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New soil-inhabiting Chaetosphaeriaceous records from Thailand

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Abstract

Soil fungi represent the most abundant and diverse taxonomic group on Earth. Tropical forest soil-based habitats contain both edaphic and climatic factors that boost fungal activities in soil. Despite having vital functions in terrestrial ecosystems, information on diversity, taxonomy, and ecological preferences of soil fungi on a global scale is lacking. This study focuses on fungal species inhabiting tropical forest soils in Krabi, Thailand. Fungal isolation was performed using the soil dilution plate method, and species delimitation was conducted via morphological characterization and phylogenetic analyses. *Chloridium gonytrichii* and *Kionochaeta microspora* are introduced herein as the soil-inhabiting records from Thailand. For each species, comprehensive descriptions and micrographs are provided.

Keywords – *Ascomycota* – *Chaetosphaeriaceae* – *Chloridium* – *Kionochaeta* – taxonomy

Introduction

Fungi are a heterogeneous group of organisms, representing a large and distinct component of microbial diversity (Naranjo-Ortiz & Gabaldon 2019, Maharachchikumbura et al. 2021). Most are cosmopolitan and feature wide geographical distribution across terrestrial and aquatic environments, including soil habitats (Coleine et al. 2018, Wu et al. 2019). They exhibit diverse lifestyles as biotrophs, endophytes, epiphytes, fungicolous, hemibiotrophs, and saprobes (Rodriguez & Redman 1997, Coleine et al. 2018, Wu et al. 2019). Global fungal diversity has been estimated to range between 2.8 to 3.8 million fungal species (Hawksworth & Lücking 2017). Recently, this has been updated by Baldrian et al. (2021), who, based on high-throughput sequencing, suggested that global fungal diversity could reach up to 6.28 million species. Nevertheless, only 1.08 million species are currently published, and it is clear that numerous species remain undescribed. One possible reason for the observed discrepancy is because fungi are cosmopolitan, featuring a wide geographical distribution across many countries on various substrates (Coleine et al. 2018, Tedersoo et al. 2020). For example, soil fungal diversity is not studied much in most Asian countries, such as Thailand (Amma et al. 2018).

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Chaetosphaeriaceae (*Chaetosphaeriales*) is a genera-rich family in *Sordariomycetes* (Réblová et al. 1999, Ho et al. 2002, Luo et al. 2019, Hyde et al. 2020). This family was proposed by Locquin (1984) but, was re-described by Réblová et al. (1999) and accommodated 20 genera. Subsequently, Maharachchikumbura et al. (2016) accepted 37 genera in *Chaetosphaeriaceae*, and currently, 43 genera are accepted in this family (Hyde et al. 2020). *Chaetosphaeriaceae* members have diverse lifestyles as endophytes, pathogens, and saprobes, but few have been recorded as fungicolous (Goh & Hyde 1996, 1998, Réblová et al. 1999, Ho et al. 2002, Maharachchikumbura et al. 2016, Sun et al. 2019, Lin et al. 2019). They commonly occur in terrestrial and aquatic environments, including soil-based substrates (Hyde et al. 2020). For instance, genera such as *Dictyochaeta*, *Menisporopsis*, and *Tainosphaeria* have been recorded from aquatic habitats. In contrast, *Adautomilanezia*, *Chaetosphaeria*, and *Thozetella* have been documented from terrestrial habitats (Hyde et al. 2020), and species in *Chetospheria*, *Chloridium*, and *Thozetella* have been recorded from soils (Domsch et al. 1993, Silva & Grandi 2013, Wu & Zhang 2013).

Link (1809) initiated *Chloridium* to accommodate *C. viride* and currently, 30 species epithets are listed for *Chloridium* (Species Fungorum 2021). *Chloridium* species are characterized by simple or proliferating, unbranched to rarely branched, dematiaceous, macronematous conidiophores (Luo et al. 2019) and represent a polyphyletic group within *Chaetosphaeriaceae* (Hyde et al. 2020). Kirk & Sutton (1985) introduced *Kionochaeta* in order to include *Kionochaeta ramifera* as the type species. Currently, 14 species are accepted in this genus (Species Fungorum 2021). Maharachchikumbura et al. (2015) placed this genus in *Chaetosphaeriaceae*, and its polyphyletic nature was discussed by Lin et al. (2019). *Kionochaeta* species have been reported on both freshwater and terrestrial habitats and are mainly saprobes on decaying leaves, seeds, and twigs (Kuthubutheen et al. 1988, Goh & Hyde 1997, Hyde & Hyde 2002, Lin et al. 2019, Hyde et al. 2020).

The objective of this study is to identify soil-inhabiting ascomycetes in tropical forests soils in Thailand. Based on morphological and multi-gene phylogenetic analyses, we report the first records of *Chloridium gonytrichii* and *Kionochaeta microspora* on soils collected from a forest in Krabi, Thailand.

Materials & methods

Samples collection, fungal isolation, and morphological characterization

Soil samples were collected from forests in Krabi Province (Southern Thailand), stored in zip-lock plastic bags, and transported to the laboratory. The fungal isolation was done using the soil dilution plate method, as described in Yasanthika et al. (2020). For morphological studies, sporulation of the fungal colonies was facilitated by alternating day and night conditions at 25 ℃. The asexual structures and mycelium were transferred from the sporulated cultures using a needle onto a glass slide containing a drop of distilled water. The fungal structures were observed in an OLYMPUS SZ61 compound microscope, and images were captured using a Canon EOS 600D digital camera mounted on a Nikon ECLIPSE 80i compound microscope. All measurements were made using the Tarosoft (R) Image Frame Work program. Photo-plates were made with Adobe Photoshop CS6 Extended version 13.0.1 (Adobe Systems, USA). Living cultures were deposited at Mae Fah Luang University Culture Collection (MFLUCC), Chiang Rai, Thailand, and dried culture specimens at Mae Fah Luang University Herbarium (Herb. MFLU).

