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Additions to wild seed and fruit fungi 2: *Parascedosporium putredinis*: a new Thailand record from *Delonix regia* seed pods

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Abstract

We are studying seed pod fungi associated with *Delonix regia* in Thailand and in this paper we provide an illustrated account of *Parascedosporium putredinis*, which is a new record for Thailand. Phylogenetic analysis of combined ITS and LSU sequence data and morphological similarities provide evidence for its identification. We illustrate the synnematal stage and lateral, solitary conidiophores of *P. putredinis* obtained *in vitro* on MEA. We also provide ITS, LSU, SSU, and *tef* sequence data for *P. putredinis*, which are deposited in GenBank.

Key words – *graphium*-like – Microascaceae – morphology – phylogeny – Sordariomycetes

Introduction

The genus *Parascedosporium* (Microascaceae, Microascales) is characterized by sympodial conidia produced from denticulate conidiogenous cells (Gilgado et al. 2007). *Parascedosporium* synanamorphs bear conidiophores that are solitary and emerge from aerial mycelium and have *graphium*-like synnemata (Gilgado et al. 2007). *Parascedosporium* was introduced by Gilgado et al. (2007) based on an authentic strain of *Graphium tectonae* C. Booth (CBS 127.84). *Graphium tectonae* was combined and renamed as *Parascedosporium tectonae* (C. Booth) Gilgado, Gené, Cano & Guarro (Gilgado et al. 2007). This strain had been isolated from *Tectona grandis* seeds in Jamaica (Lackner & de Hoog 2011). Later this species was considered as a synonym of *Parascedosporium putredinis* (Corda) M. Lackner & de Hoog (Lackner & de Hoog 2011) based on analysis of ITS sequence data and examination of the ex-type culture (CBS 127.84), and this treatment was accepted by de Beer et al. (2013). A sexual morph has not been identified for the genus (Gilgado et al. 2007, Lackner & de Hoog 2011, Wijayawardene et al. 2017).

A number of fungi have been collected from *Delonix regia* seed pods (Somrithipol et al. 2002, Sahu et al. 2003, Perera et al. 2016, 2017). One new genus, *Delonicicola*, and two new species, *Cirrenalia nigrospora* and *Delonicicola siamense*, have been described from northern Thailand (Somrithipol et al. 2002, Perera et al. 2017). A fungal succession study by Somrithipol et

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al. (2002) reported 41 micro-fungal taxa from *Delonix regia* pods in a tropical forest in Thailand. This demonstrates that pods of *Delonix regia* are an important host for microfungi. These pods are a rich substrate of lignocellulosic materials which can mainly provide substrates for the fungi (Vargas et al. 2011, Saba et al. 2014).

The objective of this study is to further identify fungi associated with fruits of *Delonix regia.* Here we report on a new record of *Parascedosporium putredinis* from *Delonix regia* pods based on molecular data, and similarities of the morphology of the asexual morph.

Materials & Methods

Sample collection, specimen examination and isolation

Dried pods of *Delonix regia* were collected from Mae Fah Luang University campus, Chiang Rai, Thailand in 2015. Specimens were examined in the laboratory and photographed with an Axio camera fitted to a Zeiss SteREO Discovery V8 stereomicroscope. Single-conidial isolates were made from fresh specimens as described in Chomnunti et al. (2014). Colonies were sub-cultured onto Malt Extract Agar (MEA) and incubated at 25°C. Cultures were incubated in dark (25°C). The asexual morph morphology was examined from the fresh specimen and cultures grown on MEA. Microscopic characters were observed using a Motic dissecting microscope (SMZ 168). Conidial structures were mounted in water and Lugol's reagent and photographed on a Canon 550D digital camera fitted to the Nikon ECLIPSE 80i compound microscope. Measurements were taken with the Tarosoft (R) Image Frame Work software. The images used for illustrating the fungus were processed with Adobe Photoshop CS5 v. 12.0 software (Adobe Systems, USA).

Herbarium materials are deposited in the Mae Fah Luang University herbarium, Chiang Rai, Thailand (MFLU). Living cultures are deposited in the Culture Collection at Mae Fah Luang University (MFLUCC). Facesoffungi number was registered as explained in Jayasiri et al. (2015).

