



Stachybotrys-like taxa from karst areas and a checklist of stachybotrys-like species from Thailand

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Abstract

During a survey of hyphomycetes from karst areas in Thailand, four stachybotrys-like taxa, viz., *Cymostachys garethjonesii* sp. nov., *Memmoniella oblongispora* sp. nov., *M. nilagirica* comb. nov. and *Stachybotrys microspora* were identified and are provided with descriptions in this paper. The new species are introduced based on morphological and molecular differences and compared with similar or related taxa. *Memmoniella nilagirica* and *Stachybotrys microspora* are new records for Thailand. An annotated checklist of stachybotrys-like taxa in Thailand is provided based on previous publications and database searches.

Key words – karst – new species – phylogeny – *Stachybotryaceae* – taxonomy

Introduction

The family *Stachybotryaceae*, typified by *Stachybotrys* Corda, was established by Crous et al. (2014) to accommodate *Myrothecium* Tode, *Peethamabra* Subram. & Bhat and *Stachybotrys* Corda. Maharachchikumbura et al. (2015, 2016) accepted four additional genera *Albosynnema* E.F. Morris, *Parasarcopodium* Melnik et al., *Sarcopodium* Ehrenb. and *Scopinella* Lév. within this family, based on morphological features and phylogenetic analysis. Lombard et al. (2016) revised this family by combining morphology and multi-locus phylogenetic analyses using *cmdA*, ITS, *rpb2*, *tef1* and *tub2* datasets. Presently, 33 genera are accepted in the family *Stachybotryaceae* (Lombard et al. 2016).

The genus *Stachybotrys* (*Stachybotryaceae*, Hypocreales) was established by Corda (1837) to accommodate *Stachybotrys atra* Corda 1837 (now known as *S. chartarum* (Ehrenb.) S. Hughes 1958) (Bisby 1943, Seifert et al. 2011). *Stachybotrys* is characterized by macronematous, mononematous, branched or unbranched conidiophores, with discrete, determinate, terminal, phialidic conidiogenous cells, with unicellular, smooth or variously ornamented conidia, produced in a slimy mass or in chains (Ellis 1971, Jong & Davis 1976, Pinruan et al. 2004b, Seifert et al.

2011, Wang et al. 2015). The genus *Stachybotrys* is widely distributed, and can commonly be found on damp paper, cotton, linen, soil and decaying plant material, and cellulose-based building material, such as drywall and wall paper in indoor environments (Ellis 1971, 1976, Whitton et al. 2001, Tang et al. 2003, Li & Yang 2004, Thongkantha et al. 2008, Izabel et al. 2010, Jie et al. 2013, Wang et al. 2015).

The genus *Memmoniella* Höhn. and *Stachybotrys* were considered to be separate genera (Bisby 1945, Jong & Davis 1976), however, Wang et al. (2015) reevaluated these genera based on morphology and phylogeny, and proposed *Memmoniella* as a synonym of *Stachybotrys*, the latter with 75 accepted species. However, Lombard et al. (2016) resurrected *Memmoniella* following combined morphology and multi-locus phylogenetic analyses using *cmdA*, ITS, *rpb2*, *tef1* and *tub2*.

The genus *Cymostachys* L. Lombard & Crous, typified by *C. fabispora* L. Lombard & Crous, was established by Lombard et al. (2016) to accommodate two stachybotrys-like species, *C. coffeicola* L. Lombard & Crous and *C. fabispora* L. Lombard & Crous, with irregularly cymosely branched conidiophores and olivaceous brown to dark brown, fabiform conidia.

Karst is the term used to describe a special style of landscape containing caves and extensive underground water systems that is developed on especially soluble rocks, such as limestone, marble, and gypsum (Ford & Williams 2007). Such areas are characterized by sinking streams, caves, enclosed depressions, fluted rock outcrops, and large springs (Ford & Williams 2007). A huge number of fungi, bacteria, lichen, algae and other microorganisms are present in the karst areas, and play an important role in karstification processes, especially the weathering and dissolution of carbonate rocks (Viles 1984, Lian et al. 2008).

During a survey of hyphomycetes from karst areas in Thailand, four stachybotrys-like species were identified and are described in this paper. Two are new species in the genera *Cymostachys* and *Memmoniella*; one is new combination and two are new records for Thailand. In addition, an annotated checklist of stachybotrys-like fungi in Thailand is provided based on previous publications and databases.

Materials & Methods

Collection and isolation of fungi

Dead litter (stems, wood, and leaves) from a variety of plants were collected during May to August 2015 from four karst areas in Thailand, viz., (1) Pha Chang (19°49'52.44", 100°01'30.64), Wiang Chai District, Chiang Rai Province; (2) Ang Kep Nam Wat Tham Khao Hin Phayanak (Wat Tham Sao Hin Payanak) (20°19'16.58"–20°19'30.12"N, 99°51'40.72"–99°51'54.50"E), Mae Sai District, Chiang Rai Province; (3) Khao Lom Muak (11°47'3.96"–11°47'11.24"N, 99°48'49.13"–99°49'0.63"E), Prachuap Khiri Khan Province and (4) Khao Lan (11°35'36"N, 99°35'38"E), Bang Saphan District, Prachuap Khiri Khan Province. Samples were taken to the laboratory in Zip-lock plastic bags for examination. The specimens were incubated in sterile moist chambers and examined from time to time using a stereo microscope (Motic SMZ 168). Fungi were removed with a needle and placed in a drop of distilled water on a slide for morphological study. Photographs of fungal structures were captured using a Nikon ECLIPSE 80i compound microscope with a Canon 450D digital camera. All measurements were made by Tarosoft (R) Image FrameWork program. Photo plates were made with Adobe Photoshop CS6 Extended version 13.0.1 (Adobe Systems, USA). Isolation onto potato dextrose agar (PDA) or malt extract agar (MEA) was performed by single spore isolation method (Chomnunti et al. 2014). Herbarium material is deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand. Cultures are deposited at Mae Fah Luang University Culture Collection (MFLUCC) and International Collection of Microorganisms from Plants (ICMP). Faces of fungi numbers are given for the reference specimens as explained in Jayasiri et al. (2015).

Table 1 GenBank accession numbers of isolates included in this study

Species	Isolate number ^a	<i>cmdA</i>	ITS	<i>rpb2</i>	<i>tef1</i>	<i>tub2</i>
<i>Cymostachys coffeicola</i>	CBS 252.76	KU846035	KU846052	KU846081	KU846097	KU846113
<i>C. coffeicola</i>	CPC 25009	– ^b	KU846053	–	–	–
<i>C. fabispora</i>	CBS 136180 = MUCL 39004 = INIFAT C93/322	KU846036	KU846054	KU846082	KU846098	KU846114
<i>C. fabispora</i>	CPC 24352	–	KU846055	KU846083	KU846099	–
<i>C. garethjonesii</i>	MFLUCC 16-0028	–	KU760375	KU760395	–	KY124126
<i>Memmoniella brunneoconidiophora</i>	CBS 109477	–	KU846138	KU846192	KU846218	KU846243
<i>M. brunneoconidiophora</i>	CBS 136191 = MUCL 43313	KU846116	KU846139	KU846193	KU846219	KU846244
<i>M. dichroa</i>	ATCC 18913 = IMI 61337	–	AF081472	–	–	–
<i>M. dichroa</i>	CBS 526.50 = ATCC 18917 = IMI 017506 = MUCL 9482	KU846117	KU846140	KU846194	KU846220	–
<i>M. dichroa</i>	CBS 123800	KU846118	KU846141	KU846195	KU846221	–
<i>M. echinata</i>	CBS 216.32	KU846119	KU846142	KU846196	KU846222	KU846245
<i>M. echinata</i>	CBS 304.54	KU846120	KU846143	KU846197	KU846223	–
<i>M. echinata</i>	CBS 343.50	KU846121	KU846144	KU846198	KU846224	KU846246
<i>M. echinata</i>	CBS 344.39	KU846122	KU846145	KU846199	KU846225	KU846247
<i>M. echinata</i>	CBS 406.80	KU846123	KU846146	KU846200	KU846226	KU846248
<i>M. echinata</i>	CBS 627.66 = IMI 045547 = NRRL 2181	KU846124	KU846147	KU846201	KU846227	KU846249
<i>M. echinata</i>	DAOMC 173162	KU846125	JN942886	KU846202	KU846228	KU846250
<i>M. echinata</i>	DAOMC 235365	KU846126	KU846149	KU846203	KU846229	KU846251
<i>M. ellipsoidea</i>	CBS 136199 = MUCL 39088	KU846127	KU846150	KU846204	KU846230	KU846252
<i>M. ellipsoidea</i>	CBS 136200 = MUCL 39089	KU846128	KU846151	KU846205	KU846231	KU846253
<i>M. ellipsoidea</i>	CBS 136201 = MUCL 39090	KU846129	KU846152	KU846206	KU846232	KU846254
<i>M. ellipsoidea</i>	CBS 136202 = MUCL 41876	–	KU846153	KU846207	KU846233	KU846255
<i>M. humicola</i>	CBS 463.74	KU846130	KU846154	KU846208	KU846234	–
<i>M. longistipitata</i>	ATCC 22699	–	AF081471	–	–	–
<i>M. longistipitata</i>	CBS 136197 = MUCL 33065	KU846131	KU846155	KU846209	KU846235	KU846256
<i>M. nilagirica</i>	MFLUCC 15-0660	KY124122	KU760374	KU760394	–	–
<i>M. oblongispora</i>	MFLUCC 15-1074	KY124123	KU760376	KU760396	–	KY124127
<i>M. oenanthes</i>	ATCC 22844 = IMI 016185	–	AF081473	–	–	–
<i>M. oenanthes</i>	CBS 388.73 = ATCC 32255	–	KU846156	KU846210	KU846236	–
<i>M. pseudonilagirica</i>	CBS 136405 = MUCL 39120	KU846132	KU846157	KU846211	KU846237	KU846257

