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New species of *Thozetella* and *Chaetosphaeria* and new records of *Chaetosphaeria* and *Tainosphaeria* from Thailand

Perera RH[1, 2], Maharachchikumbura SSN[3], Bhat JD[4], Al-Sadi AM[3], Liu JK[1], Hyde KD[2, 5, 6] and Liu ZY[1]

[1]*Guizhou Key Laboratory of Agricultural Biotechnology, Guizhou Academy of Agricultural Sciences, Xiaohe District, Guiyang City, Guizhou Province 550006, People's Republic of China*
[2]*Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[3]*Department of Crop Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, PO Box 34, Al Khoud 123, Oman*
[4]*Formerly at Department of Botany, Goa University, Goa 403206, India*
[5]*Key Laboratory for Plant Biodiversity and Biogeography of East Asia (KLPB), Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, Yunnan, China*
[6]*World Agroforestry Centre, East and Central Asia, 132 Lanhei Road, Kunming 650201, China*

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Abstract

We are studying seed and fruit-borne fungi in Thailand and in this paper report on species of *Tainosphaeria*, *Thozetella* and *Chaetosphaeria* from *Fabaceae* seed pods and decorticated wood, collected in Chiang Mai and Phang-nga provinces. Phylogenetic analysis of combined LSU, ITS and TUB sequence data provides evidence for new species of *Thozetella* and *Chaetosphaeria*. These new taxa are introduced and compared with closely related species in these genera. We provide a morphological and illustrated account of *Chaetosphaeria panamensis* isolated from *Pinus* twigs as a first record for Thailand.

Key words – *Chaetosphaeriales* – morphology – phylogeny – seed/fruit fungi – Sordariomycetes

Introduction

The hyphomycetous genus *Thozetella* Kuntze was introduced by Kuntze (1891) based on *T. nivea* (Berk.) Kuntze. The genus presently includes 19 epithets (Index Fungorum 2016) and is characterized by sporodochial or synnematous conidiomata, phialidic conidiogenesis, and aseptate conidia, with unbranched setula at each end and sterile microawns (Sutton & Cole 1983, Paulus et al. Hyde 2004). *Thozetella* species have been reported from soil and decaying plant parts in terrestrial and freshwater habitats from temperate and tropical regions (Morris 1956, Agnihothrudu 1958, 1962, Waipara et al. 1996, Sivichai et al. 2002, Allegrucci et al. 2004, Delgado-Rodrígues & Mena-Portales

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Corresponding Author: Zuo-Yi Liu – e-mail – gzliuzuoyi@163.com

2004, Paulus et al. 2004, Pinruan et al. 2007, Jeewon et al. 2009, Barbosa et al. 2011, Silva & Grandi 2011, 2013). It will be interesting to use molecular data to establish if the morphologically similar taxa from extreme regions are the same species.

Species of *Tainosphaeria* F.A. Fernández & Huhndorf are saprobes, and generally isolated from erumpent stromata of over-matured ascomycetes. The genus, typified by *T. crassiparies* F.A. Fernández & Huhndorf, is characterized by subglobose to ovoid ascomata, simple, septate paraphyses, cylindrical, pedicellate asci with an apical ring, and narrow-fusiform, septate, hyaline ascospores (Fernández & Huhndorf 2005). The asexual morph is hyphomycetous, with mononematous, unbranched conidiophores, terminating in cylindrical phialides with a collarette and ellipsoidal to clavate, or falcate, hyaline conidia (Fernández & Huhndorf 2005).

The saprobic genus, *Chaetosphaeria* Tul. & C. Tul. had been placed in *Lasiosphaeriaceae* by Barr (1990) and is presently included in *Chaetosphaeriaceae* (Chaetosphaeriales) (Réblová et al. 1999, Huhndorf et al. 2004, Maharachchikumbura et al. 2015, 2016) based on molecular data. Morphological characters of the sexual morph are simple and hardly distinguishable, while the asexual morphs characters are considered as distinctive (Gams & Holubová-Jechová 1976, Huhndorf et al. 2004). The asexual morph of *Chaetosphaeria* is hyphomycetous with macronematous or mononematous conidiophores, monophialidic or polyphialidic, hyaline, conidiogenous cells, with a distinct funnel-shaped collarette and hyaline to brown, aseptate to multi-septate, guttulate or eguttulate conidia, with or without appendages (Maharachchikumbura et al. 2016). Species in this genus are recorded on decayed plant material in terrestrial and freshwater habitats, worldwide (Ho et al. 2001, Huhndorf et al. 2001, Fernández & Huhndorf 2005, Fernández et al. 2006, Atkinson et al. 2007).

We introduce three new taxa belonging in *Chaetosphaeriaceae* collected from *Fabaceae* seed pods, with support from molecular and morphological data. New record of *Chaetosphaeria panamensis* for Thailand is also illustrated. This is the first record of *Tainosphaeria siamensis* on *Fabaceae* seed pods.

Materials and methods

Sample collection, specimen examination and isolation

Specimens were collected from Thailand during 2014 to 2015, and macroscopic and microscopic characters were observed in the laboratory. Fungal structures were observed using a Motic dissecting microscope (SMZ 168) and a Nikon ECLIPSE 80i compound microscope. Free hand sections of fungal structures were taken wherever necessary and mounted in water and Melzer's reagent for microscopic study. Photomicrography was carried out using a Canon 450D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work software. The images used for illustrating the fungi were processed with Adobe Photoshop CS5 v. 12.0 software (Adobe Systems, USA). Single ascospore/conidial colonies were established as described in Chomnunti et al. (2014). Colonies were sub-cultured on Malt Extract Agar (MEA) and incubated at room temperature at 28 °C. To induce sporulation, cultures were incubated at 28 °C in the dark.

Herbarium specimens are deposited in the Mae Fah Luang University (MFLU) herbarium, Chiang Rai, Thailand. Living cultures are deposited in the Culture Collection at Mae Fah Luang University (MFLUCC). Facesoffungi and Index Fungorum numbers were registered as explained in Jayasiri et al. (2015) and Index Fungorum (2016).

Table 1 Information on loci and PCR protocols used in the study.

Locus	Primers (Reference)	PCR conditions
ITS	ITS5/ITS4 (White et al. 1990)	[a]94 °C: 30 s, 53 °C: 30 s, 72 °C: 1.30 min (37 cycles) [b]
LSU	LR5/LR0R (Vilgalys & Hester 1990, Rehner & Samuels 1994)	[a]94 °C: 30 s, 48 °C: 30 s, 72 °C: 1.30 min (35 cycles) [b]
TUB	Bt2a/Bt2b (Glass & Donaldson 1995)	[a]94 °C: 30 s, 58 °C: 30 s, 72 °C: 1.30 min (37 cycles) [b]

[a]Initiation step of 94 °C: 5 min

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

DNA isolation, amplification and analyses

Genomic DNA was extracted from fungal colonies growing on MEA or directly from the fruiting bodies on the natural substrate, using the Ezup DNA Extraction Kit (Sangon Biotech, Shanghai, China) according to the manufacturer's protocol. Partial 28S large subunit nrDNA (LSU), the internal transcribed spacer (ITS) and partial β-tubulin (TUB) were amplified using the primer pairs and PCR protocols listed in Table 1. PCR was performed in a 25 µl reaction volume containing, 12.5 µl 2×PCR Master Mix (TIANGEN Co., China), 9.5 µl ddH_2O, 1 µl DNA (<1 µg) and 1 µl of each primer (10 µM). PCR products were viewed on 1% agarose gels and stained with ethidium bromide. Purification and sequencing of PCR products were done by Invitrogen Biotechnology Co. Ltd., Beijing, China. To ensure the integrity of the sequences, both directions of the PCR products were sequenced using the same primer pairs as used in PCR amplification. A consensus sequence for each gene region was assembled in ContigExpress (Vector NTI Suite 6.0).