DNA isolation, PCR amplification and sequencing

Fresh mycelia were scraped from colonies grown on MEA at 25 °C. Genomic DNA was extracted from mycelia, using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, P.R. China) following the manufacturer's protocol. Partial gene sequences were amplified for the 28S large subunit nrDNA (LSU), the internal transcribed spacer (ITS), 18S small subunit nrDNA (SSU) and translation elongation factor 1-alpha gene (*tef*) using the primers and conditions listed in Table 1. Polymerase Chain Reaction (PCR) amplification was performed in a 25 µl reaction volume containing, 12.5 µl of 2 × PCR Master Mix (TIANGEN Co., China), 9.5 µl ddH_2O, 5–10 ng DNA and 1 µl of each primer (10 µM). PCR products were purified and sequenced at Shanghai Sangon Biological Engineering Technology & Services Co., China. Both directions of the PCR products were sequenced using the same primer pairs as used in PCR amplification. Sequences were deposited in GenBank.

Sequence alignment and phylogenetic analysis

The sequences generated in this study were supplemented with the additional sequences obtained from GenBank (Table 2). The sequences were aligned online with the MAFFT v. 7 server (http://mafft.cbrc.jp/alignment/server/) and manually improved using BioEdit v. 7.0.5.2 (Hall 1999). Ambiguous regions were excluded from the analyses and gaps were treated as missing data. All the novel sequences were deposited in GenBank and the final alignment and tree deposited in TreeBASE, as study ID: S22966 (http://www.treebase.org/). Phylogenetic analyses were based on maximum likelihood (ML) and Bayesian inference (BI) methods.

The ML tree was regenerated using RAxML-HPC2 run on XSEDE (8.2.8) (Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) using GTR+I+G model of evolution. The optimal ML tree search was conducted with 1 000 separate runs.

Bayesian analysis was performed using MrBayes v. 3.2.0 (Ronquist & Huelsenbeck 2003). The best-fit evolutionary models for phylogenetic analyses were selected separately for ITS and

LSU gene regions using MrModeltest v. 2.2 (Nylander 2004) under the Akaike Information Criterion (AIC) implemented in both PAUP v. 4.0b10. The GTR+G and SYM+I+G models were selected for ITS and LSU respectively, and incorporated into the analysis. Two parallel analyses of each consisting of four Markov Chain Monte Carlo (MCMC) chains, run from random trees for 4 000 000 generations were sampled in every 100th generation. The distribution of log-likelihood scores was examined to determine the stationary phase for each search and to confirm whether extra runs were required to achieve convergence, using Tracer v.1.5 (Rambaut & Drummond 2007) software program. The first 20% trees, representing the burn in phase of the analyses were discarded from each run. The remaining 80% trees were used to calculate posterior probabilities (PP) in the majority rule consensus tree. Trees were viewed by FigTree v.1.4 (http://tree.bio.ed.ac.uk/software/figtree/) and edited using Microsoft PowerPoint 2010.

Table 1 PCR protocols applied for the analysed loci.

Locus	Primers (Reference)	PCR Conditions
LSU	LR5/LR0R (Vilgalys & Hester 1990, Rehner & Samuels 1994)	[a]94 °C: 30 s, 55 °C: 1 min., 72 °C: 1 min. (37 cycles)[b]
ITS	ITS5/ITS4 (White et al. 1990)	[a]94 °C: 30 s, 48 °C: 1 min., 72 °C: 1 min. (37 cycles)[b]
SSU	NS1/NS4 (White et al. 1990)	[a]94 °C: 30 s, 55 °C: 1 min., 72 °C: 1 min. (37 cycles)[b]
tef	EF1-728F/ EF1-986R (Carbone & Kohn 1999)	[a]94 °C: 30 s, 56 °C: 30 s, 72 °C: 1.30 min. (35 cycles)[b]

[a]Initiation step of 94 °C: 3 min

[b]Final elongation step of 72 °C: 7 min. and final hold at 4 °C applied to all PCR thermal cycles

Table 2 Isolates and sequences used in this study.

Name	Culture Collection No.	GenBank No.	
		ITS	LSU
Cephalotrichum stemonitis	CBS 127.22 = MUCL 4031	LM652377	AF400852
Graphium fimbriasporum	CMW5605	AY148177	KM495388
G. penicillioides	CBS 102632 = JCM 10498 (T)	AB038432	AF175961
Kernia nitida	CGMCC 3.15221	KC485066	KC485070
Lophotrichus plumbescens	NBRC 30864 (T)	LC146745	-
Microascus longirostris	CBS 196.61 = MUCL 9058 (T)	LM652421	LN851043
M. senegalensis	CBS 277.74 = IHEM 18561 (T)	LM652441	LM652523
Parascedosporium putredinis	CBS 102083 = JCM8082 (T)	HQ185348	-
P. putredinis	CBS 127.84	AY228113	AF027660
P. putredinis	CBS 108.10	HQ185347	-
P. putredinis	CBS 100392	GQ476983	-
P. putredinis	CBS 118694	AM749735	-
P. putredinis	CGMCC:3.15233	KC894850	-
P. putredinis	CMW352	HQ335312	-
P. putredinis	HSAUP052348	FJ914685	-

Table 2 Continued.