Species	Isolate number^a	<i>cmdA</i>	ITS	<i>rpb2</i>	<i>tef1</i>	<i>tub2</i>
<i>M. putrefolia</i>	CBS 101177	–	KU846158	KU846212	KU846238	KU846258
<i>M. putrefolia</i>	CBS 136171 = MUCL 41166 = INIFAT C98/65-2	KU846133	KU846159	KU846213	KU846239	KU846259
<i>Peethambarara sundara</i>	CBS 646.77	–	KU846471	KU846509	KU846531	KU846551
<i>Stachybotrys aloeticola</i>	CBS 137940 = CPC 19705	KU846570	KJ817888	KU846901	–	KJ817886
<i>S. aloeticola</i>	CBS 137941 = CPC 19706	KU846571	KJ817889	KU846902	–	KJ817887
<i>S. chartarum</i>	CBS 182.80	KU846573	KU846679	KU846904	KU847003	KU847115
<i>S. chartarum</i>	CBS 363.49	KU846575	KU846681	KU846906	KU847005	KU847117
<i>S. chartarum</i>	CBS 119366	KU846591	KU846697	KU846922	KU847021	KU847132
<i>S. chartarum</i>	CBS 119369	KU846592	KU846698	KU846923	KU847022	KU847133
<i>S. chartarum</i>	CBS 119370	KU846593	KU846699	KU846924	KU847023	KU847134
<i>S. chartarum</i>	CBS 119371	KU846594	KU846700	KU846925	KU847024	KU847135
<i>S. chlorohalonata</i>	CBS 109283	KU846622	KU846728	KU846953	KU847052	KU847163
<i>S. chlorohalonata</i>	CBS 109285	KU846623	KU846729	KU846954	KU847053	KU847164
<i>S. chlorohalonata</i>	CBS 136158 = MUCL 49910	KU846626	KU846732	KU846956	KU847056	KU847167
<i>S. limonispora</i>	CBS 128809	KU846629	KU846735	KU846959	KU847058	KU847170
<i>S. limonispora</i>	CBS 136165 = MUCL 18730	KU846630	KU846736	KU846960	KU847059	KU847171
<i>S. microspora</i>	MFLUCC 15-0830	KY124124	KU760377	KU760397	KU760392	KY124128
<i>S. microspora</i>	MFLUCC 15-1076	KY124125	KU760378	KU760398	KU760393	KY124129
<i>S. microspora</i>	ATCC 18852 = IMI 124902	–	AF081475	–	–	–
<i>S. microspora</i>	CBS 186.79	KU846631	KU846737	DQ676580	KU847060	KU847172
<i>S. phaeophialis</i>	KAS 525	KU846632	KU846738	KU846962	KU847061	KU847173
<i>S. reniformis</i>	ATCC 18839	–	AF081476	–	–	–
<i>S. reniformis</i>	CBS 976.95	KU846633	KU846739	KU846963	KU847062	KU847174
<i>S. reniformis</i>	CBS 136198 = MUCL 39087	–	KU846740	–	KU847063	–
<i>S. subsylvatica</i>	CBS 126205	KU846634	KU846741	KU846964	KU847064	KU847175
<i>Striatobotrys atypica</i>	CBS 141059 = CPC 18423	KU846646	KU846753	KU846973	KU847076	KU847187
<i>Stri. eucylindrospora</i>	CBS 203.61 = ATCC 18851 = IMI 085334 = MUCL 9483	KU846648	KU846755	KU846975	KU847078	KU847189
<i>Stri. humicola</i>	CBS 102408	KU846650	KU846759	KU846979	KU847082	KU847193
<i>Stri. oleronensis</i>	CBS 137258	–	KF777192	KU846980	KU847083	KU847194
<i>Stri. rhabdospora</i>	CBS 528.80	KU846651	KU846760	KU846981	KU847084	KU847195
<i>Stri. yuccae</i>	CBS 390.68	KU846657	KU846770	KU846989	KU847093	KU847205

ATCC, American Type Culture Collection, Manassas, United States; **CBS**, Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; **CPC**, Culture collection of Pedro Crous, housed at CBS; **DAOM**, Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada; **IMI**, International Mycological Institute, CABI-Bioscience, Egham, Boreham Lane, UK; **INIFAT**, INIFAT Fungus Collection, Ministerio de Agricultura Habana, Cuba; **KAS**, Collection of K.A. Seifert; **MUCL**, Mycothèque de l'Université Catholique de Louvain, Belgium; **NRRL**, Agricultural Research Service Culture Collection, Peoria, Illinois, United States.

^a Ex-type and ex-epitype cultures are in bold

^b No data in GenBank.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fungal mycelia grown on PDA or MEA at room temperature with the Fungal gDNA Kit (BioMIGA, USA) according to the manufacturer's instructions. The internal transcribed spacer region of ribosomal DNA (ITS), small subunit nuclear ribosomal DNA (SSU), large subunit nuclear ribosomal DNA (LSU), RNA polymerase II second largest subunit (*rpb2*), calmodulin (*cmdA*) and β -tubulin (*tub2*) genes were amplified via polymerase chain reaction (PCR) using the following primers: ITS5 and ITS4 (White et al. 1990) for ITS, NS1 and NSS4 (White et al. 1990) for SSU, LROR and LR5 (Vilgalys & Hester 1990) for LSU, rpb2H-6F2 and rpb2H-7R2 (Koster et al. 2009) for *rpb2*, CAL-228F and CAL2Rd for *cmdA* (Carbone & Kohn 1999, Groenewald et al. 2013), and Bt2a and Bt2b for *tub2* (Glass & Donaldson 1995). The PCR products were sequenced with the same primers.

Phylogenetic analyses

Original sequences from the sequencing company were checked using BioEdit version 7.0.5.3 (Hall 1999). Most of the sequences were obtained from GenBank based on previous publications Lombard et al. (2016). The remaining homogenous sequences were obtained by BLAST searches (Altschul et al. 1990) of sequences in GenBank. All sequences used in this study are listed in Table 1. Alignments for each locus were done in MAFFT v7.212 (Kato & Standley 2013) and manually verified in MEGA 6.06 (Tamura et al. 2013). Conserved blocks were selected from the initial alignments with Gblocks 0.91b (Castresana 2000). The interleaved NEXUS files were formatted with PAUP*4.0b10 (Swofford 2002) and manually formatted for Bayesian inference analyses. Bayesian inference (BI), maximum parsimony (MP) and maximum likelihood (ML) were used in this study for phylogenetic analyses. For Bayesian inference analysis, the best model of evolution was determined using MrModeltest v2 (Nylander 2004). Bayesian inference analysis was done with MrBayes v 3.2.5 (Ronquist et al. 2012). Maximum parsimony analysis was performed in PAUP*4.0b10 (Swofford 2002). Maximum likelihood analysis was performed in raxmlGUI v 1.3.1 (Silvestro & Michalak 2012). Phylogenetic trees were drawn with TreeView 1.6.6 (Page 1996).

