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Two new species of sooty moulds, *Capnodium coffeicola* and *Conidiocarpus plumeriae* in *Capnodiaceae*

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Abstract

Capnodiaceae is believed to be the largest family containing sooty mould species, the taxa of which can cause chlorosis, plant stunting disease, and marketability problems, due to black mycelium coating the surface of host. Presently, little molecular data are available for species of *Capnodiaceae* in GenBank, thus more collections and sequence data are needed to improve the understanding of genera and species boundaries in this family. "Sooty mould"-like taxa, appearing as black colonies on the surface of leaves, were collected in Chiang Rai Province, Thailand. Taxa were studied based on morphological characters and molecular analyses. A phylogenetic tree using combined LSU and ITS sequence data generated by Maximum likelihood analyses (LSU and ITS) indicated that the new species, *Capnodium coffeicola* and *Conidiocarpus plumeriae*, belong in *Capnodiaceae*. We introduce the two new species base on morphological characterization and phylogenetic analyses.

Key words – *Capnodiales* – Dothideomycetes – Phylogeny – Sooty moulds – Taxonomy

Introduction

Sooty moulds belong in seven families, which are *Antennulariellaceae* Woron., *Capnodiaceae* Höhn. ex Theiss., *Chaetothyriaceae* Hansf. ex M.E. Barr, *Coccodiniaceae* Höhn. ex O.E. Erikss., *Euantennariaceae* S. Hughes & Corlett ex S. Hughes, *Metacapnodiaceae* S. Hughes & Corlett and *Trichomeriaceae* Chomnunti & K.D. Hyde (Reynolds 1998, Winka et al. 1998, Hughes & Seifert 2012, Hyde et al. 2013, Chomnunti et al. 2014). They occur on various hosts, mainly forming a thin, superficial, network of dark mycelium on the surface of branches, flowers, fruits, leaves, and stems (Hughes 1976, Faull et al. 2002, Hyde et al. 2013, Chomnunti et al. 2014), and should not be confused with *Asterinales* M.E. Barr ex D. Hawksw. & O.E. Erikss. and *Meliolales* Gäum. ex D. Hawksw. & O.E. Erikss, which cause web-like, black colonies on leaves and cause minor damage to host plants by penetrating host cells for the uptake of nutrients (Ariyawansa et al. 2015, Hongsanan et al. 2014a, 2015a). Typically, sooty moulds reduce photosynthesis ability of plants through the mycelium coating; they can cause chlorosis under the mycelia, plant-stunting disease, low-yield, and marketability problems (Chomnunti et al. 2014).

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Sooty moulds have a wide distribution, and are most common in tropical and subtropical regions (Chomnunti et al. 2014).

Capnodiaceae is the most common family of sooty moulds, which is placed in the order *Capnodiales*, class Dothideomycetes (Batista & Ciferri 1963, Hughes 1972, Crous et al. 2009a, b, Schoch et al. 2009, Chomnunti et al. 2011, 2014, Hyde et al. 2013, Wijayawardene et al. 2014, Liu et al. 2015). This family was introduced by Höhnel (1910), and later validated by Theissen (1916) and the generic type is *Capnodium* Mont. The family is characterized by superficial, septate, dark brown hyphae, forming a thin mycelial network on the surface of hosts, bitunicate asci, the sexual and asexual morphs can be found in the same or different hosts, however, for some the sexual morph is unknown (Chomnunti et al. 2011, 2014, Hyde et al. 2013). The asexual morphs form elongated pycnidia, with short or long narrow necks, have a conspicuous oval swelling near the base, middle or apex of the pycnidia, and produce hyaline conidia inside the swollen part (Chomnunti et al. 2011). Crous et al. (2009a, b) placed three genera in *Capnodiaceae* based on their phylogenetic analyses. They also noted that Capnodiales probably contains diverse lineages, and some of these might need to be established as new families. There are presently not enough sequence data to define a new family, thus more collections and sequence data are needed. Chomnunti et al. (2011) used LSU and SSU rRNA sequence data to classify genera and species in *Capnodiaceae*, and concluded, based on morphology and phylogeny, that this family contains four genera; *Capnodium*, *Leptoxyphium* Speg., *Phragmocapnias* Theiss. & Syd. and *Scorias* Fr. Liu et al. (2015) introduced a new genus in *Capnodiaceae* (*Chaetocapnodium* Hongsanan & K.D. Hyde), and a new species of *Phragmocapnias* (*Phragmocapnias philippinensis* Hongsanan & K.D. Hyde).