Name	Culture Collection No.	GenBank No.	
		ITS	LSU
P. putredinis	**MFLUCC 15-1009**	**MH048678**	**MH048679**
P. putredinis	OP219	JN604458	-
P. putredinis	OPF44	JN663836	-
Petriella setifera	CBS 347.64	AY882346	AY884002
Pe. setifera	CBS 391.75	AY882344	AY882375
Pe. sordida	CBS 124169	GQ426957	-
Pe. sordida	CBS 184.73 (T)	AY882360	-
Petriellopsis africana	CBS 311.72 (T)	AY879797	EF151331
Pseudoscopulariopsis schumacheri	CBS 435.86 (T)	LM652455	AF400874
Scedosporium apiospermum	CBS 330.93 (T)	AY863196	AY882372
S. aurantiacum	CBS 116910 (T)	HQ231818	EF151326
S. putredinis	CBS 452.89	HQ185322	EF151329
Scopulariopsis candida	MUCL 40743 (T)	LM652484	HG380458
Trichoderma viride	DAOM JBT1003	JN942883	JN938865
Wardomyces giganteus	CBS 746.69	LM652411	LM652510
W. inflatus	CBS 216.61 (T)	LM652496	LM652553
Wardomycopsis humicola	CBS 487.66 (T)	LM652497	LM652554

(T) Ex-type/ex-epitype strains.
New strains are in bold.

Results

Phylogenetic analyses

An analysis of combined ITS and LSU sequence data was carried out for 32 selected taxa of Microascaceae and Graphiaceae, including the outgroup taxon. (Table 2). The aligned dataset comprised 1276 characters including gaps (ITS: 1–501, LSU: 502–1276). Bayesian inference analyses of the combined data set yielded a tree with similar topologies to maximum likelihood (ML) tree. The best scoring RAxML tree with a final likelihood value of -6869.025975 is illustrated (Fig. 1). The matrix comprised 441 distinct alignment patterns, with 35.80% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.238736, C = 0.265393, G = 0.274877, T = 0.220994; substitution rates AC = 1.941136, AG = 3.066672, AT = 2.506642, CG = 2.282388, CT = 7.011785, GT = 1.000000; gamma distribution shape parameter α = 0.449508.

Taxonomy

Parascedosporium putredinis (Corda) Lackner & de Hoog Figs 2–4

Facesoffungi number: FoF04482

Saprobic or pathogenic on plant material, or isolated from soil and dung. Sexual morph: Undetermined. Asexual morph from the natural substrate – Conidiomata graphium-like synnema. Synnemata 707–851 µm tall, erect, with a cylindrical stipe. Stipe 8–14 µm wide, olivaceous grey, smooth-walled, terminating in a slimy head of conidia, appearing as a slimy droplet, 31–64 µm. Conidiogenous cells 12.8–21×1.1–1.9 µm ($\bar{x}$ = 15.3×1.4 µm; n = 10), percurrent, cylindrical, smooth-walled. Conidia 4.2–6.1×2.4–3.4 µm ($\bar{x}$ = 5×2.8 µm; n = 25), oblong, hyaline, smooth-walled. Synnematal stage from the culture – Conidiomata graphium-like synnema. Synnemata 300–

555 µm tall, erect, with a cylindrical stipe. Stipe 28–41 µm wide, dark grey, smooth-walled, terminating in a slimy head of conidia, appearing as a slimy droplet, 83–255 µm. Conidiogenous cells 17–22×0.8–1.8 µm ($\bar{x}$ = 20.3×1.3 µm; n = 10), percurrent, cylindrical, hyaline, smooth-walled. Conidia 6.8–9.2×3–4.5 µm ($\bar{x}$ = 8×3.8 µm; n = 25), ovate to subcylindrical, base obtuse to subtruncate, aseptate, straight, multi-guttulate, hyaline, smooth-walled. Solitary conidiophores from culture – Conidiophores 1.5–2.1 µm wide, solitary, emerging from aerial mycelium, undifferentiated, simple, often reduced to conidiogenous cells, or irregularly branched, with branches often bearing two to five conidiogenous cells, thin-walled. Conidiogenous cells 8.6–24.4×1.7–2.5 µm ($\bar{x}$ = 14.1×2.2 µm; n = 20), cylindrical to flask-shaped, hyaline, thin-walled, denticulate, usually terminating in a cluster of two to three cylindrical denticles. Conidia 4.7–7.3×2.7–4.2 µm ($\bar{x}$ = 6.2×3.5 µm; n = 25), obovate or subglobose, hyaline, smooth, more or less thick-walled.