DNA extraction, PCR amplification, and sequencing

Fungal cultures were grown on PDA for six weeks at 25 ℃, and total genomic DNA was extracted from 50 to 100 mg of axenic mycelium from the cultures. Mycelium was ground to a fine powder with liquid nitrogen, and fungal DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) (Hangzhou, P. R. China) as mentioned in the manufacturers' instructions. Polymerase chain reactions (PCR) were performed to amplify the internal transcribed spacer region of ribosomal DNA (ITS) and large subunit nuclear ribosomal DNA region (LSU)

using the ITS5/ITS4 (White et al. 1990) and LR0R/LR5 (Vilgalys & Hester 1990) pair of primers, respectively. Amplification reactions were performed in 25 µl of total reaction, which contained 9.5 µl of sterilized water, 12.5 µl of 2 × Power Taq PCR MasterMix (Bioteke Co., China), 1 µl of each primer, and 1 µl of DNA template. PCR thermal cycle program for ITS and LSU was used following Lin et al. (2019). The quality of PCR products was checked on 1% agarose gel electrophoresis stained with ethidium bromide. PCR products were purified and sequenced by Qingke Company, Kunming City, Yunnan Province, China. Nucleotide sequences were deposited in the GenBank database (Table 1).

Sequence alignment and phylogenetic analyses

Obtained sequences were checked using BioEdit v. 7.0.5.3 (Hall 1999) and subjected to a BLAST search against the NCBI non-redundant database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Additional sequence data, including that recently published by Lin et al. (2019) and Luo et al. (2019), were downloaded from the GenBank and used for comparisons (Table 1). Alignments were performed using MAFFT v. 7.036 (http://mafft.cbrc.jp/alignment/server/index.html, Katoh et al. 2002) using the default settings and edited when necessary, using BioEdit v. 7.0.5.2 (Hall 1999).

Phylogenetic analyses were conducted to selected genera in *Chaetosphaeriaceae* to identify the taxonomic placements of our strains. Maximum likelihood (ML) and Bayesian inference (BI) were used for phylogenetic analyses. ML estimation was conducted using RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) configurated to 1,000 replicates and the model of nucleotide substitution rates GTR+I+G. Bayesian analyses were conducted with MrBayes v. 3.1.2 (Ronquist et al. 2012) to evaluate Bayesian posterior probabilities (BYPP) by Markov Chain Monte Carlo sampling (MCMC), with six independent Markov chains runs for 2,000,000 generations. Trees were sampled at every 100th generation to obtain 20,000 trees. The first 25% trees representing the burn-in phase of the analyses were discarded. The remaining trees were used to calculate the BYPP in the majority-rule consensus tree, which was visualized with the FigTree v. 1.4.0 software (Rambaut 2010) and edited in Microsoft PowerPoint (2016).

Table 1 Isolates and sequence GenBank accession numbers used in this study (newly generated sequences are indicated in bold, ex-type strains are indicated with ^{T} after the strain number).

Species name	Strain no.	GenBank accession no.	
		LSU	ITS
Adautomilanezia caesalpiniae	HUEFS 216632^{T}	NG_058594	NR_153560
Brunneodinemasporium brasiliense	CBS 112007^{T}	NG_058655	NR_137785
Chaetosphaeria innumera	SMH2748	AY017375	AY906956
Chaetosphaeria raciborskii	SMH 2017	AF466078	--
Chaetosphaeria raciborskii	SMH3119	AY436402	AY906953
Chloridium aquaticum	MFLU 11-1133	MH476567	MH476570
Chloridium aquaticum	HKAS 96226	--	--
Chloridium botryoideum	CBS 131270	MH877338	--
Chloridium botryoideum var. *botryoideum*	CBS 259.76	MH878530	--
Chloridium chloroconium	FMR 11940	KY853495	KY853435
Chloridium gonytrichii	S-360	MK835821	MK828621
Chloridium gonytrichii	HKAS:93031	MK835820	MK828620
Chloridium gonytrichii	HKAS:93053	MK835822	MK828622
Chloridium gonytrichii	SMH 3785	AF466085	--
Chloridium gonytrichii	**MFLUCC 21-0110**	**MZ771258**	**MZ771198**
Chloridium gonytrichii	MFLUCC 11-0216	MH476568	NR_158365
Chloridium gonytrichii	HGUP1805	MK372067	MK372069
Chloridium iniqualis	MR 1450	AF178564	AF178564
Chloridium lignicola	CBS 143.54	MH868806	MH857273
Chloridium pini	CPC 36627^{T}	NG_073871	NR_170050

Table 1 Continued.