Annotated checklist

An annotated checklist of the genus *Stachybotrys* in Thailand was made based on previous publications (Jeamjitt et al. 2006, Pinnoi et al. 2006, Bhilabutra et al. 2010) and the USDA fungal database (Farr & Rossman 2015). The host/substrate and location information are included in this list.

Results

Phylogenetic analyses

The aligned sequence matrix comprises *cmdA* (774 bp), ITS (612 bp), *rpb2* (721 bp), *tef1* (643 bp) and *tub2* (433 bp) sequence data for 28 taxa and one outgroup taxon with a total of 3183 total characters, of which 1181 are parsimony informative, 205 are parsimony uninformative, and 1797 characters are constant. The result of maximum parsimony (MP) analysis based on combined *cmdA*, ITS, *rpb2*, *tef1* and *tub2* sequence data is shown in Fig. 1.

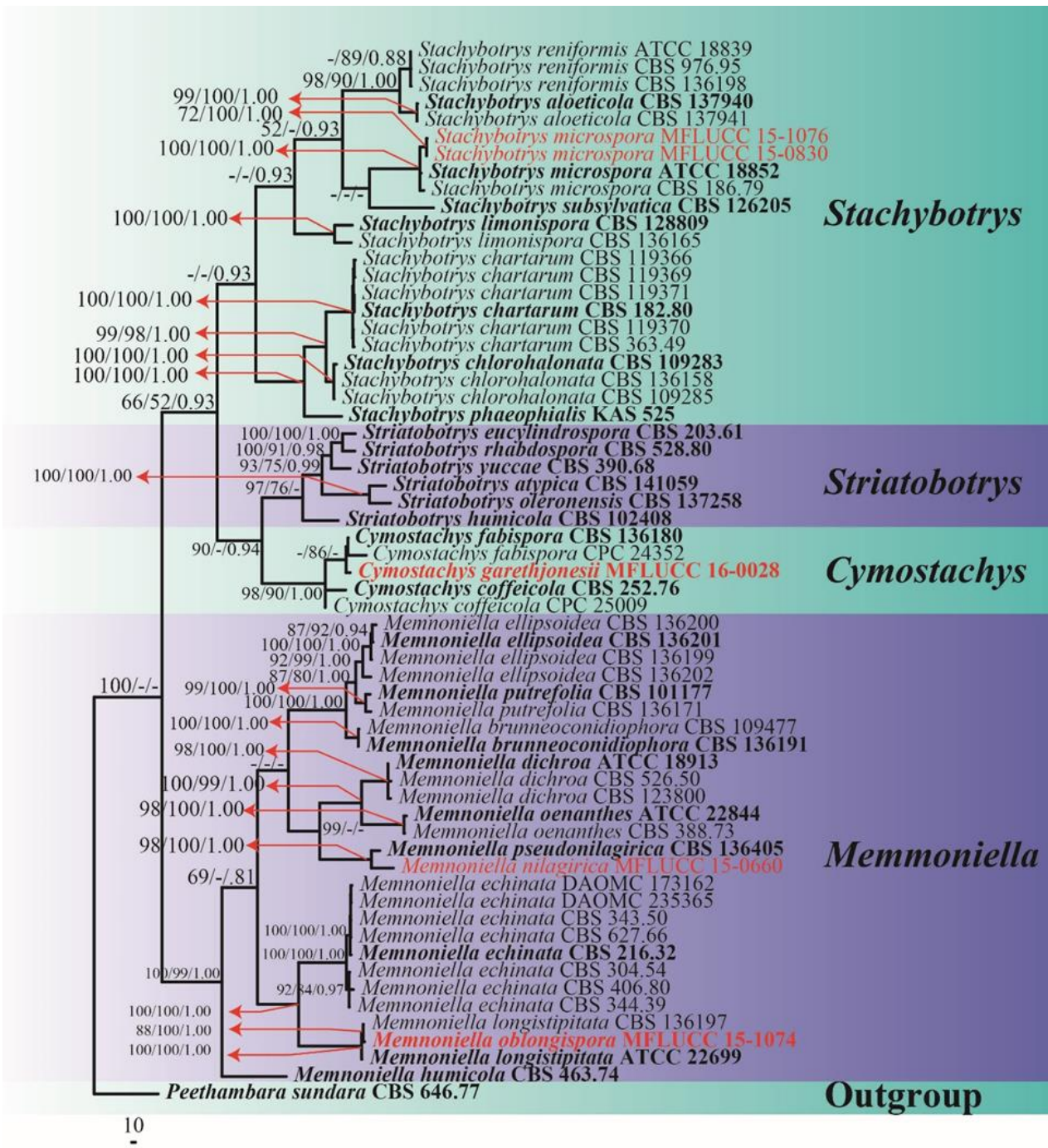


Fig. 1 – Phylogenetic tree generated from maximum parsimony (MP) analysis based on combined *cmdA*, *ITS*, *rpb2*, *tef1* and *tub2* sequence data for selected genera within the family *Stachybotryaceae*. Bootstrap support values for maximum parsimony (MP) and maximum likelihood (ML) greater than 50% and Bayesian posterior probabilities greater than 0.8 are indicated above or below the nodes as MPBS/MLBS/PP. The ex-type strains are in bold and the new isolates are in red. The tree is rooted with *Peethambara sundara* (CBS 646.77).

In the present study, we found that the strains of *S. microspora* (MFLUCC 15-1076 and MFLUCC 15-0830) form a clade together with the strain of *S. microspora* (ATCC 18852 and CBS 186.79) with 100% MP bootstrap support, 100% ML bootstrap support and 100% Bayesian posterior probabilities, sister to *S. subsylvatica* (CBS 126205). *Cymostachys garethjonesii* (MFLUCC 16-0028) forms a well-supported clade (BSMP = 98%, BSML = 90%, BYPP = 1.00) with *C. fabisporea* (CBS 136180 and CPC 24352) and *C. coffeicola* (CBS 252.76 and CPC 25009),

sister to the *Striatobotrys* clade. *Memnoniella oblongispora* (MFLUCC 15-1074) and the ex-type strain of *M. longistipitata* (ATCC 22699) grouped together with BSMP = 100%, BSML = 100% and BYPP = 1.00. *Memnoniella nilagirica* (MFLUCC 15-0660) forms a clade with the strain of *M. pseudonilagirica* (CBS 136405) with 98% MP bootstrap support, 100% ML bootstrap support and 100% Bayesian posterior probabilities.

Taxonomy

Four stachybotrys-like taxa were identified and are described below.

Cymostachys garethjonesii C.G. Lin, Yong Wang bis & K.D. Hyde, **sp. nov.**

Fig. 2

Index Fungorum number – IF 552555

Facesoffungi number – FoF 02017

Etymology – Named in honour of Professor E.B. Gareth Jones for his immense contribution to marine and tropical mycology.

Holotype – MFLU 15-3272

Colonies on PDA effuse, hairy to powdery, circular, grey to orange on top side and reverse side, attaining a diameter of 2–2.5 cm in 20 days at 25°C. *Mycelium* partly superficial and partly immersed. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. *Conidiophores* macronematous, mononematous, caespitose, erect, irregularly cymosely branched, occasionally undulating at the upper part of branches, smooth or occasionally verruculose, thick-walled, septate, hyaline at the base, light grey at the apex, bearing at its apex a crown of 2–3(–4) phialides, up to 222 µm long, 3–5 µm (\bar{x} = 3.6 µm, n = 45) wide at the base, 1.5–3 µm wide (\bar{x} = 2.3 µm, n = 40) near the apex. *Conidiogenous cells* monophialidic, discrete, determinate, terminal, clustered at the apex of conidiophores, oval, obovoid, oblong, rarely verruculose, subhyaline to grey at the base, subhyaline to light grey at the middle part, dark grey to black at the apex, 6–10.5 µm (\bar{x} = 8.3 µm, n = 60) long, 3–5 µm (\bar{x} = 4.0 µm, n = 50) wide at the widest point. *Conidia* aggregated in large, slimy, black and glistening heads, acrogenous, simple, reniform, fabiform, rounded at both ends, rough-walled, dark brown to black, unicellular, 5–9 µm (\bar{x} = 7.0 µm, n = 50) long, 3.5–6.5 µm (\bar{x} = 4.8 µm, n = 50) wide at the widest point.

