



Phylogenetic placement of *Cryptophiale*, *Cryptophialoidea*, *Nawawia*, *Neonawawia* gen. nov. and *Phialosporostilbe*

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Abstract

During a survey of freshwater fungi in China and Thailand, seven fresh collections representing four species in Chaetosphaeriaceae are described and illustrated based on morphological characters and phylogenetic analyses of combined LSU and ITS sequence data. *Cryptophiale udagawae*, *Cryptophialoidea fasciculata* and *Nawawia filiformis* were re-collected and are reported with the first sequence data. Two collections of *Phialosporostilbe* from China and Thailand are identified and introduced as a new species named *P. scutiformis* with the first sequence data for the genus. A new genus *Neonawawia* is introduced to accommodate *Ne. malaysiana*. New combinations are proposed for the genera *Neonawawia* and *Phialosporostilbe*. Descriptions and illustrations of the new taxa and identified species are provided. Reference specimens of *Cryptophiale udagawae*, *Cryptophialoidea fasciculata* and *Nawawia filiformis* are designated in this study. Phylogenetic placements of the genera *Cryptophiale*, *Cryptophialoidea*, *Nawawia*, *Neonawawia* and *Phialosporostilbe* are discussed.

Key words – 2 new taxa – asexual fungi – Chaetosphaeriaceae – reference specimens – Sordariomycetes – taxonomy

Introduction

The family Chaetosphaeriaceae was introduced by Réblová et al. (1999) to accommodate *Chaetosphaeria* and its allies. The long history of taxonomic problems of *Chaetosphaeria* has been reviewed in detail by Réblová et al. (1999) and Réblová (2000). *Chaetosphaeria* is characterized by superficial, dark globose perithecia, cylindrical asci, hyaline septate ascospores and phialidic dematiaceous asexual morphs. Ten asexual genera (*Cacumisporium*, *Catenularia*, *Chalara*, *Chloridium*, *Cylindrotrichum*, *Dictyochaeta*, *Gonytrichum*, *Menispora*, *Phialophora* and *Zanclospora*) of dematiaceous hyphomycetes producing phialidic conidia have been linked to *Chaetosphaeria* (Réblová 2000). Morphological differences between species in *Chaetosphaeria* are

often manifested among the asexual morphs, while the morphological characters of the sexual morphs show less variation which is often insufficient for their correct identification (Réblová & Winka 2000, Liu et al. 2016). Phylogenetic studies showed that *Chaetosphaeria* is not monophyletic (Réblová & Winka 2000, Liu et al. 2016, Hyde et al. 2018). Réblová et al. (1999) included seven genera in the family Chaetosphaeriaceae while presently 38 genera are accepted within the family (Lumbsch & Huhndorf 2010, Maharachchikumbura et al. 2016, Wijayawardene et al. 2018).

In this study, seven fresh collections resulted in the identification of four chaetosphaeriaceous hyphomycetes obtained from freshwater habitats in China and Thailand. A new freshwater species and three previously described species were collected and identified based on phylogenetic analyses and morphological characters. We therefore introduce *Phialosporostilbe scutiformis* as a novel taxon with an illustrated account and phylogenetic evidence. Molecular sequence data is generated for the first time for the genera *Cryptophialoidea* and *Phialosporostilbe* and for the species *Cryptophiale udagawae*, *Cryptophialoidea fasciculata* and *Nawawia filiformis*. Phylogenetic analyses based on a combined LSU and ITS sequence dataset are presented to provide further evidence for the classification of these genera, which have been sequenced, in Chaetosphaeriaceae. A new genus *Neonawawia* is introduced and new combinations are proposed for the genera *Neonawawia* and *Phialosporostilbe*.

Materials and Methods

Collection and examination of specimens

Specimens of submerged decaying twigs were collected from streams in Chiang Rai (in June 2017), Krabi (in December 2015), Trat (in April 2017) Provinces, Thailand and Guizhou (in October 2016, August 2017) Province, China. Specimens were brought to the laboratory in plastic bags and incubated in plastic boxes lined with moistened tissue paper at room temperature for one week. A Motic SMZ 168 Series dissecting microscope was used for morphological observation of the fungal structures on natural substrata. The fungal structures were collected and transferred using a syringe needle to a small drop of distilled water on a clean slide and covered with a cover glass. The fungal fruiting bodies and details were examined using a Nikon ECLIPSE 80i compound microscope and photographed with a Canon 600D/70D digital camera fitted to the microscope. Measurements were made with the Tarosoft Image Frame Work program and images used for figures were processed with Adobe Photoshop CS6 software. Single spore isolations were made onto potato dextrose agar (PDA) or water agar (WA) and later transferred onto malt extract agar (MEA) or PDA following the method of Chomnunti et al. (2014). Specimens (dry wood with fungal material) were deposited in the Herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and Herbarium of Cryptogams, Kunming Institute of Botany, Academia Sinica (HKAS), Kunming, China. Axenic cultures were deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Guizhou Culture Collection (GZCC). Facesoffungi and Index Fungorum numbers are registered as outlined in Jayasiri et al. (2015) and Index Fungorum (2018).

DNA extraction, PCR amplification and sequencing

Isolates were grown on PDA/MEA medium at 25°C for one month. Fungal mycelium was scraped off and transferred to a 1.5 ml microcentrifuge tube using a sterilized lancet for genomic DNA extraction. Ezup Column Fungi Genomic DNA Purification Kit (Sangon Biotech, China) was used to extract DNA following the manufacturer's instructions. LSU and ITS gene regions were amplified using the primer pairs LR0R with LR5 or LR7 (White et al. 1990) and ITS5 or ITS1 with ITS4 (Vilgalys & Hester 1990). The amplifications were performed in a 25 µl reaction volume containing 9.5 µl ddH₂O, 12.5 µl 2 × Taq PCR Master Mix with blue dye (Sangon Biotech, China), 1 µl of DNA template and 1 µl of each primer (10 µM). The amplification condition for LSU and ITS consisted of initial denaturation at 94°C for 3 min; followed by 40 cycles of 45 s at 94°C, 50 s at 56°C and 1 min at 72°C and a final extension period of 10 min at 72°C. Purification and sequencing

of PCR products were carried out using the above-mentioned PCR primers at Sangon Biotech (Shanghai) Co. Ltd. in China.