Species name	Strain no.	GenBank accession no.	
		LSU	ITS
Chloridium salinicola	MFLU 19-1238	MN017890	MN047125
Chloridium sp.	HGUP1806	MK372068	MK372070
Chloridium submersum	MFLUCC 16-1344 [T]	NG_073788	NR_171867
Chloridium virescens	NRRL 37636	--	GU183124
Chloridium virescens var. *caudigerum*	CBS 152.53	MH868678	MH857142
Chloridium virescens var. *chlamydosporum*	CBS 345.67	MH870689	MH858992
Chloridium virescens var. *virescens*	CBS 239.75B	MH878291	--
Conicomyces pseudotransvaalensis	GS20	LC001708	LC001710
Cryptophiale udagawae	MFLU 18-1497	MH758211	MH758198
Cryptophiale udagawae	MFLU 18-1498	MH758210	MH758197
Cryptophialoidea fasciculata	MFLUCC 17-2119	MH758208	MH758195
Dendrophoma cytisporoides	CBS 223.95	JQ889289	JQ889273
Dictyochaeta simplex	CBS 966.69	AF178559	AF178559
Dictyochaeta simplex	MFLU 19 0202	MN104620	MN104609
Dinemasporium morbidum	CBS 129.66[T]	NG_059110	NR_137788
Dinemasporium polygonum	CBS 516.95[T]	NG_059109	NR_137786
Dinemasporium pseudoindicum	CBS 127402	MH876021	JQ889277
Ellisembia brachypus	HKUCC 10555	DQ408563	--
Eucalyptostroma eucalypti	CPC 28764[T]	KY173500	KY173408
Eucalyptostroma eucalypti	CPC 28748	KY173499	KY173407
Infundibulomyces cupulatus	BCC11929	EF113979	
Infundibulomyces sp.	NR 2006a	EF113980	EF113977
Kionochaeta castaneae	GZCC 18-0025	MN104621	MN104610
Kionochaeta ivoriensis	CBS 374.76[T]	NG_063387	NR_160149
Kionochaeta microspora	GZCC 18-0036	MN104618	MN104607
Kionochaeta microspora	**MFLUCC 21-0109**	**MZ771246**	**MZ770858**
Kionochaeta ramifera	MUCL 39164	MW144404	MW144421
Lecythothecium duriligni	CBS 101317	AF261071	--
Leptosporella arengae	MFLUCC 15-0330[T]	MG272246	MG272255
Leptosporella gregaria	SMH4673	HM171287	--
Menispora tortuosa	CBS 214 56	MH869135	MH857588
Menispora tortuosa	AFTOL-ID 278	AY544682	KT225527
Menisporopsis breviseta	MFLU 19-0212	MN104623	MN104612
Menisporopsis dushanensis	MFLU 19-0213[T]	NG_070470	NR_166299
Morrisiella indica	HKUCC 10827	DQ408578	--
Multiguttulispora sympodialis	MFLU 19-0218	MN104617	MN104606
Nawawia filiformis	MFLU 18-1500	MH758209	MH758196
Nawawia filiformis	MFLU 18-1501	MH758206	--
Neopseudolachnella acutispora	MAFF 244358[T]	NG_059404	NR_154223
Neopseudolachnella uniseptata	MAFF 244360[T]	NG_059406	NR_154225
Paliphora intermedia	CBS 896.97[T]	NG_057766	NR_160203
Phaeostalagmus cyclosporus	CBS 663.70	MH871680	MH859892
Phaeostalagmus cyclosporus	CBS 312.75	MH872661	--
Phialosporostilbe sp.	MFLU 18-1502	MH758207	MH758194
Phialosporostilbe sp.	HKAS 102205	MH758212	MH758199
Polynema podocarpi	CPC 32761	MH327833	MH327797
Pseudodinemasporium fabiforme	CPC 24781	KR611906	KR611889
Pseudodinemasporium fabiforme	MAFF 244361	AB934044	AB934068
Pseudolachnea fraxini	CBS 113701[T]	NG_057956	NR_155628
Pseudolachnea hispidula	MAFF 244364	AB934047	AB934071
Pseudolachnea hispidula	MAFF 244365	AB934048	AB934072
Pseudolachnea sp.	AM09.1	--	KM246165
Pseudolachnella asymmetrica	MAFF 244366[T]	NR_154276	AB934049

Table 1 Continued.

Species name	Strain no.	GenBank accession no.	
		LSU	ITS
Pseudolachnella complanata	MAFF 244371^{T}	NG_059409	NR_154278
Pseudolachnella scolecospora	MAFF 244379	AB934062	AB934086
Pyrigemmula aurantiaca	CPC 18063	HM241692	HM241692
Pyrigemmula aurantiaca	CPC 18064	HM241693	HM241693
Rattania setulifera	GUFCC 15501	HM171322	GU191794
Sporoschisma hemipsila	MFLUCC15-0615	KX358074	--
Sporoschisma sp.	JY-2016a	KX358077	KU557563
Stanjehughesia vermiculata	HKUCC 10840	DQ408570	--
Striatosphaeria codinaeaphora	MR 1230	AF178546	AF178546
Striatosphaeria codinaeaphora	SMH 1524	AF466088	--
Tainosphaeria jonesii	GZCC 16-0053	KY026056	KY026059
Tainosphaeria jonesii	GZCC 16-0065^{T}	KY026057	KY026060
Thozetella nivea	--	EU825200	EU825201
Thozetella tocklaiensis	CBS 378.58	MH869349	MH857817
Verhulstia trisororum	CBS 143234^{T}	MG022160	MG022181
Zanclospora iberica	FMR 11584^{T}	KY853544	KY853480
Zanclospora iberica	FMR 12186	KY853545	KY853481

Results

Phylogenetic analysis

The combined LSU and ITS alignment comprised 90 strains of *Chaetosphaeriaceae*. *Leptosporella arengae* (MFLUCC 15-0330) and *L. gregaria* (SMH4673) were selected as the outgroup taxa. A best-scoring ML tree (Fig. 1) had a final ML optimization likelihood value of -16395.584097. The matrix had 694 distinct alignment patterns, with 13.59% undetermined characters or gaps and estimated base frequencies as follows; A = 0.225233, C = 0.266477, G = 0.310020, T = 0.198270; substitution rates AC = 1.626817, AG = 2.175528, AT = 1.814828, CG = 0.762626, CT = 7.023644, GT = 1.000000; proportion of invariable sites I = 0.450438; gamma distribution shape parameter α = 0.535850. Both ML and Bayesian inferences (BYPP) presented similar topology at the generic relationships.

Our phylogenetic analysis indicates that *Chloridium* and *Kionochaeta* are polyphyletic within *Chaetosphaeriaceae* (Fig. 1). Their polyphyletic nature has been illustrated in previous taxonomy studies as well (Luo et al. 2019, Hyde et al. 2020, Réblová et al. 2021). *Chloridium* species are grouped in three different clades (*Chloridium* 1–3), and *Kionochaeta* nested in two different clades (*Kionochaeta* 1 and 2) in *Chaetosphaeriaceae* (Fig. 1). Comparatively, *Chloridium* clade 1 has a higher number of species (*C. aquaticum*, *C. caudigerum*, *C. chlamydosporum, C. chloroconium, C. gonytrichii, C. inaequalis, C. salinicola, C. submersum* and *C. virescens*) than *Chloridium* clade 2 (*C. lignicola* and *C. pini*) and clade 3 (*C. botryoideum*). Réblová et al. (2016) and Hyde et al. (2020) indicated that the type species of *Chloridium*, *C. viride* is congeneric with the type species of *Melanopsammella*, *M. inaequalis*. Thus, Hyde et al. (2020) included *Chloridium inaequalis* in their phylogenetic tree. Our collection, MFLUCC 21-0110 clustered with *C. gonytrichii* isolates (S-360, HKAS:93031, MFLUCC 11-0216, SMH 3785) with 100% ML and 1.00 BYPP support, within *Chloridium* clade 1 (Fig. 1). *Chloridium gonytrichii* isolates show a sister relationship to *C. aquaticum* (MFLU 19-1238), *C. salinicola* (MFLU 11-1133), and *Chloridium* sp. (HGUP 1806) with 91% ML and 0.99 BYPP support (Fig. 1). Our other collection (MFLUCC 21-0109) formed a well-supported clade with *Kionochaeta microspora* (GZCC 18-0036) with 100% ML and 1.00 of BYPP support, which is located in *Kionochaeta* clade 1. In this clade, *Kionochaeta microspora* is clustered with *K. castaneae* and the generic type, *K. ramifera* (Fig. 1) (Réblová et al. 2021). *Kionochaeta* clade 2 contains single species, *K. ivoriensis* (Fig. 1).

