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Two new Sordariomycetes records from forest soils in Thailand

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Abstract

Forest soils contain relatively high levels of fungal diversity compared to other soil types and are primarily comprised of pathogens, saprobes or mutualists. This study was conducted to investigate the fungal diversity of mixed deciduous forest soils in Thailand. Fungi were isolated using a dilution plate method and are illustrated, described and subjected to combined phylogenetic analyses (maximum likelihood and Bayesian analyses). We herewith report *Beltraniella fertilis* and *Stachybotrys subcylindrospora* for the first time from mixed deciduous forest soils of northern Thailand.

Key words – *Beltraniella fertilis* – dilution plate method – phylogeny – soil fungi – *Stachybotrys subcylindrospora*

Introduction

Soil is a highly diversified and unique habitat for various types of microorganisms (Bridge & Spooner 2001, Pangging et al. 2019, Wei et al. 2020). A large array of taxonomic and functional groups of fungi are found in soil ecosystems. These fungal groups have saprobic, pathogenic and mutualistic (mycorrhizal) nutritional modes, all of which play major roles in plant growth and nutrient cycling in forest ecosystems (Taylor & Sinsabaugh 2015, Shi et al. 2019). Besides Basidiomycetes, soil fungal taxa largely belong to Sordariomycetes, which are widely distributed across Thailand forest soils (Tedersoo et al. 2014, Amma et al. 2018). In our study, we report two species of Sordariomycetes, belonging to Beltraniaceae and Stachybotryaceae, from mixed deciduous forest (dominated by Dipterocarpaceae) soils in northern Thailand.

Beltraniella was established by Subramanian (1952) with *B. odinae* as the type species. Most species of *Beltraniella* were reported as asexual morphs. *Pseudomassaria carolinensi*s (sexual

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morph) was previously linked to *B. portoricensis* (asexual morph) based on morphological support by Hodges & Barr (1971). Subsequently, *B. carolinensis* was proposed as a new combination with *P. carolinensis*, supported by the phylogenetic analyses of Jaklitsch et al. (2016). Castañeda-Ruiz et al. (1996) provided a key for *Beltraniella* species. Shirouzu et al. (2010) provided a synopsis for all accepted species in *Beltraniella*. The genus is currently accommodated in Beltraniaceae (Xylariales, Xylariomycetidae) with 28 species epithets from various hosts and habitats (i.e. fallen decomposing leaves, soil and submerged woods in freshwater) in tropical and subtropical regions of the world (Pirozynski 1963, Kirk 1981, Goh & Hyde 1996, Sakayaroj et al. 2005, Duong et al. 2008, Lin et al. 2017a, b, Hyde et al. 2020, Index Fungorum 2020, Wijayawardene et al. 2020). Four species of *Beltraniella* (*B. amoena*, *B. fertilis*, *B. japonica* and *B. portoricensis*) have been identified, and recorded from Brazil (Gusmão et al. 2000, Marques et al. 2007, Magalhães et al. 2011). Magalhães et al. (2011) recorded *B. fertilis* and *B. portoricensis* on endemic plants of Atlantic forests. Dos Santos et al. (2014) also recorded *B. botryospora* and *B. portoricensis* associated with the leaf litter of *Inga thibaudiana*, *Myrcia splendens* and *Pera glabrata* along with the first report of *B. botryospora* from the Atlantic forest in Brazil. *Beltraniella fertilis* was erected by Heredia et al. (2002) as a saprobe on leaf litter from Mexico. Lin et al. (2017b) recorded the asexual morph of *B. fertilis* on leaf litter from Thailand.

Stachybotrys was introduced by Corda (1837) and typified with *Stachybotrys atra* (= *S. chartarum*) which belongs to Stachybotryaceae (Hypocreales, Sordariomycetes) (Bisby 1943, Seifert et al. 2011, Crous et al. 2014). *Memnoniella* and *Stachybotrys* were initially identified as two distinct genera in Stachybotryaceae (Bisby 1945, Jong & Davis 1976). Lombard et al. (2016) supported this using multi-locus sequence analysis and presented the genera *Memnoniella* and *Stachybotrys* as well-supported distinct clades in the phylogenetic tree. However, Wang et al. (2015) suggested *Stachybotrys* as a synonym of *Memnoniella* based on morphological and phylogenetic similarities. Pinruan et al. (2004) provided a key for *Stachybotrys* that includes 50 species. *Stachybotrys* are found on damp papers, cotton, linen, soil, litter and other indoor environments as saprobes or pathogens (Ellis 1971, 1976, Whitton et al. 2001, Thongkantha et al. 2008, Jie et al. 2013, Wang et al. 2015). Most *Stachybotrys* species have been described from asexual morphs, and only *S. oleronensis* is described as the sexual morph with morpho-molecular support (Lechat et al. 2013, Crous et al. 2014). Recently, *Melanopsamma* and *Ornatispora* were linked as the sexual morphs of *Stachybotrys* (Lechat et al. 2013, Wang et al. 2015). *Stachybotrys chartarum* is common in soil and cellulose-based building materials (Wang et al. 2015). Wu & Zhang (2010) isolated *S. jiangziensis* and *S. xigazenensis* from forest soil in China. Furthermore, Jie et al. (2013) introduced *S. subcylindrospora* from forest soils in China.

Beltraniella has mainly been recorded from plant litter whereas some species of *Stachybotrys* reported from soils (Lin et al. 2016, 2017a, b, Lee et al. 2019, Hyde et al. 2020). Most of these species exhibit a saprobic life mode while a few species are known to be plant pathogens (Wang et al. 2015, Lin et al. 2017a, b, Hyde et al. 2020). Soil fungal communities are poorly investigated using morpho-molecular analyses (Wu et al. 2013, Shi et al. 2019). Compared to other regions of East Asia, numerous studies relating to soil fungi have been conducted in China (Yang & Insam 1991, Wu et al. 2013, Shi et al. 2019). However, studies on taxonomic diversity, molecular phylogeny, chemistry and geographic distribution of soil-inhabiting fungi in Thailand are still limited (Amma et al. 2018). Thus, it is important to improve our knowledge of fungi and fungal communities from soils in Thailand as this knowledge could contribute towards an improved scientific understanding of fungi in tropical soils, improved forest management systems and enhanced agricultural practices (Amma et al. 2018, Khuna et al. 2019). In this study, we carried out an investigation of soil-inhabiting microfungi in Thailand. We present the first report of *Beltraniella fertilis* from mixed deciduous forest soils and the first country record of *Stachybotrys subcylindrospora* from soils in northern Thailand.