The sequences generated in this study were supplementary with the additional sequences obtained from GenBank (Table 2) selected based on recent publications on this group (Crous et al. 2012, Maharachchikumbura et al. 2015, 2016). The sequence data were aligned online with the MAFFT v. 7 server (http://mafft.cbrc.jp/ alignment/server/) and manually adjust using MEGA6 v. 6.0 where necessary (Tamura et al. 2011). Phylogenetic analyses were based on Bayesian inference (BI) and maximum likelihood (ML) analysis. ML analyses was performed using RAxML GUI v. 1.3 (Silvestro & Michalak 2011). The optimal ML tree search was conducted with 1 000 separate runs, using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing likelihood scores with the GTRGAMMA nucleotide substitution model. Bayesian analysis was performed using MrBayes v. 3.2.0 (Huelsenbeck & Ronquist 2001). The best-fit evolutionary models for phylogenetic analyses were selected separately for LSU, ITS and TUB gene regions using MrModeltest v. 2.2 (Nylander 2004). The GTR+I+G model was selected for each gene separately, and incorporated into the analysis. Two parallel analyses of each consisting of six Markov Chain Monte Carlo (MCMC) chains, run from random trees for 5 000 000 generations were sampled every 100 generations resulting in 20 000 total trees. The first 10 000 trees, representing the burn in phase of the analyses were discarded from each run. The remaining trees were used to calculate posterior probabilities (PP) in the majority rule consensus tree. ML bootstrap values (>50%) (ML) and Bayesian posterior probabilities (>90%) (PP) are provided (Fig 1). Trees were viewed by FigTree v1.4 (http://tree.bio.ed.ac.uk/software/figtree/) and edited using Microsoft PowerPoint 2016.

Table 2 GenBank accession numbers of the isolates used in this study

Taxon	Collection/Isolate no.	GenBank accession no.		
		LSU	ITS	TUB
Brunneodinemasporium brasiliense	CBS 112007	JQ889288	JQ889272	-
Chaetosphaeria abietis	CBS 427.83	-	AF178541	-
C. acutata	CBS 101312	AF178553	AF178553	-
C. albida	PDD 92537	EU037898	EU037890	-
C. bombycina	PDD 92538	-	EU037892	-
C. caesariata	SMH 279	AF466060	-	AF466020
C. callimorpha	CBS 525.88	AF178555	AF178555	-
C. capitata	SMH 3239	AF466061	-	AF466021
C. chalaroides	SMH 2223	AF466063	-	AF466024
C. chloroconia	MR 1119	-	AF178542	-
C. chlorotunicata	SMH 1565	AF466064	-	AF466025
C. ciliata	ICMP 18253	GU180637	-	-
C. conirostris	SMH 2183	AF466066	-	AF466027
C. cubensis	SMH 3258	AF466067	-	AF466028
C. curvispora	ICMP 18255	GU180636	-	-
C. cylindrospora	SMH 3568	AY017373	-	-
C. decastyla	SMH 2629	AF466068	-	AF466029
C. dilabens	CBS 712.88	AF178557	AF178557	-
C. ellisii	SMH3860	-	AY906944	-
C. fennica	CBS 101641	AF178562	AF178562	-
C. fuegiana	ICMP 15153	EF063574	-	-
C. fusiformis	CBS 101429	AF178554	AF178554	-
C. garethjonesii	MFLUCC 15-1012	KY212759	KY212751	KY212755
C. hebetiseta	SMH 2729	AF466069	AY906955	AF466030
C. inaequalis	MR 1450	AF178564	AF178564	-
C. innumera	SMH 2748	AY017375	AY906956	AF466018
C. jonesii	MFLUCC 15-1015	KY212761	KY212753	KY212757
C. lapaziana	SMH 3043	-	AY906947	-
C. lateriphiala	SMH 3320	AF466072	-	AF466033
C. lentomita	MR 1265	AF178548	AF178548	-
C. lignomollis	SMH 3015	AF466073	EU037896	AF466034
C. longiseta	SMH 1725	AF279416	-	AF466035
C. luquillensis	SMH 2973	AF466074	-	AF466037
C. metallicans	PDD 92539	EU037899	EU037893	-
C. minuta	SMH3396	AF466075	-	AF466038
C. myriocarpa	MUCL 34784	AF466076	-	AF466039
C. panamensis	SMH 3596	-	AY906948	-
C. panamensis	MFLUCC 15-1011	KY212760	KY212752	KY212756
C. preussii	CBS 262.76	AF178561	AF178561	-
C. pygmaea	UPSC 2523	AF466077	-	AF466040
C. raciborski	SMH 2017	AF466078	AY906949	AF466041
C. rivularia	CBS 127686	KR347357	KR347356	-
C. sylvatica	SMH 2893	AF279419	-	AF466043