Conidiocarpus is the asexual morph of *Phragmocapnias* which was introduced by Woronichin in Jaczewski (1917); the type species is *Conidiocarpus caucasicus* Woron. However, Batista & Ciferri (1963) cited *C. penzigii* Woron. which was introduced in 1926, as the type species (Hughes 1976). There are several publications noting that the genus *Conidiocarpus* was introduced in 1926 based on the type species *C. penzigii*, thus it was synonymized under *Phragmocapnias* (sexual morph) in many publications. Bose et al. (2014) followed the discussion in Hughes (1976), and they transferred species in *Phragmocapnias* to *Conidiocarpus* based on the rules of nomenclatural priority. There are 11 species of, and 386 hits for *Phragmocapnias* in Index Fungorum (2016) and Google respectively and 10 species of, and 106 hits for *Conidiocarpus*. We therefore agree with Bose et al. (2014), that *Conidiocarpus* should be used over *Phragmocapnias*, for these linked genera. With the exception of *Phragmocapnias philippinensis*, all species in *Phragmocapnias* were transferred to *Conidiocarpus* by Bose et al. (2014). Thus herein we synonymize *Phragmocapnias philippinensis* under *Conidiocarpus philippinensis* (Hongsanan & K.D. Hyde) Hongsanan & K.D. Hyde.

In this study, we introduce two new species, *Capnodium coffeicola* and *Conidiocarpus plumeriae* in *Capnodiaceae*. The new taxa are compared morphologically with other species in *Capnodiales*. The introductions of *Capnodium coffeicola* and *Conidiocarpus plumeriae* are also supported by phylogenetic analyses of the LSU and ITS sequence data.

Materials & Methods

Collections, isolation and morphology

Specimens with “Sooty mould”-like taxa were collected in Chiang Rai Province, Thailand, and observed under a stereomicroscope. Ascomata were studied by free-hand section, and their morphology studied under a compound microscope (Nikon 80i), slides were preserved in lactoglycerol after photographing. Measurements were determined using Tarosoft (R) Image Frame Work v. 0.9.7. Single spore isolation was carried out using the methods in Chomnunti et al. (2014). Type specimens of the new species are deposited in the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand, and ex-type cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC), and in Kunming Institute of Botany (KIB). Faces of fungi numbers

and Index Fungorum numbers are provided as explained in Jayasiri et al. (2015) and Index Fungorum (2016).

DNA isolation, amplification and sequencing

DNA was extracted from mycelium using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, P.R. China); following the instructions. The conditions for the polymerase chain reaction (PCR) were determined using the primer pairs LROR/LR5 to amplify the large subunit region (LSU), and ITS1/ITS4 to amplify the internal transcribed spacer region (ITS). The amplification was carried with setting times and temperatures for the initial denaturation and the final extension period following Hongsanan et al. (2014b, 2015b). PCR products were checked on 1% agarose electrophoresis gels, and sequenced by Majorbio Co., China. Sequences generated for the new species are deposited in GenBank.

Phylogenetic analysis

Thirty-six sequences were downloaded from GenBank to supplement the dataset. *Davidiella tassiana* was selected as outgroup taxon (Table 1). The data set, including the new species, *Capnodium coffeicola* and *Conidiocarpus plumeriae*, were aligned by using MAFFT (Katoh et al. 2009), and checked manually using Bioedit (Hall 1999). Maximum likelihood analysis was carried out in raxmlGUIv.0.9b2 (Silvestro & Michalak 2012). The search strategy was set to bootstrapping and the analysis performed using the GTRGAMMAI model. The number of replicates was inferred using the stopping criterion (Pattengale et al. 2009). The bootstrap values expressed from 1,000 repetitions by RAxML analysis which are equal or greater than 50% are given to the left of each node (Fig. 1). The model of evolution was performed in MrModeltest 2.2 (Nylander 2008). Posterior probabilities (PP) were set by MCMC sampling in MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001, Zhaxybayeva & Gogarten 2002), following the details in Cai et al. (2006, 2008) and Hongsanan et al. (2014a, b). Posterior probabilities values (PP) from Bayesian analysis which are equal or greater than 0.90 are given the right of each node (Fig. 1). Phylogenetic trees were viewed using MEGA v5.2.1 (Tamura et al. 2011).