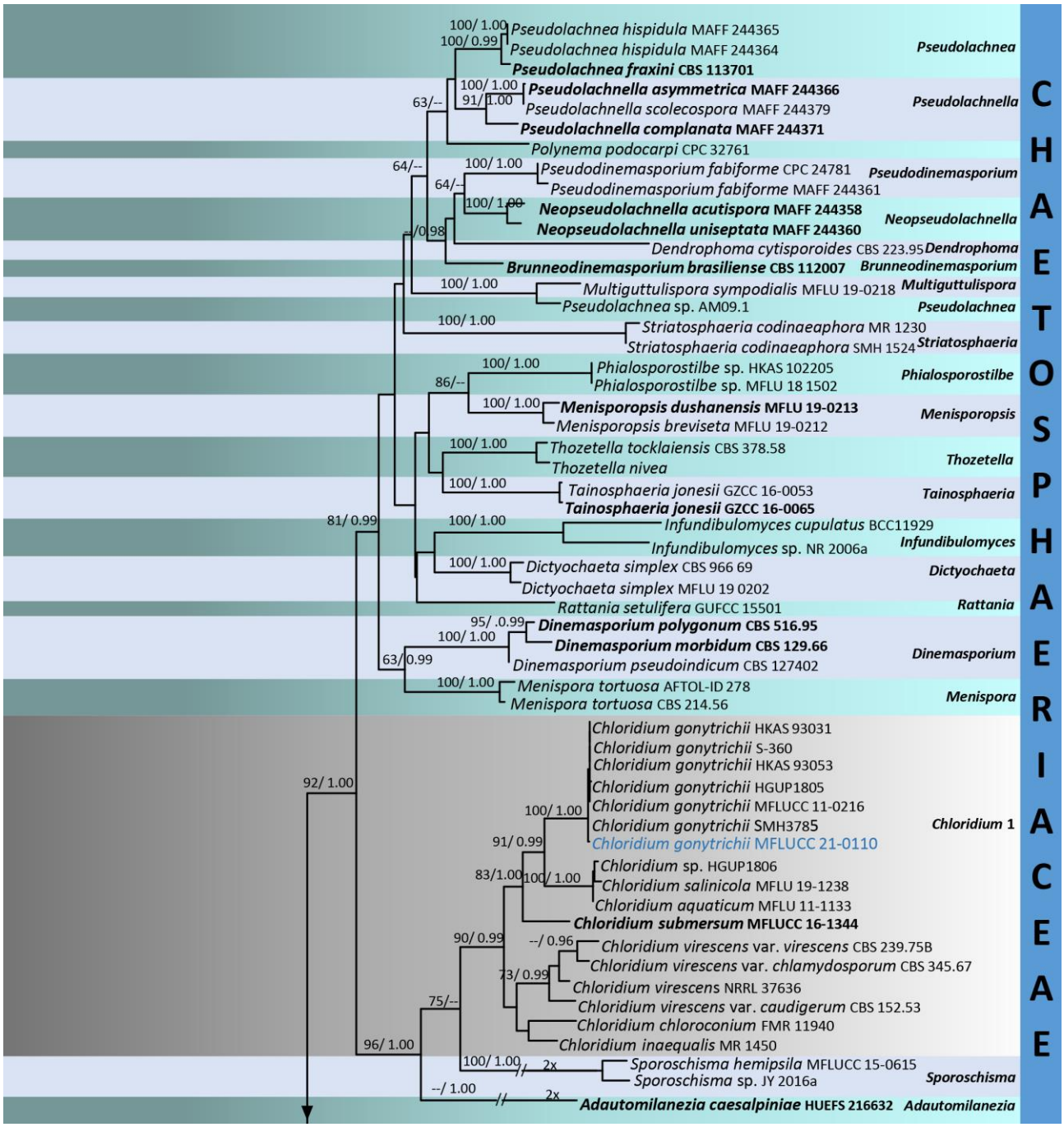

Fig. 1 – Phylogenetic tree generated from the maximum likelihood analysis based on combined LSU and ITS gene sequence data for the selected genera in the family *Chaetosphaeriaceae*. Bootstrap support values of maximum likelihood greater than 60% and Bayesian posterior probabilities (BYPP) greater than 0.95 are indicated above the nodes. Newly added strains are in blue and ex-type strains are in black bold. The tree is rooted to *Leptosporella arengae* (MFLUCC 15-0330) and *L. gregaria* (SMH4673).