Taxon	Collection/Isolate no.	GenBank accession no.		
		LSU	ITS	TUB
C. talbotii	EXP0560F	-	DQ914666	-
C. tropicalis	SMH 1267	AF279418	-	AF466044
C. vermicularioides	MR 1148	AF178550	AF178550	-
Chloridium vermicularioides = "Melanopsammella vermicularioides"	FC404	AF466087	-	AF466052
Chloridium gonytrichii *= "Melanopsammella gonytrichii"*	SMH 3785	AF466085	-	AF466051
C. lignicola	CBS 143.54	AF178544	AF178544	-
Codinaea acaciae	CPC 249122 = CBS 139907	-	KR476732	-
Codinaeopsis gonytrichoides	CBS 593.93	-	AF178556	-
Dendrophoma cytisporoides	CBS 223.95	JQ889289	JQ889273	-
Dictyochaeta fertilis	CBS 624.77	-	AF178540	-
D. simplex	ICMP 14613	-	EF029193	-
Dinemasporium pseudoindicum	CBS 127402	JQ889293	JQ889277	-
D. pseudostrigosum	CBS 717.85	JQ889294	JQ889278	-
D. pseudostrigosum	CBS 825.91	JQ889295	JQ889279	-
D. strigosum	CBS 520.78	JQ889298	JQ889282	-
Exserticlava vasiformis	TAMA 450	AB753846	-	-
Gelasinospora tetrasperma	CBS 178.33 = AFTOL-ID 1287	DQ470980	AY681178	AY681212
Infundibulomyces cupulata	BCC11929	EF113979	EF113976	-
Lecythothecium duriligni	CBS 101317	AF261071	-	-
Menispora ciliata	CBS 122132	-	EU488737	-
M. manitobaensis	-	-	EU488738	-
M. pulviscula = "Zignöella pulviscula"	MUCL 15710	AF466090	-	AF466059
M. pulviscula = "Zignöella pulviscula"	SMH 3289	AF466091	-	AF466058
M. pulviscula = "*Zignöella pulviscula"*	MR 1120	-	AF178543	-
M. tortuosa	AFTOL-ID 278 = DAOM 231154	AY544682	KT225527	-
M. tortuosa	CBS 214.56	-	AF178558	-
Neopseudolachnella acutispora	HHUF 29727	AB934041	AB934065	-
N. magnispora	HHUF 29977 = MAFF 244359	AB934042	AB934066	-
N. uniseptata	HHUF 29728 = MAFF 244360	AB934043	AB934067	-
Pseudolachnea fraxini	HHUF 28762	AB934045	AB934069	-
P. fraxini	CBS 113701	JQ889301	JQ889287	-
P. hispidula	HHUF 30118	AB934048	AB934072	-
Pyrigemmula aurantiaca	CPC 18063 = CBS 126743	-	HM241692	-
Rattania setulifera	HM171322	HM171322	GU191794	-
Sordaria fimicola	CBS 508.50	AY681160	AY681188	AY681228
Sporoschisma aotearoae = "Melanochaeta aotearoae"	SMH 3551	AF466082	-	AF466048
S. hemipsila = "*Melanochaeta hemipsila"*	SMH2125	AY346292	-	AF466049

Taxon	Collection/Isolate no.	GenBank accession no.		
		LSU	ITS	TUB
Striatosphaeria codinaeophora	SMH 1524	AF466088	-	AF466055
Tainosphaeria crassiparies	SMH 1934	AF466089	-	AF466056
T. siamensis	MFLUCC 15-0607	KY212758	KY212750	-
T. siamensis	MFLU 16-2644	KT167896	KT167897	-
Thozetella acerosa	BP 5970	-	AY330996	-
T. boonjiensis	BP 2383	-	AY330995	-
T. boonjiensis	BRIP 29318 = BP 2334	-	AY330994	-
T. cristata	LAMIC0153/13	-	KJ183032	-
T. fabacearum	MFLUCC 15-1020	KY212762	KY212754	-
T. falcata	BP F715	-	AY331003	-
T. gigantea	BP F712	-	AY331002	-
T. gigantea	BRIP 29202 = BP F709	-	AY331001	-
T. havanensis	HER3-4	-	JQ717009	-
T. havanensis	ICMP:14173	-	EF029184	-
T. nivea	-	EU825200	EU825201	-
T. pinicola	RJ-2008	EU825195	EU825197	EU825199
T. queenslandica	BRIP 29164 = BP F415	-	AY330997	-
T. queenslandica	BP F612	-	AY330998	-
Umbrinosphaeria caesariata	CBS 102664	AF261069	-	-

•Ex-type strains are in bold.

Results

Phylogenetic analyses

Phylogenetic analysis of combined LSU, ITS and TUB sequence data was carried out to clarify the phylogenetic placement of our strains within *Chaetosphaeriaceae* (Table 2). The combined LSU, ITS and TUB dataset consisted of 99 isolates representing 86 species including two outgroup taxa. The aligned dataset comprised 2236 characters (LSU: 1–799, ITS: 800–1303 and TUB: 1304–2236) including gaps. The Bayesian inference yielded trees with similar topologies to support the same terminal clades as obtained from maximum likelihood analysis. The ML trees generated from RAxML are illustrated in Fig 1.

In the phylogenetic analysis, our isolate MFLU 16-2644 clustered together with the ex-type strain of *Tainosphaeria siamensis*, and formed a sister group to *T. crassiparies*, which is the generic type. *Tainosphaeria*, which is basal to *Thozetella*, is phylogenetically related to *Thozetella* in the phylogenetic tree (Fig 1). The new isolate *Thozetella fabacearum* clusters within the genus and is closely related to *T. falcata*, *T. cristata* and *T. havanensis* in the phylogenetic tree. New species *Chaetosphaeria garethjonesii* shows a greater phylogenetic affinity to *C. albida* and *C. bombycina* (Fig 1). Our newly collected strain MFLUCC 15-1011 clustered with the ex-type strain of *Chaetosphaeria panamensis* (SMH3596) (100% ML/PP). *Chaetosphaeria jonesii* (MFLUCC 15-1015) clustered with the *C. tropicalis* (SMH1267), being close to *C. lateriphiala* F.A. and *C. sylvatica* (Fig 1).

Fig. 1 – Phylogram generated from maximum likelihood analysis of combined LSU, ITS and TUB sequence data from species of *Chaetosphaeriaceae*. Maximum likelihood bootstrap support values greater than 50% and Bayesian posterior probabilities (PP) above 90% are shown near the nodes. The new isolates are in blue and ex-type strains in bold. The tree is rooted with *Gelasinospora tetrasperma* and *Sordaria fimicola.*

Taxonomy

Chaetosphaeria panamensis Huhndorf & F.A. Fernández Figs 2, 3
FOF 02657

Saprobic on dead wood. Sexual morph – *Ascomata* 180–230 µm diameter, 195–285 µm high, scattered, sparse, superficial, not collapsing when dry, reddish-brown to dark brown, setose, rough-walled, papillate. *Setae* scattered over entire ascomata, dark brown, stiff, pointed, 60–85 µm long. *Peridium* 28–45 µm thick, with cells of *textura globosa* in surface view, 2-layerd in longitudinal section, inner layer 6–10 cells thick, comprising small, brown to dark brown, elongate to polygonal cells, with setae arising from inner layer, outer layer 4–6 cells thick, composed of large isodiametric to polygonal, brown to dark brown cells. *Paraphyses* 4–4.8 µm wide at the base, tapering towards the apex, numerous, septate. *Asci* 120–140×10–11.3 ($\overline{x}$ = 130.8×10.6, n = 10) µm, 8-spored, unitunicate, arising from the basal hymenium, cylindrical, rounded at the apex, with a J-, apical ring. *Ascospores* 60–73×3–4.1 ($\overline{x}$ = 68.2×3.5, n = 20) µm, filiform, loosely fasciculate, straight or slightly curved, 7-septate, without constrictions at the septum, rounded at both ends, apical end broader than the basal end, hyaline, smooth walled. Asexual morph from the culture – colonies on MEA cream coloured to light brown. *Conidiophores* macronematous, mononematous, flexuous, septate, branched, smooth, pale to moderately brown. *Conidiogenous cells* 4.3–5.3×1.5–2.1 µm, phialidic, flask-shaped to clavate, arising on septate, brown conidiophores, hyaline to light brown, guttulate, terminating with a flared conspicuous, cup-shaped collarette. *Conidia* 14.6–17×10–14.8 ($\overline{x}$ = 15.6×12.3, n = 20) µm, enteroblastic, hyaline, 1-celled, globose to subglobose or ellipsoid, guttulate. *Aleuriospore-like cells* absent.

