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Fissuroma (Aigialaceae: Pleosporales) appears to be hyperdiverse on Arecaceae: evidence from two new species from southern Thailand

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ABSTRACT

Thailand and other tropical regions have high fungal diversity. Our investigation and examination of microfungi on palms (Arecaceae) revealed two new ascomycetous species of *Fissuroma*. *Fissuroma arengae* and *F. wallichiae* spp. nov. are introduced using morphological and phylogenetic evidence. The novel species have coriaceous ascomata, cylindrical-clavate asci and ascospores with a distinct and thin mucilaginous sheath. *Fissuroma arengae* is similar to *F. wallichiae* but can be distinguished by minor morphology, host substrate and gene base-pair differences. Phylogenetic analyses of combined LSU, ITS, SSU, *tef1-α* and *rpb2* sequence data showed that these strains grouped within *Fissuroma*, further confirming this genus as monophyletic. The two new species are described and illustrated to support their taxonomic placement. *Fissuroma* appears to be a highly diverse genus often occurring on palms. It is likely that more research will result in numerous new taxa being discovered.

Keywords: morphology, palm fungi, phylogeny, Thai fungi, two novel taxa

Introduction

To help understand the diversity of microfungi, we have been investigating the fungi on palms, Pandanaceae and grasses (Taylor & Hyde 2003; Hyde *et al.* 2007; Whitton *et al.* 2012; Thambugala *et al.* 2017; Goonasekara *et al.* 2018; Tibpromma *et al.* 2018). Our studies have constantly revealed new taxa, and would suggest that the estimated 2.2–3.8 million fungal species (Hawksworth & Lücking 2017) is certainly not an excessive number. In Thailand, the diversity has been shown to be extremely high with up to 96 % of species collected being new (Hyde *et al.* 2018). The high diversity of novel fungi on palms have been revealed in several studies (Hyde 1997; Fröhlich & Hyde 1999; 2000; Yanna *et al.* 2001; Pinnoi *et al.* 2006; Pinruan *et al.* 2007; Konta *et al.* 2017; Zhang *et al.* 2019). In one study, taxa on two adjacent palms of different genera had less than 6 % of overlapping species indicating the remarkable diversity likely to be harbored by different genera (Jones *et al.* 2014).

Aigialaceae was introduced by Suetrong *et al.* (2009) with three genera *Aigialus*, *Ascocratera* and *Rimora*. Aigialaceae members are characterized by carbonaceous ascomata without papilla, trabeculate pseudoparaphyses, cylindrical asci with apical rings and ascospores with a sheath or gelatinous appendages around the apical cells (Liu *et al.* 2011; Zhang *et al.* 2012; Hyde *et al.* 2013). Wijayawardene *et al.*

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(2018) accepted five genera in Aigialaceae, namely *Aigialus*, *Ascocratera*, *Fissuroma*, *Neoastrosphaeriella* and *Rimora*. Recently, *Posidoniomyces* was introduced by Vohník *et al.* (2019). Thus, six genera are currently accepted in Aigialaceae.

Fissuroma was established with *F. maculans* as the type species (Liu *et al.* 2011), and is characterized by black ascomata, opening with a slit-like ostiole, trabeculate pseudoparaphyses (*sensu* Liew *et al.* 2000), obclavate to cylindrical asci, and hyaline, fusiform, 1-septate ascospores (Liu *et al.* 2011; Phookamsak *et al.* 2015; Tennakoon *et al.* 2018). The asexual morph of *Fissuroma* is coelomycetous, being pleurophomopsis-like (Liu *et al.* 2011; Phookamsak *et al.* 2015). Currently, twenty *Fissuroma* species are accepted based on both morphology and phylogeny (Niranjan & Sarma 2018; Tennakoon *et al.* 2018; Wanasinghe *et al.* 2018; Zhang *et al.* 2020).

In this study, *Fissuroma arengae* and *F. wallichiae* are introduced as new species, from dead petioles of *Arenga* and *Wallichia* (Arecaceae), respectively. Their phylogenetic position was determined based on maximum likelihood, maximum parsimony and Bayesian inference of a combined LSU, ITS, SSU, *tef1-α* and *rpf2* sequence dataset. Descriptions, illustrations and molecular data are provided to compare the new species with related taxa.

Materials and methods

Collection and isolation

Decayed rachides/petioles were collected from Thailand (Krabi and Phang-Nga Provinces) in 2014 on *Arenga pinnata* and *Wallichia* sp. Information on the environment, geographic location and host was recorded. The fungal taxa were identified based on morphological characteristics and phylogenetic analyses. Isolations and specimen examinations were conducted following the methods provided by Konta *et al.* (2016). Samples were taken to the laboratory in ziplock bags and morphological characteristics were observed using a Motic SMZ 168 series stereo-microscope. Free-hand sections were made using a razor blade and placed on a droplet of water on a glass slide. Morphological characters were observed and photo-micrographed using a Nikon ECLIPSE80i compound microscope with a fitted Canon 600D digital camera. Measurements were determined using an Image Framework program. Photo-plates were made by Adobe Photoshop CS6. Pure cultures were obtained using single ascospores isolation method (Chomnunti *et al.* 2014). Ascospore mass was transferred to a drop of sterile water on a flame-sterilized slide. The ascospore suspension was spread on a petri-dish containing malt extract agar (MEA) and incubated at 25-28 °C overnight. Germinating ascospores were transferred to fresh MEA dishes. Two palm samples were collected and three isolates were obtained from each sample. Holotype specimens and ex-type cultures were deposited in the herbarium of Mae Fah Luang University (MFLU) and Mae Fah Luang Culture Collection (MFLUCC) at Mae Fah Luang University, Chiang Rai, Thailand. Facesoffungi and Index Fungorum numbers were registered as outlined in Jayasiri *et al.* (2015) and Index Fungorum (2020).