Phylogenetic analyses

The taxa included in the phylogenetic analyses were selected and obtained from previous studies and GenBank (Réblová & Winka 2000, Crous et al. 2016, Liu et al. 2016, Ma et al. 2016, Konta et al. 2017, Hyde et al. 2018). LSU and ITS gene regions were used for the combined sequence data analyses. Sequences were optimized manually to allow maximum alignment and maximum sequence similarity. The sequences were aligned using the online multiple alignment program MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/>) (Kato & Standley 2013). The alignments were checked visually and improved manually where necessary.

Phylogenetic analysis of the sequence data consisted of maximum likelihood (ML) using RAxML-HPC v.8 (Stamatakis 2006, Stamatakis et al. 2008) on the XSEDE Teragrid of the CIPRES science Gateway (<https://www.phylo.org>) (Miller et al. 2010) with rapid bootstrap analysis, followed by 1000 bootstrap replicates. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTRGAMMA substitution model.

The program MrModeltest v.2.3 (Nylander 2008) was used to infer the appropriate substitution model that would best fit the model of DNA evolution for the combined dataset for Bayesian inference analysis with GTR+G+I substitution model selected. Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v.3.2 (Ronquist & Huelsenbeck 2003). Six simultaneous Markov chains were run for 1 million generations, with trees sampled every 100 generations (resulting in 10000 trees). The first 2000 trees, representing the burn-in phase of the analyses were discarded and the remaining 8000 trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree (Larget & Simon 1999).

The resulting trees were printed with FigTree v. 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>), and the layout was created in Microsoft Powerpoint for Mac v. 15.19.1. The alignment of phylogenetic analyses and phylogenetic trees were deposited in TreeBASE (www.treebase.org, submission number 23182). Sequences generated in this study were submitted to GenBank (Table 1).

Table 1 Isolates and sequences used in this study (newly generated sequences are indicated in bold with new taxa in red, ex-type strains are indicated with ^T after the strain number).

Species	Source	GenBank accession no.	
		LSU	ITS
<i>Adautomilanezia caesalpiniae</i>	CC-LAMIC 102/12 ^T	KU170671	KX821777
<i>Anacacumisporium appendiculatum</i>	HMAS 245593 ^T	KT001553	KT001555
<i>Anacacumisporium appendiculatum</i>	HMAS 245602	KT001554	KT001556
<i>Brunneodinemasporium brasiliense</i>	CBS 112007 ^T	JQ889288	JQ889272
<i>Brunneodinemasporium jonesii</i>	GZCC 16-0050 ^T	KY026055	KY026058
<i>Chaetosphaeria innumera</i>	SMH 2748	AY017375	AY906956
<i>Chaetosphaeria pygmaea</i>	MR 1365	AF178545	AF178545
<i>Chloridium lignicola</i>	CBS 143.54 ^T	AF178544	AF178544
<i>Codinaeopsis gonytrichoides</i>	CBS 593.93	AF178556	AF178556
<i>Cryptophiale hamulata</i>	MFLUCC 18-0098	MG386756	–
<i>Cryptophiale udagawae</i>	MFLUCC 18-0422	MH758211	MH758198
<i>Cryptophiale udagawae</i>	MFLUCC 18-0428	MH758210	MH758197
<i>Cryptophialoidea fasciculata</i>	MFLUCC 17-2119	MH758208	MH758195
<i>Dendrophoma cytisporoides</i>	CBS 223.95 ^T	JQ889289	JQ889273
<i>Dictyochoaeta siamensis</i>	MFLUCC 15-0614 ^T	KX609952	KX609955

Table 1 Continued.

Species	Source	GenBank accession no.	
		LSU	ITS
<i>Dictyochaeta simplex</i>	CBS 966.69	AF178559	AF178559
<i>Dinemasporium decipiens</i>	CBS 592.73	JQ889291	JQ889275
<i>Dinemasporium morbidum</i>	CBS 129.66 ^T	JQ889296	JQ889280
<i>Echinosphaeria canescens</i>	SMH 4791	AY436403	–
<i>Ellisembia brachypus</i>	HKUCC 10555	DQ408563	–
<i>Eucalyptostroma eucalypti</i>	CBS 142074 = CPC 28764 ^T	KY173500	KY173408
<i>Eucalyptostroma eucalypti</i>	CBS 142075 = CPC 28748	KY173499	KY173407
<i>Exserticlava vasiformis</i>	TAMA 450	AB753846	–
<i>Helminthosphaeria clavariarum</i>	SMH 4609 ^T	AY346283	–
<i>Infundibulomyces cupulata</i>	BCC 11929 ^T	EF113979	EF113976
<i>Infundibulomyces oblongisporus</i>	BCC 13400 ^T	EF113980	–
<i>Lasiosphaeria ovina</i>	SMH 4605	AY436413	AY587923
<i>Lecythothecium duriligni</i>	CBS 101317	AF261071	–
<i>Leptosporella arengae</i>	MFLUCC 15-0330 ^T	MG272246	MG272255
<i>Leptosporella gregaria</i>	SMH 4290 ^T	AY346290	–
<i>Linocarpon arengae</i>	MFLUCC 15-0331 ^T	MG272247	–
<i>Linocarpon cocois</i>	MFLUCC 15-0812 ^T	MG272248	–
<i>Melanopsammella gonytrichii</i>	SMH 3785	AF466085	–
<i>Melanopsammella vermicularioides</i>	FC 404	AF466087	–
<i>Menispora tortuosa</i>	DAOM 231154 = AFTOL-ID 278	AY544682	KT225527
<i>Menispora tortuosa</i>	CBS 214.56	AF178558	AF178558
<i>Menisporopsis theobromae</i>	MFLUCC 15-0055	KX609954	KX609957
<i>Morrisiella indica</i>	HKUCC 10827	DQ408578	–
<i>Nawawia filiformis</i>	MFLUCC 16-0853	MH758206	–
<i>Nawawia filiformis</i>	MFLUCC 17-2394	MH758209	MH758196
<i>Neonawawia malaysiana</i>	CPC 16757 = CBS 125544 ^T	GU229887	GU229886
<i>Neopseudolachnella magnispora</i>	MAFF 244359 ^T	AB934042	AB934066
<i>Neopseudolachnella uniseptata</i>	MAFF 244360 ^T	AB934043	AB934067
<i>Phialosporostilbe scutiformis</i>	MFLUCC 17-0227^T	MH758207	MH758194
<i>Phialosporostilbe scutiformis</i>	MFLUCC 18-1288	MH758212	MH758199
<i>Pseudodinemasporium fabiforme</i>	MAFF 244361 ^T	AB934044	AB934068
<i>Pseudolachnea fraxini</i>	CBS 113701 ^T	JQ889301	JQ889287
<i>Pseudolachnea hispidula</i>	MAFF 244364	AB934047	AB934071
<i>Pseudolachnella asymmetrica</i>	MAFF 244366 ^T	AB934049	AB934073
<i>Pseudolachnella botulispora</i>	MAFF 244367 ^T	AB934050	AB934074
<i>Pyrigemmula aurantiaca</i>	CBS 126743 ^T	HM241692	HM241692
<i>Pyrigemmula aurantiaca</i>	CBS 126744	HM241693	HM241693
<i>Rattania setulifera</i>	GUFCC 15501	HM171322	GU191794
<i>Ruzenia spermoides</i>	SMH 4606	AY436422	–
<i>Sporoschisma hemipsilum</i>	SMH 2125	AY346292	–
<i>Sporoschisma palauense</i>	MFLUCC 15-0616 ^T	KX358075	KX505870
<i>Stanjehughesia vermiculata</i>	HKUCC 10840	DQ408570	–
<i>Striatosphaeria codinaeophora</i>	SMH 1524	AF466088	–

