicus* (Arecaceae)	Niranjan & Sarma 2018
N. aquatica (MFLU 18-1392, holotype)	Cylindric-clavate or obclavate	84–112 × 14–19 ($\bar{x}$ = 98 × 15.7)	Hyaline, grayish brown and verrucose at maturity, fusiform, 1-septate	31–37 × 5–8 ($\bar{x}$ = 34 × 6.4)	Freshwater / submerged wood in freshwater	Bao et al. 2019
N. aquatica (MFLU 19-0816, MFLU 19-0819, reference specimens)	Cylindric-clavate or obclavate	90–120 × 10–20 ($\bar{x}$ = 111 × 16)	Hyaline, becoming brown and verrucose when mature, fusiform, 1-septate	30–40 × 5–8 ($\bar{x}$ = 35.5 × 6.5)	Terrestrial/ *Metroxylon sagu* (Arecaceae)	This study
N. krabiensis (MFLU 11-1148, holotype)	Obclavate	85–135 × 15–23 ($\bar{x}$ = 100 × 18)	Hyaline, becoming brown and verrucose when mature, fusiform, 1-septate	32–40 × 6–9 ($\bar{x}$ = 35.5 × 7)	Terrestrial/ *Metroxylon sagu* (Arecaceae), *Elaeis guineensis* (Arecaceae)	Liu et al. 2011
N. sribooniensis (MFLU 17-1254, holotype)	Cylindric-clavate or obclavate	90–110 × 15–18 ($\bar{x}$ = 99.5 × 16.9)	Hyaline, fusiform, 1-septate, smooth-walled	30–40 × 5–7 ($\bar{x}$ = 37.8 × 6.3)	Terrestrial / *Calamus rotang* (Arecaceae)	Wanasinghe et al. 2018
N. phoenicis (MFLU 19-0807, holotype)	Obclavate	110–130 × 15–22 ($\bar{x}$ = 121 × 19.5)	Hyaline, becoming brown and verrucose when mature, fusiform, 1–3-septate	40–60 × 6–11 ($\bar{x}$ = 49 × 8)	Mangrove / *Phoenix paludosa* (Arecaceae)	This study

Culture characteristics – Colonies growing well on PDA media and attaining a diameter about 3–4 cm after 21 days at 25°C, flattened colony, slightly radiating with concentric ring of cottony mycelium at edge of colony, obverse gray-green, reverse dark green.

Material examined – THAILAND, Ranong Province, Ranong District, Ngao, Ngao mangrove forest research center, on the petiole of *Phoenix paludosa* Roxb. (Arecaceae), immersed in mangrove mud and water, 7 December 2016, S.N. Zhang, SNT59 (MFLU 19-0807, holotype; HKAS105449, isotype), ex-type living culture MFLUCC 18-1477.

Notes – *Neoastrosphaeriella phoenicis* was collected from a mangrove habitat, and is different from other species by having hyaline to pale brown, 1–3-septate, guttulate, verrucose ascospores (Fig. 5, Table 3). *Neoastrosphaeriella phoenicis* morphologically resembles *N. alankrithabeejae* (Niranjan & Sarma 2018), which presents two pseudo-septa but lacks molecular data. However, *Neoastrosphaeriella phoenicis* differs from *N. alankrithabeejae* in having smaller, obclavate asci (110–130 × 15–22 µm vs. 132–154.5 × (19.2–) 21–32.5 µm) and 3-septate ascospores (Fig. 5o). The phylogenetic result (Fig. 1) showed that *N. phoenicis* clustered together with other *Neoastrosphaeriella* taxa which formed a monophyletic clade, while the isolate of *N.*

phoenicis formed a distinct lineage (with 29 nucleotide differences across the 813 nucleotides of LSU sequence data) in *Neoastrosphaeriella* clade and can be recognized as a new species.