DNA extraction and amplification (PCR)

Genomic DNA was extracted from fungal mycelium using the Biospin Fungus Genomic DNA extraction Kit (BioFlux, P.R. China) following the manufacturer's protocol. The partial nucleotide genes were subjected to PCR amplification and sequencing of the large subunit (28S, LSU) (Vilgalys & Hester 1990), the internal transcribed spacer (ITS) (White *et al.* 1990), the small subunit (18S, SSU) (White *et al.* 1990), the translation elongation factor 1-alpha (*tef1-α*) (Rehner 2001; Rehner & Buckley 2005) and the RNA polymerase II second largest subunit (*rpb2*) (Liu *et al.* 1999). For primers and conditions see Table 1.

The total volume of PCR mixtures for amplification were 25 µl containing 8.5 µl ddH_2O, 12.5 µl 2× Easy Taq PCR SuperMix (mixture of Easy Taq TM DNA Polymerase, dNTPs and optimized buffer (Beijing Trans Gen Biotech Co., Beijing, P.R. China), 2 µl of DNA template, 1 µl of each forward and reverse primers (10 pM). The quality of PCR products was checked on 1 % agarose gel electrophoresis stained with 4S green nucleic acid (Life Science Products & Services, Shanghai, P.R. China). Purification and sequencing of PCR products were carried out by Sangon Biotech Co., Shanghai, P.R. China. The resulting fragments were sequenced in both forward and reverse directions. The DNA sequences generated were analysed. Consensus sequences were computed using SeqMan software. The new sequences generated in this study were deposited in GenBank (Tab. 2).

Phylogenetic analyses

The sequences generated in this study were subjected to BLAST search in GenBank to identify closely related sequences. Sequence data retrieved from GenBank and recent publications were used as references (Tennakoon *et al.* 2018; Wanasinghe *et al.* 2018; Zhang *et al.* 2020). Sequences of the LSU, ITS, SSU, *tef1-α* and *rpb2* were analysed individually and in combination. A total of 44 taxa were used for the phylogenetic analyses. *Astrosphaeriella fusispora*

Table 1. Details of genes/loci with PCR primers and PCR conditions.

Genes/loci	PCR primers (forward/reverse)	PCR conditions
LSU, ITS, SSU, *tef1-α*	LR5/LR0R, ITS5/ITS4, NS4/NS1, EF1-983F/EF1-2218R	a; 95 °C: 30 s, 55 °C: 50 s, 72°C: 30 s (35 cycles); c
rpb2	*fRPB2*-5f/*fRPB2*-7cR	b; 95 °C: 1 min, 54 °C: 2 min, 72 °C: 1.5 min (35 cycles); c

a Initiation step of 95 °C: 3 min. b Initiation step of 95 °C: 5 min. c Final elongation step of 72 °C: 10 min and final hold at 4 °C.

(MFLUCC 10-0555) and *A. neofusispora* (MFLUCC 11-0161) were selected as the outgroup taxa. Absent sequence data (*i.e.* ITS, *tef1-α*, *rpb2* sequence data) in the alignments were treated as missing data. Sequence alignments were carried out with MAFFT v.6.864b (Katoh & Standley 2013) and were manually improved where necessary. The single gene datasets were combined using Mega7 (Kumar *et al.* 2016). Data were converted from fasta to nexus and PHYLIP format with Alignment Transformation Environment online, https://sing.ei.uvigo.es/ALTER/ (Glez-Peña *et al.* 2010). The tree topologies obtained from single gene sequence data were compared prior to the combined gene analysis for checking the incongruence in the overall topology of the phylogenetic tree.

Maximum likelihood (ML) analysis was performed using RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis *et al.* 2008; Stamatakis 2014) in the CIPRES Science Gateway platform (Miller *et al.* 2010) with GTRGAMMA model

Table 2. GenBank accession numbers of sequences used in phylogenetic analysis.