Table 1 Continued.

Species	Source	GenBank accession no.	
		LSU	ITS
<i>Striatosphaeria codinaeophora</i>	MR 1230	AF178546	AF178546
<i>Synaptospora plumbea</i>	SMH3962	KF765621	–
<i>Tainosphaeria crassiparies</i>	SMH 1934	AF466089	–
<i>Tainosphaeria siamensis</i>	MFLUCC15-0607 ^T	KX609953	KX609956
<i>Thozetella nivea</i>	–	EU825200	EU825201
<i>Thozetella pinicola</i>	–	EU825195	EU825197
<i>Umbrinosphaeria caesariata</i>	CBS 102664	AF261069	–
<i>Zanclospora iberica</i>	FMR 11584 = CBS 130426 ^T	KY853544	KY853480
<i>Zanclospora iberica</i>	FMR 12186	KY853545	KY853481
<i>Zignoella pulviscula</i>	MUCL 15710	AF466090	–
<i>Zignoella pulviscula</i>	SMH 3289	AF466091	–

Phylogenetic results

Seven isolates of asexual fungi obtained from the incubated specimens of submerged wood were identified in the family Chaetosphaeriaceae. The analyzed dataset consisted of combined LSU (848 bp) and ITS (547 bp) sequence data (a total of 1395 characters including gaps) for 69 taxa in Chaetosphaeriales with *Lasiosphaeria ovina* (SMH 4605) as the outgroup taxon. The Bayesian tree is shown in Fig. 1.

Genera in Chaetosphaeriaceae sequenced in Réblová & Winka (2000), Lumbsch & Huhndorf (2010), Crous et al. (2012), Hashimoto et al. (2015a, b), Liu et al. (2015, 2016), Maharachchikumbura et al. (2015), and the recent sequenced genera *Adautomilanezia* (Ma et al. 2016), *Anacacumisporium* (Crous et al. 2016), *Cryptophiale* (Hyde et al. 2018) and *Eucalyptostroma* (Crous et al. 2016) were included in our analyses. The analyzed ML and Bayesian trees were similar in topology and did not differ significantly. Of our seven isolates, two were identified as *Cryptophiale udagawae* (MFLUCC 18-0422 and MFLUCC 18-0428) and clustered together with *C. hamulata* (MFLUCC 18-0098) with good support. *Cryptophialoidea fasciculata* (MFLUCC 17-2119) clustered close to the genera *Cryptophiale*, *Morrisiella* and *Stanjehughesia*. Two isolates of *Nawawia filiformis* (MFLUCC 17-2394 and MFLUCC 16-0853) were close to the genera *Exserticlava*, *Umbrinosphaeria* and *Zanclospora*. Two isolates of *Phialosporostilbe scutiformis* (MFLUCC 17-0227 and MFLUCC 18-1288) clustered together and they were well-supported as a sister clade to *Menisporopsis theobromae* (MFLUCC 15-0055). In addition, *Neonawawia malaysiana* (CPC 16757) clustered as a sister clade to the family Chaetosphaeriaceae.

Taxonomy

Cryptophiale udagawae Pirozynski & Ichinoe, Can. J. Bot. 46(9): 1126 (1968)

Fig. 2

Index Fungorum number: IF329371; Facesoffungi number: FoF04866

Saprobic on decaying plant substrates. Asexual morph: *Colonies* effuse, hairy, scattered, glistening, with conspicuous branches at the apex of conidiophores and slimy mass of conidia. *Mycelium* mostly immersed, partially superficial. *Conidiophores* macronematous, mononematous, solitary, erect, straight or slightly curved, dark brown, smooth, thick-walled, septate below the branches, (90–)100–130(–140) × (4.5–)5.2–6.5(–7.2) μm (\bar{x} = 115 × 5.8 μm, n = 20), dichotomously branching towards the apex and generally above the fertile region, with 2–3 dichotomous divisions tapering towards the apex; terminal branches 11.5–38 × 2.8–4.5 μm. Fertile region from middle up to the first dichotomy, 37–63 × 11–16 μm, consisting of two rows of phialides one on each side of the conidiophore with each cell narrowly ellipsoid. *Conidiogenous cells* not observed. *Conidia* hyaline, smooth, clavate, aseptate, with a short appendage at the base, (18.5–)20.5–24.5(–26.2) ×

1.4–2.6 μm ($\bar{x} = 22.9 \times 2.0 \mu\text{m}$, $n = 45$). Sexual morph: Undetermined.