Materials & Methods

Sample collection, fungal isolation and morphological characterization

Soil samples were collected from a mixed deciduous forest (dominated by Dipterocarpaceae) in Chiang Mai Province, Thailand during the dry season (March 2019). The samples were taken to the laboratory using zip-lock plastic bags within an icebox and kept in cold storage at 4°C until the isolation process started. The soil dilution plate method was used to isolate soil fungal strains (Aziz & Zainol 2018, Lee et al. 2019). Ten grams of soil was dissolved in 100 mL of sterilized distilled water, and the sample was shaken thoroughly. One milliliter of the initial solution was transferred to a vial containing 9 mL of sterilized distilled water and thoroughly mixed. One milliliter of the diluted solution taken from the second vial was transferred to a third vial containing 9 mL of sterilized distilled water. Similarly, the soil sample was diluted six times (with sterilized distilled water) from 10^{-1} until 10^{-6}. From each dilution, 0.1 mL of the solution was transferred into a Petri dish containing, antibiotic treated-sterilized PDA (Potato Dextrose Agar) medium and uniformly spread on the surface of the medium using a sterilized glass spreader. The plates were sealed and incubated at 25°C for 2–3 days, until colony formation. As the colonies appeared, hyphal tips from each colony were transferred into fresh PDA plates to get pure fungal culture. The pure cultures were incubated at 25°C to facilitate sporulation. Conidial structures with mycelium were removed with a needle and placed in a drop of distilled water on a slide for morphological study. Photomicrographs of fungal structures were captured using an OLYMPUS SZ61 compound microscope, and images were recorded with 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. Dried culture specimens were deposited at Mae Fah Luang University Herbarium (Herb. MFLU). Faces of Fungi numbers were registered as described in Jayasiri et al. (2015).

DNA extraction, PCR amplification and sequencing

Fungal isolates were grown on PDA for six weeks at 25°C, and total genomic DNA were extracted from 50 to 100 mg of axenic mycelium from the growing 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) according to the manufacturers' instructions. Polymerase chain reactions (PCR) conducted to amplify the internal transcribed spacer region of ribosomal DNA (ITS) and large subunit nuclear ribosomal DNA region (LSU) by using ITS5/ITS4 (White et al. 1990), and LR0R/LR5 primers, respectively (Vilgalys & Hester 1990). Other protein-coding gene regions were amplified as follows: the β-tubulin (tub2) gene region amplified using primers Bt2a and Bt2b (Glass & Donaldson 1995), and calmodulin (cmdA) using primers CAL-228F (Carbone & Kohn 1999) and CAL2Rd (Groenewald et al. 2005). PCR amplification process was conducted in a 25 µL PCR mixture containing 12.5 µL of 2×Power Taq PCR MasterMix (a premixed ready-to-use solution, composed of 0.1 Units/µL Taq DNA Polymerase, 500 µM dNTP Mixture each (dATP, dCTP, dGTP, dTTP), 20 mM TrisHCl pH 8.3, 100 Mm KCl, 3 mM MgCl2, stabilizer and enhancer), 1 µL of each primer (10 µM), 2 µL genomic DNA extract and 8.5 µL double distilled water. The PCR thermal cycle for the amplification of LSU and ITS regions was programmed initially at 94°C for 3 mins., followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 50 s, elongation at 72°C for 1 min., and a final extension at 72°C for 10 min (Lin et al. 2016, 2017b). The PCR conditions for the amplification of the cmdA was initiated at 94°C for 5 min and 40 cycles were executed. Each cycle consists of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and elongation at 72°C for 30 s. A final elongation was at 72°C for 7 min. (Groenewald et al. 2005). Amplification of the β-tubulin region started at 94°C for 1 min and the temperature was reduced to around 58–68°C for 1 min, and later increased to 72°C for 1 min. This process was repeated for 32 cycles under the above

conditions, and 5–10s of extension time was maintained in each cycle (Glass & Donaldson 1995). All PCR products were visualized using an ethidium bromide (EtBr) staining on 1.2% agarose gels. Successful PCR products were sent for the sequencing at Qingke Company, Kunming City, Yunnan Province, P. R. China.

Sequence alignment

Obtained sequences were subjected to a BLAST search in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). BLAST search results and initial morphological studies supported that our isolates belonged to *Beltraniella* and *Stachybotry*s. Additional sequence data, based on previously published material, were obtained from GenBank (Table 1) (Jie et al. 2013, Crous et al. 2014, Lin et al. 2016, Lombard et al. 2016, Lin et al. 2017b, Hyde et al. 2020). The single gene and multigene alignments were automatically done by MAFFT v. 7.036 (http://mafft.cbrc.jp/alignment/server/index.html, Katoh et al. 2002) using the default settings and later refined where necessary, using BioEdit v. 7.0.5.2 (Hall 1999).