Species	Strains	GenBank accession numbers					References
		LSU	ITS	SSU	*tef1-α*	*rpb2*	
Aigialus grandis T	BCC 18419	GU479774	-	GU479738	GU479838	GU479813	Suetrong *et al.* 2009
A. grandis T	BCC 20000	GU479775	-	GU479739	GU479839	GU479814	Suetrong *et al.* 2009
A. mangrovis	BCC 33563	GU479776	-	GU479741	GU479840	GU479815	Suetrong *et al.* 2009
A. parvus	BCC 32558	GU479779	-	GU479743	GU479843	GU479818	Suetrong *et al.* 2009
A. parvus	BCC 18403	GU479778	-	GU479744	GU479842	GU479817	Suetrong *et al.* 2009
A. rhizophorae	BCC 33572	GU479780	-	GU479745	GU479844	GU479819	Suetrong *et al.* 2009
A. rhizophorae	BCC 33573	GU479781	-	GU479746	GU479845	GU479820	Suetrong *et al.* 2009
Ascocratera manglicola T	JK 5262C	GU301799	-	GU296136	-	GU371763	Schoch *et al.* 2009
A. manglicola T	HHUF 30032	GU479783	-	GU479748	GU479847	GU479822	Suetrong *et al.* 2009
A. manglicola T	BCC 09270	GU479782	-	GU479747	GU479846	GU479821	Suetrong *et al.* 2009
Astrosphaeriella fusispora T, E	MFLUCC 10-0555	KT955462	-	KT955443	KT955425	KT955413	Phookamsak *et al.* 2015
A. neofusispora E	MFLUCC 11-0161	KT955463	-	KT955444	KT955426	KT955418	Phookamsak *et al.* 2015
Fissuroma aggregatum	KT 984	AB524591	-	AB524450	AB539105	AB539092	Tanaka *et al.* 2009
F. aggregatum	KT 767	AB524590	-	AB524449	-	-	Tanaka *et al.* 2009
F. arengae E	**MFLUCC 15-0325A**	**MN726232**	**MN726238**	**MN726244**	**MN937554**	**-**	**This study**
F. arengae	**MFLUCC 15-0325B**	**MN726233**	**MN726239**	**MN726245**	**MN937555**	**-**	**This study**
F. arengae	**MFLUCC 15-0325C**	**MN726234**	**MN726240**	**MN726246**	**MN937556**	**-**	**This study**
F. bambusae E	MFLUCC 11-0160	KT955468	-	KT955448	KT955430	KT955417	Phookamsak *et al.* 2015
F. bambusae	MFLUCC 11-0198	KT955469	-	KT955449	KT955431	KT955449	Phookamsak *et al.* 2015
F. calami E	MFLUCC 13-0836	MF588993	-	MF588983	MF588975	-	Wanasinghe *et al.* 2018
F. caryotae	MFLUCC 16-1383	MN712335	MN735992	MN699322	MN744228	-	Zhang *et al.* 2020
F. caryotae E	MFLU 17-1253	MF588996	-	MF588986	MF588979	-	Wanasinghe *et al.* 2018
F. maculans T, E	MFLUCC 10-0886	JN846724	JN846710	JN846734	-	-	Liu *et al.* 2011
F. maculans	MFLUCC 10-0887	JN846725	JN846712	JN846736	-	-	Phookamsak *et al.* 2015
F. maculans	MFLUCC 10-0888	JN846726	JN846713	JN846737	-	-	Phookamsak *et al.* 2015
F. maculans	MFLUCC 11-0023	JN846728	JN846714	JN846738	-	-	Phookamsak *et al.* 2015
F. neoaggregatum E	MFLUCC 10-0554	KT955470	-	KT955450	KT955432	KT955412	Phookamsak *et al.* 2015
F. neoaggregatum	MFLUCC 13-0227	KT955471	-	KT955451	KT955433	KT955407	Phookamsak *et al.* 2015
F. palmae E	MFLU 19-0820	MN712336	-	-	MN744229	-	Zhang *et al.* 2020
F. taiwanense E	FU30861	MG189605	-	MG189607	MG252072	-	Tennakoon *et al.* 2018
F. taiwanense	FU30862	MG189606	-	MG189608	MG252073	-	Tennakoon *et al.* 2018
F. thailandicum	MFLUCC 11-0189	KT955472	-	KT955452	KT955434	KT955423	Phookamsak *et al.* 2015
F. thailandicum E	MFLUCC 11-0206	KT955473	-	KT955453	KT955435	KT955415	Phookamsak *et al.* 2015
F. wallichiae	**MFLUCC 15-0315A**	**MN726235**	**MN726241**	**MN726247**	**MN953045**	**-**	**This study**
F. wallichiae E	**MFLUCC 15-0315B**	**MN726236**	**MN726242**	**MN726248**	**MN953046**	**MN915108**	**This study**
F. wallichiae	**MFLUCC 15-0315C**	**MN726237**	**MN726243**	**MN726249**	**MN953047**	**-**	**This study**
Neoastrosphaeriella aquatica E	MFLUCC 18-0209	MK138829	MK138710	MK138789	-	-	Bao *et al.* 2019
N. krabiensis T	MFLUCC 11-0022	JN846727	JN846711	JN846735	-	-	Liu *et al.* 2011
N. krabiensis T, E	MFLUCC 11-0025	JN846729	-	JN846739	-	-	Liu *et al.* 2011
N. sribooniensis E	MFLUCC 13-0834	MF588997	-	MF588987	MF588977	-	Wanasinghe *et al.* 2018
Posidoniomyces atricolor T, E	BRK-21	MK656107	MK656111	MK656115	-	-	Vohník *et al.* 2019
P. atricolor E	BRK-97	MK656110	MK656114	MK656118	-	MK656935	Vohník *et al.* 2019
Rimora mangrovei T	JK 5246A	GU301868	-	GU296193	-	GU371759	Schoch *et al.* 2009
R. mangrovei T	JK 5437B	GU479798	-	GU479765	-	-	Schoch *et al.* 2009

Notes: Newly generated sequences are in bold; T denotes the type species of the genus; E denotes the ex-type culture.

and set as 1,000 bootstrap replicates. Bayesian analysis was performed at CIPRES using Bayesian analysis on XSEDE (v.3.2.6) as part of the "MrBayes on XSEDE" tool (Huelsenbeck & Ronquist 2001; Miller *et al.* 2010). GTR+I+G model was selected by using MrModelTest 2.2 (Nylander 2004) under the Akaike information criterion (AIC) as the best-fit models of the combined dataset for maximum likelihood and Bayesian analysis (Nylander 2004). Bayesian posterior probabilities (BYPP) were determined by Markov chain Monte Carlo sampling (MCMC) in MrBayes on XSEDE v.3.2.6. Six simultaneous Markov chains were run for 815,000 generations and trees were sampled every 1,000th generation. MCMC heated chain was set with a "temperature" value of 0.20. All sampled topologies beneath the asymptote (25 %) were discarded as part of a burn-in procedure; the remaining trees (816) were used for calculating posterior probabilities in the majority rule consensus tree. Maximum parsimony (MP) analysis was carried out with PAUP v 4.0b10 (Swofford 2002). Statistical supports for branches of the most parsimonious tree were estimated using maximum parsimony bootstrap analysis with 1,000 bootstrap replicates (Felsenstein 1985). All characters were unordered and of equal weight, and gaps were treated as missing data. Descriptive tree statistics