Culture characters – Reaching 20–27 mm within 7 days on MEA, at 25 °C, circular, with sparse aerial mycelium, and smooth, margins lobed, white from above, reverse gray in the center, becoming white towards the margin. Aerial mycelium produced simple conidiophores within 7 days at the margins. Synnemata produced on MEA within 30 days. Colonies becoming dark gray, and slightly granular at the centre due to the abundance of synnemata.

Material examined – THAILAND, Chiang Rai Province, Mae Fah Luang University campus, on a dried seed pod of *Delonix regia* (Boj. ex Hook.) Raf. (Fabaceae), 14 December 2015, R.H. Perera, RHP 130 (MFLU 17-0736), dry culture on MEA, MFLU 18-0516; living culture, MFLUCC 15-1009. GenBank: MH048678 (ITS), MH048679 (LSU), MH048680 (SSU), MH048681 (*tef*).

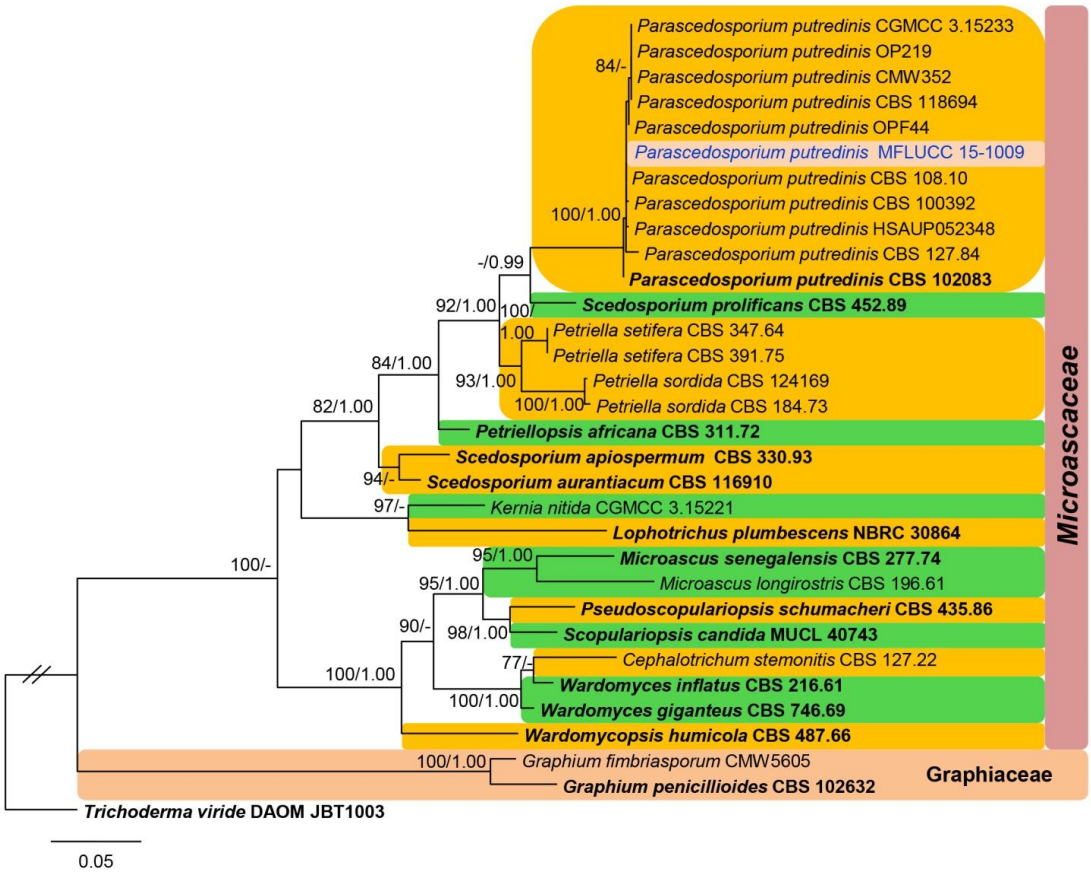

Fig. 1 – Maximum likelihood tree resulting from analysis of combined ITS and LSU sequence data of selected taxa of Microascaceae and Graphiaceae. Maximum likelihood bootstrap support values (ML≥75%) and Bayesian posterior probabilities (PP≥0.99) are shown near the nodes. The new isolate in blue and ex-type strains in bold. The scale bar represents the expected number of nucleotide substitutions per site. The tree is rooted to *Trichoderma viride*.