Table 1 Taxa used in the phylogenetic analyses with the corresponding GenBank accession numbers. Type strains are indicated as superscript T and newly generated strains are indicated in **bold**

Species	Strain Number	Gene bank Accession number			
		cmdA	ITS	LSU	tub2
Achroiostachys betulicola	CBS 136397	KU845772	KU845792	--	KU845753
A. humicola	CBS 868.73	KU845779	KU845799	--	KU845760
Alfaria caricicola	CBS 113567^{T}	KU845976	KU845983	--	KU846014
Al. ossiformis	CBS 324.54	KU845977	KU845984	--	KU846015
Amphisphaeria flava	MFLUCC 18-0361^{T}	--	NR-168782	NG-06858	--
Am. sorbi	MFLUCC 13-0721^{T}	--	NR-153531	KP744475	--
Beltrania pseudorhombica	CPC 23656	--	KJ869158	KJ869215	--
B. rhombica	CPC 27482	--	KX519521	KX519515	--
Beltraniella acaciae	CPC 29498^{T}	--	NR-147685	NG-066374	--
Be. brevis	DS 2--23	--	MN252876	MN252883	--
Be. brevis	DS 2--21	--	MN252877	MN252884	--
Be. carolinensis	9502 (IFO)	--	-	DQ810233	--
Be. endiandrae	CBS 137976^{T}	--	NR-148073	NG-058665	--
Be. fertilis	MFLUCC 17-2138	--	MF580248	MF580255	--
Be. fertilis	MFLUCC 19-0487	--	MT215489	MT215539	--
Be. fertilis	MFLUCC 17-2136	--	MF580246	MF580253	--
Be. fertilis	MFLUCC 17-2137	--	MF580247	MF580254	--
Be. fertilis	**MFLUCC 20-0119**	--	**MT835158**	**MT835156**	--
Be. humicola	CBS 203.64	--	MH858416	MH870044	--
Be. pandanicola	MFLUCC 18-0121^{T}	--	MH275049	MH260281	--
Be. portoricensis	NFCCI 3993	--	KX519516	KX519522	--
Be. portoricensis	CBS 856.70	--	MH859981	MH871777	--
Be. pseudoportoricensis	CBS 145547^{T}	--	NR-165552	NG-067875	--
Be. ramosiphora	LCG 10-2	--	MG717500	MG717502	--
Be. thailandica	MFLUCC 16-0377^{T}	--	NR-168175	NG-068824	--
Beltraniopsis longiconidiophora	MFLUCC 17-2139^{T}	--	NR-158353	NG-066200	--
Be. neolitseae	CBS 137974	--	NR-148072	NG-058664	--
Brevistachys globosa	CBS 397.73	KU846023	KU846037	--	KU846100

Table 1 Continued.

Species	Strain Number	Gene bank Accession number			
		cmdA	**ITS**	**LSU**	**tub2**
Be. lateralis	CBS 141058^{T}	KU846027	KU846043	--	KU846106
Cymostachys coffeicola	CBS 252.76	KU846035	KU846052	--	KU846113
C. fabispora	CBS 136180^{T}	KU846036	KU846054	--	KU846114
Globobotrys sansevieriicola	CBS 138872^{T}	--	KR476717	--	KR476794
Grandibotrys pseudotheobromae	CBS 136170^{T}	--	KU846135	--	KU846241
G. xylophila	CBS 136179^{T}	KU846115	KU846137	--	--
Hemibeltrania cinnamomi	NFCCI 3997	--	KX519517	KX519523	--
H. cinnamomi	MFLUCC 17-2141	--	MF580251	MF580258	--
Melanopsamma pomiformis	CBS 325.90	KU846031	KU846048	--	KU846111
M. xylophila	CBS 100343	KU846034	KU846051	--	--
Memnoniella brunneoconidiophora	CBS 136191^{T}	KU846116	KU846139	--	KU846244
Me. echinata	CBS 216.32	KU846119	KU846142	--	KU846245
Me. ellipsoidea	CBS 136199	KU846127	KU846150	--	KU846252
Me. humicola	CBS 136197	KU846131	KU846155	--	KU846256
Me. pseudonilagirica	CBS 136405	KU846132	KU846157	--	KU846257
Parapleurotheciopsis caespitosa	CBS 519.93^{T}	--	MH862437	NG-066263	--
P. inaequiseptata	MUCL 41089	--	EU040235	EU040235	--
Peethambara sundara	CBS 646.77	--	KU846471	--	KU846551
Porobeltraniella porosa	NFCCI 3995	--	KX519519	KX519525	--
Po. porosa	NFCCI 3996	--	KX519520	KX519526	--
Pseudobeltrania lauri	CPC 33589^{T}	--	NR-166309	NG-068311	--
Ps. ocoteae	CPC 26219^{T}	--	NR-138416	NG-067305	--
Sirastachys castanedae	CBS 164.97	KU846553	KU846658	--	KU847094
Stachybotrys aloeticola	CBS 137940^{T}	KU846570	KJ817888	--	KJ817886
S. aloeticola	CBS 137941	KU846571	KJ817889	--	KJ817887
S. chartarum	CBS 129.13	--	KM231858	--	KM232127
S. chartarum	CBS 177.42	--	KU846678	--	KU847114
S. chartarum	CBS 182.80	--	KU846679	--	KU847115
S. chlorohalonata	CBS 109283	KU846622	KU846728	--	KU847163
S. chlorohalonata	CBS 109285	KU846623	KU846729	--	KU847164
S. chlorohalonata	DAOMC 235557	KU846644	KU846751	--	KU847185
S. dolichophialis	DAOMC 227011	KU846628	KU846734	--	KU847169
S. limonispora	CBS 128809	KU846629	KU846735	--	KU847170
S. limonispora	CBS 136165	KU846630	KU846736	--	KU847171
S. microspore	ATCC 18852	--	AF081475	--	--
S. microspore	CBS 186.79	KU846631	KU846737	--	KU847172
S. phaeophialis	KAS 525	KU846632	KU846738	--	KU847173
S. reniformis	ATCC 18839	--	AF081476	--	--
S. reniformis	CBS 976.95	KU846633	KU846739	--	KU847174
S. reniformis	CBS 136198	--	KU846740	--	--
S. subcylindrospora	HGUP 0201^{T}	--	KC305354	--	--
S. subcylindrospora	**MFLUCC 20-0120**	**MT861049**	**MT835159**	--	**MT861048**
S. subsylvatica	CBS 126205	KU846634	KU846741	--	KU847175
Striatobotrys eucylindrospora	CBS 203.61^{T}	KU846648	KU846755	--	KU847189
Subramaniomyces podocarpi	CPC 32031^{T}	--	NR-156659	NG-066201	--

Note: -- refers “no data in GenBank”

Phylogenetic analyses

Two phylogenetic analyses were conducted separately for Beltraniaceae and Stachybotryaceae to identify the taxonomic placements of our strains. Maximum likelihood (ML) trees were generated using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008) in the CIPRES Science Gateway platform (Miller et al. 2010). For each tree, parameters were set including 1000 replicates with the GTR+I+G model of nucleotide substitution rates. Bayesian analyses were conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronqvist 2001) to evaluate Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). In each analysis, two parallel runs were conducted using the default settings with the following adjustments: Six simultaneous Markov chains run for 2,000,000 generations, trees were sampled at every 100^{th} generation, and 20,000 trees were obtained. The first 4,000 trees, representing the burn-in phase of the analyses were discarded. The remaining 16,000 trees were used to calculate the PP in the majority rule consensus tree. Phylograms were visualized with FigTree v1.4.0 program (Rambaut 2010) and reorganized in Microsoft power point.