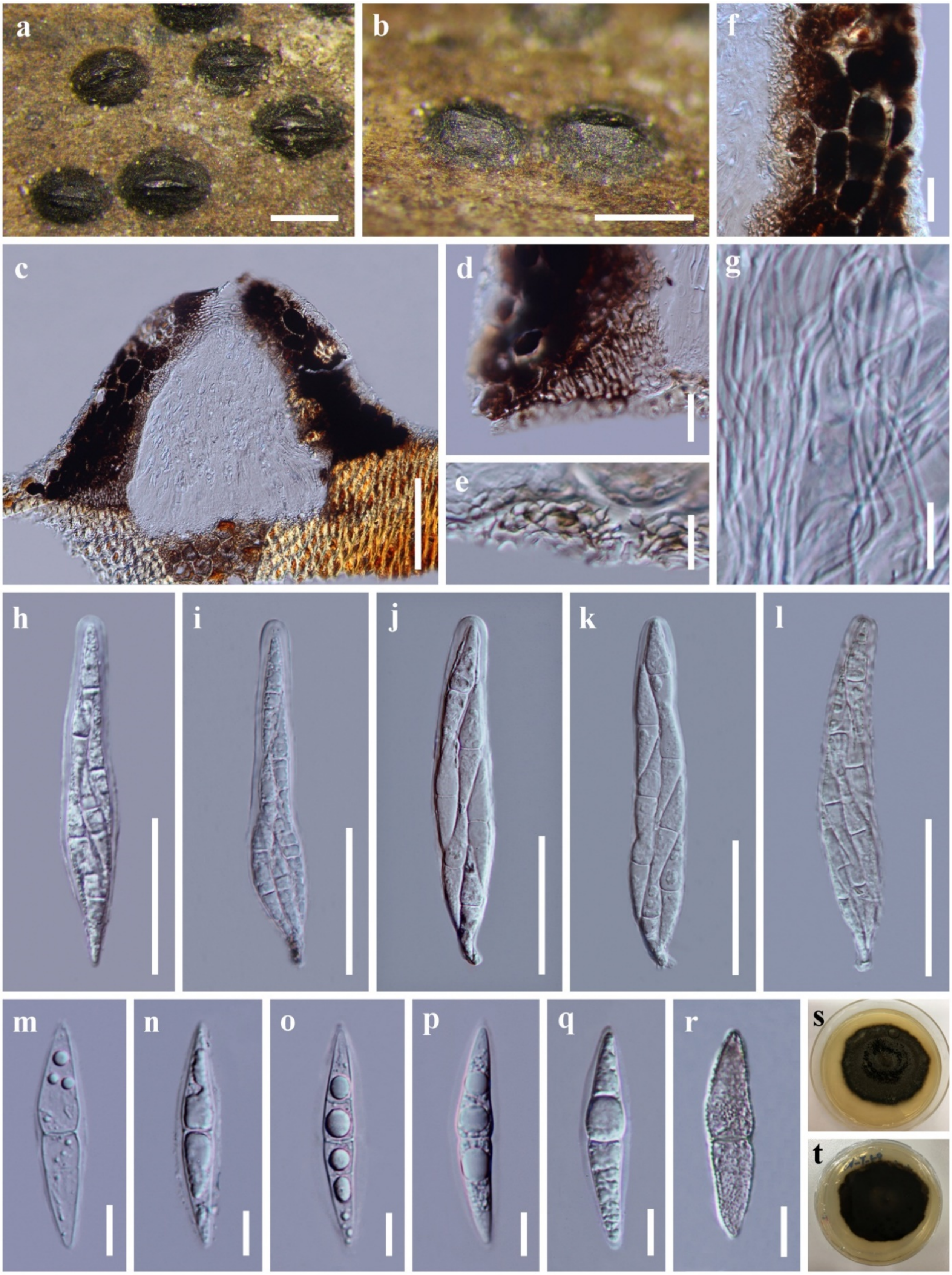

Figure 5 – *Neoastrosphaeriella phoenicis* (MFLU 19-0807, holotype). a, b Appearance of ascomata on host surface. c Vertical section of ascoma. d–f Structure of peridium. g Trabeculate

pseudoparaphyses. h–l Asci. m–r Ascospores. s, t Colony on PDA. Scale bars: a, b = 500 µm, c = 100 µm, h–l = 50 µm, d, f = 20 µm, e, g, m–r = 10 µm.

Discussion

Fissuroma and *Neoastrosphaeriella* were established by Liu et al. (2011) in a phylogenetic study of *Astrosphaeriella sensu lato* in Aigialaceae. *Neoastrosphaeriella* was considered to be different from *Fissuroma* in having smaller obclavate asci and brown verrucose ascosproes, and the two genera were distinguished by the shape (cylindric-clavate, obclavate) of asci, and the colour (hyaline, brown) and ornamentation (smooth, verrucose) of ascospores (Liu et al. 2011). However, the morphological characteristics of the two genera overlap when more taxa have been included in subsequent studies (Phookamsak et al. 2015, Niranjan & Sarma 2018, Tennakoon et al. 2018, Wanasinghe et al. 2018, Bao et al. 2019). For example, brown mature ascospores were found in *Fissuroma fissuristoma* (≡ *Astrosphaeriella fissuristoma*) (Phookamsak et al. 2015) and *F. palmae* (this study), and cylindric-clavate asci were found in *N. alankrithabeejae* (Niranjan & Sarma 2018) and *N. aquatica* (Bao et al. 2019). Morphological features may differ depending on the state (mature or immature) of the specimen being observed, while DNA sequence data is more objective. The comparison of nucleotides difference of TEF1-α region was commonly used for delineating *Fissuroma* species, such as the identification of *F. bambusae*, *F. neoaggregata*, *F. thailandicum* (Phookamsak et al. 2015); *F. calami* and *F. caryotae* (Wanasinghe et al. 2018).

There are twelve *Fissuroma* and five *Neoastrosphaeriella* species described, including the new members *Fissuroma palmae* and *Neoastrosphaeriella phoenicis* in this study. Seven of the *Fissuroma* species are from terrestrial palms, and all the *Neoastrosphaeriella* species have been collected from palms (Tables 2, 3). It is possible to find the same species (e.g. *F. maculans*) from different palm hosts, and also discover the morphologically similar but different species (e.g. *F. calami* vs. *N. sribooniensis*) from the same palm host.

Members of *Neoastrosphaeriella* have been found from terrestrial and freshwater habitats. We report a new species, *N. phoenicis,* which was collected from the decayed petiole of *Phoenix paludosa* (mangrove date palm) that grows in soft mangrove mud and salty water. Thus, the species *N. phoenicis* can be considered as manglicolous, and additional mangrove habitat for the genus *Neoastrosphaeriella* is reported.

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