Notes – Our new isolate (MFLUCC 15-1009) clustered with other *Parascedosporium putredinis* strains in the phylogenetic tree (Fig. 1). Differences of two bases were noted in the ITS region between our new isolate and the ex-type (CBS 102083). The new collection produced synnemata and lateral solitary conidiophores (in culture) similar to the holotype of *P. putredinis* (Figs 2–4). Our new *P. putredinis* isolate is slightly different from the holotype in possessing larger conidia (6.8–9.2×3–4.5 vs. 5.5–7.5×2.5–3.5 μm) (Gilgado et al. 2007). By considering the morphological and molecular data we designate our new collection as *P. putredinis*.

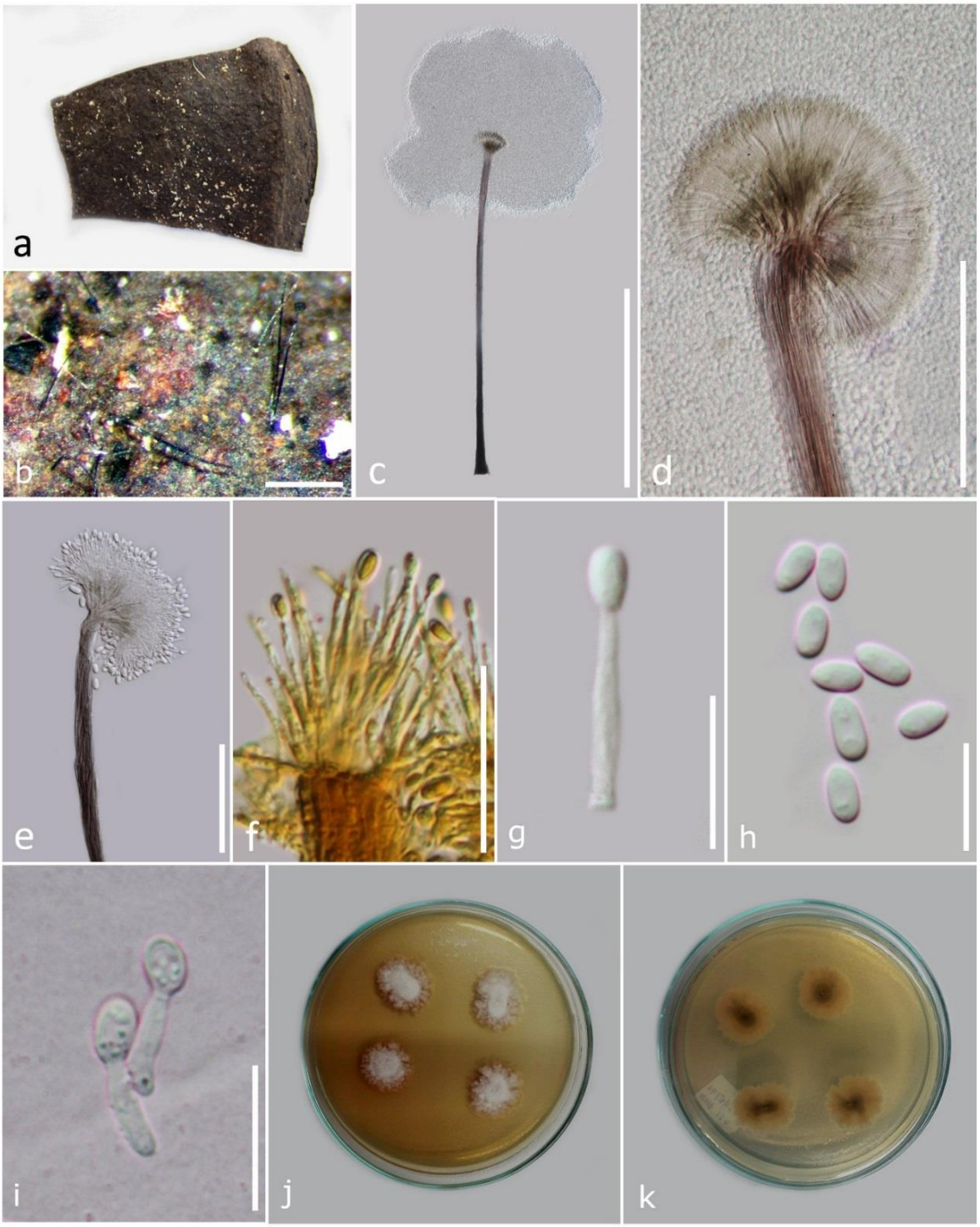

Fig. 2 – *Parascedosporium putredinis* (MFLU 17-0736). a Herbarium material. b Conidiomata on the host substrate. c Conidiophore with conidia. d, e Close up of the apex of the stipe. f, g Conidiogenous cells with conidia. h Conidia. i Germinating conidia. j, k Colonies on MEA. Scale bars: b, c = 500 μm, d = 100 μm, e = 50 μm, f = 20 μm, g–i = 10 μm.