Results

Phylogenetic analysis of Beltraniaceae

The tree inferred from a RAxML analysis belonging to Beltraniaceae genera (Fig. 1). The combined LSU and ITS alignment comprised 30 strains of Beltraniaceae and the outgroup comprised two taxa, *Amphisphaeria flava* (MFLUCC 18-0361) and *A. sorbi* (MFLUCC 13-0721). Combined alignment as well as the individual alignments were analyzed. A best scoring RAxML tree (Fig. 1) has a final ML optimization likelihood value of –4404.133370. The matrix had 276 distinct alignment patterns, with 6.41% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.252948, C = 0.211572, G = 0.267559, T = 0.267921; substitution rates AC = 1.018207, AG = 2.648344, AT = 1.48916, CG = 0.752809, CT = 6.303354, GT = 1.00; proportion of invariable sites I = 0.672359; gamma distribution shape parameter α = 0.612156. Both trees of ML and BYPP analyses were similar in topology at the generic relationships, which is in agreement with previous studies based on multi-gene phylogeny of Lin et al. (2017b) and Hyde et al. (2020). Our isolate *Beltraniella fertilis* (MFLUCC 20-0119) clustered with *Beltraniella fertilis* (MFLUCC 17-2136, MFLUCC 17-2137, MFLUCC 17-2138, MFLUCC 19-0487) with 82% ML and 0.95 PP support.

Phylogenetic analysis of Stachybotryaceae

A best scoring RAxML tree resulted from the phylogenetic analyses of the combined calmodulin-ITS-tub2 alignment that comprised 40 strains of Strachybotryaceae and the outgroup taxon *Peethambara sundara* (CBS 646.77). The final ML optimization likelihood value of the best scoring RAxML tree (Fig. 2) is –15993.261619. The matrix had 870 distinct alignment patterns, with 24.41% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.221214, C = 0.301303, G = 0.257589, T = 0.219895; substitution rates AC = 1.284061, AG = 3.421068, AT = 1.422903, CG = 0.919545, CT = 4.917557, GT = 1.00; proportion of invariable sites I = 0.44495; gamma distribution shape parameter α = 1.165007. Trees of ML and BYPP were similar in topology in generic relationships, which is in agreement with previous studies by Jie et al. (2013), Lombard et al. (2016) and Hyde et al. (2020). Our isolate (MFLUCC 20-0120) is grouped with the type strain *Stachybotrys subcylindrospora* (HGUP 0201) with strong statistical support (ML = 98%, PP = 1.00).