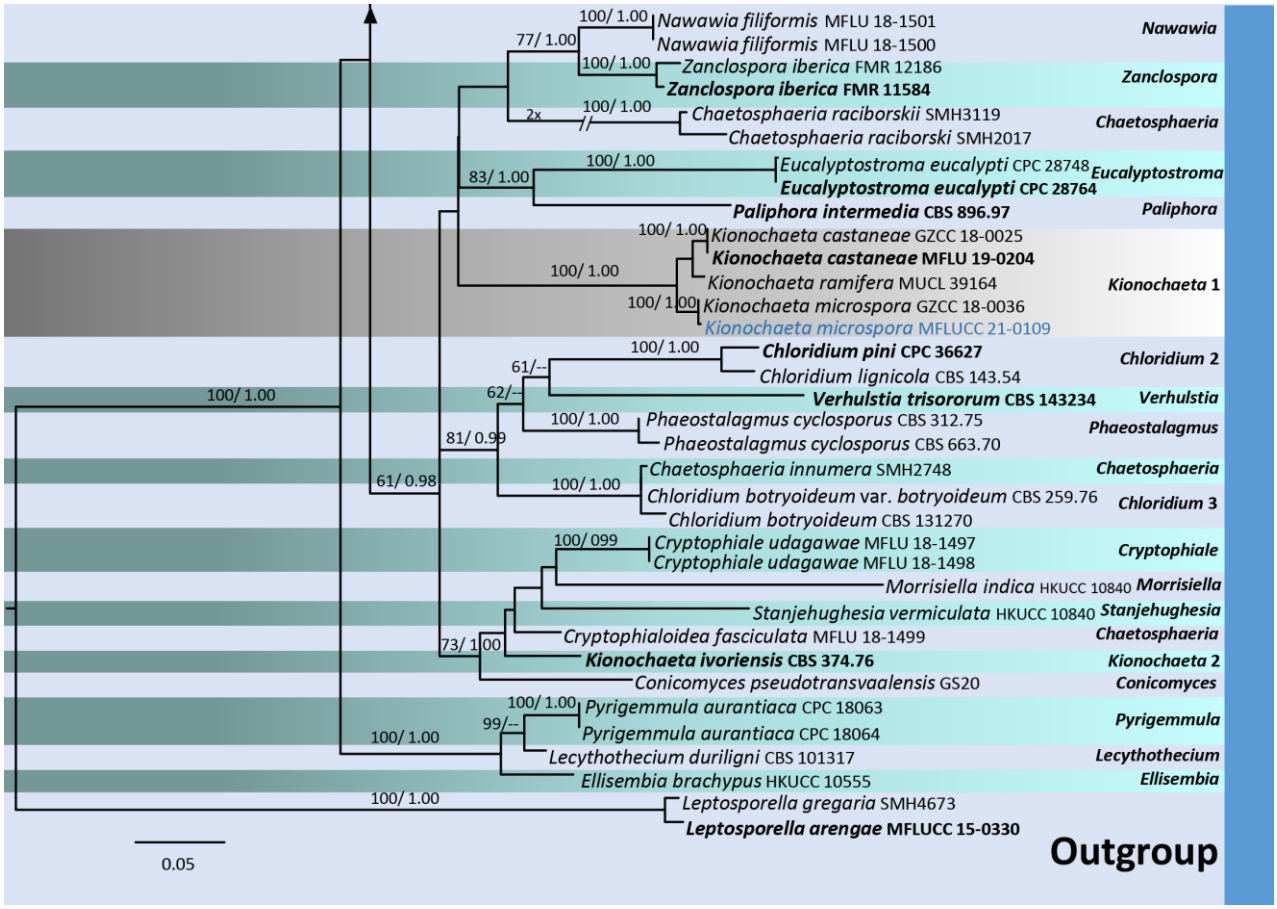

Fig. 1 – continued.

Taxonomy

Chloridium gonytrichii Réblová & Seifert, IMA Fungus 7: 134 (2016)..... Fig. 2

Index Fungorum number: IF 816827; Facesoffungi number: FoF 05463,

≡ *Melanopsammella gonytrichii* F.A. Fernández & Huhndorf, Fungal Diversity 18: 42 (2005)

Culture characteristics – *Colonies* on PDA, from above: whitish gray in the center with white concentric zones at the initial stage, reaching a diam. of 2–3 cm in 7 days, becoming grayish brown, with reverse becoming grayish brown with grayish center at 25 °C when mature. Colony smooth to hairy at surface, effuse, raised, with circular margin, sometimes with gray to black erect, flexuous synnemata. *Mycelium* 2–4 µm ($\overline{x}$ = 3 µm) wide, superficial, composed of septate hyphae, hyaline to sub-hyaline when immature, latter becoming branched and melanized, sporulated after 4 weeks. Sexual morph: Previously reported by Fernández & Huhndorf (2005). Asexual morph: *Conidiophores* 100–250 × 2–4 µm ($\overline{x}$ = 175 × 3 µm, n = 20), hyaline to sub hyaline, macronematous, mononematous, solitary, septate, unbranched, percurrently 2–5 times proliferating, with monophialidic aperture at the apex, with 2–5 intercalary percurrent phialides. *Conidiogenous cells* phialidic, cylindrical to lageniform, each with multiple enteroblastic conidiogenous loci producing conidia. *Conidia* 2.5–3.5 × 2.5–3 µm ($\overline{x}$ = 3× 2.8 µm, n = 20), globose to subglobose, aseptate, hyaline, with minutely rough surface.

Material examined – Thailand, Krabi Province, Khao Phanom District, Na Khao 8.3811N, 98.9286 E, in tropical forest soil, 28 April 2019, E. Yasanthika, B103 (MFLU 21-0142), living culture (MFLUCC 21-0110).

Notes – As examined morphological characteristics largely overlap with *Chloridium gonytrichii* isolates (SMH3785, HKAS 93031, and HKAS 93053), we accordingly report our collection (MFLU 21-0142) as a new record of *C. gonytrichii* from soil-based habitats in Thailand. Our isolate (MFLUCC 21-0110) resembles *Chloridium gonytrichii* in having macronematous, mononematous, solitary, multi-septate, unbranched, percurrently proliferating conidiophores,

phialidic, cylindrical to lageniform conidiogenous cells, and globose to sub-globose and aseptate conidia (Luo et al. 2019). Multi-locus phylogeny (LSU and ITS) also indicates that our collection grouped with *C. gonytrichii* isolates in a strongly supported clade (100% ML, 1.00 BYPP). *Chloridium gonytrichii* was initially introduced by Réblová et al. (2016), which was previously known as *Melanopsammella gonytrichii* (Fernández & Huhndorf 2005). *Chloridium gonytrichii* seems to have cosmopolitan distribution since it has been reported from both terrestrial and freshwater habitats (Fernández & Huhndorf 2005, Wei et al. 2018, Luo et al. 2019). Balami et al. (2021) recorded *Chloridium gonytrichii* from soils of agricultural land in Nepal by high throughput sequencing technology. We provide the first record of soil-inhabiting *C. gonytrichii* (MFLUCC 21-0110) in Thailand, with morpho-molecular descriptions.