Material examined – Thailand, Prachuap Khiri Khan, Bang Saphan District, Khao Lan, 11°35'36"N 99°35'38"E, on decaying *Dracaena* sp. leaf, 29 July 2015, Chuan-Gen Lin, KLM 10-1 (MFLU 15-3272, **holotype**), ex-type living culture MFLUCC 16-0028.

Notes – In the phylogenetic tree generated from MP analysis based on combined *cmdA*, ITS, *rpb2*, *tef1* and *tub2* sequence data, *Cymostachys garethjonesii* (MFLUCC 16-0028) clusters within *Cymostachys* with good support (Fig. 1).

This new species is different from *C. coffeicola* and *C. fabispora* by having longer and narrower conidiophores, smaller conidiogenous cells, and slightly larger conidia. A crown of 2–3(–4) phialides are borne at the apex of each conidiophore in the new species, whereas, the crown contains 2–6 phialides in *C. coffeicola* and *C. fabispora*. *Cymostachys garethjonesii* is similar to the description of *S. sinuatophora* in Matsushima (1971) and Wang et al. (2015) as both have reniform conidia and sometimes *C. garethjonesii* has undulating conidiophores. However, the conidiogenous cells of *C. garethjonesii* (3–5 µm) are smaller than those of *S. sinuatophora* (5–7 µm), and the conidia are also smaller (5–9 × 3.5–6.5 µm vs. 8–12 × 6–7 µm).

The most significant difference between *C. garethjonesii* and *S. sinuatophora* is the different branching of the conidiophores. Conidiophores of *C. garethjonesii* are irregularly cymosely branched, which is the distinct feature of the genus *Cymostachys*, while, conidiophores of *S. sinuatophora* are repeatedly, alternately branched. Jong and Davis (1976) proposed *S. sinuatophora* and *S. reniformis* as synonyms of *S. nephrospora*, however, Pinruan et al. (2004a), Wang et al. (2015) and Lombard et al. (2016) proposed *S. sinuatophora* to be a distinct species, because of its repeatedly, alternately branched, undulating to coiling conidiophores, which are not known for other stachybotrys-like species

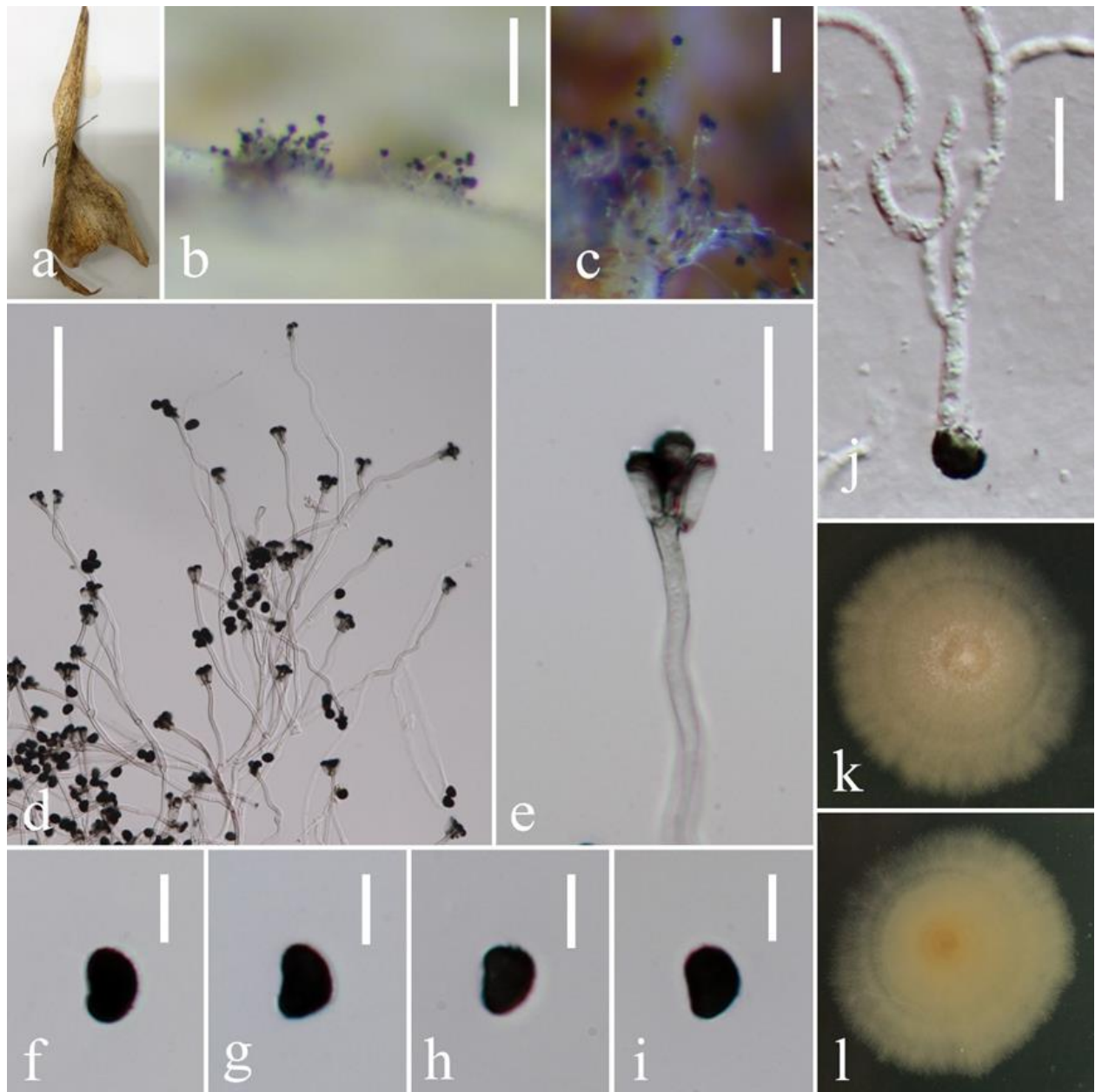


Fig. 2 – *Cymostachys garethjonesii* (MFLU 15-3272, holotype) **a.** Host leaves. **b, c.** Conidiophores on the host surface. **d.** Conidiophores and conidia. **e.** Conidiogenous cells. **f–i.** Conidia. **j.** Germinating conidium. **k, l.** 40-day old colonies on PDA, k from above, l from below. – Scale bars: b = 200 μ m, c–d = 100 μ m, e, j = 20 μ m, f–i = 10 μ m.

Memnoniella oblongispora C.G. Lin, McKenzie, Yong Wang bis & K.D. Hyde, **sp. nov.** Fig. 3

Index Fungorum number – IF 552085

Facesoffungi number – FoF 02081

Etymology – In reference to the oblong conidia.

Holotype – MFLU 15-3269

Colonies on PDA, effuse, gelatinous, circular, white to grey from center to the edge from above, light yellow to grey from center to the edge from below, attaining a diameter of 2.5–3.5 cm in 21 days at 25°C. *Mycelium* partly superficial and partly immersed. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. *Conidiophores* macronematous, mononematous, erect, simple, straight or flexuous, mostly unbranched, but sometimes branched, smooth, thick-walled, septate, bearing at its apex a crown of phialides, hyaline at the base, olive grey at the apex,

85–180 μm (\bar{x} = 125.6 μm , n = 20) long, 6.5–12 μm (\bar{x} = 9.5 μm , n = 20) wide at the base, tapering to 2.5–5 μm (\bar{x} = 3.9 μm , n = 30) at the narrowest point near the apex, sometimes swelling again below the conidiogenous cells. *Conidiogenous cells* monophialidic, discrete, determinate, terminal, clustered at the apex of conidiophores, ellipsoidal, clavate, cylindrical, the outer ones somewhat curved, smooth, hyaline to olive green, 9–14 μm (\bar{x} = 11 μm , n = 40) long, 4–6.4 μm (\bar{x} = 5 μm , n = 40) wide at the widest point. *Conidia* aggregated in large, slimy, black and glistening heads, acrogenous, simple, oblong, verrucose, hyaline when young, olive green to black when mature, unicellular, 8.5–12 μm (\bar{x} = 10.1 μm , n = 80) long, 4.5–7 μm (\bar{x} = 5.8 μm , n = 80) wide at the widest point.