Culture characters – Twenty-one day old colonies on MEA, 55–58 mm diameter, mostly immersed, with floccose superficial hyphae, margins effuse, light brown from above, reverse dark brown, producing asexual morph after 60 days of incubation in the dark at 28 °C.

Material examined – THAILAND, Chiang Mai Province, garden of Mushroom Research Center, on decorticated twig of *Pinus* (*Pinaceae* Spreng. ex F.Rudolphi), 12 July 2015, R.H. Perera, RHP 116 (MFLU 16-1014), living culture, MFLUCC 15-1011.

Notes – Our newly collected strain (MFLUCC 15-1011) clustered with the ex-type strain of *Chaetosphaeria panamensis* (SMH 3596), collected from wood fragment in Panama. The ascomata, size of asci, ascospores and asexual morph of our strain is typical of *C. panamensis* in the shape and size (Fernández & Huhndorf 2005) and the ITS molecular data is identical to SMH3596, which is the only gene available for the ex-type. Here, we provide additional LSU, ITS and *TUB* sequence data for the species. This is the first record of *C. panamensis* for Thailand.

Chaetosphaeria garethjonesii R.H. Perera, Maharachch. & K.D. Hyde, **sp. nov.** Fig. 4
Index Fungorum IF552536
FOF 02658

Etymology – In honor of E.B.G. Jones for his work on marine fungi.

Saprobic on a *Fabaceae* seed pod. Sexual morph – *Ascomata* brown to dark brown, not collapsing when dry, ovoid to broadly obpyriform, 191–250 µm diameter, 240–257 µm high, superficial, scattered to gregarious, setose, slightly papillate. *Setae* scattered over entire ascomata, dark brown, stiff, pointed, 38–47 µm. *Peridium* cells 21–59 µm thick, with cells *textura globosa* in surface view, 3-layered in longitudinal section, with inner layer 2–3 cells thick, composed of light brown to hyaline elongate cells, with middle layer 4–6 cells thick, composed of small, brown to

Fig. 2 – *Chaetosphaeria panamensis* (MFLU 16-1014 a, b). Appearance of ascomata on host substrate. c. Section of ascoma. d. Paraphyses. h, i. Asci. j. Ascospores. k. Germinating ascospore. m, n. Colony on MEA (above and below). Bars – c = 100 µm, d, e = 50 µm, f–l = 20 µm.

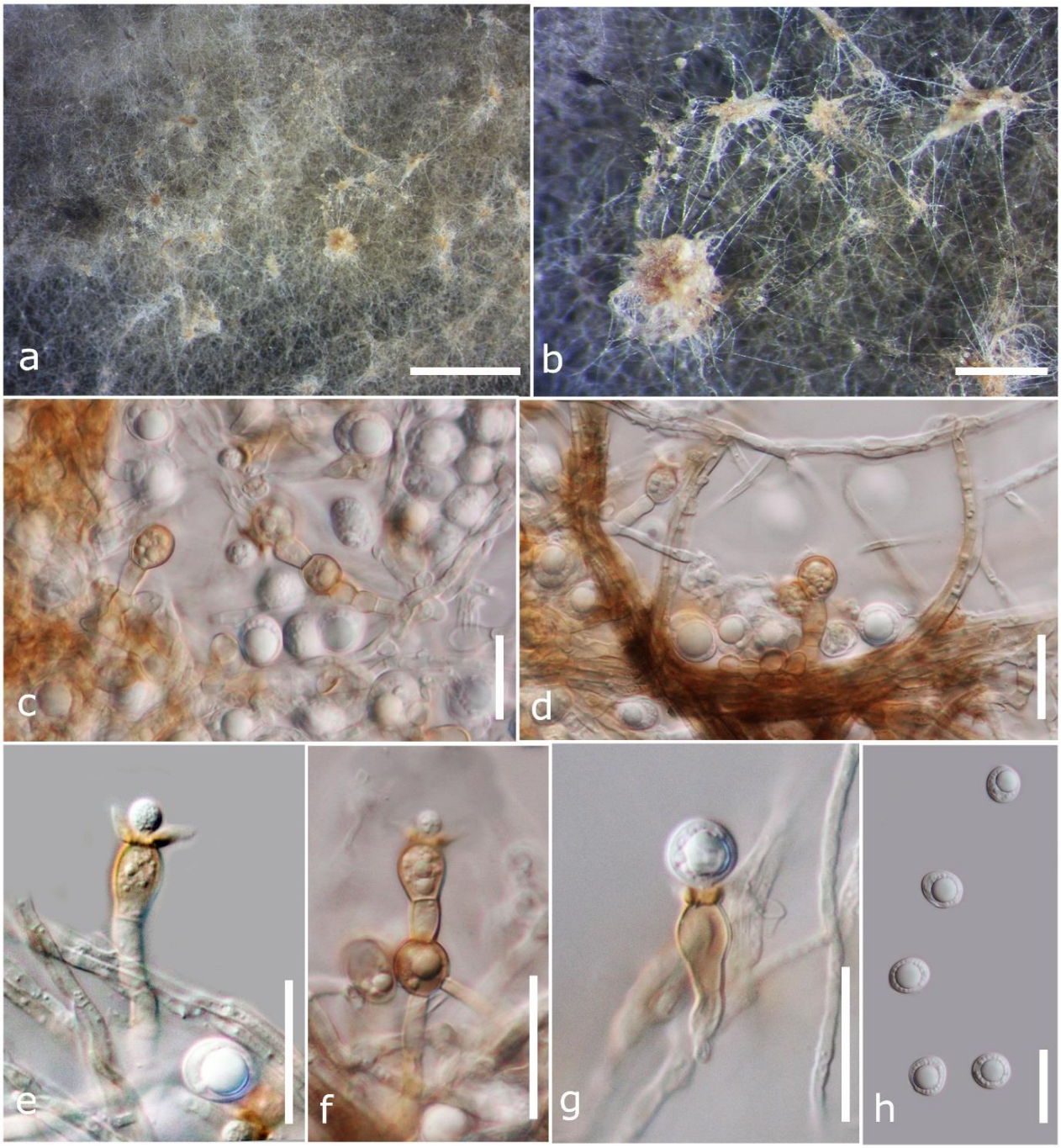

Fig. 3 – *Chaetosphaeria panamensis.* asexual morph (MFLUCC 15-1011 a, b) Sporulation in MEA. c–g. Conidiophores with conidia. h. Conidia. Bars – a = 2 mm, b = 500 µm, c–h = 20 µm.

dark brown, elongate to polygonal cells, with outer layer 2–3 cells thick, composed of large isodiametric to polygonal, brown cells. *Paraphyses* 5.6–8.6 *µm* wide, of variable length, simple, sparse, hyaline, unbranched, tapering towards the apex, septate. *Asci* 120–152×10.7–13.3 ($\overline{x}$ =137×12 µm, n = 20) µm, 8-spored, unitunicate, cylindrical, pedicellate, with rounded apex, with a thin, J-, apical ring. *Ascospores* 63.3–75×2.3–3.7 ($\overline{x}$ = 69.5×3.1 µm, n = 30) µm, fasciculate, straight to gently curved, with rounded ends, slightly tapering to base, 7-septate, sometimes slightly constricted at septa, hyaline, smooth-walled, without a gelatinous sheath. Asexual morph **–** Undetermined.