Results

Phylogenetic analyses

The large subunit ribosomal (LSU) and Internal transcribed spacer (ITS) sequences from 38 isolates of *Capnodiaceae*, *Dissoconiaceae, Euantennariaceae* and *Mycosphaerellaceae,* were included in the phylogenetic analysis; *Davidiella tassiana* is used as the outgroup taxon (Fig. 1). In the tree, the family *Capnodiaceae* is placed within *Capnodiales* under Dothideomycetes. The *Capnodiaceae* clade comprised 29 strains which belong in *Antennariella* Bat. & Cif., *Capnodium* Mont., *Conidioxyphium* Bat. & Cif., *Leptoxyphium* Speg., *Microxiphium* (Harv. ex Berk. & Desm.) Thüm., *Phragmocapnias*, *Polychaeton* (Pers.) Lév., and *Scorias* Fr. (98% ML, 1.0 PP). The *Leptoxyphium* clade comprised three strains of *Leptoxyphium* including *Microxiphium citri* (100% ML, 1.0 PP), this result is similar to previous studies (Chomnunti et al. 2014, Liu et al. 2015). Four *Capnodium* strains clustered with species of *Conidioxyphium* and *Microxiphium* (97% ML and 1.0 PP), furthermore five strains of *Scorias* are basal. *Capnodium coffeicola* clustered in a moderately supported clade within the genus *Capnodium*, and is separated from the other species. The new taxon *Conidiocarpus plumeriae* is closely related to *C. betle* (Syd. et al.) T. Bose, with high bootstrap support, but is a distinct species (100% ML, 1.0 PP). Two representative strains of *Dissoconiaceae* clustered together with high bootstrap support (100% ML, 1.0 PP) and are related to *Capnodiaceae* (78% ML). Six strains of *Mycosphaerellaceae* grouped with high bootstrap support (100% ML, 1.0 PP) and are closely related to the two strains of *Euantennariaceae* (91% ML, 0.99 PP). These three families form a sister group to *Capnodiaceae* within the order *Capnodiales*.

Table 1 Taxa used in the phylogenetic analysis with GenBank accession numbers (LSU and ITS) and species voucher/culture numbers.