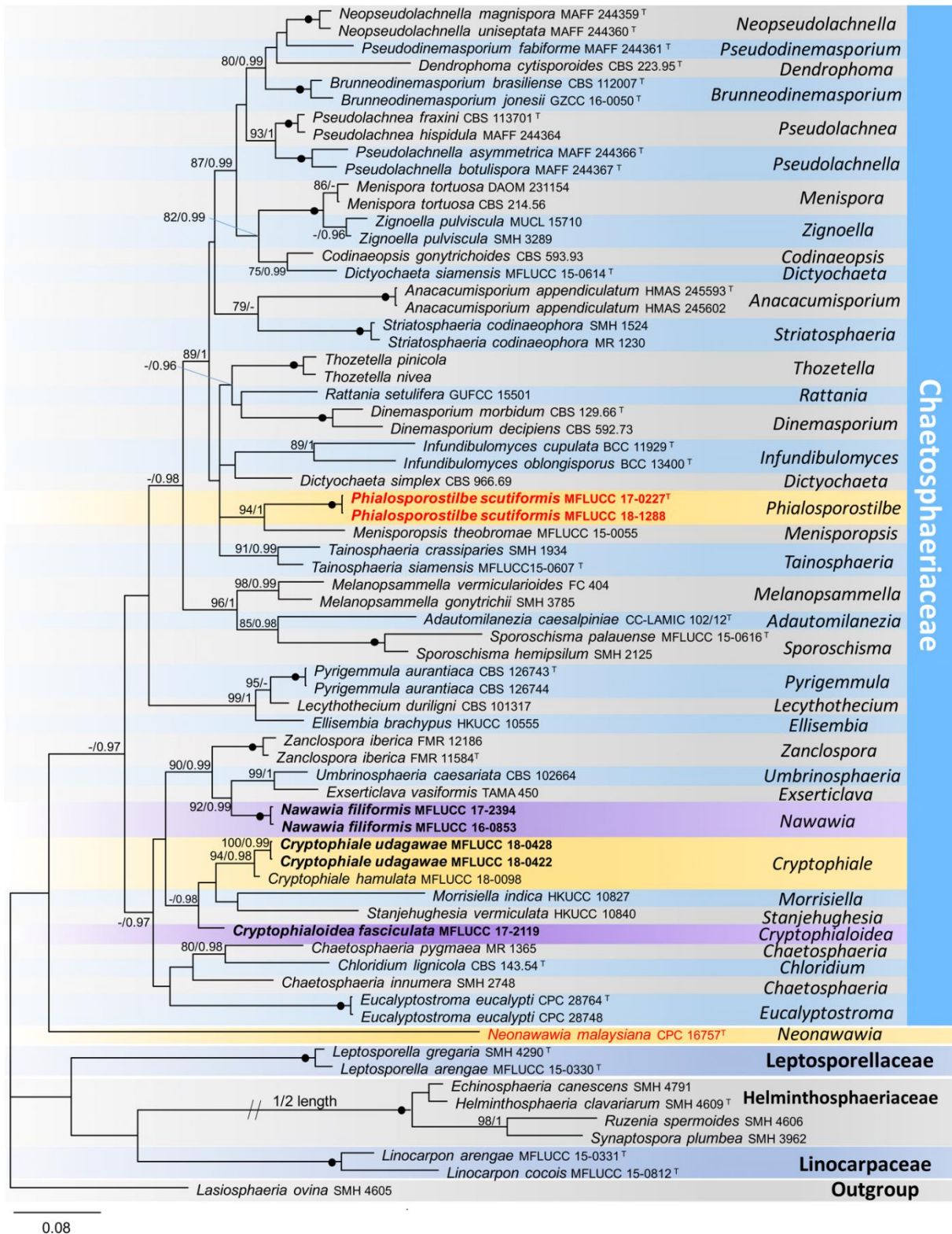


Figure 1 – Consensus phylogram (50%) majority rule resulting from a Bayesian analysis of a combined LSU and ITS sequence alignment of Chaetosphaeriales. ML bootstrap proportion greater than 75% and Bayesian posterior probabilities above 0.95 are indicated above the nodes as BP/PP. The scale bar represents the expected number of changes per site. The tree is rooted with *Lasio-sphaeria ovina* (SMH 4605). The strain numbers are noted after the species names with ex-type strains indicated with ^T. The new collections are in bold with the new taxa in red. Branches with 100% ML BP and 1.0 PP are shown as black nodes.



Figure 2 – *Cryptophiale udagawae* (MFLU 18-1497 reference specimen) a Colonies on woody substrate. b–e Conidiophores with conidiogenous region. f Conidiogenous region. g–j Conidia. k Germinated conidium. l, m Culture, l from above, m reverse. Scale bars: b–e = 40 μm , f, g, k = 30 μm , h–j = 10 μm .

Cultural characteristics – Conidia germinating on PDA within 24 h and germ tubes produced from the base and the upper part. Colonies on MEA medium, reaching 5–10 mm diam. in a week at

25°C, in natural light, circular, with fluffy, dense, brown mycelium on the surface; in reverse dark brown.

Material examined – CHINA, Guizhou Province, Dushan District, 25°57.9'N, 107°39'E, on decaying wood submerged in a freshwater stream, 26 August 2017, J Yang, SG 19-1 (MFLU 18-1497 **reference specimen designated here**, HKAS 102153), living culture MFLUCC 18-0422, additional sequence of SSU: MH758205; *ibid* SG 41-1 (MFLU 18-1498), living culture MFLUCC 18-0428, additional sequence of SSU: MH758204.

Notes – Pirozynski (1968) established the genus *Cryptophiale* with the type species *C. kakombensis* Pirozynski and the second species *C. udagawae*. The genus is characterized by setiform, unbranched or apically dichotomous or verticillate conidiophores with a subapical or apical fertile region, monophialidic, obscured conidiogenous cells in two rows shielded by a plate of modified cells and hyaline, unicellular to multiseptate conidia produced in slimy masses on one side of the conidiophore (Pirozynski 1968, Seifert et al. 2011). Presently, 22 epithets are listed under the genus *Cryptophiale* in Index Fungorum (October 2018). *Cryptophiale secunda* Kuthubutheen & B. Sutton and *C. manifesta* B. Sutton & Hodges, however, have been transferred to the genus *Cryptophialoidea* (Kuthubutheen & Nawawi 1987, 1994b). Thus, 20 species are accepted in *Cryptophiale* (Goh & Hyde 1996, Whitton et al. 2012). *Cryptophiale* is morphologically similar to *Cryptophialoidea* and *Paracryptophiale*. *Cryptophialoidea* was separated from *Cryptophiale* in having obvious monophialidic conidiogenous cells arranged only on one side of the conidiophore and not covered by a shield of sterile cells (Kuthubutheen & Nawawi 1987, Whitton et al. 2012). *Paracryptophiale* resembles *Cryptophiale* in having setiform conidiophores and typical cryptophiale-like conidiogenous cells aggregated into a fertile region with shield cells. The major difference separating the two genera is the multicellular, dictyosporous conidia produced by *Paracryptophiale* (Kuthubutheen & Nawawi 1994a). Generic keys or synopses to *Cryptophiale*, *Cryptophialoidea* and *Paracryptophiale* were provided by Goh & Hyde (1996), Delgado et al. (2005), Marques et al. (2008) and Whitton et al. (2012). In a study of microfungi associated with palms, Hyde et al. (1999) reported the connection of *Cryptophiale udagawae* and *C. kakombensis* with their sexual morphs *Chaetosphaeria hongkongensis* K.D. Hyde, Goh, J.E. Taylor & J. Fröhl. and *Chaetosphaeria saltuensis* K.D. Hyde, Goh, J.E. Taylor & J. Fröhl., respectively on host substrate. Hyde et al. (2018) provided the first sequence data for the genus *Cryptophiale* and showed its placement within the family Chaetosphaeriaceae.