Culture characters – Twenty-one day old colonies on MEA 60–76 mm diameter, flat, lacking aerial mycelium, margins effuse, dark brown at margins, gray to white at the center from above, reverse dark brown.

Material examined – THAILAND, Chiang Mai Province, on a *Fabaceae* seed pod, 19 July 2015, R.H. Perera, RHP 115 (MFLU 16-1019, holotype), ex-type living culture, MFLUCC 15-1012.

Notes – *Chaetosphaeria garethjonesii* is a distinct species in the genus as supported by molecular and morphological characters. In the phylogenetic tree, *C. garethjonesii* is sister to *C. albida* T.J. Atk. et al. and *C. bombycina* T.J. Atk. et al. (Fig 1). *Chaetosphaeria garethjonesii* differs from *C. albida* and *C. bombycina* in ascomatal morphology. *Chaetosphaeria albida* and *C. bombycina* are characterized by almost white or light fawn-grey, translucent or reflective ascomatal with walls lacking setae, while *C. garethjonesii* has brown to dark brown ascomata with setose, rough walls (Atkinson et al. 2007). Ascospore septation in *C. albida* is also less than that in *C. bombycina* (7 vs. 11; Atkinson et al. 2007).

Chaetosphaeria jonesii R.H. Perera, Maharachch. & K.D. Hyde, **sp. nov.** Fig. 5

Index Fungorum IF552574

FOF 02659

Etymology – In honour of E.B.G. Jones for his work on tropical mycology.

Saprobic on wood. Sexual morph **–** *Ascomata* 102–250 µm diameter, 142–263 µm high, superficial, arranged in clusters, subglobose to globose, dark brown, surface rough, ostiolate, surrounded by setae. *Ostiole* periphysate. *Setae* brown, multi-septate, sinuous, cylindrical, with a rounded apex, arising from base of ascomata and abundant on the substrate. *Peridium* composed of dark brown cells of *textura angularis* in surface view, 11–16 µm thick in longitudinal section, 2 layered, inner layer 3–5 cells thick, composed of hyaline elongate cells of *textura angularis*, with outer layer 4–7 cells thick, composed of brown to dark brown, globose to polygonal cells. *Paraphyses* 3.9–4.6 µm wide, sparse, simple, septate. *Asci* 69–90×8.5–11 ($\bar{x}$ = 80×10 µm, n = 20) µm, 8-spored, unitunicate, cylindrical-clavate, short-pedicellate, with a thin, J-, apical ring. *Ascospores* 16.2–17.7×2.8–3.6 ($\bar{x}$ = 19.5×3.6 µm, n = 20) µm, overlapping biseriate, hyaline to pale brown, fusiform, 3-septate, curved, smooth-walled. Asexual morph – Undetermined.

Material examined – THAILAND, Chiang Mai, on decorticated wood, 5 August 2015, S. Boonmee, RHP 121 (MFLU 16-1020, holotype), ex-type living culture, MFLUCC 15-1015.

Notes – Maximum likelihood and Bayesian analyses of combined LSU, ITS and *TUB* sequence data shows, *Chaetosphaeria jonesii* (MFLUCC 15-1015) clustered with the ex-type strain of *C. tropicalis* F.A. Fernández & Huhndorf (SMH 1267) which was earlier collected from Puerto Rico, on a wood fragment. We observed that the asci (69–90×8.5–11 vs. 100–138×10–12.5 µm; Fernández & Huhndorf 2005) and ascospores (16.2–17.7×2.8–3.6 vs. 19–26×3.2–6.3 µm; Fernández & Huhndorf 2005) of our strain are smaller than *C. tropicalis. Chaetosphaeria jonesii* is also phylogenetically and morphologically close to *C. lateriphiala* F.A. Fernández & Huhndorf and *C. sylvatica* F.A. Fernández & Huhndorf (Fig 1). However, the ascomata (102–250×142–263 µm), asci (69–90×8.5–11 µm) and ascospores (16.2–17.7×2.8–3.6 µm) of *C. jonesii* are smaller than those of *C. lateriphiala* (ascomata: 200–248×234–307 µm, asci: 95–113×10–12.5 µm, ascospores: 18–24×4.5–6 µm; Fernández & Huhndorf 2005). *Chaetosphaeria jonesii* also can be distinguished from *C. sylvatica* by its smaller ascomatal size (102–250×142–263 µm vs. 264–302×269–292 µm; Fernández & Huhndorf 2005), smaller asci (69–90 µm vs. 95–115 µm; Fernández & Huhndorf

Fig 4 – *Chaetosphaeria garethjonesii* (MFLU 16-1019, holotype a–c). Appearance of ascomata on host substrate. d. Section of ascoma. e. Peridium with ascomatal setae. f. Peridium. g. Paraphyses. h, i. Asci. j, k. Ascospores. l. Germinating ascospore. m, n. Colony on MEA (above and below views). Bars – b = 500 µm, d = 100 µm, e = 50 µm, f–i = 20 µm, j, k = 50 µm, l = 20 µm.

2005) and thinner ascospores (2.8–3.6 vs. 4–5.5; Fernández & Huhndorf 2005). By taking into account the morphological and molecular data we introduce *Chaetosphaeria jonesii* as a new species.

Tainosphaeria siamensis J. Yang, K.D. Hyde & J.K. Liu Fig. 6

Saprobic on seeds and wood. Sexual morph – Undetermined. Asexual morph – *Colonies* on natural substrate, effuse, superficial, hairy, dark brown, in groups. *Conidiophores* mononematous, macronematous, subcylindrical, flexuous or straight, septate, unbranched, brown, becoming light brown towards the apex, smooth-walled, tapering to a terminal single phialide, 5–6.4 µm at the base. *Conidiogenous cells* phialidic cylindrical to clavate, light brown, smooth walled, with a periclinal thickened, flared, collarette. *Collarette* funnel-shaped, 4–6.2 µm wide at the opening, hyaline. *Conidia* 17.5–20.5×3.2–3.4 (12.5×1.8) µm, enteroblastic, naviculate to fusiform or cylindrical, equilateral, hyaline, both ends obtuse to subobtusely rounded, sometimes basal end truncate, with single setula at both ends, 7.8–9.1 µm long.

Culture characters – Cultures were not obtained.

Material examined – THAILAND, Chiang Mai Province, on a *Fabaceae* seed pod, 20 December 2015, R.H. Perera, DeloL 8 (MFLU 16-2644).