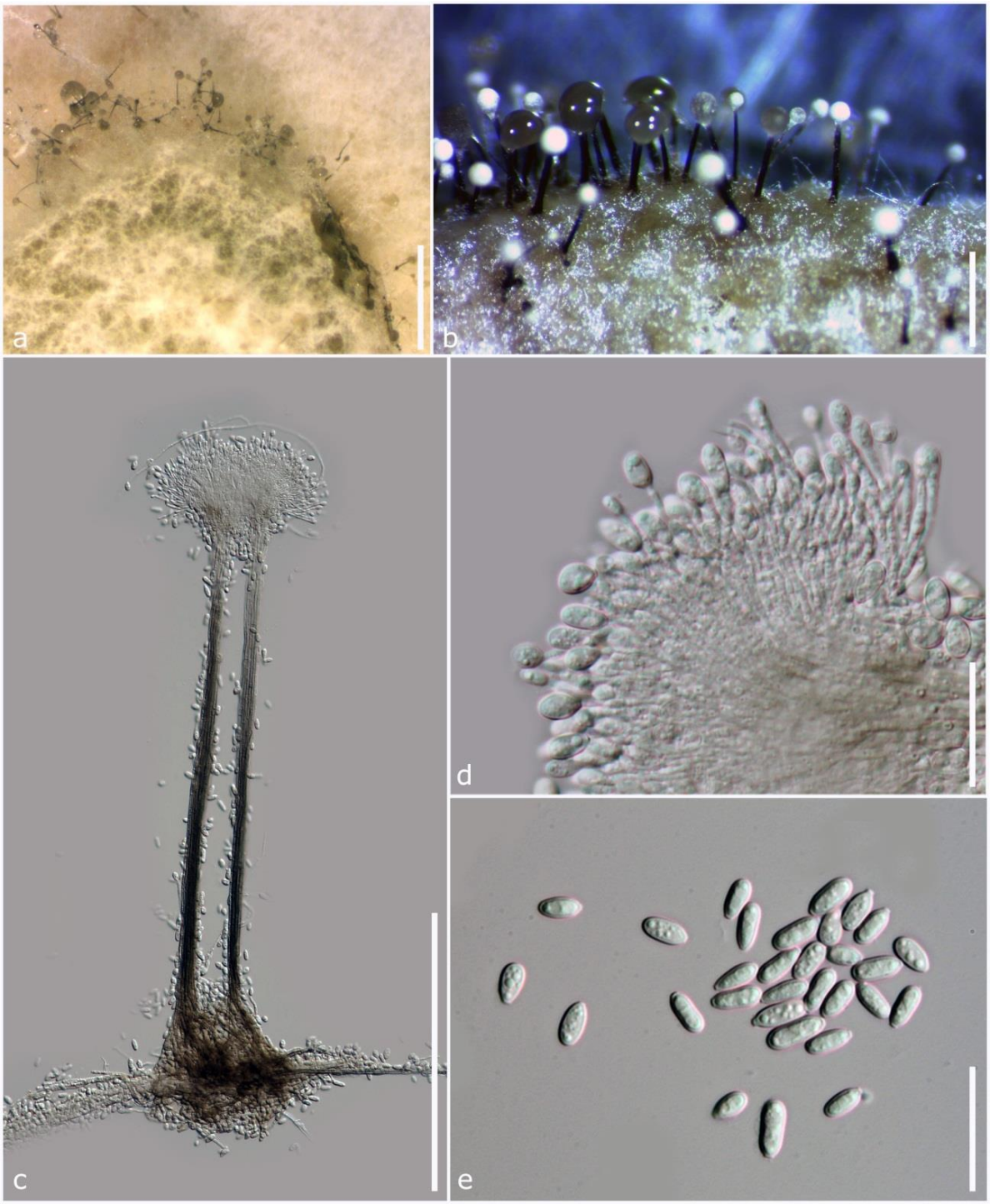

Fig. 3 – *Parascedosporium putredinis* graphium stage on MEA (MFLU 18-0516). a, b Synnemata on MEA. c Synnemata. d Conidiophores with conidia. e Conidia. Scale bars: a = 1 mm, b = 500 μm, c = 200 μm, d, e = 20 μm.