In this study, phylogenetic analyses of combined LSU and ITS sequence data showed that *Cryptophiale* is related to *Cryptophialoidea*, *Morrisiella* and *Stanjehughesia*. *Cryptophiale udagawae* is a sister taxon to *C. hamulata* Whitton, K.D. Hyde & McKenzie. Specimens observed in this study agree with the original diagnosis given in Pirozynski (1968). The conidiogenous regions of our collections (37–63 × 11–16 µm) are smaller than that in the holotype (70–100 × 15–27 µm), but similar in size to the later collections by Kuthubutheen & Sutton (1985) and Marques et al. (2008). Unfortunately, the inconspicuous phialidic conidiogenous cells were not observed in our collections. We therefore provide the first sequence data for *Cryptophiale udagawae* and designate a reference specimen for the species (*sensu* Ariyawansa et al. 2014).

Cryptophialoidea fasciculata Kuthubutheen & Nawawi, Mycol. Res. 98(6): 686 (1994) Fig. 3
Index Fungorum number: IF362165; Facesoffungi number: FoF04867

Saprobic on decaying plant substrates. Asexual morph: *Colonies* effuse, hairy, scattered, dark brown to black, glistening, with slimy mass of conidia on one side of the conidiophores. *Mycelium* mostly immersed, composed of smooth, septate, branched, pale brown to mid brown hyphae. *Conidiophores* macronematous, mononematous, erect, solitary, straight or flexuous, thick-walled, smooth, septate, subulate, dark brown, 185–236 × 4.8–6.2 µm. *Conidiogenous* region about halfway up and restricted only to the middle third of the conidiophore, comprising a row of phialides that are mostly closely arranged in discrete bundles and that all open on only one side of the conidiophore, 56–70 × 10.5–12.5 µm. *Conidiogenous cells* monophialidic, intercalary, determinate, lageniform, extruded through minute pores on only one side of the conidiophores, light olivaceous brown, 7.2–

11.2 × 3.0–4.8 μm (\bar{x} = 9.5 × 3.8 μm, n = 20). *Conidia* hyaline, smooth-walled, 0–1-euseptate, falcate, with the base slightly narrower than the apex, sometimes guttulate, 26.5–33.4 × 1.2–2.4 μm (\bar{x} = 29.7 × 1.7 μm, n = 60). Sexual morph: Undetermined.



Figure 3 – *Cryptophialoidea fasciculata* (MFLU 18-1499 reference specimen). a Colonies on woody substrate. b, c Conidiophores with conidiogenous cells and conidia. d Conidiophores. e–i Conidiogenous cells. j–m Conidia. n Germinated conidium. o, p Culture, o from above, p reverse. Scale bars: a = 100 μm, b–d = 50 μm, e–i, n = 20 μm, j = 30 μm, k–m = 10 μm.

Cultural characteristics – Conidia germinating on PDA within 24 h and germ tubes produced from the base and the upper part. Colonies on MEA medium, reaching 5–10 mm diam. in a week at 25°C, in natural light, circular, with fluffy, dense, brown mycelium in the middle and sparse mycelium in the narrow outer ring on the surface with entire margin; in reverse dark brown.

Material examined – THAILAND, Trat Province, Amphoe Ko Chang, 12°08'N, 102°38'E, on decaying wood submerged in a freshwater stream, 27 April 2017, YZ Lu, YJT 8-1 (MFLU 18-1499 reference specimen designated here), living culture MFLUCC 17-2119, SSU sequence: MH758202; *ibid* YJT 29-1 (HKAS 102154), living culture MFLUCC 17-2131 (sequence unavailable).

Notes – The generic concept of the genus *Cryptophiale* was restricted in Kirk & Sutton (1985) to those fungi with sessile conidiogenous cells borne unilaterally and submedianly on a setiform conidiophore and obscured by a shield of sterile cells. However, *Cryptophiale secunda* is characterized by a single row of conspicuous phialides closely arranged on only one side of the conidiophore and located about halfway up the conidiophore without a protected shield of sterile cells (Kuthubutheen & Sutton 1985, Kuthubutheen & Nawawi 1987). Thus, Kuthubutheen & Nawawi (1987) proposed the segregation of *Cryptophiale secunda* in a new genus *Cryptophialoidea*. Based on the generic limitation, *Cryptophiale manifesta* was also transferred to *Cryptophialoidea* with polyphialidic conidiogenous cells, which are distinct from the monophialidic conidiogenous cells of other species in *Cryptophialoidea* (Kuthubutheen & Nawawi 1994b). Presently, five species are accepted in *Cryptophialoidea* (Index Fungorum 2018).

Cryptophialoidea fasciculata was re-collected in this study with the first sequence data for the genus. Our collection of *Cryptophialoidea fasciculata* is well-matched with the morphological characters in the original diagnosis of the holotype (Kuthubutheen & Nawawi 1994b), except the conidiogenous cells in our collection ($7.2\text{--}11.2 \times 3\text{--}4.8 \mu\text{m}$) are smaller than that in the holotype ($12\text{--}18 \times 4\text{--}6 \mu\text{m}$) and conidia in our collection ($26.5\text{--}33.4 \times 1.2\text{--}2.4 \mu\text{m}$) are slightly larger than that in the holotype ($22\text{--}29 \times 1.5\text{--}2 \mu\text{m}$). *Cryptophialoidea fasciculata* is unique by its discrete bundle-like conidiogenous cells. Due to the close phylogenetic relationship between *Cryptophiale* and *Cryptophialoidea*, molecular data of more taxa are required to confirm the separation of the two genera. In this study, sequence data is provided for *Cryptophialoidea fasciculata* and a reference specimen is designated for the species (*sensu* Ariyawansa et al. 2014).