Notes – DNA was extracted directly from the colonies growing on the natural substrate. Phylogenetic analysis of combined LSU, ITS and *TUB* sequence data confirms our strain clusters with the ex-type strain of *Tainosphaeria siamensis* which was isolated from Thailand (Liu et al. 2016). It is similar to the ex-type strain of *T. siamensis* in conidiophore morphology, conidial size and shape. *Tainosphaeria siamensis* resembles the asexual morph of *T. crassiparies*, in terms of similar conidiophore morphology and conidial appendages. It can be distinguished from the latter by equilateral (vs. inequilateral) larger conidia (vs. 10.5–14.8×2–3 µm) and conidiogenesis (phialidic vs. percurrent) (Fernández & Huhndorf 2005, Liu et al. 2016).

Thozetella fabacearum R.H. Perera & K.D. Hyde, **sp. nov.** Figs 7, 8

Index Fungorum IF552537

FOF 02660

Etymology – Referring to the host family, *Fabaceae.*

Saprobic on a *Fabaceae* seed pods. Sexual morph – Undetermined. Asexual morph from the natural substrate – *Colonies* effuse, superficial, sporodochial, white. *Sporodochia* cylindrical or subulate, scattered, sessile, superficial, of cream white mass. *Microawns* 33–60 µm long, 3–5.3 µm wide, visible as small hairs in mass on the natural substrate, aseptate, smooth-walled, thick-walled, hyaline, variously-shaped, sigmoid or sickle-shaped, sometimes L-shaped. *Conidia* 13.2–17.2×1.9–2.4 ($\bar{x}$ = 15.5×2.1 µm, n = 30) µm, falcate, inequilateral, with rounded apical end, truncate at basal end, hyaline, smooth-walled, with a single filiform setula at each end, 3.6–9 µm long. Asexual morph from the culture – *Conidiomata* funnel-shaped, synnematous, abundant, elongated, brown to dark brown, with hyaline to ash apices, branched. *Conidiophores* macronematous, brown, cylindrical, septate, branched, densely compacted along the synnemata axis, smooth-walled. *Conidiogenous* cells 1.7–2.8 µm wide, phialidic, hyaline, smooth-walled, thick-walled. *Microawns* 32.8–56.4 µm long, 1.7–2.7 µm wide, aseptate, smooth-walled, hyaline, variously shaped, predominantly L-shaped, sometimes sigmoid or sickle-shaped. *Conidia* 11.6–14.7×2.2–3.4 ($\bar{x}$ = 13.3×2.6 µm, n = 20) µm, naviculate to fusiform or ellipsoid, inequilateral, with rounded apical end, truncate at basal end, hyaline, smooth-walled, with a single filiform setula at each end, 2.1–3.4 µm long.

Culture characters – 21 day old colonies on MEA 35 mm, margins effuse, gray to dark-brown, flat, lacking aerial mycelium, producing synnemata after 150 days of incubation in the dark at 28 °C, reverse dark-brown to black.

Fig. 5 – *Chaetosphaeria jonesii* (MFLU 16-1020, holotype) a. Herbarium specimen. b, c. Appearance of ascomata on host substrate. d. Section of ascoma. e. Peridium. f. Peridium in surface view. g. Setae. h. Paraphyses. i, j. Immature and mature asci. k. Close up of apical asci in Melzer's reagent. l. Ascospores. m. Germinating ascospore. Bars – b, c = 2 mm, d = 100 µm, e = 50 µm, f–o = 20 µm.

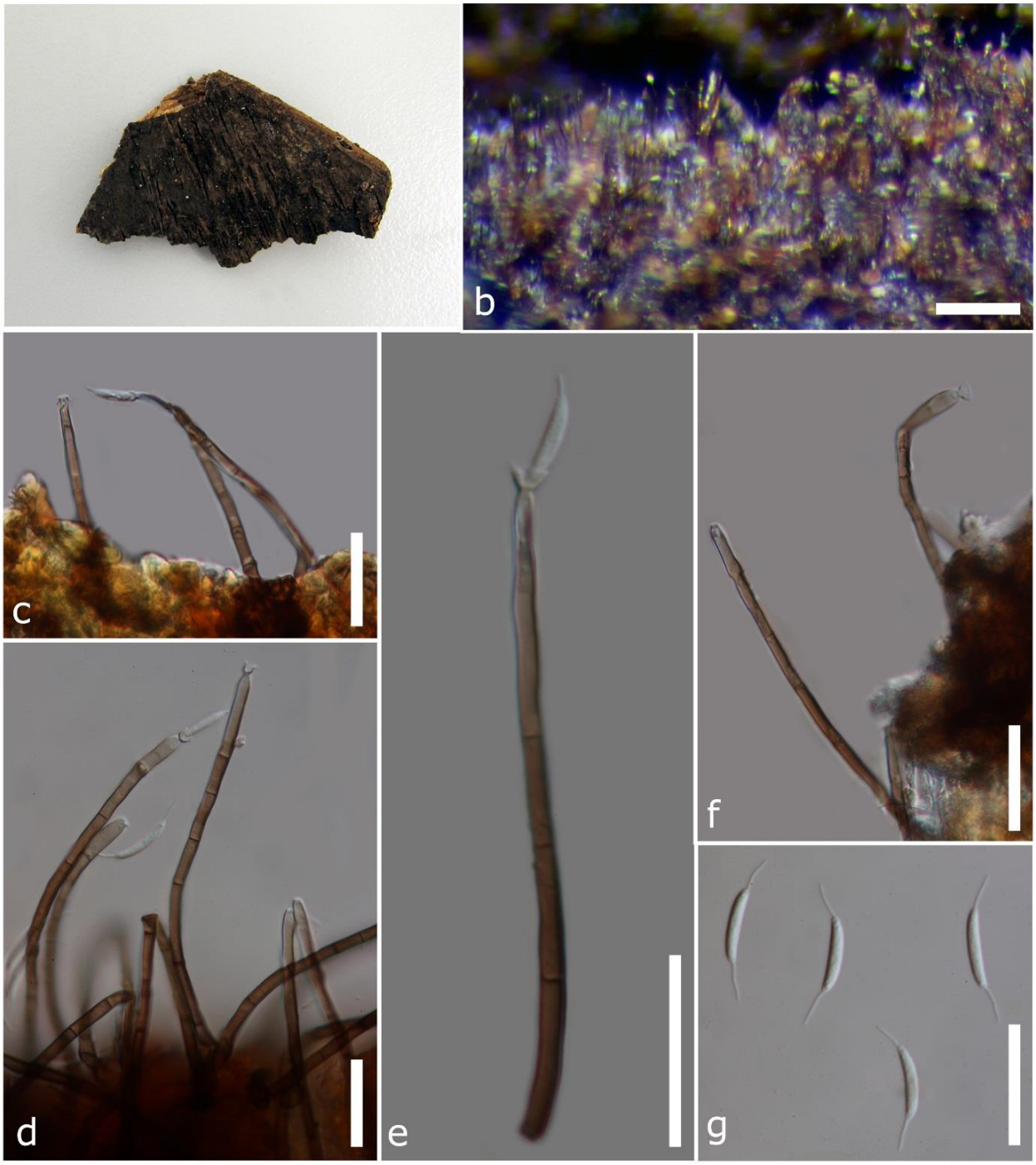

Fig. 6 – *Tainosphaeria siamensis* **(**MFLU 16-2644 a). Herbarium specimen. b. Conidiophores on host substrate. c–e. Conidiophore with developing conidium. f. Apical phialides. g. Conidia. Bars – b = 50 µm, c–g = 20 µm.