Species	Voucher/culture numbers	Accession numbers	
		LSU	ITS
Antennariella placitae	CBS 124785	GQ303299	GQ303268
Capnodium coartatum	MFLUCC10-0069	JN832614	-
Capnodium coartatum	MFLUCC10-0070	JN832615	-
Capnodium coffeae	CBS 147.52	GU214400	AJ244239
Capnodium coffeicola	MFLUCC15-0206	**KU358920**	**KU358921**
Chaetocapnodium siamensis	MFLUCC13-0778	KP744479	-
Conidioxyphium gardeniorum	CPC 14327	GU301807	-
Davidiella tassiana	CBS 399.80	-	AJ244227
Dissoconium aciculare	CBS 204.89	GU214419	AY725520
Leptoxyphium cacuminum	MFLUCC10-0049	JN832602	have
Leptoxyphium kurandae	CPC:17274	JF951170	JF951150
Leptoxyphium madagascariense	CBS 124766	GQ303308	GQ303277
Microxyphium aciculiforme	CBS 892.73	GU301847	-
Microxyphium citri	CBS 451.66	GU301848	-
Microxyphium theae	CBS 202.30	GU301849	GU296178
Mycosphaerella ellipsoidea	CBS:110843	GQ852602	AY725545
Mycosphaerella endophytica	CBS:114662	GQ852603	DQ302953
Mycosphaerella punctiformis	CBS 113265	NG027571	KF442502
Pallidocercospora irregulariramosa	CBS 111211	KF902053	KF901706
Conidiocarpus philippinensis	MFLUCC12-0110	KP744503	-
Conidiocarpus plumeriae	MFLUCC15-0205	**KU358918**	**KU358919**
Conidiocarpus siamensis	MFLUCC10-0053	JN832606	KU358922
Conidiocarpus siamensis	MFLUCC10-0061	JN832607	KU358923
Conidiocarpus siamensis	MFLUCC10-0062	JN832612	KU358924
Conidiocarpus siamensis	MFLUCC10-0063	JN832608	KU358925
Conidiocarpus siamensis	MFLUCC10-0064	JN832609	KU358926
Conidiocarpus siamensis	MFLUCC10-0065	JN832610	KU358927
Conidiocarpus siamensis	MFLUCC10-0074	JN832611	KU358928
Polychaeton citri	CBS 116435	GU214469	GU214649
Ramichloridium apiculatum	CBS 156.59	EU041848	EU041791
Rasutoria pseudotsugae	rapssd	EF114704	EF114687
Rasutoria tsugae	ratstk	EF114705	EF114688
Scorias leucadendri	CBS 131318	JQ044456	JQ044437
Scorias spongiosa	AFTOL-ID 1594	DQ678075	-
Scorias spongiosa	CBS 325.33	-	GU214696
Scorias mangiferae	MFLUCC15-0230	KT588603	KT588604

Conidiocarpus philippinensis (Hongsanan & K.D. Hyde) Hongsanan & K.D. Hyde, **comb. nov.**
Index Fungorum: IF551807
Facesoffungi number: FoF01771

≡ *Phragmocapnias philippinensis* Hongsanan & K.D. Hyde, in Liu et al., Fungal Diversity: 172:69 (2015)

Notes – This species was introduced in Liu et al. (2015) as *Phragmocapnias philippinensis*. Phylogenetic analyses indicated that it was placed in *Capnodiaceae*, however it did not cluster with others species in *Phragmocapnias sensu stricto* (Liu et al. 2015); the result is similar to our study. Based on morphology, Liu et al. (2015) stated that *P. philippinensis* is most similar to other species in *Phragmocapnias*, but differs in having 5-septate ascospores without a hyaline sheath, thus they introduced it as a new species. Bose et al. (2014) synonymized *Phragmocapnias* under *Conidiocarpus*, which was the oldest name of these linked genera. They transferred species of *Phragmocapnias* to *Conidiocarpus*, thus, herein we synonymize *P. philippinensis* under *C. philippinensis*. This may require new genus when more related species are found, but for the present we place it in *Conidiocarpus sensu lato.*