Nawawia filiformis (Nawawi) Marvanová, Trans. Br. mycol. Soc. 75(2): 227 (1980) Fig. 4

Index Fungorum number: IF113008; Facesoffungi number: FoF04868

≡ *Clavatospora filiformis* Nawawi, Trans. Br. mycol. Soc. 61(2): 390 (1973)

Saprobic on decaying plant substrates. Asexual morph: Colonies on the natural substratum effuse, hairy, scattered, dark brown, with glistening mass of conidia at the apex of conidiophores. Mycelium partly immersed, partly superficial, composed of pale brown, septate hyphae. Conidiophores macronematous, mononematous, single or in small groups, erect, straight, rarely geniculate, smooth, mid or dark brown, becoming paler towards the apex, septate, $(49\text{--})77\text{--}215(\text{--}236) \times 4.1\text{--}5.9 \mu\text{m}$. Conidiogenous cells integrated, terminal, monophialidic, pale brown, smooth, cylindrical or lageniform, often with up to three percurrent proliferations. Conidia hyaline, round-tetrahedral, pentahedral or pyramidal, aseptate, smooth, thin-walled, guttulate, viewed from above triangular or quadrangular, $8.5\text{--}16.2 \mu\text{m}$ ($\bar{x} = 12.3 \mu\text{m}$, $n = 35$) long of the side, with an obconical truncate base, with an appendage at the apex of each corner. Sexual morph: Undetermined.

Cultural characteristics – Conidia germinating on PDA within 24 h and germ tubes produced from the base. Colonies on MEA medium slow-growing, reaching 5–10 mm diam. in a month at 25°C, in natural light, circular, with fluffy, dense, brown mycelium on the surface with entire margin; in reverse dark brown.

Material examined – THAILAND, Trat Province, Amphoe Ko Chang, 12°08'N, 102°38'E, on decaying wood submerged in a freshwater stream, 27 April 2017, YZ Lu, YJT 23-2 (MFLU 18-1500 reference specimen designated here, HKAS 102155), living culture MFLUCC 17-2394, additional sequence of SSU: MH758203; THAILAND, Krabi Province, on decaying wood submerged in a freshwater stream near Morakot Lake, 15 December 2015, S. Tibpromma, Site 6-6-4 (MFLU 18-

1501), living culture MFLUCC 16-0853, additional sequence of SSU: MH758200.



Figure 4 – *Nawawia filiformis* (MFLU 18-1500 reference specimen). a Colonies on woody substrate. b Conidiophore with mass of conidia on woody substrate. c–f Conidiophores. g Conidial formation. h, i Conidiogenous cells. j, k Conidia. l Germinated conidium. m, n Culture, m from above, n reverse. Scale bars: a = 200 μ m, b–d, f = 50 μ m, e, g, j–l = 30 μ m, h, i = 20 μ m.

Notes – The genus *Nawawia* was established by Marvanová (1980) with *N. filiformis* (as *Clavatospora filiformis*) transferred from *Clavatospora*. *Nawawia* is characterized by having brown, macronematous conidiophores, monophialidic conidiogenous cells sometimes with percurrent proliferations and hyaline, turbinate-tetrahedral to obpyramidal conidia with the blunt corners at the distal end each provided with a distinct hair-like appendage. Goh et al. (2014) provided a synopsis for the genus with six known species: *N. dendroidea* K.D. Hyde, Goh & Steinke, *N. filiformis*, *N. malaysiana* Crous & S.S. Lee, *N. nitida* Kuthub., G.M. Liew & Nawawi, *N. quadrisetulata* Goh, W.Y. Lau & K.C. Teo and *N. sasae-kurilensis* Melnik & K.D. Hyde. Subsequently, Peng et al. (2016) introduced the seventh species *N. oviformis* J. Peng & Z.F. Yu. Among the seven species, five are reported from aquatic habitats on submerged wood or leaves, with *N. malaysiana* and *N. sasae-kurilensis* found in terrestrial habitats (Nawawi 1973, Kuthubutheen et al. 1992, Hyde et al. 1996, Mel'nik & Hyde 2006, Crous et al. 2009, Goh et al. 2014, Peng et al. 2016). Sequence data is only available for *N. malaysiana* which is the only coelomycetous species within the genus. However, Crous et al. (2009) provided the BLAST results of the LSU sequence of *N. malaysiana* without carrying out a phylogenetic analysis. *Nawawia dendroidea* already provided the difference from other species in the genus in having synnematos conidiophores, which match the generic concept of *Phialosporostilbe*. The type species *Nawawia filiformis* has been reported worldwide (Nawawi 1973, Wu & McKenzie 2003, Pinnoi et al. 2006, Raja et al. 2009, Sudheep & Sridhar 2011), and it is the only species in the genus which produced a *Chloridium* synanamorph (conidia ellipsoid, without appendage) based on cultural studies (Nawawi 1973, Wu & McKenzie 2003).

Morphological characters of our collection agree with the original diagnosis and previous reports of *Nawawia filiformis*. *Nawawia filiformis* morphologically resembles *N. quadrisetulata* in having brown, mononematous conidiophores, monophialidic conidiogenous cells with a collarette and hyaline turbinate-tetrahedral to obpyramidal conidia. However, conidia of *N. filiformis* have 3–4 (mostly 3) appendages while those in *N. quadrisetulata* have 4–5 (mostly 4) appendages. The first sequence data of the species is generated in this study. Phylogenetic analyses showed that two isolates of *N. filiformis* clustered together and they are close to the genera *Exserticlava*, *Umbrinosphaeria* and *Zanclospora*. In this study, sequence data is provided for *Nawawia filiformis* and a reference specimen is designated for the species (*sensu* Ariyawansa et al. 2014).

Neonawawia J. Yang, K.D. Hyde & J.K. Liu, gen. nov.

Index Fungorum number: IF555449; Facesoffungi number: FoF05180

Etymology – Referring to the conidial similarity to the genus *Nawawia*.

Conidiomata sporodochial, semicupulate. *Mycelium* consisting of pale brown, smooth to somewhat roughened, septate, branched hyphae. *Conidiophores* hyaline to pale brown, smooth, subcylindrical, aggregated, branched, rarely loose or solitary, septate, or reduced to clavate cells giving rise to 7–8 conidiogenous cells radiating from the apex. *Conidiogenous cells* terminal, monophialidic, hyaline to pale brown, smooth, subcylindrical to somewhat ampulliform, tapering towards the apex. *Conidia* in olivaceous, slimy masses, solitary, hyaline, smooth, lobed, with 4–6 projections, aseptate, with a hair-like appendage at each corner, truncate at the base.

Type species – *Neonawawia malaysiana*.

Neonawawia malaysiana (Crous & S.S. Lee) J. Yang, K.D. Hyde & J.K. Liu, comb. nov.