Material examined – THAILAND, Phang-nga Province, Mueang Phang-nga District, Tham Nam Phut, on a *Fabaceae* seed pod, 7 December 2014, M. Dayarathne RHP 126 (MFLU 16-1021, holotype), ex-type living culture, MFLUCC 15-1020.

Notes – In this paper, we introduce *Thozetella fabacearum* as a new species which was collected from Phang-nga Province, Thailand. Maximum likelihood and Bayesian analyses of combined LSU, ITS and *TUB* sequence data (Fig 1) showed that *T. fabacearum* is closest to *T. falcata* B.C. Paulus et al. and *T. cristata* Piroz. & Hodges with moderate support. *Thozetella falcata*

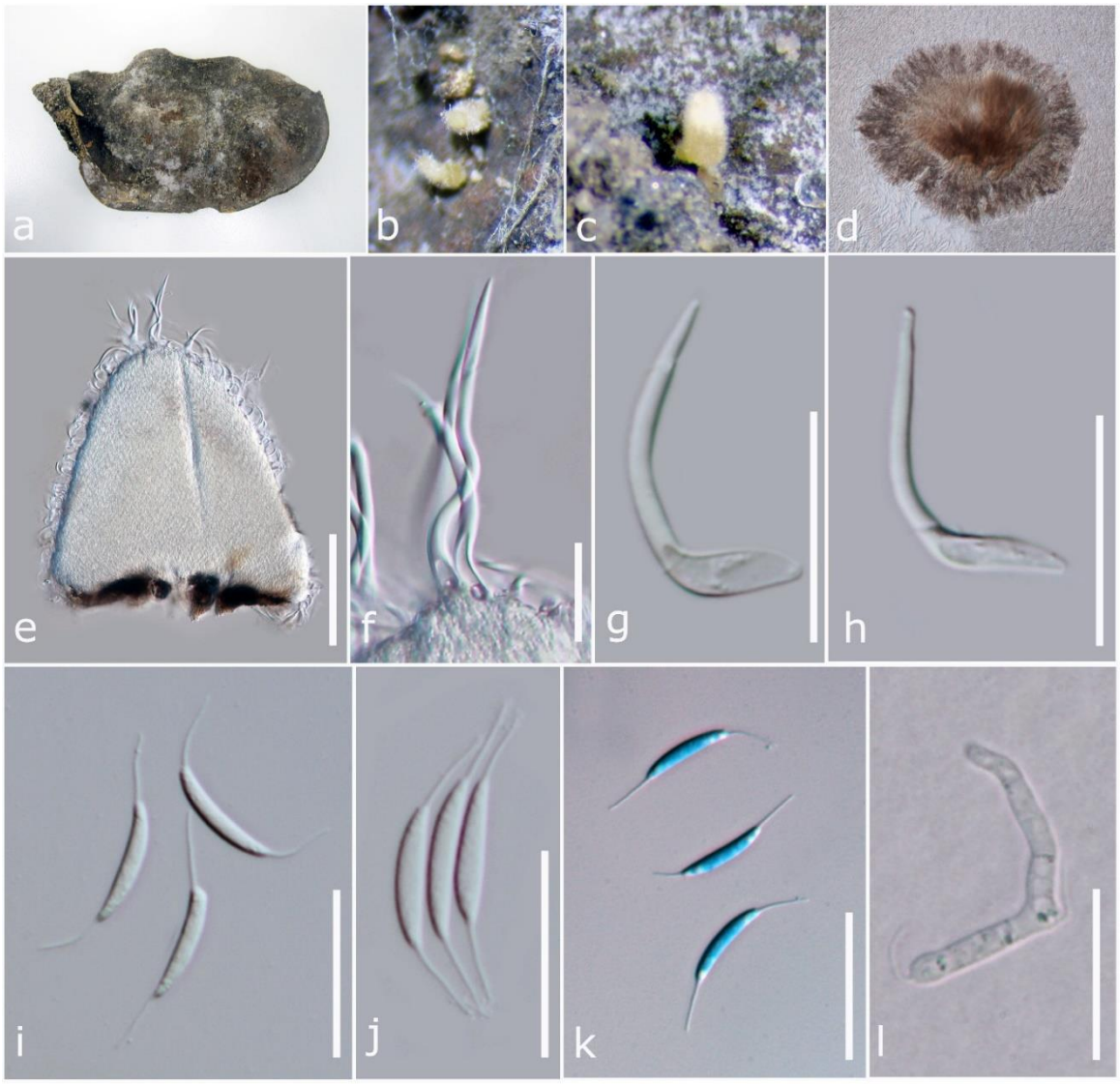

Fig. 7 – *Thozetella fabacearum* (MFLU 16-1021, holotype) a. Herbarium specimen. b, c. Appearance of conidiomata on host substrate. d. Synnemata, circular in shape. e. Section through synnemata. f–h. Microawns. i–k. Conidia (k in Cresol blue). l. Germinating conidium. Bars – d = 100 µm, e–k = 20 µm, l = 10 µm.

only has ITS sequence data for comparison. Therefore, it is not well-separated from *Thozetella fabacearum* in the phylogeny. However, *T. fabacearum* can easily be distinguished from *T. falcata* by the conidiomatal morphology and shorter microawns (33–60 vs. 40–95 µm) (Paulus et al. 2004). *Thozetella fabacearum* produce sporodochia on the natural substrate and synnemata in the culture, while *T. falcata* and *T. cristata* produce synnemata on the natural substrate. *Thozetella tocklaiensis* (Agnihothr.) Piroz. & Hodges is the only *Thozetella* species producing sporodochia on the natural substrate and synnemata in culture (Barbosa et al. 2011, Paulus et al. 2004). However, *T. fabacearum* differs from *T. tocklaiensis* in having larger conidia (13.2–17.2×1.9–2.4 vs. 9–13×1.5–3 µm) and smaller microawns (33–60×3–5.3 vs. 18–38×1.5 µm) (Agnihothrudu 1958, Pirozynski & Hodges CS Jr 1973). Although, the microawns size in *T. fabacearum* and *T. cristata* overlap, *T. fabacearum* differs in having larger conidia (13.2–17.2×1.9–2.4 µm as compared to 11.5–14.5×2.3–2.7 µm in *T. cristata*) (Barbosa et al. 2011).