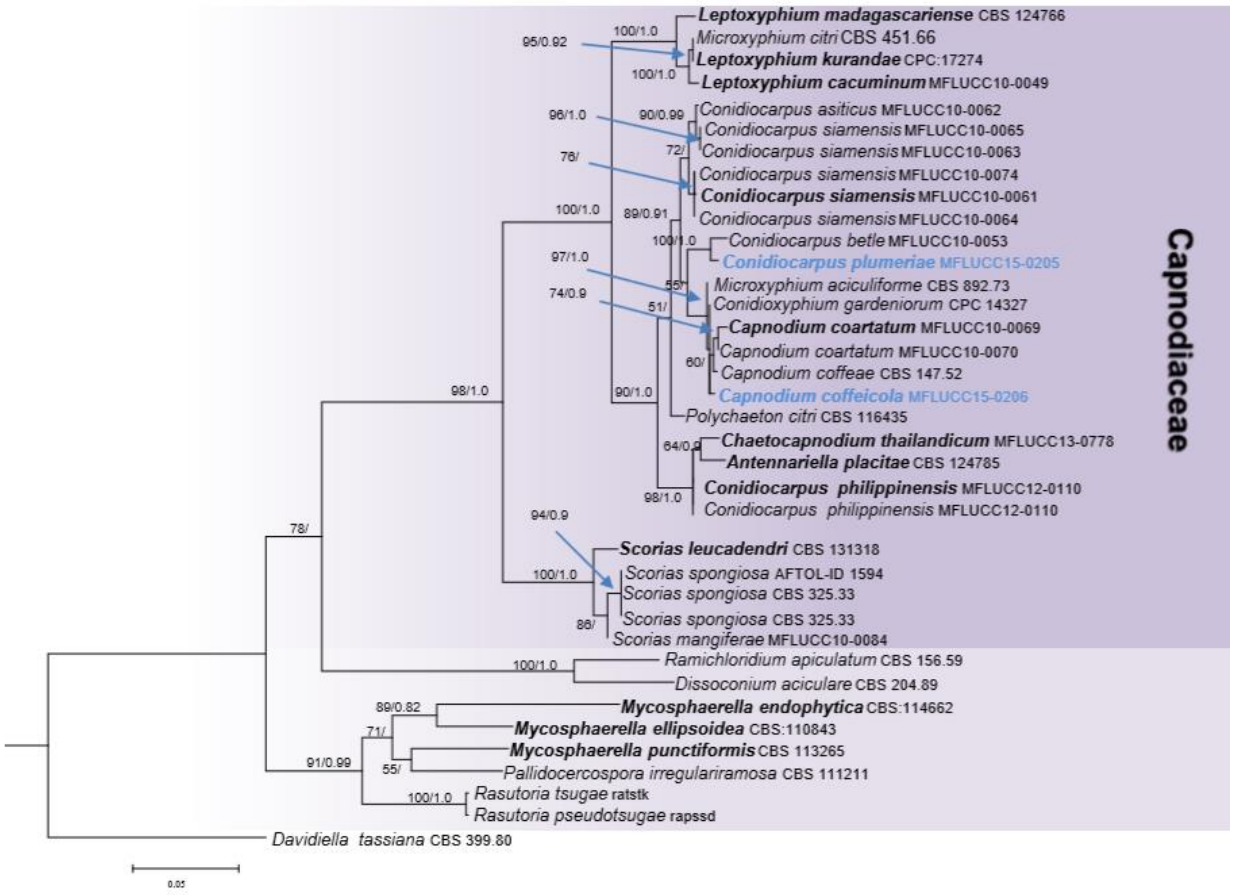

Fig. 1 – RAxML maximum likelihood phylogenetic tree generated from analysis of combined LSU and ITS sequence data. The first set of numbers above the nodes are RAxML bootstrap values equal or greater than 50%. The second set of numbers above the nodes are Bayesian posterior probabilities, with values above 0.9 shown. Strain numbers are indicated after species names. Ex-types strains are in black bold, new sequence data are in blue bold.

Capnodium coffeicola Hongsanan & K.D. Hyde, **sp. nov.** Fig. 2
Index Fungorum number: IF551802
Facesoffungi number: FoF01765

Etymology – *coffeicola* referring to the host on which the taxon was found.

Saprobic on sugary exudates from *Coccus* sp. (*Coccidae*, Insecta) growing on the surface of leaves, branches, and stems of *Coffea* sp. *Thallus* thin, dark brown, easily removed from the host surface, composed of cylindrical hyphae. *Superficial hyphae* 3–5 µm wide ($\overline{x}$=4 µm, n=20), septate, constricted at the septum, branched, brown to dark brown, with subcylindrical hyphal cells. **Sexual morph**: Undetermined. **Asexual morph**: *Pycnidia* 165–178 µm long ($\overline{x}$=170 µm, n=10), superficial, scattered or gregarious, blackish brown, cylindrical, swollen at the central part, 14–16 µm diam. ($\overline{x}$=34 µm, n=10), stalk black, 19–24 long × 18–23 µm diam. ($\overline{x}$=23 × 21 µm, n=20), wall comprising mostly cylindrical cells, the swollen part producing conidia inside. *Ostiole* 14–16 µm diam. ($\overline{x}$=15 µm, n=10), surrounded by hyaline hyphae, 23–26 × 2–3 µm ($\overline{x}$=25 × 2.5 µm, n=20). *Conidiogenous* cells formed on the inner cell walls of the swollen part. *Conidia* 5–7 × 1–3 µm ($\overline{x}$=6 × 2 µm, n=20), cylindrical to oblong, ends round, hyaline, smooth-walled.