Material examined – THAILAND, Chiang Rai, Mae Sai District, Ang Kep Nam Wat Tham Khao Hin Phayanak (Wat Tham Sao Hin Payanak), 20°19'16.58"–20°19'30.12"N, 99°51'40.72"–99°51'54.50"E, on decaying *Quercus* sp. leaf, 19 June 2015, Chuan-Gen Lin, WTSP 17-2 (MFLU 15-3269, **holotype**), ex-type living culture MFLUCC 15-1074.

Notes – Morphologically this species is most similar to *M. dichroa* (Grove) L. Lombard & Crous, *M. longistipitata* D.W. Li, Chin S. Yang, Vesper & Haugland and *S. chartarum*, but it can be distinguished from *M. longistipitata* and *S. chartarum* in having wider and rarely branched conidiophores. In addition, *M. longistipitata* can simultaneously produce both subspherical to spherical catenate conidia or oblong to ovoid conidia aggregated in slimy masses (Li et al. 2003). *M. dichroa* is similar to *M. oblongispora* in conidial size and shape (Bisby and Ellis 1949), but it has hyaline and unbranched conidiophores, while the conidiophores of *M. oblongispora* are olive grey at the apex and sometimes branched. Jong and Davis (1976) re-examined 50 strains of *Stachybotrys* and *Memmoniella*, including the culture of *M. dichroa* ATCC 18913, which was isolated by M. B. Ellis and further studied by Bisby & Ellis (1949). Jong and Davis described the conidial shape of *M. dichroa* were ovate (Jong & Davis 1976), whereas, the conidia of *M. oblongispora* are oblong.

From the phylogenetic trees of Bayesian inference (BI), maximum parsimony (MP) and maximum likelihood (ML) analyses based on combined *cmdA*, ITS, *rpb2*, *tef1* and *tub2* sequence data (Fig. 1), this species is closest to *M. longistipitata*, however, it can be distinguished from *M. longistipitata* by the morphological characteristics outlined above.

Lombard et al. (2016) cited a strain (CBS 136197 = MUCL 33065) as *M. longistipitata* without a description. In our study, this strain was in the same clade as *M. oblongispora* with high support and we suggest that it is a second isolate of *M. oblongispora*.

Memmoniella nilagirica (Subram.) C.G. Lin, Yong Wang bis & K.D. Hyde, **comb. nov.** Fig. 4

≡ *Stachybotrys nilagirica* Subram., Proc. Indian Acad. Sci., Pl. Sci. 46: 331 (1957)

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Colonies on PDA effuse, hairy, white from above, light yellow from below, attaining a diameter of 5–7 cm in 30 days at 25°C. *Mycelium* partly superficial and partly immersed. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. *Conidiophores* macronematous, mononematous, erect, simple, straight or flexuous, rarely branched, smooth, thick-walled, septate, bearing at its apex a crown of phialides, hyaline at the base, olive grey at the apex, 185–350 μm long, 9–22 μm wide at the base, tapering to 4–6.5 μm near the apex. *Conidiogenous cells* monophialidic, discrete, determinate, terminal, clustered at the apex of conidiophores, obovate, ellipsoidal, clavate or reniform, smooth, subhyaline, 13–20 \times 3.5–11.5 μm . *Conidia* aggregated in large, slimy, black and glistening heads, acrogenous, simple, spherical, tuberculate, dark brown, unicellular, 18–23 μm (\bar{x} = 20.6 μm , n = 35) diam.

Material examined – THAILAND, Chiang Rai Province, Wiang Chai District, Pha Chang, 19°49'52.44", 100°01'30.64", on decaying wood, 18 May 2015, Chuan-Gen Lin, PCP 9-33-1 (MFLU 15-3267), living culture MFLUCC 15-0660.

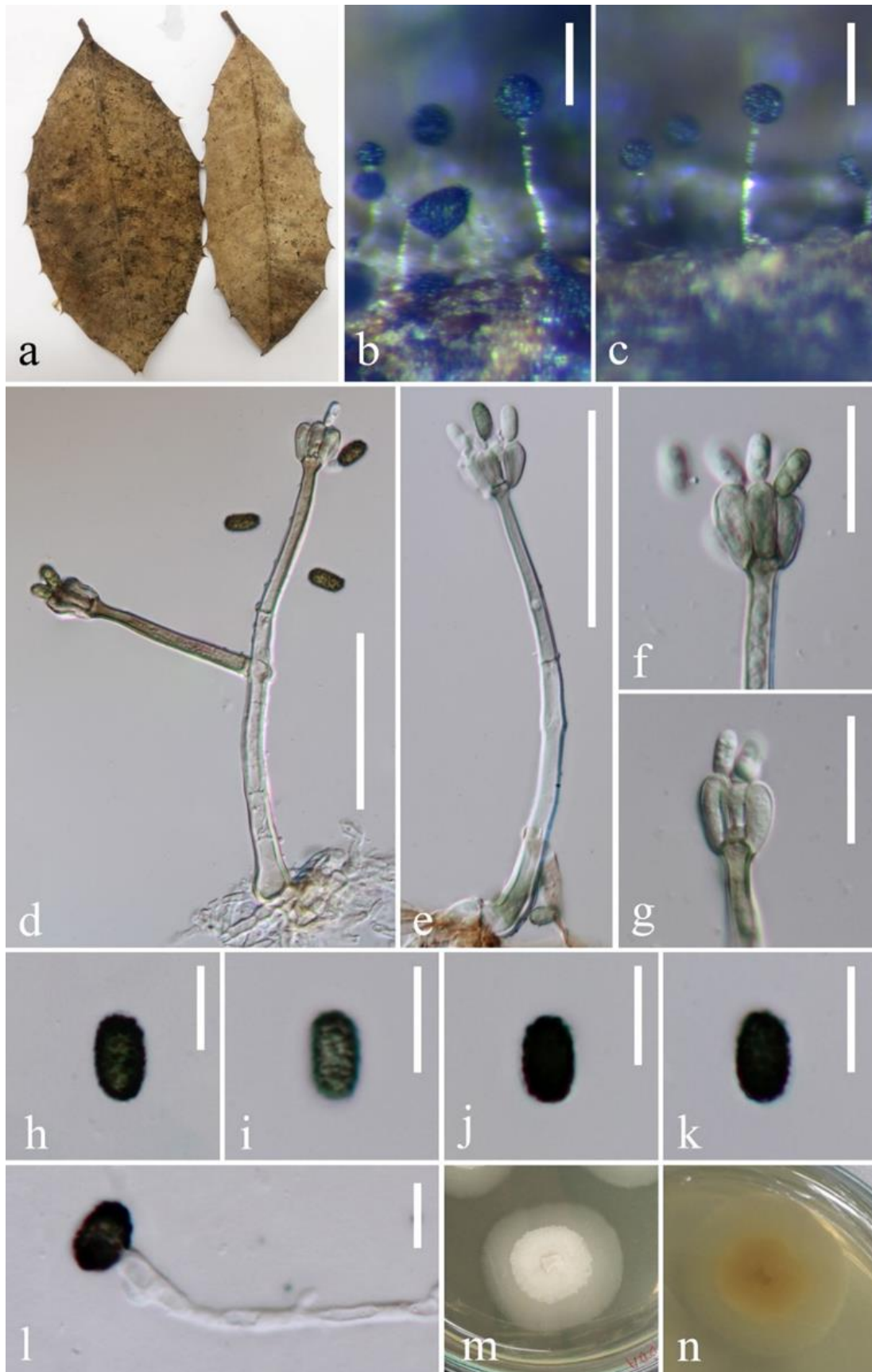


Fig. 3 – *Memnoniella oblongispora* (MFLU 15-3269, holotype) **a**. Host leaves. **b–c**. Conidiophores on the host surface. **d–e**. Conidiophores and conidia. **f–g**. Conidiogenous cells and conidia. **h–k**. Conidia. **l**. Germinating conidium. **m–n**. 25-day old colonies on PDA, m from above, n from below. – Scale bars: b–c = 100 μ m, d–e = 50 μ m, f–g = 20 μ m, h–l = 10 μ m.