Discussion

Parascedosporium putredinis has been isolated from different countries with different habitats. Strains have been isolated from living plant leaves, seeds of *Tectona grandis*, other plant debris and soil or dung. In the Center of Excellence in Fungal Research (Mae Fah Luang University), we are investigating diversity of microfungi on wild seeds and fruits in Thailand. However, this is the first record of *P. putredinis* on *Delonix regia* seed pods and first record from Thailand. It is possible that *P. putredinis* is a species complex but this requires further study with

more genes. Currently There are only ITS and SSU sequence data available for *P. putredinis* in GenBank. Our study provides additional LSU and *tef* sequences for future studies.

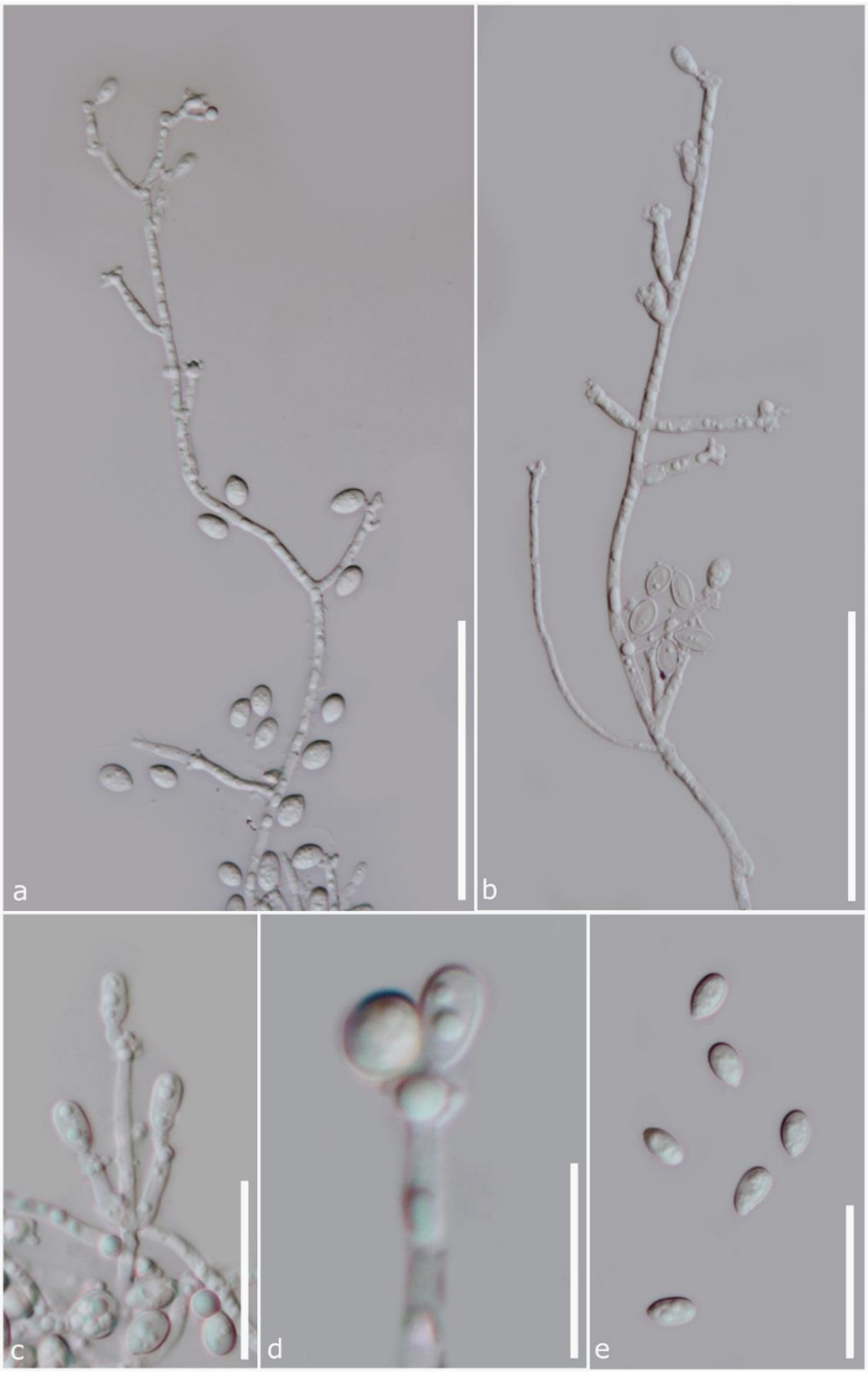

Fig. 4 – *Parascedosporium putredinis* solitary conidiophores on MEA (MFLU 18-0516). a, b Aerial hyphae with simple or branched conidiophores producing sympodial conidia. c, d Lateral, cylindrical conidiogenous cells with conidia. e Conidia. Scale bars: a, b = 50 μm, c = 20 μm, d = 10 μm, e = 20 μm.