Culture characters – Conidia germinating on PDA at 25–28°C for 12 h with dark, hyphae germinating from the conidia, septate, constricted at the septum, hyaline to grayish at the beginning, and become black to greenish later. Colonies slow growing, reaching 2 cm diam. after 5 days on PDA, colony superficial to erumpent, sometimes hyphae growing downwards and immersed into media, surface verrucose, velvety, branched at the margin, asexual structures produced in PDA after 3 days.

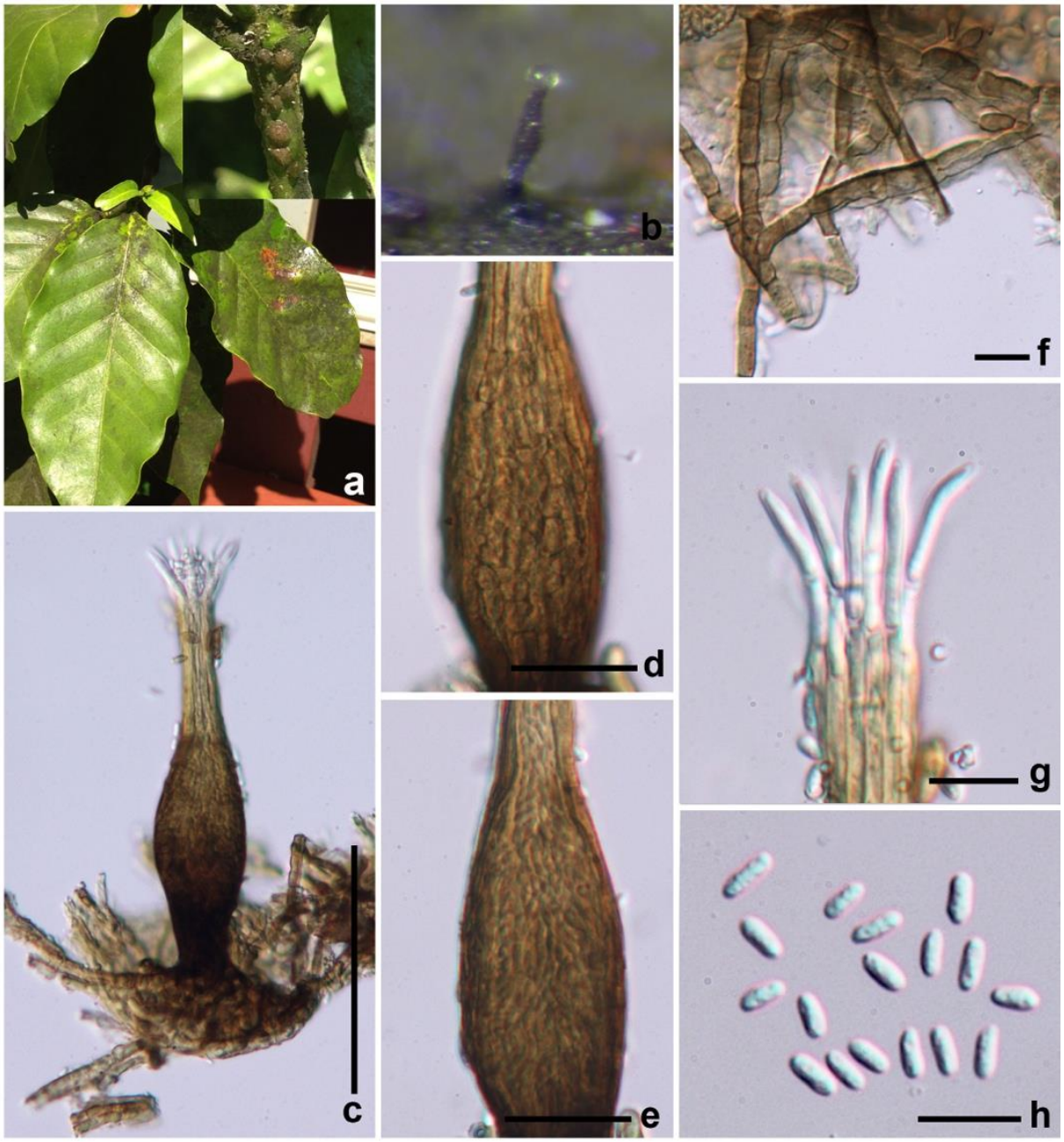

Fig. 2 – *Capnodium coffeicola* (holotype). a Substrate. b Pycnidia on surface of leaves. c Pycnidia when viewed in squash mount. d, e Cells wall of swollen part when viewed in squash mounts. f Septate hyphae constricted at the septum. g Ostiole surrounded by hyaline hyphae. k Unicellular conidia. – Bars c=100 µm, d, e=20 µm, f–h j=10 µm.

Material examined – Thailand, Chiang Rai, Tasud, Mae Fah Luang University, AD2 building on leaves of *Coffea* sp., 4 January 2015, S. Hongsanan, PST2-1 (MFLU 15-3565, **holotype**); *ibid.* (**isotype** in KIB) – ex-type living culture in MFLUCC 15-0206.

Notes – *Capnodium coffeicola* is most typical of *C. coartatum* Chomnunti & K.D. Hyde, but it has pycnidia with short and black stalks at the base, and is swollen at the central part, and cylindrical to oblong conidia, while *C. coartatum* has long and brown pycnidia, which are swollen at the base, lacks black and short stalks, and has ellipsoidal conidia. In addition, *C. coffeicola* is also similar to some species in the genus *Conidiocarpus*, based on its pycnidia being swollen at the central part of the pycnidium and a stalk in the lower swollen part. However, *Capnodium coffeicola* has a very short stalk and darkened lower swollen part. Phylogenetic analyses demonstrates that *C. coffeicola* belongs in *Capnodium*, within the family *Capnodiaceae.*