Notes – The specimen observed in this study agrees with the descriptions given in the literature (Subramanian 1957, Izabel et al. 2010). *Memmoniella nilagirica* is characterized by having large globose and tuberculate conidia. Several species of *Stachybotrys* s.l. produce globose conidia. However, *M. nilagirica* produces larger conidia (18–23.2 µm) (Subramanian 1957, Wang et al. 2015), which distinguishes it from *M. leprosa* R.F. Castañeda (7–12 µm) (Wang et al. 2015), *M. stilboidea* Munjal & J.N. Kapoor (4–5.5 µm, also developing synnemata) (Ellis 1976), *S. crassa* Marcha (16–18 µm) (Wang et al. 2015), *S. globosa* P.C. Misra & S.K. Srivast. (4.5–8 µm) (Misra & Srivastava 1982), *S. jiangziensis* Y.M. Wu & T.Y. Zhang (6–9 µm) (Wu & Zhang 2010), *S. mexicanus* J. Mena & Heredia (9–12.5 µm, colorless) (Wang et al. 2015), *S. microspora* (B.L. Mathur & Sankhla) S.C. Jong & E.E. Davis (5–6 µm) (Jong & Davis 1976), *S. ruwenzoriensis* Matsush (6–8 µm) (Matsushima 1985), *S. sphaerospora* Morgan-Jones & R.C. Sinclair (11–12 µm) (Morgan-Jones & Sinclair 1980), *S. subreniformis* Q.R. Li & Y.L. Jiang (6–9.5 × 4.5–7.5 µm) (Li & Jiang 2011) and *S. variabilis* H.F. Wang & T.Y. Zhang (4–20 × 3–13 µm) (Wang & Zhang 2009).

Memmoniella nilagirica (MFLUCC 15-0660) forms a clade together with the strains of *M. pseudonilagirica* L. Lombard & Crous (CBS 136405) with 98% MP bootstrap support, 100% ML bootstrap support and 100% Bayesian posterior probabilities within the *Memmoniella* clade (Fig. 1). *Memmoniella pseudonilagirica*, which is morphologically similar to *M. nilagirica*, was introduced by Lombard et al. (2016). It can be distinguished from *M. nilagirica* by its longer conidiophores and smaller conidia (Lombard et al. 2016).

Accordingly, in this study, we propose the synonymy of *Stachybotrys nilagirica*, under the new combination *Memmoniella nilagirica*, based on its morphology and phylogenetic analysis (Fig. 1). This is the first report of *M. nilagirica* in Thailand. We also provide ITS, SSU, LSU, RPB2 and TEF1 sequence data.

Stachybotrys microspora (B.L. Mathur & Sankhla) S.C. Jong & E.E. Davis, Mycotaxon 3(3): 448 (1976) Fig. 5

≡ *Stachybotrys atra* var. *microspora* B.L. Mathur & Sankhla, Sci. Cult.: 93 (1966)

Colonies on MEA effuse, hairy, circular, grey to dark on top side, light yellow to dark on reverse, attaining a diameter of 2–2.5 cm in 14 days at 25°C. *Mycelium* mostly superficial. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. *Conidiophores* macronematous, mononematous, erect, simple, straight or flexuous, irregularly branched or sympodially branched, sometimes unbranched, smooth, often covered with dark granules near the apex, thick-walled, septate, branches of the conidiophore hyaline at the base, dark grey at the apex, bearing at its apex a crown of phialides, 29–61 µm long (\bar{x} = 38.9 µm, n = 26), 2.5–4.5 µm wide (\bar{x} = 3.4 µm, n = 39) at the base, tapering to 1–2.5 µm wide (\bar{x} = 1.7 µm, n = 23) near the apex. *Conidiogenous cells* monophialidic, discrete, determinate, terminal, clustered at the apex of conidiophores, obovoid, the outer ones somewhat curved, smooth, light grey, 6–9.5 µm long (\bar{x} = 7.2 µm, n = 37), 3–5 µm wide (\bar{x} = 4.1 µm, n = 35) at the widest point. *Conidia* unicellular aggregated in large, slimy, black and glistening heads, acrogenous, simple, when young elliptical or pyriform, rounded at the apex and rounded or truncate at the base, 5.5–8 µm long (\bar{x} = 6.9 µm, n = 37), 3–4.5 µm wide (\bar{x} = 3.9 µm, n = 38), becoming globose, 4–6 µm (\bar{x} = 5.2 µm, n = 50) in diameter at maturity, roughened, dark olive grey to black.

Material examined – Thailand, Prachuap Khiri Khan, Khao Lom Muak, 11°47'3.96"–11°47'11.24"N, 99°48'49.13"–99°49'0.63"E, on decaying wood, 29 July 2015, Chuan-Gen Lin, KLM 3-2 (MFLU 15-3270), living culture MFLUCC 15-0830; Thailand, Prachuap Khiri Khan, Khao Lom Muak, 11°47'3.96"–11°47'11.24"N, 99°48'49.13"–99°49'0.63"E, on decaying small shrubs, 29 July 2015, Chuan-Gen Lin, KLM 16-1 (MFLU 16-0883), living culture MFLUCC 15-1076.

Notes – Among the species that produce more or less globose or spherical conidia, *M. nilagirica* and *S. globosa* P.C. Misra & S.K. Srivast. are most similar to *S. microspora*. *Memmoniella nilagirica* has larger globose conidia (18–23 µm), but *S. globosa* is very similar to *S.*

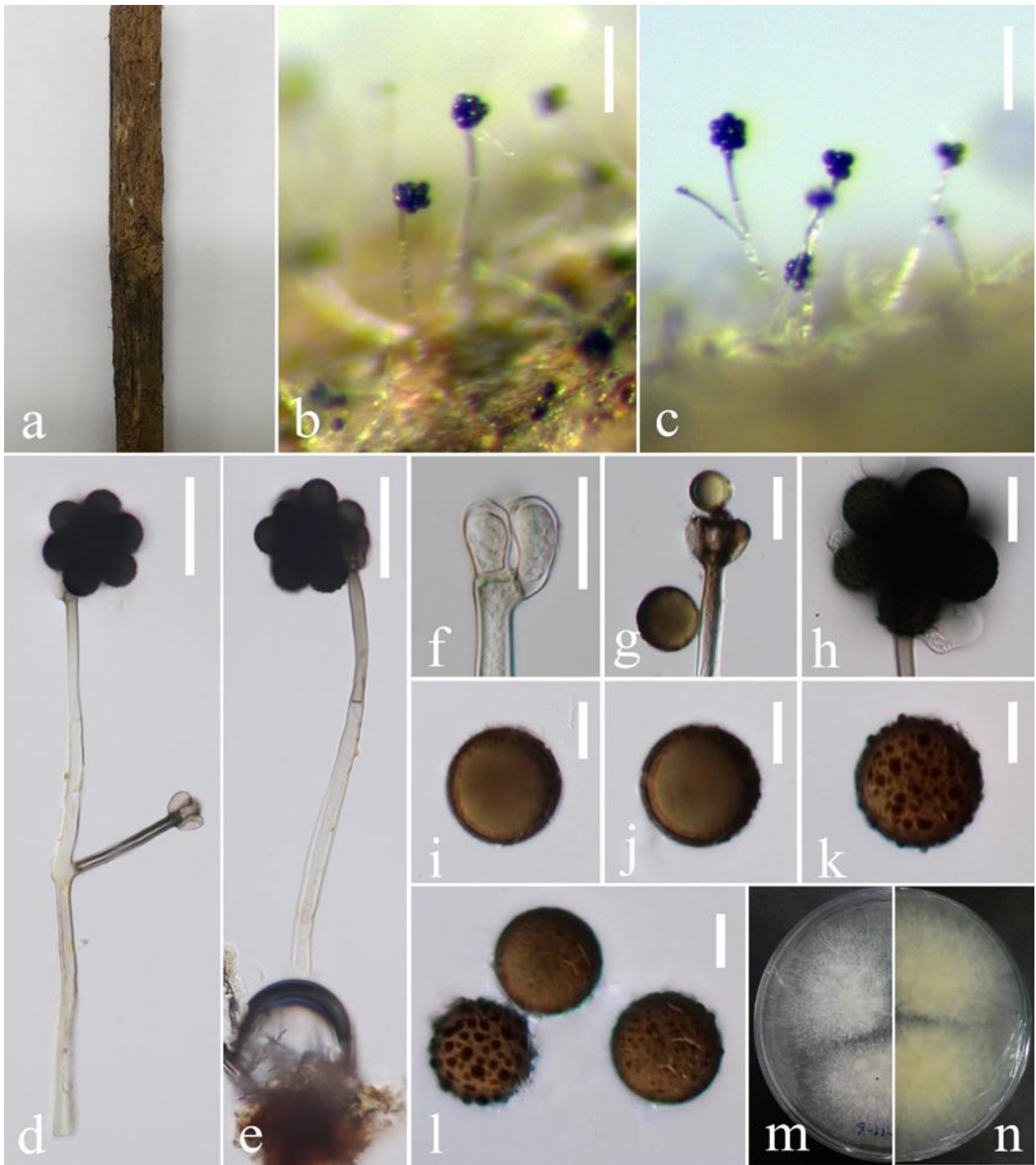


Fig. 4 – *Memnoniella nilagirica* (MFLU 15-3267) **a**. Host material, **b**, **c**. Conidiophores on the host surface. **d**, **e**. Conidiophores and conidia. **f–h**. Conidiogenous cells and conidia. **i–l**. Conidia. **m**, **n**. 28-day old colonies on PDA, m from above, n from below. – Scale bars: b–c = 100 μ m, d–e = 50 μ m, f–h = 20 μ m, i–l = 10 μ m.