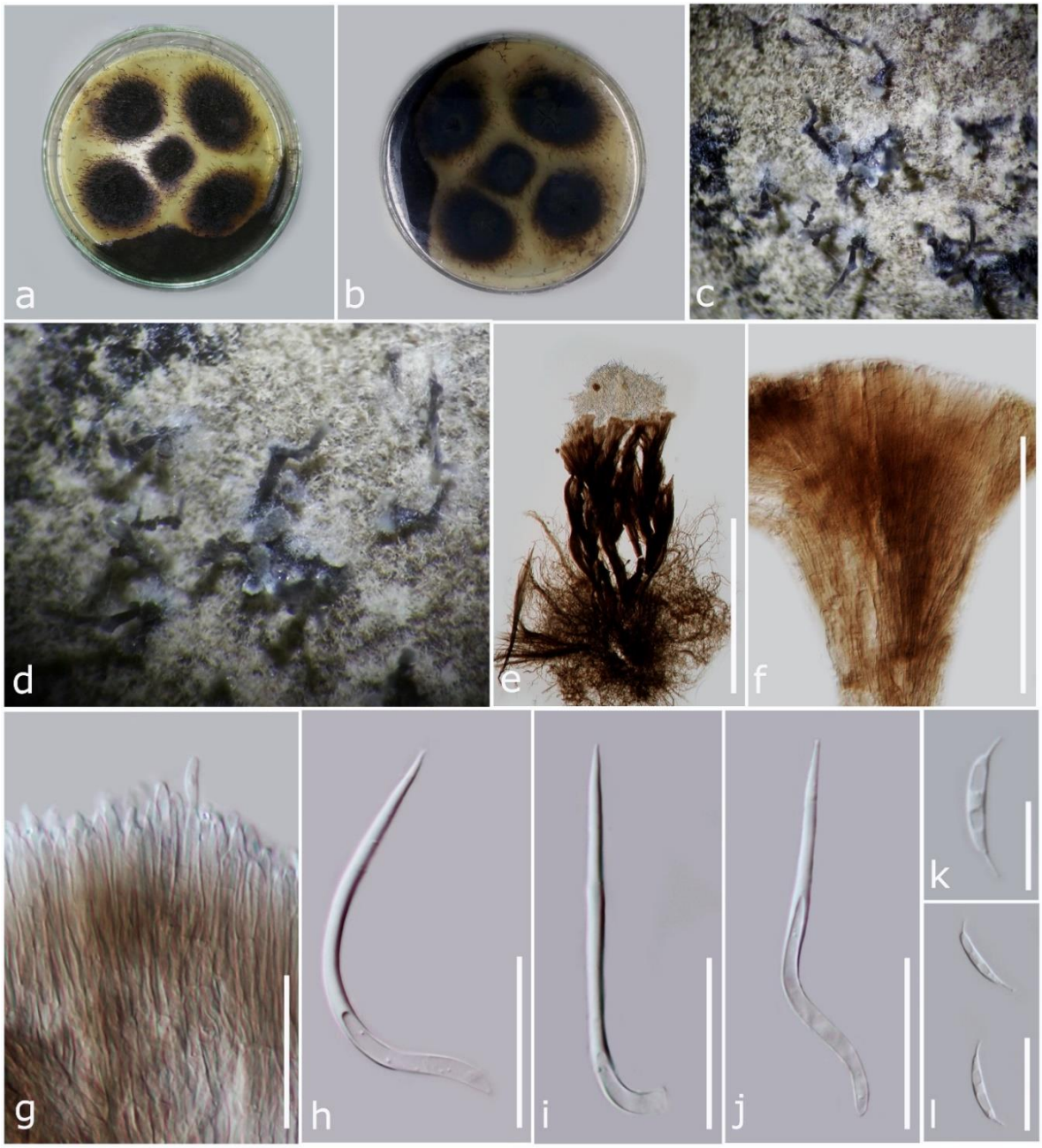

Fig. 8 – *Thozetella fabacearum* in culture (MFLUCC 15-1020) ex-type culture a, b. Sporulation on MEA. c, d. Appearance of conidiomata on MEA. e. Synnemata. f. Closely packed conidiophores. g. Conidiogenous cells. h–j. Microawns. k, l. Conidia. Bars – e = 500 µm, f = 100 µm, g–j = 10 µm, k = 10 µm, l = 20 µm.

Discussion

Fabaceae (legume family) is the third largest family of flowering plants which is diverse and cosmopolitan (Gepts et al. 2005). They are economically important as legume crops, medicinal plants and fodder (Gepts et al. 2005, Gao et al. 2010). *Fabaceae* members are associated with rhizobial symbionts and play important role in the environment by fixing nitrogen (Gepts et al. 2005). The fruit pod is composed of a single seed-bearing carpel that splits open along two seams. Legume pods contain cellulose, hemicelluloses and lignin which can also provide substrates for fungal growth (Paula et al. 2011). Two new taxa *Tainosphaeria crassiparies* and *Cirrenalia nigrospora* were

described from seed pods of *Hymenaea* and *Delonix regia* respectively (Somrithipol et al. 2002, Fernández & Huhndorf 2005). Moreover, a number of chaetosphaeriaceous taxa (ie. *Chloridium macrocladum* (=*Gonytrichum macrocladum*), *Chloridium* sp., *Dictyochaeta* sp., *Sporoschisma nigroseptatum, S. saccardoi, S. hemipsila* and *Thozetella nivea*) have been isolated from *Fabaceae* seed pods in Thailand (Somrithipol et al. 2002).

The family *Chaetosphaeriaceae* M.V. Locq. was proposed without any description by Locquin (1984) to accommodate five genera including *Chaetosphaeria, Zignöella* Sacc, *Niesslia* Auersw., *Rhagadostoma* Körb, and *Loramyces* W. Weston. This was not considered as a validly published family (Hawksworth & David 1989 – Art. 36.1, Grueter et al. 1994). Réblová et al. (1999) therefore re-introduced the family based on *Chaetosphaeria* Tul. & C. Tul., to accommodate six other genera: *Ascocodinaea, Melanochaeta, Melanopsammella, Porosphaerella, Porosphaerellopsis* and *Striatosphaeria*. Réblová et al. (1999) maintained *Chaetosphaeriaceae* in Sordariales based on morphology. Huhndorf (2004) placed the family in Chaetosphaeriales, based on LSU sequence data. Subsequently more genera were added to the family and, currently, there are 38 accepted genera (Index Fungorum 2016, Liu et al. 2016, Maharachchikumbura et al. 2016). Maharachchikumbura et al. (2016) noted the taxonomic confusion of genera in the family and the importance of a monograph with molecular support for accepted genera.

In this study, we introduce one new species of *Thozetella*, two new species of *Chaetosphaeria*, and one new record of *Chaetosphaeria* for Thailand. Here we illustrate *Tainosphaeria siamensis*, which is the first record on *Fabaceae* seed pods. Fungal identifications are based on morphology and analysis of combined LSU, ITS and *TUB* sequence data. *Thozetella fabacearum* is distinct from other *Thozetella* species, since it produces sporodochia on natural substrates and synnemata *in vitro* on MEA. This has only been observed in *Thozetella tocklaiensis* (Agnihothr.) Piroz. & Hodges (Paulus et al. 2004, Barbosa et al. 2011). *Chaetosphaeria* species seems to be polyphyletic within the family, and these finding are similar to those obtained by Jeewon et al. (2009) and Crous et al. (2012) using ITS and LSU sequence data. The generic type strain of *Chaetosphaeria* (*C. innumera*) lacks ex-type sequence data. Therefore, it is essential to re-collect, epitypify (*sensu* Ariyawansa et al. 2014) and sequence the types of genera to resolve the phylogenetic confusion.

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