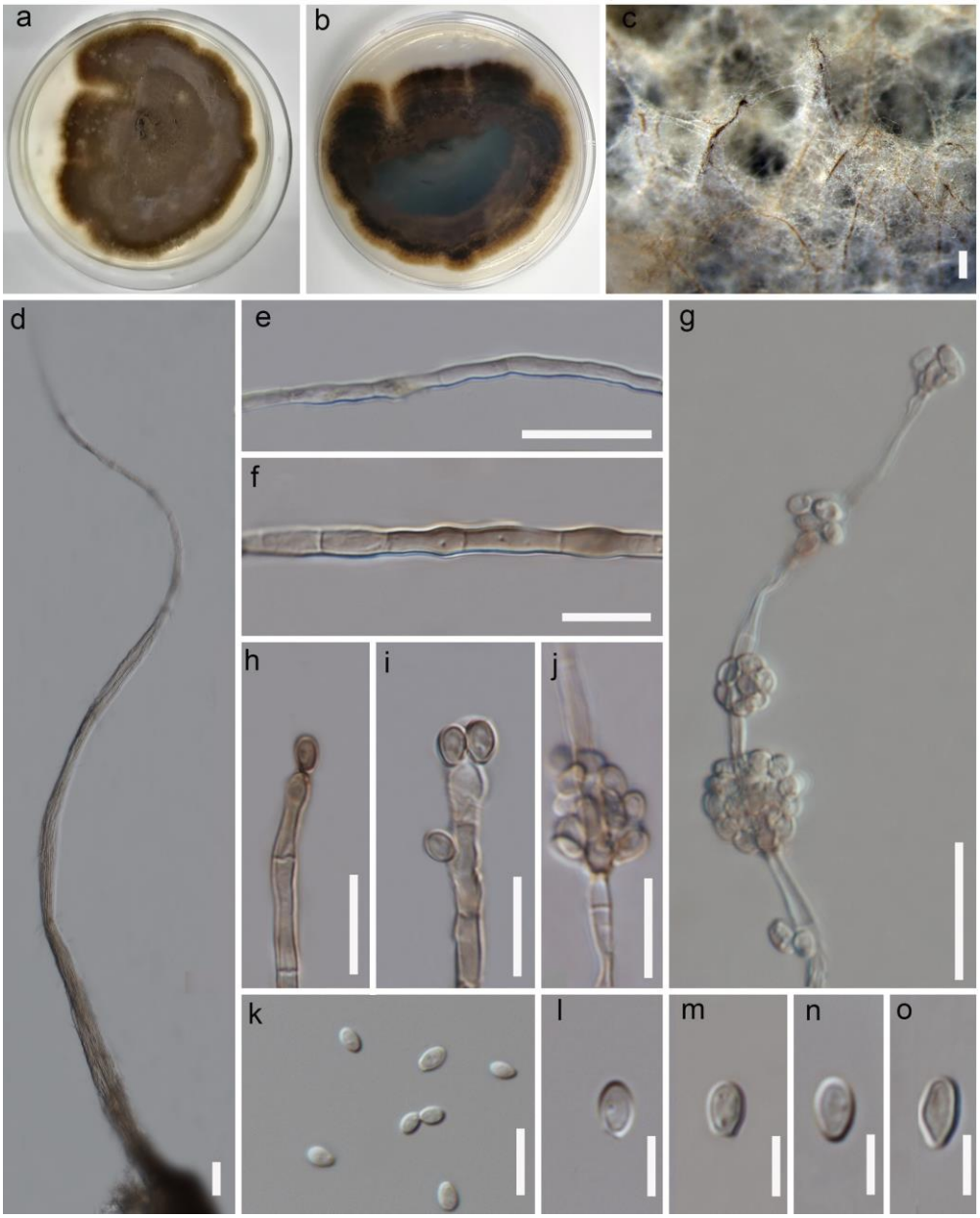

Fig. 2 – ***Chloridium gonytrichii*** (MFLUCC 21-0110) a Colony from above (on PDA). b Colony from below (on PDA). c Sporulated colony with conidial attachments on the mycelium. d Erect young immature synnema. e Immature septate hyphae. f Mature septate melanized hyphae. g Macronematous conidiophore with intercalary percurrent conidiogenous cells. h–j Conidiogenesis on the conidiophore. k–o Conidia. Scale bars: c = 200 μm, d = 25 μm, e, g = 20 μm, f, h–k = 10 μm, l–o = 5 μm.

Kionochaeta microspora C.G. Lin & K.D. Hyde, Mycosphere 10: 678 (2019)..... Fig. 3

Index Fungorum number: IF556708; Facesoffungi number: FoF 06289

Culture characteristics – *Colonies* on PDA at 25 ℃, become 2–3 cm diameter after 7 days, appearing in white become olive-green to dark green at the center, rind-like at the edge when mature, reverse yellowish-white at the center with olive-green to dark green rind at the edge, semi immersed to superficial, setae present and have a slimy raised surface with a filiform margin. *Mycelium* immersed to semi immersed, hyaline, producing immature aseptate, smooth-surfaced hyphae, becoming septate, less-branched, course-surfaced hyphae, sporulated after 4 weeks. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiophores* 40–45.5 × 2–3 ($\overline{x}$ = 43 × 2.5) μm, macronematous, mononematous, caespitose, erect, straight or slightly curved, smooth-walled, thick-walled, unbranched, septate, hyaline, cylindrical at the apex or middle fertile region when present 12–21 × 2–4.5 μm. *Conidiogenous cells* 3–4.5 × 2–3 ($\overline{x}$ = 3.8 × 2.5) μm, arising from the fertile region at the apex, hyaline monophialidic, discrete, determinate, terminal, narrowly ellipsoid, ampulliform. *Conidia* 2–4.5 × 1.5–3 μm ($\overline{x}$ = 3.3 × 2.3 μm, n = 25), hyaline, spherical to ovate, and aseptate, smooth-walled.