microspora in that it produces elliptical or pyriform conidia when young that become globose at maturity. Because of the similarity in the conidial shape and size between *S. microspora* and *S. globosa* (4.5–8 μ m) (Misra & Srivastava 1982), Wang et al. (2015) proposed these two species should be further studied to determine if they are conspecific. This is the first report of *S. microspora* in Thailand.

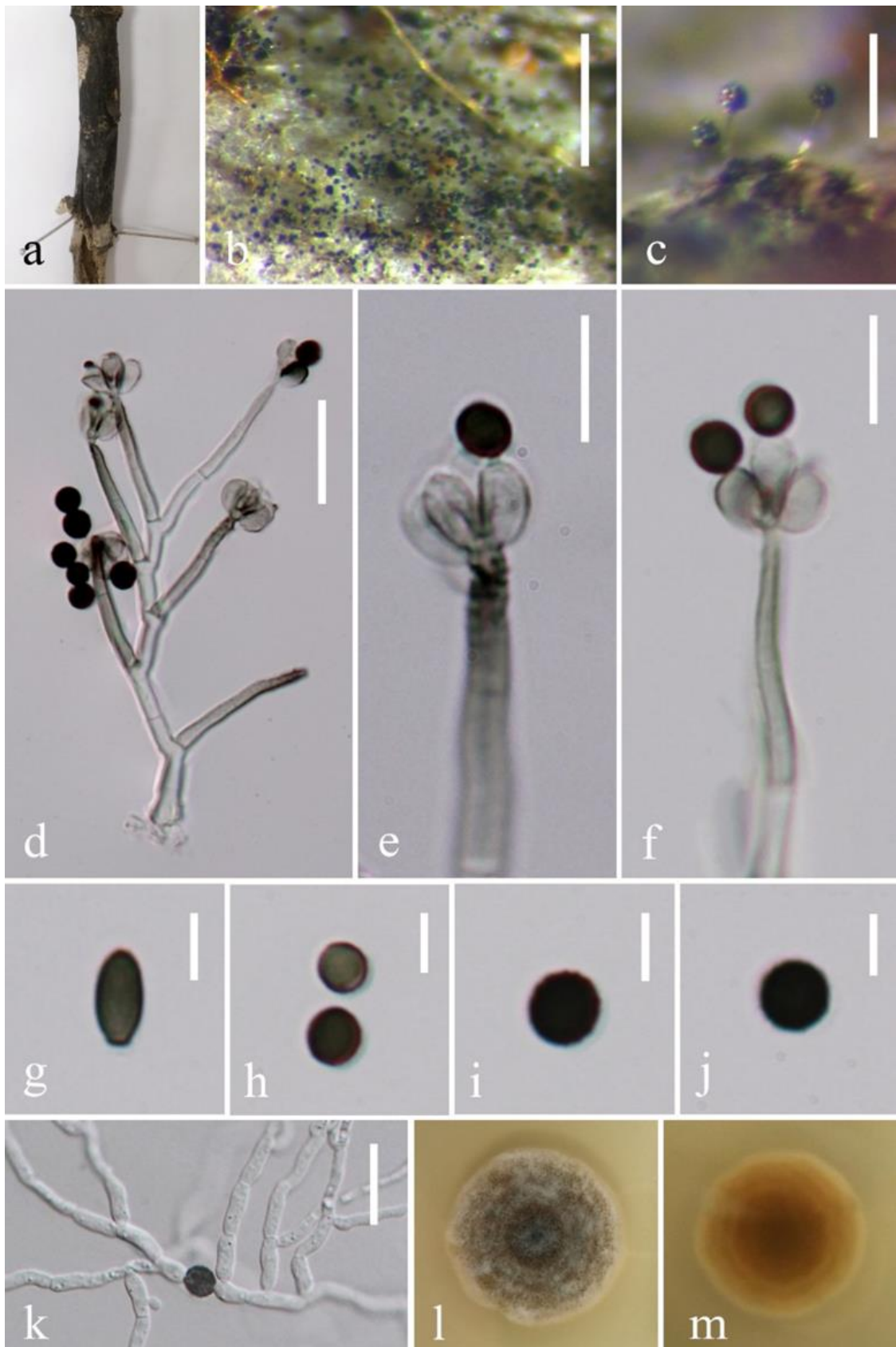


Fig. 5 – *Stachybotrys microspora* (MFLU 15-3270) **a.** Host. **b, c.** Conidiophores on the host surface. **d.** Conidiophores and conidia. **e, f.** Conidiogenous cells and conidia. **g–j.** Conidia. **k.** Germinating conidium. **l, m.** 10-day old colonies on MEA, l from above, m from below. – Scale bars: b = 500 μ m, c = 100 μ m, d = 20 μ m, e–f = 10 μ m, g–j = 5 μ m, k = 20 μ m.

Annotated checklist of stachybotrys-like fungi in Thailand

- Brevistachys subsimplex*** (Cooke) L. Lombard & Crous, in Lombard, Houbraken, Decock, Samson, Meijer, Réblová, Groenewald & Crous, *Persoonia* 36: 185 (2016)
On *Thysanoleana latifolia*, Doi Suthep-Pui National Park (Bhilabutra et al. 2010).
On *Musa acuminata*, Doi Suthep Pui National Park (Photita et al. 2003b, Farr & Rossman 2015).
- Cymostachys garethjonesii*** C.G. Lin, Yong Wang bis & K.D. Hyde
On decaying *Dracaena* sp. leaf, Khao Lan, Bang Saphan District, Prachuap Khiri Khan (This study).
- Memnoniella dichroa*** (Grove) L. Lombard & Crous, in Lombard, Houbraken, Decock, Samson, Meijer, Réblová, Groenewald & Crous, *Persoonia* 36: 196 (2016)
On *Musa acuminata*, Thailand (Photita et al. 2003a, Farr & Rossman 2015).
- Memnoniella echinata*** (Riv.) Galloway, *Trans. Brit. Mycol. Soc.* 18: 165. (1933)
On *Delonix regia*, Khao Yai National Park (Somrithipol et al. 2002).
On cow dung, Saraburi, Maung District, KUFC 3089 (Jeamjitt et al. 2006).
On decaying leaves of *Dracaena lourieri*, Chiangdao National Park, Chiang Mai Province (Thongkantha et al. 2008).
On *Thysanoleana latifolia*, Doi Suthep-Pui National Park (Bhilabutra et al. 2010).
- Memnoniella levispora*** Subram., *J. Indian bot. Soc.* 33: 40 (1954)
On dead twig of *T. grandis*, Mae Chan District, Chiang Rai Province (Doilom et al. 2017).
- Memnoniella nilagirica*** (Subram.) C.G. Lin, Yong Wang bis & K.D. Hyde
On decaying wood, Pha Chang Park, Wiang Chai District, Chiang Rai Province (This study).
- Memnoniella oenanthes*** (M.B. Ellis) L. Lombard & Crous, in Lombard, Houbraken, Decock, Samson, Meijer, Réblová, Groenewald & Crous, *Persoonia* 36: 199 (2016)
On fallen leaves of *Ficus*, Chiang Mai Province (Wang et al. 2008).
- Memnoniella oblongispora*** C.G. Lin, McKenzie, Yong Wang bis & K.D. Hyde
On decaying *Quercus* sp. leaf, Ang Kep Nam Wat Tham Khao Hin Phayanak (Wat Tham Sao Hin Phayanak), Mae Sai District, Chiang Rai Province (This study).
- Stachybotrys albipes*** (Berk. & Broome) S.C. Jong & Davis, *Mycotaxon* 3(3): 425 (1976) (= *Melanopsamma pomiformis*)
On palm *Eleiodoxa conferta*, Sirindhorn Research and Nature Study Center (Sirindhorn Peat Swamp Forest), Narathiwat Province (Pinnoi et al. 2006).
- Stachybotrys bambusicola*** Rifai, *Trans. Br. mycol. Soc.* 47(2): 270 (1964)
On *Licuala longicalycata*, Sirindhorn Peat Swamp Forest in southern Thailand (06°12'N 101°57'E, elevation 0–4 m) (Pinruan et al. 2007).
- Stachybotrys chartarum*** (Ehrenb.) S. Hughes, *Can. J. Bot.* 36: 812 (1958)
Cultivated lands soil, Eastern Thailand (Thamsurakul et al. 1985).
On toad dung, Bangkok, Bang Sue District, KUFC 3207 (Jeamjitt et al. 2006).
On *Dracaena lourieri*, Chiangdao National Park in Chiang Mai Province (Thongkantha et al. 2008).
- Stachybotrys elegans*** (Pidopl.) W. Gams, *Compendium of Soil Fungi* (London): 746 (1980)
On fallen leaves of *Ficus*, Chiang Mai Province (Wang et al. 2008).
- Stachybotrys microspora*** (B.L. Mathur & Sankhla) S.C. Jong & E.E. Davis, *Mycotaxon* 3(3): 448 (1976)
On decaying wood, Khao Lom Muak, Prachuap Khiri Khan (This study).
On decaying subshrubs, Khao Lom Muak, Prachuap Khiri Khan (This study).
- Stachybotrys nephrospora*** Hansf., *Proc. Linn. Soc. London* 155: 45 (1943) [1942–43]
On *Musa acuminata*, Doi Suthep Pui National Park (Photita et al. 2003b).
On fallen leaves of *Ficus*, Chiang Mai Province (Wang et al. 2008).
On *Musa acuminata*, Thailand (Photita et al. 2003a, Farr & Rossman 2015).
On dead needles of *Pinus khasya*, Thailand (Tokumasu et al. 1990, Whitton et al. 2001, Farr & Rossman 2015).