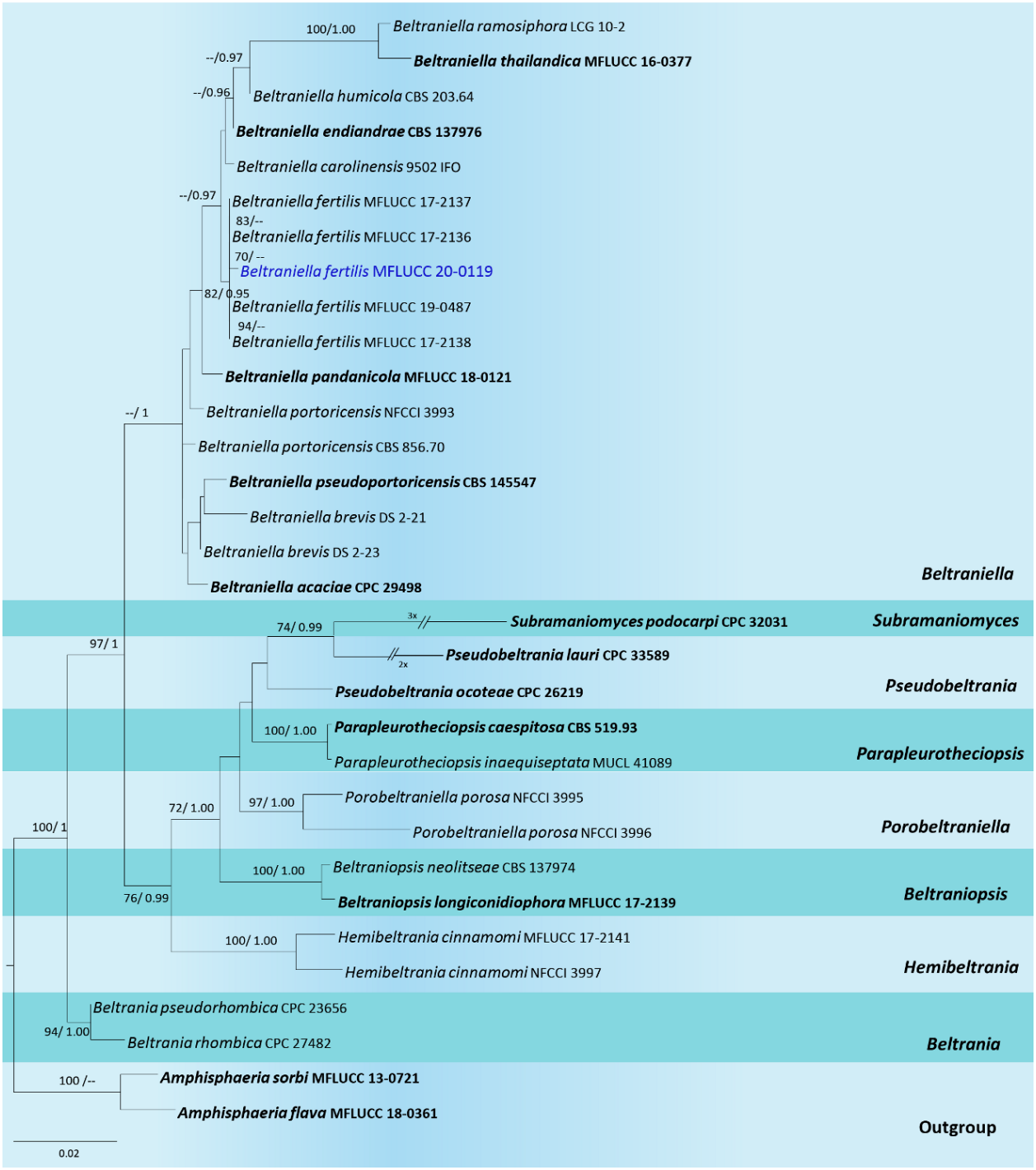

Fig. 1 – Maximum likelihood tree revealed by RAxML from an analysis of combined LSU-ITS matrix of *Beltraniella*, showing the phylogenetic position of *Beltraniella fertilis* (MFLUCC 20-0119). ML bootstrap supports equal to or greater than 60% and Bayesian posterior probabilities (PP) equal or greater than 0.95 are indicated above the nodes as (ML/ PP). The tree is rooted with *Amphisphaeria flava* (MFLUCC 18-0361) and *A. sorbi* (MFLUCC 13-0721). The ex-type strains are in bold and the new isolate of this study is in blue. The scale bar represents the expected number of nucleotide substitutions per site.

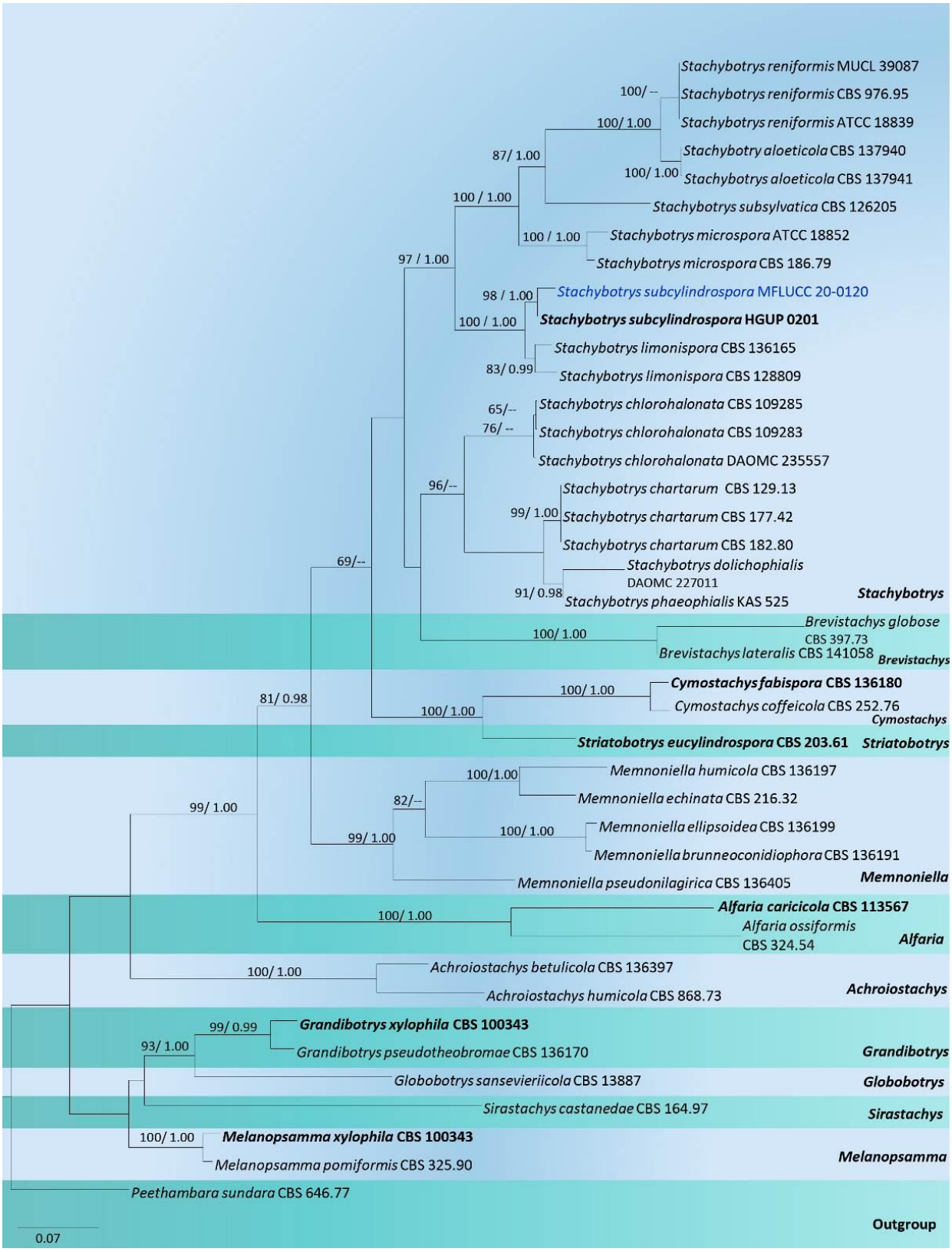

Fig. 2 – Maximum likelihood tree revealed by RAxML from an analysis of combined cmdA -ITS-tub2 matrix of Stachybotryaceae showing the phylogenetic position of *Stachybotrys subcylindrospora* (MFLUCC 20-0120). ML bootstrap supports equal to or greater than 60% and Bayesian posterior probabilities (PP) equal to or greater than 0.95 are indicated at the nodes as (ML/PP). The tree is rooted with *Peethambara sundara* (CBS 646.77). The ex-type strains are in bold and the new isolate of this study is in blue. The scale bar represents the expected number of nucleotide substitutions per site.