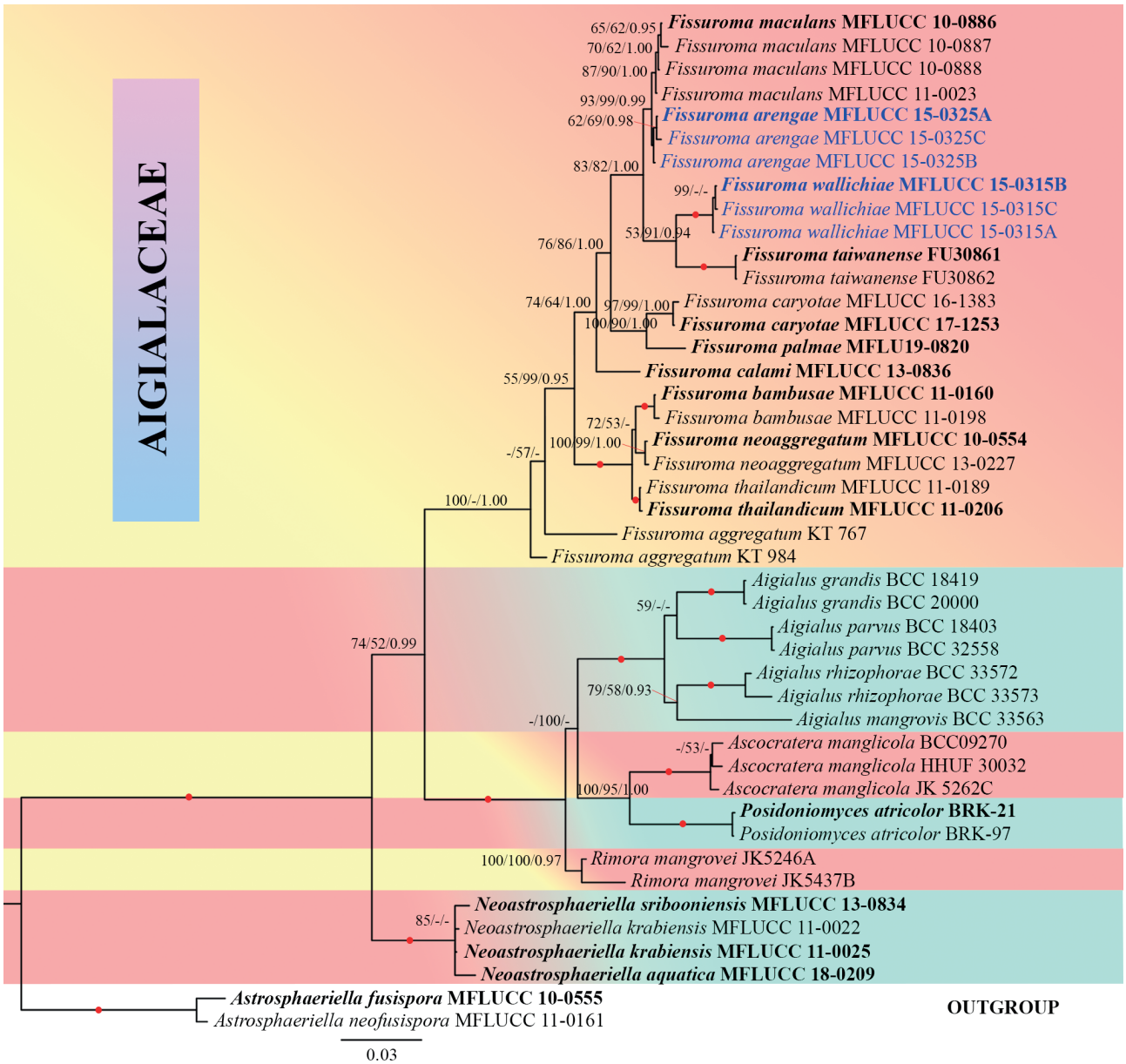

Figure 1. Bayesian inference tree based on a combined dataset of LSU, ITS, SSU, *tef1-α* and *rpb2* partial sequences. Bootstrap support values for maximum likelihood (ML), maximum parsimony (MP) higher than 50 % and Bayesian posterior probabilities (BYPP) greater than 0.90 are given above each branch respectively. Branches with 100 % ML, 100 % MP and 1.00 BYPP are shown with a red dot. Novel taxa are in blue. Ex-type strains are in bold. The tree is rooted to *Astrosphaeriella fusispora* (MFLUCC 10-0555) and *A. neofusispora* (MFLUCC 11-0161) (Astrosphaeriellaceae).

for parsimony (tree length [TL], consistency index [CI], retention index [RI], relative consistency index [RC] and homoplasy index [HI]) were calculated for trees generated under different optimality criteria. Bootstrap support values for ML, MP and BYPP are given near to each node (Fig. 1).

The phylogenetic trees were configured in FigTree v1.4.0 (Rambaut 2012) and edited using Microsoft Office PowerPoint 2010 and Adobe Photoshop CS6 (Adobe Systems, USA). The alignments and respective phylogenetic trees were deposited in TreeBASE, submission ID: 25410 (http://www.treebase.org/).

Results

Phylogenetic analyses

The combined multigene dataset comprised 44 taxa from seven genera (*Aigialus*, *Ascocratera*, *Fissuroma*, *Neoastrosphaeriella*, *Posidoniomyces*, *Rimora* (Aigialaceae), and *Astrosphaeriella* (Astrosphaeriellaceae) in Pleosporales (Tab. 2). The RAxML analysis of the combined dataset yielded the best-scoring tree (Fig. 1) with a final ML optimization likelihood value of -18651.882128. The alignment comprised a total of 5,088 characters including gaps. The matrix had 1,359 distinct alignment patterns, with 36.76 % undetermined characters or gaps, 3,872 constant characters and 207 parsimony-uninformative characters. Estimated base frequencies were: A = 0.249050, C = 0.245449, G = 0.278730, T = 0.226771; substitution rates AC = 1.424415, AG = 3.977296, AT = 0.940998, CG = 1.292848, CT = 9.900102, GT = 1.000000; gamma distribution shape parameter α = 0.179440. Tree-Length = 0.781448. Maximum parsimony analysis of the remaining 1,009 parsimony-informative characters resulted in 1,000 trees with TL = 2261, CI = 0.677, RI = 0.843, RC = 0.571, HI = 0.323. Bayesian posterior probabilities from MCMC were evaluated with a final average standard deviation of the split frequency of 0.009698. The Bayesian analysis resulted in a tree with similar topology and clades as the ML and MP trees. The bootstrap values for ML and MP greater than 50 % and BYPP more than 0.90 are given at the nodes. Phylogenetic analyses of combined LSU, ITS, SSU, *tef1-α* and *rpb2*, showed that the two novel species of *Fissuroma* clustered in a single clade within Aigialaceae.