- Stachybotrys palmae* Pinruan, in Pinruan, McKenzie, Jones & Hyde, *Fungal Diversity* 17: 146 (2004)
 On *Licuala longicalycata*, Sirindhorn Peat Swamp Forest in southern Thailand (06°12'N 101°57'E, elevation 0–4 m) (Pinruan et al. 2007).
 On decaying rachis of *Licuala longicalycata* Furtado, Sirindhorn Peat Swamp Forest, Narathiwat, BBH U. Pinruan (Wah 35) (holotype) (Pinruan et al. 2004b).
- Stachybotrys parvispora* S. Hughes, *Mycol. Pap.* 48: 74 (1952)
 On *Thysanoleana latifolia*, Doi Suthep-Pui National Park (Bhilabutra et al. 2010).
 On *Magnolia liliifera*, an evergreen forest in Doi Suthep-Pui, Chiang Mai (N 18° 48' 18.73", E 98° 54' 47.28", elevation, 107 m) (Monkai et al. 2013).
 On *Magnolia liliifera*, Thailand (Promputtha et al. 2004, Farr & Rossman 2015).
- Stachybotrys renispora* P.C. Misra, *Mycotaxon* 4(1): 161 (1976)
 On fallen leaves of *Ficus*, Chiang Mai Province (Wang et al. 2008).
 On dead twig of *T. grandis*, Chiang Dao District, Chiang Mai Province (Doilom et al. 2017)
- Stachybotrys ruwenzoriensis* Matsush., *Matsush. Mycol. Mem.* 4: 17 (1985)
 On *Musa acuminata*, Doi Suthep Pui National Park (Photita et al. 2003b).
 On *Musa acuminata*, Thailand (Photita et al. 2003a, Farr & Rossman 2015).
- Stachybotrys sansevieriae* G.P. Agarwal & N.D. Sharma, in Sharma & Agarwal, *J. Indian bot. Soc.* 53(1–2): 78 (1974)
 On fallen leaves of *Ficus*, Chiang Mai Province (Wang et al. 2008).
- Stachybotrys suthepensis* Photita, P. Lumyong, K.D. Hyde & McKenzie, in Photita, Lumyong, McKenzie, Hyde & Lumyong, *Cryptog. Mycol.* 24(2): 149 (2003)
 On dead petioles of *Musa acuminata*, Doi Suthep Pui National Park, PDD 74601 (holotype) (Photita et al. 2003a).
 On *Musa acuminata*, Doi Suthep Pui National Park (Photita et al. 2003b).
- Stachybotrys theobromae* Hansf., *Proc. Linn. Soc. London* 155: 45 (1943) [1942–43]
 On *Musa acuminata*, Doi Suthep Pui National Park (Photita et al. 2003b).
 On *Dracaena lourieri*, Chiangdao National Park, Chiang Mai Province (Thongkantha et al. 2008).
 On *Musa acuminata*, Thailand (Photita et al. 2003a, Farr & Rossman 2015).

Discussion

In this study, the stachybotrys-like taxa *Cymostachys Garethjonesii*, *Memnoniella oblongispora*, *M. nilagirica* and *Stachybotrys microspora* were identified from decaying wood and leaf material collected from karst areas in Thailand. Of these, *C. Garethjonesii* and *M. oblongispora* are new species and *M. nilagirica* is new combination introduced in this study and *M. nilagirica* and *S. microspora* are new records for Thailand. Meanwhile, *Memnoniella nilagirica* was sequenced for the first time. An annotated checklist of stachybotrys-like taxa in Thailand totalling 19 species is provided based on previous publications and database.

ITS, SSU, LSU, EF1- α and RPB2 sequence data have been used for the phylogenetic analysis of the stachybotrys-like genera in previous studies (Haugland & Heckman 1998, Haugland et al. 2001, Koster et al. 2009, Wang et al. 2015). In this study, the phylogeny of selected genera within the family *Stachybotryaceae* is inferred from sequence data (*cmdA*, ITS, *rpb2*, *tef1* and *tub2*) and a phylogenetic tree is provided to infer the phylogenetic position of these four strains within the family *Stachybotryaceae*. Within the genera *Stachybotrys* s.l. and *Memnoniella*, several species are problematic, thus in need of further study. For example, the type species *S. chartarum* is a species complex.

Several asexual / sexual connections have been reported for *Stachybotrys* species. *Melanopsamma pomiformis* (Pers.) Sacc. was reported to have an *S. albipes* (Berk. & Broome) S.C. Jong & Davis asexual morph (Booth 1957). *Stachybotrys gamsii* (K.D. Hyde et al.) Yong Wang bis, et al. was reported to have a *Didymostilbe aurantiospora* Seifert & G. Okada asexual morph (Hyde et al. 1999). The connections of asexual / sexual of *Ornatisspora nepalensis* Whitton et al. –

Stachybotrys nepalensis and *Ornatisspora nova-zealandiae* Whitton et al. – *S. freycinetiae* McKenzie were reported, as both specimens from *Pandanaceae* were surrounded by a hyphal subiculum made of fertile *Stachybotrys* conidiophores, in addition, the setae of *Ornatisspora nepalensis* can become fertile conidiophores of *Stachybotrys* species surrounding the ascomata (Whitton et al. 2012, Wang et al. 2015). Wang et al. (2015) therefore synonymized *Ornatisspora nepalensis* and *Ornatisspora nova-zealandiae* under *Stachybotrys*. Now *Stachybotrys* comprises several genera these taxa need recollecting and sequencing to establish their affinities.

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This paper is dedicated to Professor E.B. Gareth Jones in celebration of his 80th birthday and thanking him for his contribution to mycology. We would like to thank Prof. Shaun Pennycook (Landcare Research Manaaki Whenua, New Zealand) for advising on the fungal names. The research is supported by the National Natural Science Foundation of China (No. NSFC 31560489), the International Scientific Cooperated Project of Guizhou Province (No[2013]7004, Fundamental Research on Science and Technology, Ministry of Science and Technology of China (2014FY120100), and the grant [JD2014018] from Education Department of Guizhou Province.

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