Taxonomy

Beltraniella fertilis Heredia, R.M. Arias, M. Reyes & R.F. Castañeda, Fungal Diversity 11: 100 (2002) Fig. 3

Index Fungorum number: IF489903; Faces of fungi number: FoF 03632

Colonies on PDA pale white, reaching a diam. of 2–3 cm in 4 days at 25ºC, flat and circular-shaped, pale brown, smooth at surface and produce highly branched melanized hyphae with brownish exudates in old cultures, after 16 weeks, with conidiophores forming on the mycelium; reverse light yellow to dark brown. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. *Mycelium* mostly immersed in the substratum, composed of septate, branched subhyaline hyphae. *Setae* numerous, erect, straight or flexuous, unbranched, single or in small groups, thick-walled, verrucose, dark brown at the base, paler at apex, 61.7–149.8 µm long, 2.5–7 µm wide ($\bar{x}$ = 113 × 4 µm, n = 30) at the base, tapering to a pointed apex. *Conidiophores* macronematous, sometimes setiform; single, straight, septate, partly verrucose, thick-walled to smooth-walled, 6.4–91.5 µm long, 2.3–6.5 µm wide ($\bar{x}$ = 37 µm × 3.4, n = 30), sometimes branched at the apical region, dark brown to sub hyaline at the swollen base, paler and slightly tapering towards a pointed apex. *Conidiogenous cells* holoblastic, monoblastic to polyblastic, integrated, terminal. *Conidia* solitary to aggregated, acrogenous, simple, dry, straight, smooth, thin-walled, biconic, turbinate to pyriform, rostrate to pointed at proximal end, rounded at distal end, hyaline to sub hyaline, 5–15 µm long, 2–6.5 µm wide ($\bar{x}$ = 10.11 × 3.97 µm, n = 30) in the broadest part.

Distribution – Atlantic forests, Brazil, Mexico, Thailand

Known hosts – Dead leaves of *Mangifera indica* and *Parinari alvimii*

Material examined – Thailand, Chiang Mai Province, Mae Tang district, Ban Pa Deng, Mushroom Research Center, N 19º 07’ 13.7”, E 98º 43’ 52,9”, 905 m, in forest soil (dominated by Dipterocarpaceae), 20th March 2019, Erandi Yasanthika, Erscm11 (MFLU 20-0506), living culture MFLUCC 20-0119.

Notes – *Beltraniella fertilis* was introduced by Heredia et al. (2002) based on morphological characteristics. The sequence data for this species was provided by Lin et al. (2017b) for strains MFLUCC 17-2136, MFLUCC 17-2137 and MFLUCC 17-2138 isolated from decaying leaves collected in Thailand. Most of *Beltraniella* strains in GenBank have only ITS and LSU sequence data. In the combined phylogenetic tree (LSU-ITS) of Beltraniaceae (Fig. 1), species delineation within *Beltraniella* has low bootstrap support. This low support can be due to the lack of protein-coding gene sequences, or else *B. fertilis* may be a species complex (Jeewon & Hyde 2016). Existing strains of *Beltraniella fertilis* (MFLUCC 17-2136, MFLUCC 17-2137, MFLUCC 17-2138 and MFLUCC 19-0487) and our isolate (MFLUCC 20-0119) clustered with good support (ML = 82%, PP = 0.95) in the phylogenetic tree. Our strain (MFLU 20-0506) is similar to the holotype of *B. fertilis* (CB712XAL) in having numerous setae in the colony, setiform conidiophores and solitary to aggregated, acrogenous, simple, dry, straight, smooth, thin-walled, biconic, turbinate to pyriform, rostrate to pointed at the proximal end, rounded at the distal end, hyaline to sub-hyaline conidia. However, compared to Heredia et al. (2002) and Lin et al. (2017b), the size of conidia is different and conidial formation from separating cells was not observed in our study. These morphological variations can be resulted due to the effects of different environmental conditions (Francisco et al. 2019). Based on both morphological characteristics and multigene phylogenetic analysis, we report this collection as *B. fertilis* from forest soils in Thailand.

Stachybotrys subcylindrospora C.Y. Jie, Y.L. Jiang, D.W. Li, E.H.C. McKenzie & Yong Wang bis, Mycological Progress 12 (4): 695 (2012) Fig. 4

Index Fungorum Number: IF821464; Facesoffungi number: FoF08728

Colonies on PDA, initially with abundant white to brownish aerial mycelium, mostly superficial, immersed at margins, forming lobate shaped edge reaching a diam. of 2–3 cm in 7 days at 25ºC; becoming amber to brownish with wrinkled granulated surface. After 3 weeks, conidiophores forming on the aerial mycelium becoming slimy with grey to black conidial masses;

reverse brownish center and amber at periphery. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. *Conidiophores* macronematous, mononematous, single or in groups, determinate, thin-walled, simple to irregularly branched, erect to slightly curved, hyaline to sub-hyaline, uniseptate, smooth, 22–70 µm long, 2–6 µm wide ($\bar{x}$ = 50 × 4 µm, n = 30) with curved base, terminating in phialidic conidiogenous cells. *Phialides* in groups of 3–6 on the apices of conidiophores, discrete, clavate to subclavate, hyaline, smooth, 6–11 µm long and 2–6 µm wide ($\bar{x}$ = 9 × 5 µm, n = 30), with conspicuous collarettes. *Conidia* acrogenous, aggregated in slimy masses, aseptate, cylindrical or subcylindrical, thick-walled, truncated at base, rounded at the apex, hyaline when young, becoming subhyaline to brown at maturity, verruculose, 6–11 µm long, 4–6 µm wide ($\bar{x}$ = 10 × 4.5 µm, n = 30); young conidia bear delicate irregular to circular striations and become 1–3 guttulate when mature.