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

Index Fungorum number: IF557045, Facesoffungi number: FoF06903, Fig. 2

Etymology: Epithet refers to host genus, *Arenga*.

Holotype: MFLU 15-0300.

Saprobic on dead rachis of *Arenga pinnata*. Sexual morph: *Ascomata* 510–970 µm long, in vertical section 175–350 µm high, 425–870 µm diam., dark brown, coriaceous, solitary, scattered, gregarious, hemispherical, semi-immersed, immersed beneath host epidermis, appearing as raised areas. *Ostioles* central, apapillate, with carbonaceous, thin, slit-like opening. *Peridium* 40–76 µm wide at sides, 8–23 µm wide at base, dark brown to black, thick-walled, of *textura prismatica*, poorly developed at the base, thick at sides towards the apex. *Hamathecium* composed of dense, 1.1–2.7 µm wide, trabeculate, hyaline pseudoparaphyses, anastomosing at the apex, embedded in a gelatinous matrix. *Asci* 110–170 × 20–30 µm ($\bar{x}$ = 133 × 24 µm, n = 30), 8-spored, bitunicate, fissitunicate, cylindrical to obclavate, short pedicellate, narrow and rounded apex, with a small ocular chamber. *Ascospores* 40–55 × 10–16 µm ($\bar{x}$ = 48 × 14 µm, n = 30), overlapping, 1–2-seriate, fusiform, hyaline, smooth-walled, tapering to pointed apices, 1-septate, septum near median, constricted at the septum, straight to curved, surrounded by a thin distinctive sheath, 1.5–3.8 µm wide ($\bar{x}$ = 2.6 µm, n = 30), smooth-walled, with two large guttules near septum, and two minute guttules towards ends of ascospores. Asexual morph: Undetermined. Appressoria: not formed. Distribution: Thailand.

Culture characteristics: Colonies on MEA reaching 20 mm diam. after two weeks at 25–30 °C, colonies medium dense, circular, convex, surface slightly rough with edge entire, effuse, velvety to hairy, margin well-defined, colony from above, white to cream at the margin, brown at the center, not producing pigments in culture.

Material examination: THAILAND, Phang-Nga Province, on dead rachis and leaflet of *Arenga pinnata* (Wurmb) Merr. (Arecaceae), 5 December 2014, Sirinapa Konta, PHR07f (MFLU 15-0300, holotype); ex-type living culture, MFLUCC 15-0325A; ibid. MFLUCC 15-0325B and MFLUCC 15-0325C

Notes: *Fissuroma arengae* is similar to *F. maculans* in having ascomata with slit-like ostioles and hyaline, fusiform, didymosporous ascospores. However, *F. arengae* has larger asci (110–170 × 20–30 µm vs. 65–125 × 10–17 µm) and ascospores (40–55 × 10–16 µm vs. 29–38 × 4–8 µm). In addition, the ascospores of *F. arengae* are smooth whereas those of *F. maculans* are verruculose (Liu *et al.* 2011). In phylogenetic analyses, *F. arengae* formed a separate branch as a sister clade to *F. maculans* with high support (93 % ML, 99 % MP, 0.99 BYPP, Fig. 1). Comparison of the LSU, SSU and ITS genes of *F. arengae* and *F. maculans* showed minimal nucleotide differences; 2/841 bp (0.24 %) in LSU, 1/992 bp (0.1 %) in SSU and 3/530 bp (0.57 %) in ITS (Tab. 3). Thus, a comparison of base pairs of LSU, SSU and ITS cannot separate all species of *Fissuroma* (Tab. 3).

Fissuroma wallichiae Konta & K.D. Hyde., sp. nov.

Index Fungorum number: IF557046, Facesoffungi number: FoF06904, Fig. 3

Etymology: Epithet refers to host genus, *Wallichia*.

Holotype: MFLU 15-0290.

Saprobic on dead petioles of *Wallichia* sp. Sexual morph: *Ascomata* 360–870 µm long, in vertical section 170–330 µm high, 400–650 µm diam., dark brown, coriaceous, solitary, scattered, gregarious, hemispherical, semi-immersed,