Index Fungorum number: IF 555450; Facesoffungi number: FoF05181

≡ *Nawawia malaysiana* Crous & S.S. Lee, *Persoonia* 23: 195 2009

Holotype – MALAYSIA, Sarawak, on leaves of *Eucalyptus urophylla*, 20 April 2009, S.S. Lee, CBS H-20345, ex-type living cultures CPC 16757 = CBS 125544, CPC 16758.

Notes – The BLAST results of the LSU sequence showed that *Nawawia malaysiana* was close to several *Chaetosphaeria* species (Crous et al. 2009). It has similar conidial ontogeny and conidial morphology to *Nawawia*, and this taxon was accommodated in the genus while no other molecular data were available. Goh et al. (2014) questioned the taxonomic placement of *N. malaysiana* in the genus as the species differs from the other six species in being sporodochial, producing conidia that

are distinctly smaller and fusoid to ellipsoid in side-view, and it was isolated from the apex of a conidiomatal spore mass of a foliicolous *Satchmopsis* species. It is likely that *N. malaysiana* was misclassified based on the phylogenetic results and it should not belong to the genus. In this study, phylogenetic analyses indicated that the type species *N. filiformis* grouped together with the genera *Exserticlava*, *Umbrinosphaeria* and *Zanclospora*. *Nawawia malaysiana*, however, formed a sister clade to the family Chaetosphaeriaceae. We therefore introduce a new genus *Neonawawia* to accommodate this taxon and treat it as genera *incertae sedis* in the order Chaetosphaeriales.

Phialosporostilbe scutiformis N.G. Liu, J. Yang & K.D. Hyde, sp. nov.

Figs 5–6

Index Fungorum number: IF555325; Facesoffungi number: FoF04869

Etymology – Referring to the shield-shaped conidia from front view.

Saprobic on decaying plant substrates. Asexual morph: *Colonies* effuse, scattered, dark brown, with masses of conidia on the apex of conidiophores. *Mycelium* partly immersed, partly superficial. *Setae* simple, central, solitary, erect, dark brown or dark olivaceous, paler at the apex, rarely fertilizable, lower part encased by the compacted conidiophores, (250–)380–500(–540) × 4.2–5.2 μm (\bar{x} = 435 × 4.8 μm, n = 15), wider than each conidiophore. *Conidiophores* macronematous, synnematos, erect, solitary, septate, smooth, dark brown or dark olivaceous, becoming paler towards the apex, cylindrical, slightly tapering at the apical part, (175–)245–415(–460) × 3.3–4.8 μm (\bar{x} = 330 × 4 μm, n = 20) of the single conidiophore, 14–22(–30) μm (\bar{x} = 18.5 μm, n = 15) wide of the synnemata, often splaying out along the sides of the upper half of the synnemata, slightly undulated at the splayed part. *Conidiogenous cells* monophialidic, terminal, integrated, diverging from the main axis of the synnema, pale brown, subhyaline towards the apex, cylindrical to cylindrical-clavate, 35–45 μm long, 3.5–4.7 μm wide at the base, 1.9–3.0 μm wide at the tip, with an inconspicuous apical collarete. *Conidia* round-tetrahedral, pyramidal, smooth, aseptate, thin-walled, guttulate, viewed from above triangular, (5.5–)6–8.2(–8.7) μm (\bar{x} = 7.0 μm, n = 45) long of each above edge, 7.5–11.6 μm (\bar{x} = 9.5 μm, n = 40) long of the side, with an appendage 3.5–7.5 μm (\bar{x} = 5.6 μm, n = 40) long at each corner. Sexual morph: Undetermined.

Cultural characteristics – Conidia germinating on PDA within 24 h and germ tubes produced from the base. Colonies on MEA medium, reaching 5–10 mm diam. in a week at 25°C, in natural light, circular, with dense, yellowish-brown mycelium in the middle and yellow mycelium in the outer ring on the surface with undulate margin; in reverse mid brown.

Material examined – CHINA, Guizhou Province, Anshun city, Gaodang village, 26°4.2'N, 105°41.8'E, on decaying wood submerged in Suoluo river, 19 October 2016, J Yang, GD 12-6 (MFLU 18-1502 holotype, HKAS 102156 isotype); ex-type living cultures MFLUCC 17-0227, GZCC 17-0043, additional sequence of SSU: MH758201; THAILAND, Chiang Rai Province, on decaying wood submerged in a stream, 16 June 2017, NG Liu, CR004-1 (HKAS 102205 paratype), living culture MFLUCC 18-1288.

Notes – The genus *Phialosporostilbe* was introduced by Sierra & Portales (1985) based on the type species *P. turbinata* Mercado & J. Mena. It is characterized by synnematos conidiophores, monophialidic conidiogenous cells and subhyaline, turbinate or cordiform conidia with each bearing three apical setulae (Sierra & Portales 1985). Subsequently, another four species have been included in the genus, namely *P. catenata* Sureshkumar, Sharath, Kunwar & Manohar., *P. gregarioclava* Shirouzu & Y. Harada, *P. setosa* Bhat & W.B. Kendr., and *P. yadongensis* Y.M. Wu & T.Y. Zhang from different habitats and countries (Bhat & Kendrick 1993, Shirouzu & Harada 2004, Sureshkumar et al. 2005, Wu & Zhang 2009). The genus *Phialosporostilbe* resembles *Nawawia* in having macronematous conidiophores, monophialidic conidiogenous cells and hyaline conidia similar in shape. However, *Nawawia* has mononematous conidiophores, while *Phialosporostilbe* is characterized by synnematos conidiophores. The two genera have ever been considered to be congeneric (Bhat & Kendrick 1993, Hyde et al. 1996, Shirouzu & Harada 2004) due to the similar conidial ontogeny and conidial morphology.

The first sequence data for the genus is generated for *Phialosporostilbe scutiformis* in this study. Phylogenetic analyses indicated that *P. scutiformis* clustered as a sister clade to *Menisporopsis*

theobromae S. Hughes with good support. The two strains of *P. scutiformis* (MFLUCC 17-0227 and MFLUCC 18-1288), isolated from specimens material collected in China and Thailand respectively, are regarded as the same species based on the molecular data. It is shown that *Phialosporostilbe* and *Nawawia* are phylogenetically distinct genera.