Distribution – Hainan Province in China and Thailand

Known hosts – Tropical primeval rain forest soil

Material examined – Thailand, Chiang Mai Province, Mae Tang district, Ban Pa Deng, Mushroom Research Center, N 19^{O} 07' 13.7", E 98^{O} 43' 52,9", 905 m, in forest soil (dominated by Dipterocarpaceae), 20th March 2019, Erandi Yasanthika, Er202 (MFLU 20-0505); living culture MFLUCC 20-0120

Notes – In the multigene phylogenetic analysis (Calmodulin-ITS-β-tubulin) of *Stachybotrys*, our strain (MFLUCC 20-0120) grouped with the type strain of *S. subcylindrospora* (HGUP 0201). Our isolate is morphologically similar to *S. subcylindrospora* (HGUP 0201) in having cylindrical conidia. However, conidia of *S. subcylindrospora* (HGUP 0201) have irregular striations on the surface (Jie et al. 2013), while immature conidia of our strain (MFLUCC 20-0120) bears circular to irregular surface striations and 1–3 guttules at maturity. These changes may be due to an adaptation to withstand diverse environmental stresses (Francisco et al. 2019). Based on both morphological characteristics and multigene phylogenetic analysis, we report our collection (MFLUCC 20-0120) as *S. subcylindrospora* from forest soils in Thailand.

Discussion

Beltraniella fertilis recorded on dead leaves of *Mangifera indica* and *Parinari alvimii* (Heredia et al. 2002, Magalhães et al. 2011). *Beltraniella fertilis* has been recorded from both living plants and dead or decomposing leaves (Heredia et al. 2002, Marques et al. 2007). Therefore, we presume that *B. fertilis* can switch its life mode from endophytic to saprobic based on the availability of nutrients (Promputtha et al. 2010). Lin et al. (2017b) recorded this species on dead leaves from Thailand and provided molecular data for the first time. We report on *B. fertilis* from forest soils in Thailand for the first time. In this study, *B. fertilis* was isolated by soil dilution plating method. The sporulation barely occurred on PDA (25ºC) as very few spores were formed even after 3–4 months of incubation.

Beltraniella fertilis and *B. botryospora* are morphologically similar in having two types of conidiophores (long setiform and short non-setiform), polyblastic conidiogenesis, separating cells and turbinate conidia (Shirouzu et al. 2010). However, *B. fertilis* has short setae and narrower conidia that can be distinguished from *B. botryospora* (Lin et al. 2017b).

Stachybotrys subcylindrospora was previously reported from tropical primeval rain forest soil of Hainan Province in China (Jie et al. 2013). Based on phylogenetic inference of ITS sequence data by Jie et al. (2013) and Lombard et al. (2016), *S. subcylindrospora* is closely related to *S. limonispora*, *S. sansevieriae* and *S. zeae*. However, this is the first study that uses combined cmdA, ITS and tub2 sequence data for *S. subcylindrospora*, which further validates the phylogenetic placement of the taxa within the genus.

Stachybotrys limonispora is phylogenetically adjacent to *S. subcylindrospora*, and both species reported from the soil. However, *S. limonispora* is morphologically distinct from *S. subcylindrospora* in having limoniform conidia (Lombard et al. 2016). Jie et al. (2013) mentioned that *S. subcylindrospora* has cylindrical conidia similar to *S. eucylindrospora* and *S. longispora*. However, *S. longispora* contains smooth conidia that are different from *S.*

subcylindrospora. Conidia in *S. eucylindrospora* contain longitudinal striations, while *S. subcylindrospora* shows circular to irregular striations in immature conidia and 1–3 guttules when mature. This is the first geographical record of *S. subcylindrospora* from forest soils of Thailand.