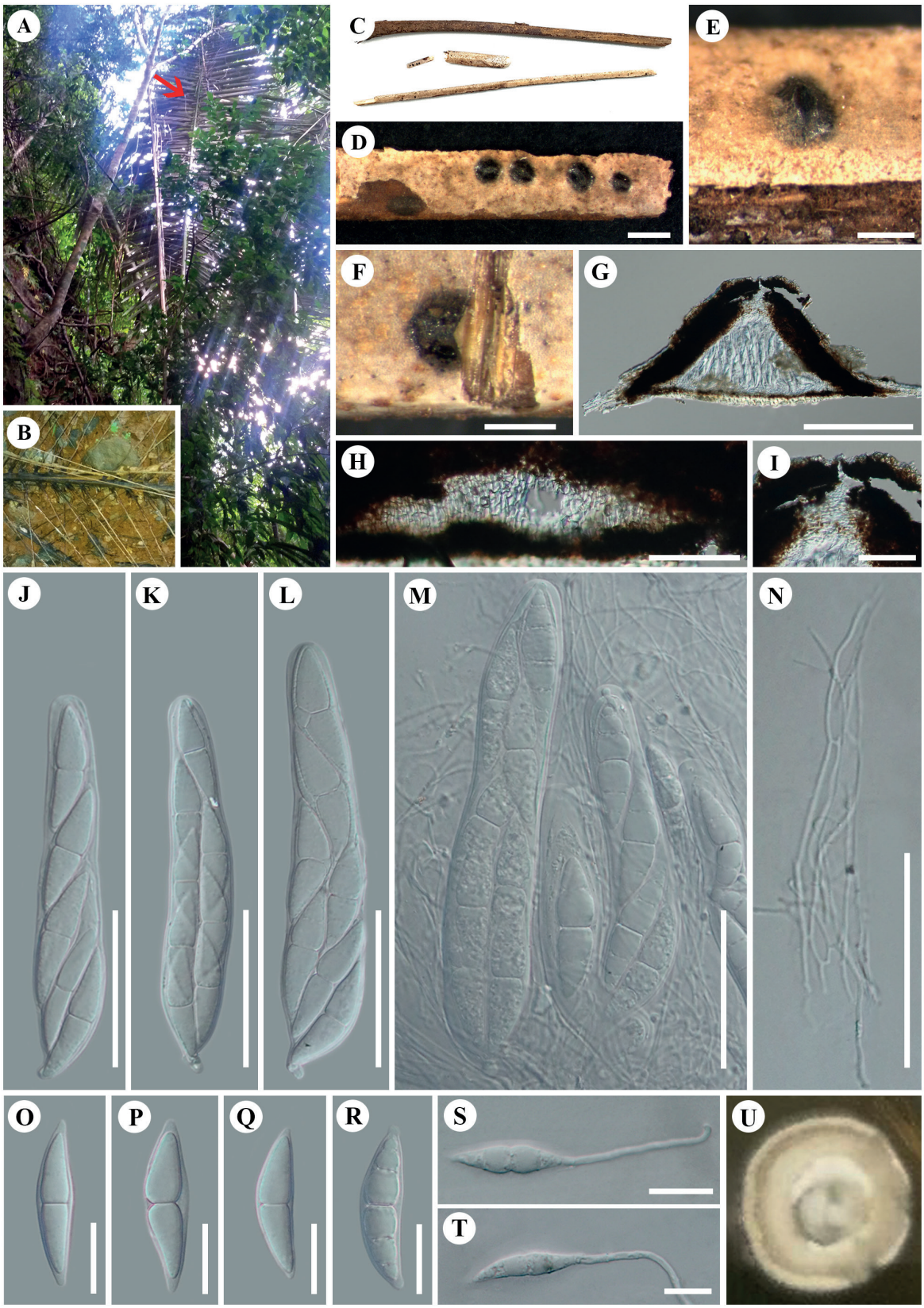

Figure 2. *Fissuroma arengae* (MFLU 15-0300, holotype). **A, B**: The forest in Phang-nga Province. **C**: Palm samples (*Arenga pinnata*, Arecaceae). **D**: Appearance of ascomata on host. **E**: Close-up of ascoma. **F**: Vertical cut of ascoma. **G**: Vertical section of ascoma. **H**: Section of peridium. **I**: Close-up of ostiole. **J–M**: Asci and ascospores with pseudoparaphyses. **N**: Pseudoparaphyses. **O–R**: Ascospores. **S, T**: Germinated ascospores. **U**: Colony on MEA. bars: D = 1,000 µm, E, F = 500 µm, G = 200 µm, H–N = 50 µm, O–T = 20 µm.