Material examined – Thailand, Krabi Province, Khao Phanom District, Na Khao, 8.3811N, 98.9286 E, in tropical forest soil, 28 April 2019, E. Yasanthika, ES1 (MFLU 21-0143, living culture (MFLUCC 21-0109).

Notes – The morphology of our collection (MFLU 21-0143) resembles *Kionochaeta microspora* (MFLU 19-0206) in having monophialidic, discrete, determinate, terminal, rarely intercalary, narrowly ellipsoid conidiogenous cells and hyaline, smooth, aseptate slimy conidia (Lin et al. 2019). Multi-gene phylogeny (LSU and ITS) indicates that our isolate (MFLUCC 21-0109) nested with *Kionochaeta microspora* isolates (GZCC 18-0036) in a well-supported clade (100% ML, 1.00 BYPP). Therefore, based on both morphology and phylogenetic evidence, we account for our isolate (MFLUCC 21-0109) as *K. microspora*, collected from the forest soils in Thailand. *Kionochaeta microspora*, which was isolated from decaying wood in China, was initially introduced by Lin et al. (2019). Therefore, we report our collection (MFLUCC 21-0109) as the first record of soil-inhabiting *Kionochaeta microspora*.

Discussion

Soil fungi are a diverse taxonomic group on the planet, and among them, *Ascomycota* features widespread distribution in soils worldwide (Tedersoo et al. 2014). They play vital roles as decomposers, mutualists, parasites, and/or pathogens in the ecosystem, providing a prominent contribution to key terrestrial processes, such as decomposition of organic materials and nutrient cycling (Bridge & Spooner 2001, Taylor & Sinsabaugh 2014). Further, some soil fungal strains and their secondary metabolites are valuable sources for biotechnological development (Stefani et al. 2015). Despite their importance in ecosystems and biotechnology, information regarding the soil fungal diversity and their ecological features on a global scale remains scarce. One possible reason could be limitations in traditional culture-based approaches and isolation techniques (Tedersoo et al. 2014, Wu et al. 2019). Giri et al. (2005) pointed out that only <5% of soil fungi are culturable. To overcome these limitations, morphological studies coupled with DNA sequence-based molecular approaches have been used to survey soil fungal diversity across various terrestrial ecosystems and countries worldwide (Wu et al. 2019). Moreover, fungal activities in tropical soils are comparatively high because of the beneficial climate and edaphic factors present in such ecosystems (Amma et al. 2018). It is worth considerable attention to identify soil fungal diversity in poorly studied countries, such as Thailand. Ito et al. (2001) studied the diversity of fungi inhabiting tropical mangrove forest soils in Thailand and reported more than 20 fungal taxa. We assume that there will be high soil fungal diversity in Thailand because of the tropical climatic conditions, and up to 96% of fungal species found in Northern Thailand are new to science (Amma et al. 2018, Hyde et al. 2018).