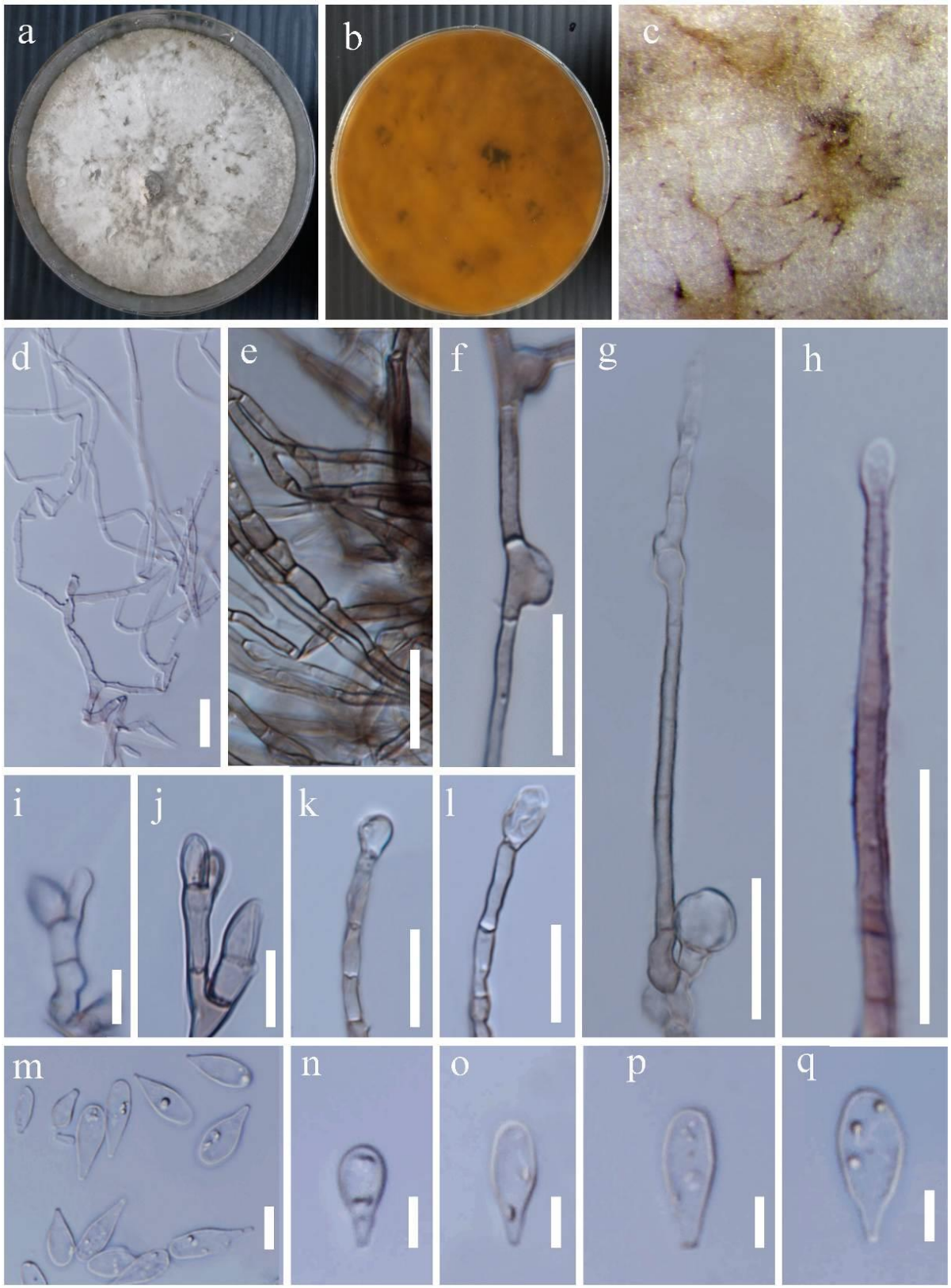

Fig. 3 – *Beltraniella fertilis* (MFLUCC 20-0119). a Mature colony on PDA after 16 weeks with the sporulation. b Reverse of the colonies on PDA after 16 weeks. c Sporulation of the colony with conidial attachments on the mycelium. d Immature aseptate hyphae. e Mature pigmented, septate

hyphae. f Chlamydospores on the mycelium. g Verrucose pigmented setae arising from the mycelium. h Conidiogenesis on the setae. i–l Conidiogenesis on the conidiophore. m–q Conidia. Scale bars: g = 25 µm d, h, i = 20 µm, e, f, j, k, l, m = 10 µm, n–q = 5 µm.

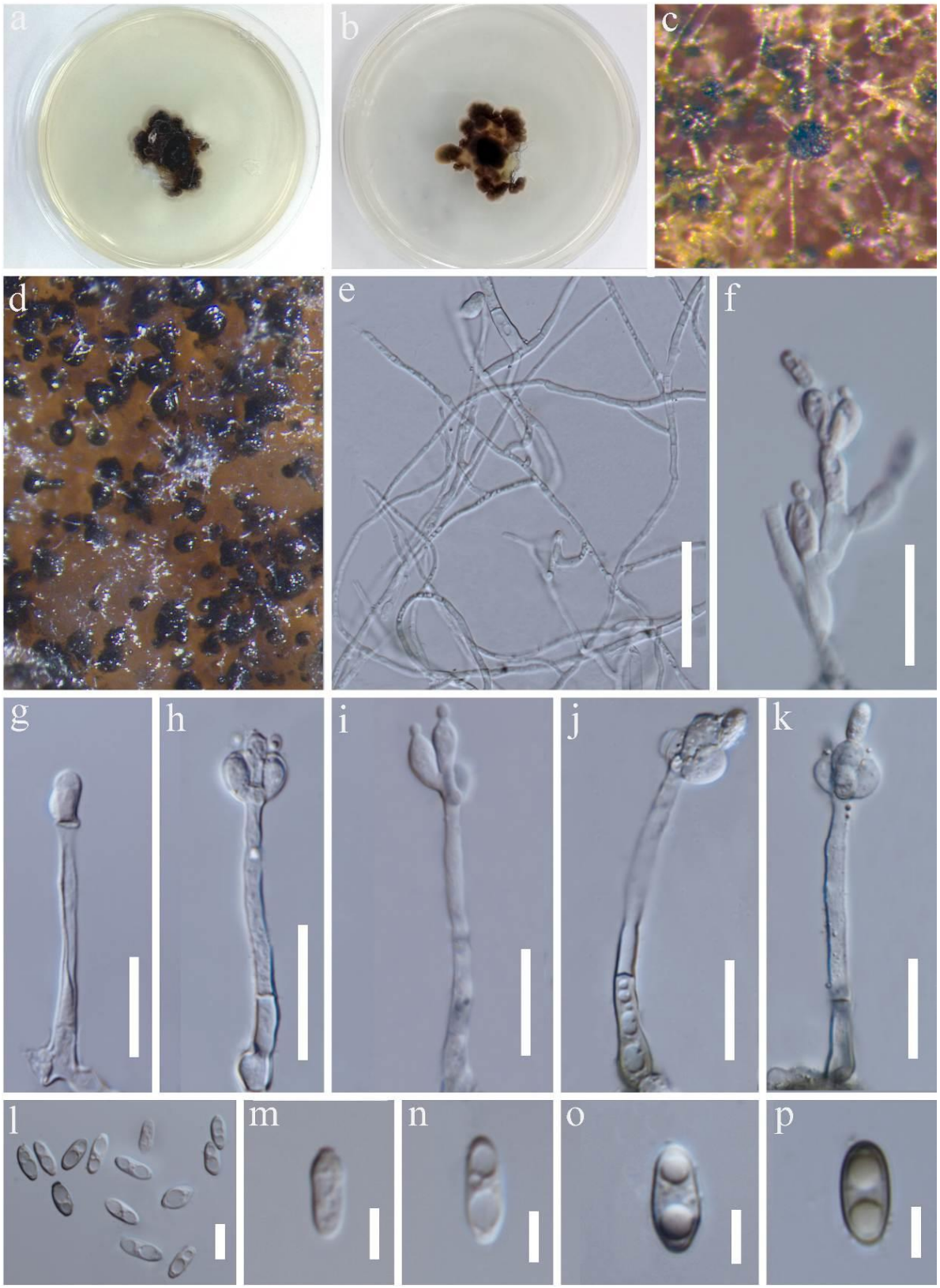

Fig. 4 – *Stachybotrys subcylindrospora* (MFLUCC 20-0120). a Mature colony on PDA after 3 weeks with the sporulation. b Reverse of the colonies on PDA after 3 weeks. c, d Sporulation of the colony appear grey to black with conidial attachments on the mycelium. e Mycelium with aseptate

hyphae. f–k Conidiogenesis cells and conidia attached on the conidiophore. l–p Conidia. Scale bars: e– k = 20 μm, l = 10 μm, m–p = 5μm

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