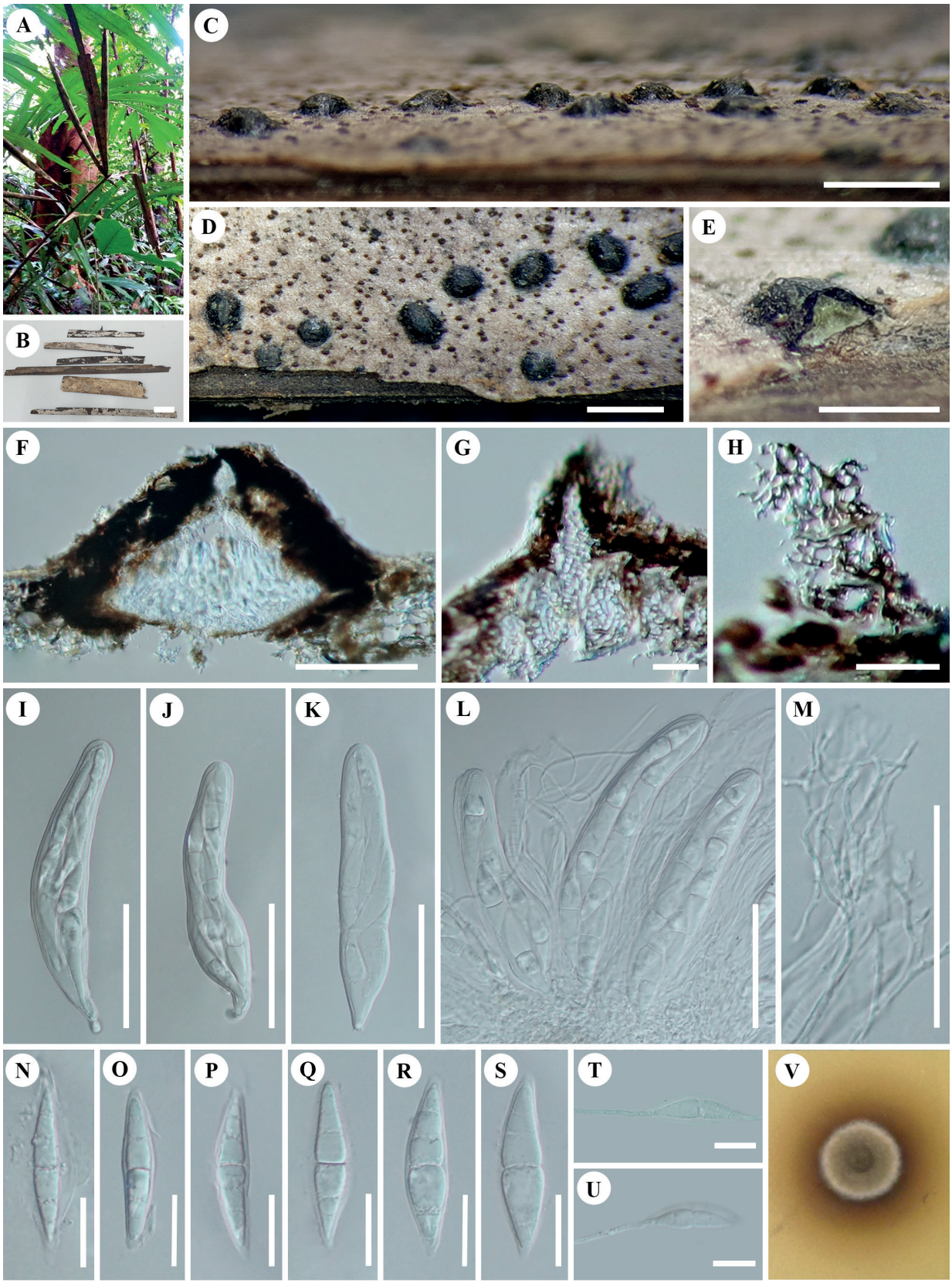

Figure 3. *Fissuroma wallichiae* (MFLU 15-0290, holotype). **A**: The forest in Krabi Province. **B**: Palm samples (*Wallichia* sp., Arecaceae). **C, D**: Appearance of ascomata on host. **E**: Vertical cut of ascoma. **F**: Vertical section of ascoma. **G**: Close-up of ostiole. **H**: Section of peridium. **I–L**: Asci and ascospores with pseudoparaphyses. **M**: Pseudoparaphyses. **N–S**: Ascospores. **T, U**: Germinated ascospores. **V**: Colony on MEA. bars: B = 3 cm, C, D = 1,000 µm, E = 500 µm, F = 100 µm, G, H, N–U = 20 µm, I–M = 50 µm.

immersed beneath host epidermis, appearing as raised areas. *Ostioles* central, apapillate, with carbonaceous, thin, slit-like opening. *Peridium* 30–95 µm wide at sides, 19–75 µm wide at base, dark brown to black, thick-walled, of *textura prismatica*, poorly developed at the base, thick at sides towards the apex. *Hamathecium* composed of dense, 1–2.4 µm wide, trabeculate, hyaline pseudoparaphyses, anastomosing at the apex, embedded in a gelatinous matrix. *Asci* 105–150 × 16–30 µm ($\bar{x}$ = 123 × 24 µm, n = 30), 8-spored, bitunicate, fissitunicate, cylindrical to obclavate, short pedicellate, narrow and rounded apex, with a small ocular chamber. *Ascospores* 40–55 × 6–20 µm ($\bar{x}$ = 48 × 10 µm, n = 30), overlapping, 1–3-seriate, fusiform, hyaline, smooth-walled, tapering to pointed apices, 1-septate, septum nearly median, constricted at the septum, straight to curved, surrounded by a thin distinctive sheath, 1.2–4.1 µm wide ($\bar{x}$ = 2.5 µm, n = 30), smooth-walled, with two large guttules near septum, and two minute guttules towards ends of ascospores. Asexual morph: Undetermined. Appressoria: not formed. Distribution: Thailand.

Culture characteristics: Colonies on MEA reaching 10–20 mm diameter after 2 weeks at 25–30 °C, colonies medium dense, circular, convex, surface slightly rough with edge entire, effuse, velvety to hairy, margin well-defined, colony from above, white to cream at the margin, brown to dark greenish at the center, producing brown pigments in the agar.

Material examination: THAILAND, Krabi Province, on dead petioles of *Wallichia* sp. (Arecaceae), 3 December 2014, Sirinapa Konta, KBC03b (MFLU 15-0290, holotype); ex-type living culture, MFLUCC 15-0315B; ibid. MFLUCC 15-0315A and MFLUCC 15-0315C.

Notes: Phylogenetically, *F. wallichiae* is closely related to *F. taiwanense* with statical support (53 % ML, 91 % MP, 0.94 BYPP, Fig. 1). *Fissuroma wallichiae* resembles *F. taiwanense* in having ascomata with slit-like ostioles and hyaline, fusiform, 1-septate ascospores. *F. wallichiae* differs from *F. taiwanense* by having larger ascomata (in vertical section part), with dark brown to black-walled peridium, thin a mucilaginous sheath without club-shaped appendages at ends of ascospores (Tennakoon *et al.* 2018). Comparison of the LSU, SSU and *tef1-α* nucleotides of *F. wallichiae* and *F. taiwanense* showed a high difference in nucleotide bases; 12/904 bp (1.32 %) in LSU, 11/1029 bp (1.06 %) in SSU and 34/892 bp (3.81 %) in *tef1-α* (Tab. 4). In addition, *F. wallichiae* was found on a palm in Thailand while *F. taiwanense* was found on *Hedychium coronarium* (Zingiberaceae) in Taiwan. We also compared base pairs difference of *F. wallichiae* to all species of *Fissuroma* that are available in GenBank (Tab. 4).

Discussion

Liu *et al.* (2011) introduced *Fissuroma* with *F. maculans* as type species and *F. aggregatum*. Subsequently, another seven species were introduced based on morphology and molecular data (Phookamsak *et al.* 2015; Wanasinghe *et al.* 2018; Tennakoon *et al.* 2018; Zhang *et al.* 2020). *Fissuroma kavachabeejae* and *F. microsporum* were introduced using morphology (Niranjan & Sarma 2018). Most recently, *F. palmae* was described by Zhang *et al.* (2020). *Fissuroma* species have been recorded on two host families (Arecaceae, Poaceae) of which eight from twenty species were found on Arecaceae (*F. calami*, *F. caryotae*, *F. fissuristoma*,

Table 3. A comparison of the nucleotide polymorphisms of *Fissuroma arengae* (MFLUCC 15-0325) for all species in *Fissuroma*.