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References

Carbone I, Kohn LM. 1999 – A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91, 553–556.

Chomnunti P, Hongsanan S, Hudson BA, Tian Q et al. 2014 – The sooty moulds. Fungal Diversity 66, 1–36.

de Beer ZW, Seifert KA, Wingfield MJ. 2013 – A nomenclator for ophiostomatoid genera and species in the Ophiostomatales and Microascales. Ophiostomatoid fungi: Expanding frontiers. Utrecht, CBS-KNAW Fungal Biodiversity Centre. CBS Biodiversity Series 12, 261–268.

Gilgado F, Gené J, Cano J, Guarro J. 2007 – Reclassification of *Graphium tectonae* as *Parascedosporium tectonae* gen. nov., comb. nov., *Pseudallescheria africana* as *Petriellopsis africana* gen. nov., comb. nov. and *Pseudallescheria fimeti* as *Lophotrichus fimeti* comb. nov. International Journal of Systematic and Evolutionary Microbiology 57, 2171–2178.

Hall TA. 1999 – BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98.

Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat J et al. 2015 – The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. Fungal Diversity 74, 3–18.

Lackner M, de Hoog GS. 2011 – *Parascedosporium* and its relatives: phylogeny and ecological trends. IMA Fungus 2, 39–48.

Miller MA, PfeifferW, Schwartz T. 2010 – Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: In: Proceedings of the gateway computing environments workshop (GCE) 14 Nov 2010. Institute of Electrical and Electronics Engineers, New Orleans, LA, pp 1–8.

Nylander JAA. 2004 – MrModeltest 2.0. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Perera RH, Maharachchikumbura SSN, Bhat JD, Al-Sadi AM et al. 2016 – New species of *Thozetella* and *Chaetosphaeria* and new records of *Chaetosphaeria* and *Tainosphaeria* from Thailand. Mycosphere 7, 1301–1321.

Perera RH, Maharachchikumbura SS, Jones EG, Bahkali AH et al. 2017 – *Delonicicola siamense* gen. and sp. nov. (Delonicicolaceae fam. nov., Delonicicolales ord. nov.), a saprobic species from *Delonix regia* seed pods. Cryptogamie, Mycologie. 38, 321–340.

Rambaut A, Drummond AJ. 2007 – Tracer v. 1.4. Available at: http://beast.bio.ed.ac.uk/Tracer

Rehner SA, Samuels GJ. 1994 – Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycological Research 98, 625–634.

Ronquist F, Huelsenbeck JP. 2003 – MrBayes 3: Bayesian phylogenetic inference under mixed models Bioinformatics 19, 1572–1574.

Saba N, Jawaid M, Paridah MT. 2014 – Lignocellulosic Materials as the Potential Source of Catalyst In Biomass and Bioenergy (pp. 247–274). Springer International Publishing.

Sahu RK, Agarwal VK, Sachan IP. 2003 – Detection of fungi associated with the seeds of gulmohur (*Delonix regia* Boj. Hook.) Raf., Bioved 14(1/2), 37–40.

Somrithipol S, Jones EBG, Hywel-Jones NL. 2002 – Fungal diversity and succession on pods of *Delonix regia (Leguminosae)* exposed in a tropical forest in Thailand. In: *Fungal Succession* (eds. KD Hyde and EBG. Jones). Fungal Diversity 10, 131–139.

Stamatakis A. 2014 – RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.

Vargas AM, Cazetta AL, Garcia CA, Moraes JC et al. 2011 – Preparation and characterization of activated carbon from a new raw lignocellulosic material: Flamboyant (*Delonix regia*) pods. Journal of Environmental Management 92, 178–184.

Vilgalys R, Hester M. 1990 – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. The Journal of Bacteriology 172, 4238–4246.

White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), PCR Protocols: a guide to methods and applications. Academic Press, New York 315–322.

Wijayawardene NN, Hyde KD, Tibpromma S, Wanasinghe DN et al. 2017 – Towards incorporating asexual fungi in a natural classification: checklist and notes 2012–2016. Mycosphere 8, 1457–1555.