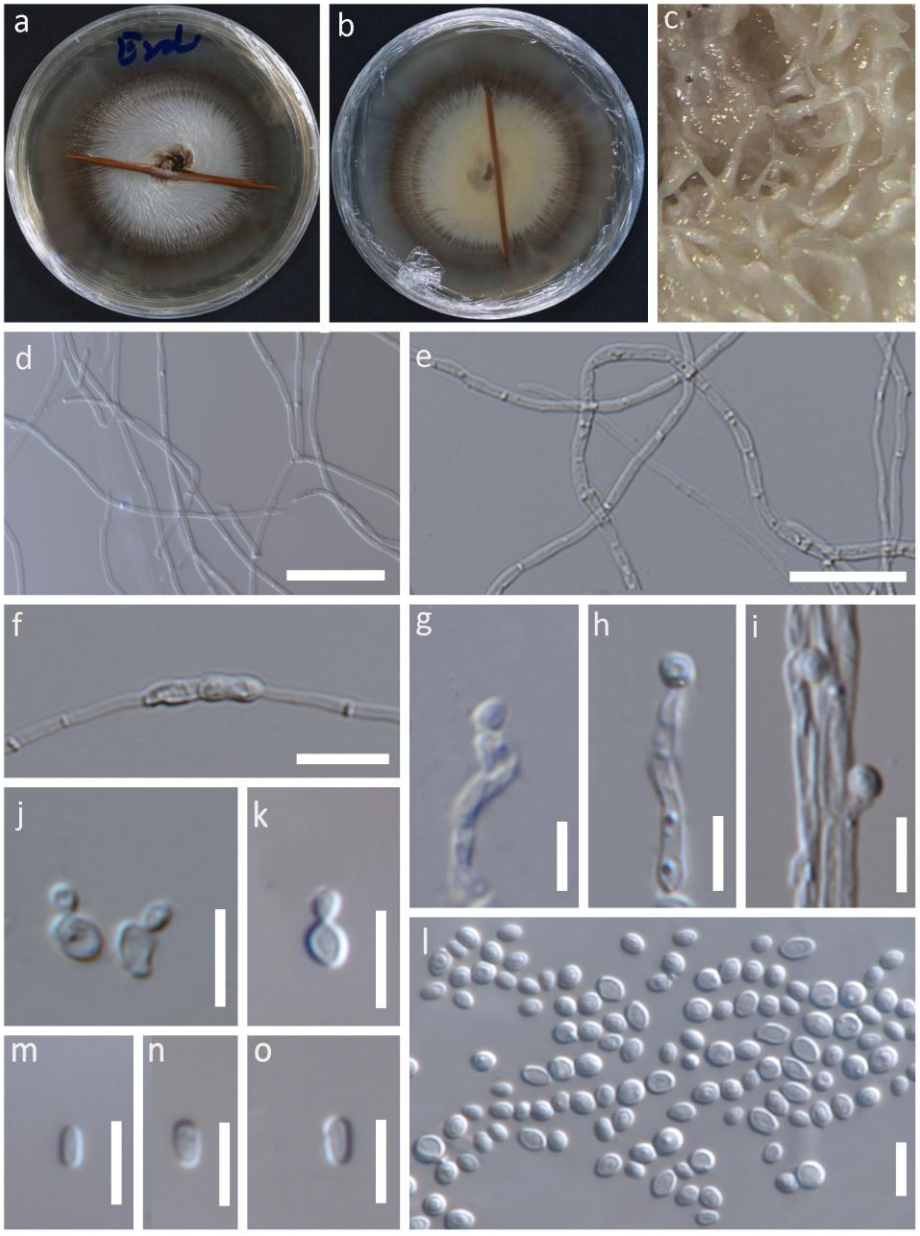

Fig. 3 – ***Kionochaeta microspora*** (MFLUCC 21-0109) a Colony from above (on PDA). b Colony from below (on PDA). c Sporulated colony with conidial attachments on the mycelium. d Immature hyphae. e Mature septate course-surfaced hyphae. f Chlamydospore on the mycelium. g–i Conidiogenesis on the conidiophores. j, k Conidia attached to detached phialides. l–o Conidia Scale bars: d = 25 μm, e = 20 μm, f = 10 μm, g–o = 5 μm.

Studies related to soil fungal taxonomy, community compositions, and biodiversity in Thailand are still lacking (Corlett et al. 2014, Sato et al. 2015, Amma et al. 2018, Shi et al. 2019). The reasons for this situation are the edaphic and climatic complexities limiting the investigation of soil fungal species present in this region (Amma et al. 2018, Shi et al. 2019). Furthermore, continued deforestation has resulted in the loss of natural habitats of soil fungi and the lack of knowledge on soil microbial communities (Hansen et al. 2013, Tedersoo et al. 2014, McGuire et al. 2015). Despite the challenges, several researchers have made considerable efforts to resolve soil fungal taxonomy in Thailand by using high-throughput (HTS) techniques (Herrmann et al. 2016, Amma et al. 2018, Kitisin et al. 2021). However, most HTS studies have limitations in identifying fungi at the species level (Tedersoo et al. 2014, Wu et al. 2019). Because this technique targets only a short gene region (generally ITS1 or ITS2) featuring high variability, it causes difficulty in the

sequence alignments (Tedersoo et al. 2020). Therefore, to obtain better insights into species diversity of soil, a combination of both approaches (morphology and molecular data) is needed in future studies (Wu et al. 2019).

This study provided both morphological and molecular phylogenetic analyses to describe and report for the first time two *Chaetosphaeriaceae* fungal species (*Chloridium gonytrichii* - MFLUCC 21-0110 and *Kionochaeta microspora* - MFLUCC 21-0109) inhabiting the tropical forest soils of southern Thailand (Figs. 2, 3). Phylogenetic study of the recorded species clustered them with previously registered isolates with high statistical support (Fig. 1), in agreement with previous multi-gene phylogeny investigations into *Chaetosphaeriaceae* (Lin et al. 2019, Luo et al. 2019, Hyde et al. 2020). When compared with the type materials, our isolates (*C. gonytrichii* - MFLUCC 21-0110 and *K. microspora* - MFLUCC 21-0109) showed the size and shape differences in conidial morphology (Lin et al. 2019, Luo et al. 2019). Fernández & Huhndorf (2005) reported that *C. gonytrichii* (SMH3785) is characterized with ellipsoid and light green conidia while our collection is characterized with globose to subglobose, aseptate and hyaline conidia. Conidia observed in the type material of *K. microspora* (MFLU 19-0206) are lunate, cylindrical or clavate (Lin et al. 2019) while our isolate (MFLUCC 21-0109) has spherical to ovate conidia. These morphological deviations within a fungal species can result from their physiological adaptations against ecological factors and growth conditions (Francisco et al. 2019).

Réblová et al. (2016) synonymized the species belonging to *Melanopsammella* under *Chaetosphaeria* and *Chloridium.* Subsequently, all species distributed in *Chloridium*, *Gonytrichum*, and *Melanopsammella* are treated as *Chloridium* (Hyde et al. 2020). The sexual morph of *Chloridium* has broadly ovoid to globose ascomata containing eight-spored asci. In addition, *Chloridium paucisporum* and *C. virescens* are recorded as endophytes in this genus (Rashmi et al. 2019). Wei et al. (2018) introduced saprobic species of *Chloridium* from a freshwater habitat in Thailand (*C. aquaticum*). Both sexual and asexual morphs of *C. gonytrichii* have been previously reported on decorticated wood in Puerto Rico (Fernández & Huhndorf 2005). The asexual morph of this species was also recorded in submerged decaying woods in China and Thailand (Wei et al. 2018, Luo et al. 2019) (Fig. 1).

Kionochaeta castaneae was introduced by Lin et al. (2019) from the decaying shell of *Castanea mollissima* in China, and *K. pughii* was isolated from decaying *Dipterocarpaceae* seeds in Thailand (Pittayakhajonwut et al. 2002, Lin et al. 2019). Up to date, *K. microspora* has been recorded only from decaying wood in China (Lin et al. 2019). Lin et al. (2019) provided a synopsis of *Kionochaeta* species.

Chaetosphaeriaceae species play important ecological roles in their habitats as they contribute to nutrient cycling and ecosystem functioning. Thus, many species in this family possess the ability to decompose lignocellulose substrates in woody litter and release nutrients (Palmer et al. 1997, Yuen et al. 1998, Hyde et al. 2016, Liu et al. 2016). As both *C. gonytrichii* and *K. microspora* have been previously recorded from decaying wood, we suggest that the presence of these species in soils resulted from the host jumping during the decomposition process (Promputtha et al. 2010). Some species in this family have the ability to produce useful secondary metabolites (Yamaguchi et al. 2005, Krohn et al. 2008, Hashimoto et al. 2015). Pittayakhajonwut et al. (2002) described *Kionochaeta pughii* as a source for producing 'pughiinin A' and 'pycnidione', which contains anti-plasmodium activity against *Plasmodium falciparum* and anti-cancer activity. Thus, the taxonomic investigation of our study is important for forming the basis of further mycological studies that focus on biotechnology and biodiversity in Thailand. We suggest, for future studies to explore the diversity of soil-inhabiting fungi across different geographic regions of Thailand.

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