Species	Strain number	LSU	ITS	SSU	*tef1-α*	References
F. aggregatum	KT984	2.4 % (21/856 bp)	-	1.0 % (10/988 bp)	6.87 % (65/945 bp)	Tanaka *et al.* 2009
F. bambusae	MFLUCC 11-0160	4.34 % (35/805 bp)	-	1.24 % (10/808 bp)	7.82 % (68/869 bp)	Phookamsak *et al.* 2015
F. calami	MFLUCC 13-0836	0.87 % (7/807 bp)	-	0.66 % (7/1057 bp)	5.5 % (48/872 bp)	Wanasinghe *et al.* 2018
F. caryotae	MFLU 17-1253, MFLUCC 16-1383 (for ITS)	0.84 % (7/830 bp)	9.67 % (54/558 bp)	0.57 % (6/1057 bp)	4.13 % (36/872 bp)	Wanasinghe *et al.* 2018; Zhang *et al.* 2020
F. maculans	MFLUCC 10-0886	0.24 % (2/841 bp)	0.57 % (3/530 bp)	0.1 % (1/992 bp)	-	Liu *et al.* 2011
F. neoaggregatum	MFLUCC 10-0554	4.15 % (33/795 bp)	-	1.56 % (15/964 bp)	7.22 % (63/872 bp)	Phookamsak *et al.* 2015
F. palmae	MFLU 19-0820	1.64 % (14/853 bp)	-	-	4.62 % (30/648 bp)	Zhang *et al.* 2020
F. thailandicum	MFLUCC 11-0206	4.12 % (348/825 bp)	-	1.23 % (12/937 bp)	8.57 % (66/872 bp)	Phookamsak *et al.* 2015
F. wallichiae	MFLUCC 15-0315	0.22 % (2/901 bp)	2.11 % (13/615 bp)	0 % (0/1049 bp)	1.48 % (15/1014 bp)	This study
F. taiwanense	FU30861	1.1 % (10/903 bp)	-	1.1 % (11/1028 bp)	3.36 % (30/892 bp)	Tennakoon *et al.* 2018

Notes: '-' do not have sequence; '0' no base pair similarity; base pair differences included gaps.

Table 4. A comparison of the nucleotide polymorphisms of *Fissuroma wallichiae* (MFLUCC 15-0315) for all species in *Fissuroma*.

Species	Strain number	LSU	SSU	*tef1-α*	ITS	*rpb2*	References
F. aggregatum	KT984	2.79 % (24/861 bp)	1.1% (10/988 bp)	7.13 % (67/939 bp)	-	7.83 % (84/1072 bp)	Tanaka *et al.* 2009
F. arengae	MFLUCC 15-325	0.22 % (2/901 bp)	0 % (0/1049 bp)	1.48 % (15/1014 bp)	2.11 % (13/615 bp)	-	This study
F. bambusae	MFLUCC 11-0160	4.59 % (37/805 bp)	1.24 % (10/808 bp)	7.94 % (69/869 bp)	-	8.4 % (69/819 bp)	Phookamsak *et al.* 2015
F. calami	MFLUCC 13-0836	1.11 % (9/809 bp)	0.86 % (9/1036 bp)	6.42 % (56/872 bp)	-	-	Wanasinghe *et al.* 2018
F. caryotae	MFLU 17-1253, MFLUCC 16-1383 (for ITS)	1.08 % (9/829 bp)	0.76 % (8/1055 bp)	4.58 % (40/872 bp)	9.13 % (51/558 bp)	-	Wanasinghe *et al.* 2018; Zhang *et al.* 2020
F. maculans	MFLUCC 10-0886	0.49 % (4/809 bp)	0.1 % (1/992 bp)	-	2.63 % (14/531 bp)	-	Liu *et al.* 2011
F. neoaggregatum	MFLUCC 11-0206	4.4 % (35/795 bp)	1.35 % (13/963 bp)	7.56 % (66/872 bp)	-	9.27 % (76/819 bp)	Phookamsak *et al.* 2015
F. palmae	MFLU 19-0820	1.64 % (14/851 bp)	-	4.62 % (30/648 bp)	-	-	Zhang *et al.* 2020
F. taiwanense	FU30861	1.32 % (12/904 bp)	1.06 % (11/1029 bp)	3.81 % (34/892 bp)	-	-	Tennakoon *et al.* 2018
F. thailandicum	MFLUCC 11-0206	4.36 % (36/824 bp)	1.17 % (11/937 bp)	7.91 % (69/872 bp)	-	8.66 % (71/819 bp)	Phookamsak *et al.* 2015

Notes: '-' do not have sequence; '0' no base pair similarity; base pair differences included gaps.

F. kavachabeejae, *F. maculans*, *F. microsporum*, *F. palmae*) and this supports that *Fissuroma* is hyperdiverse on palms (Liu *et al.* 2011; Phookamsak *et al.* 2015; Niranjan & Sarma 2018; Wanasinghe *et al.* 2018; Zhang *et al.* 2020). All known species have been recorded from Asian countries (Brunei, China, India, Japan, Taiwan, Thailand), and only from terrestrial habitats.

Morphological characters of *F. arengae* and *F. wallichiae* fit well with the description of the generic type of *Fissuroma*. Our phylogenetic analysis also supported the new taxa placement within *Fissuroma*. A comparison of nucleotide base pairs in Tables 3 and 4 which showed that the protein-coding gene regions (*tef1-α*, *rpb2*) have a high percentage of base pair differences. A comparison of LSU and ITS nucleotides revealed a high percentage of base pair differences for some species, while SSU showed few differences (< 1.6 %) among species (Tabs. 3, 4). The phylogeny recovered herein also agrees with that previously established for *Fissuroma* species in Aigialaceae (Tennakoon *et al.* 2018; Wanasinghe *et al.* 2018; Zhang *et al.* 2020). However, the phylogenetic analysis of combined LSU, ITS and SSU sequence data could not separate species of *Fissuroma* well. Thus, for species-level identification of *Fissuroma* it is better to use protein-coding genes such as *tef1-α* and *rpb2*; *rpb2* sequences are required to confirm the monophyly of these species.

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