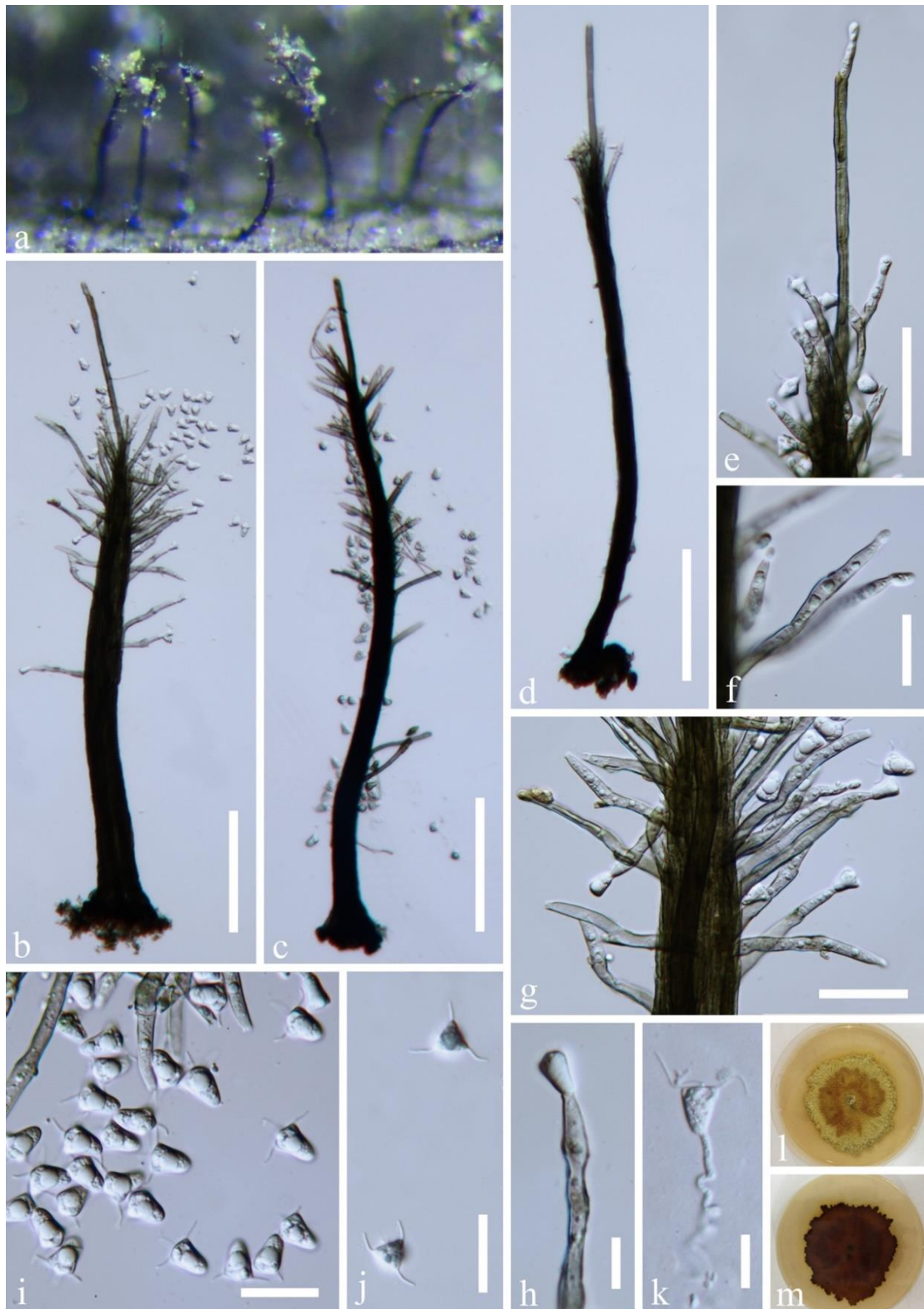


Figure 5 – *Phialosporostilbe scutiformis* (MFLU 18-1502 holotype, collected from China). a Colonies on woody substrate. b–d Conidiophores. e–h Conidiogenous cells with conidia. i, j Conidia. k Germinated conidium. l, m Culture, l from above, m reverse. Scale bars: b–d = 100 μ m, e = 50 μ m, f, i, j = 20 μ m, g = 30 μ m, h, k = 10 μ m.



Figure 6 – *Phialosporostilbe scutiformis* (HKAS 102205 paratype, collected from Thailand). a Specimen. b–c Colonies on substrate. d–e Fruiting bodies. f–g Conidiogenous cells and Conidia. h–k Conidia. Scale bar: b = 200 μm , c = 100 μm , d–e = 50 μm , f = 20 μm , g–k = 10 μm .

Phialosporostilbe scutiformis is morphologically similar to *P. turbinata* and *Nawawia* (*Phialosporostilbe*) *dendroidea* in having tree-like structures of conidiophores and similar conidial shape and size. However, *P. scutiformis* is distinguished from the two species by its conspicuous seta

which is erect in the center of the lower conidiophores. *Phialosporostilbe scutiformis* resembles *Menisporopsis theobromae* in having synnematosus conidiophores with a conspicuous central seta, terminal, monophialidic conidiogenous cells producing hyaline, aseptate conidia with appendages. However, conidiophores of *M. theobromae* are closely compacted towards the apex and halfway up the seta, while in *P. scutiformis* they are 1/4 or 1/5 lower on the seta and splay out at the upper part to form the tree-like shape. Moreover, *M. theobromae* produces clavate to cylindrical conidia which are slightly curved with hyaline appendages at both ends, but *P. scutiformis* has round-tetrahedral, pyramidal conidia bearing three appendages with one at each corner.

Phialosporostilbe dendroidea (K.D. Hyde, Goh & T. Steinke) J. Yang & K.D. Hyde, comb. nov.

Index Fungorum number: IF555326; Facesoffungi number: FoF04870

≡ *Nawawia dendroidea* K.D. Hyde, Goh & T. Steinke, Mycol. Res. 100(7): 810 (1996).

Holotype – South Africa, Durban, Palmiet River, on submerged pieces of stems of *Phragmites*, Nov. 1994, K. D. Hyde & T. Steinke, KDH 2157, BRIP 22870.

Notes – *Nawawia dendroidea* was introduced within *Nawawia* due to the consideration that *Phialosporostilbe* is congeneric with *Nawawia*, then the name *Nawawia* has priority over *Phialosporostilbe* (Hyde et al. 1996). Phylogenetic studies have indicated that *Nawawia* and *Phialosporostilbe* are phylogenetically distinct. *Nawawia dendroidea* differs from other *Nawawia* species in having synnematosus conidiophores, which match the generic concept of *Phialosporostilbe*. To be in line with the phylogenetic result and generic concept, we therefore transfer *N. dendroidea* to the genus *Phialosporostilbe*.

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