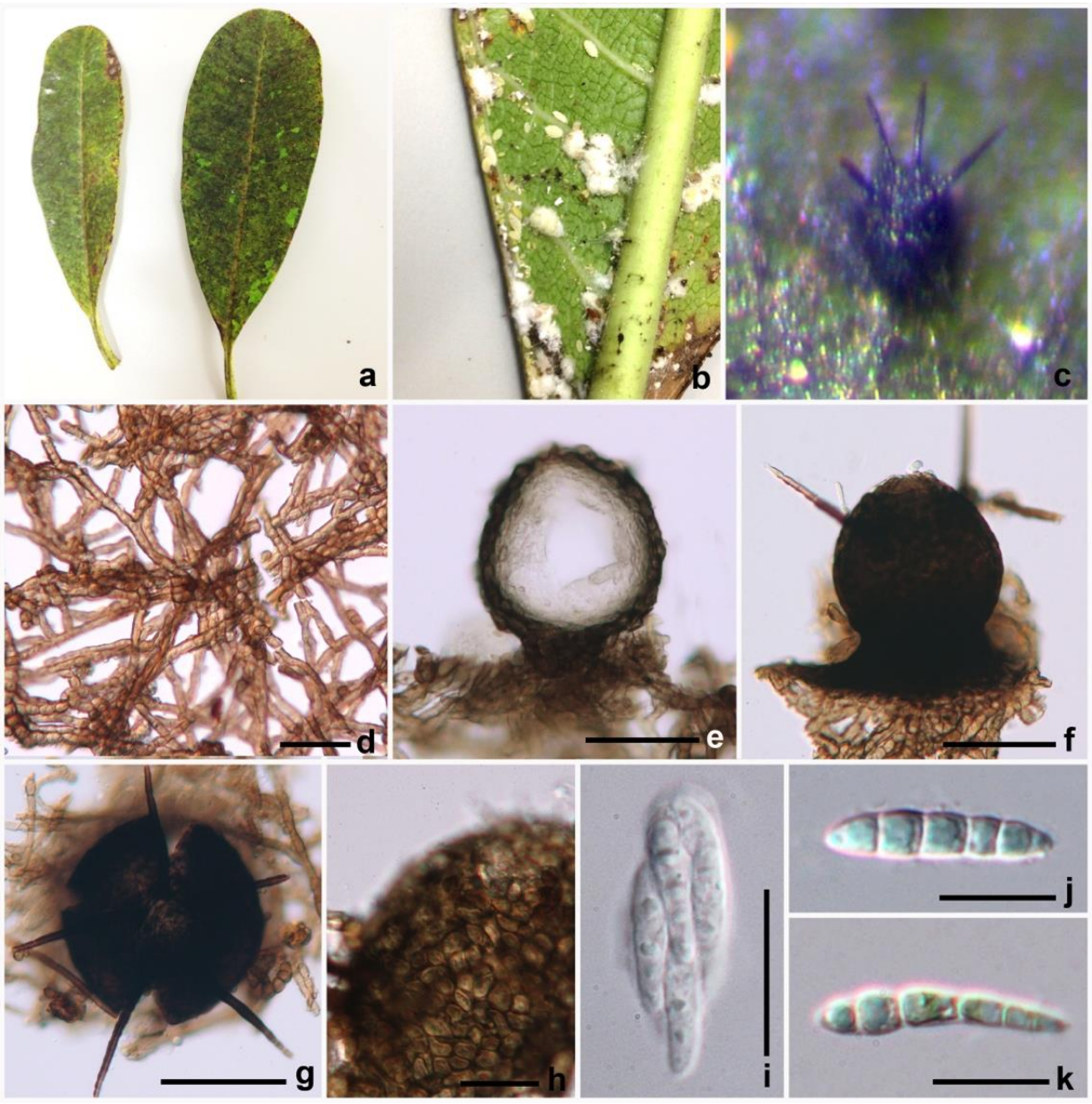

Fig. 3 – *Conidiocarpus plumeriae* (holotype). a Substrate. b Mealy bug on the lower surface of leaves. c Solitary ascomata on the surface of leaves. d Hyphal networks of *C. plumeriae*. e Section through ascoma. f, g Ascomata when viewed in squash mounts. h Upper wall when viewed in squash mounts. i Asci with 8-spores. j Ascospores. k Ascospores in Melzer's reagent. – Bars e-g=50 µm, d, h, i=20 µm, k, j=10 µm.

Conidiocarpus plumeriae Hongsanan & K.D. Hyde, **sp. nov.** Fig. 3
Index Fungorum IF551805
Facesoffungi number FoF01766

Etymology – *plumeriae* referring to the host on which the taxon was found.

Saprobic on sugary exudates from *Pseudococcus* sp. (*Pseudococcidae*, Insecta), growing on the upper surface of *Plumeria* sp. *Thallus* thin, dark brown, easily removed from the host surface, composed of cylindrical hyphae. *Superficial hyphae* 5 *µm* wide, branched, septate, slightly constricted and dark at the septum, pale brown to brown **Sexual morph**: *Ascomata* 90–95 µm diam. ($\bar{x}$=94 µm, n=10), superficial, solitary, subglobose, narrowly rounded above, constricted at the base, dark brown to black, ostiole present at maturity, thin-walled, with 3–4 ascomatal setae at the upper part of ascomata. *Ascomatal setae* 87–91 × 4–6 µm ($\bar{x}$=89 × 5 µm, n=10), aseptate, dark brown to reddish brown, but pale brown to hyaline at the apex. *Peridium* 10–13 µm ($\bar{x}$=11 µm,

n=10), comprising cells of *textura angularis*, inner layer hyaline, outer layer dark brown to reddish brown. *Hamathecium* lacking pseudoparaphyses. *Asci* 37–42 × 13–16 µm ($\bar{x}$=39 × 14 µm, n=10), 8-spored, bitunicate, fissitunicate, subcylindrical to obovoid, short pedicellate or sometimes apedicellate, ocular chamber not observed. *Ascospores* 19–25 × 4–6 µm ($\bar{x}$=22 × 5 µm, n=10), bi to tri-seriate, cylindrical to clavate, 5-septate, slightly constricted at the septum, with narrow ends, somewhat tapering towards the base, hyaline, smooth-walled. **Asexual morph**: Undetermined.

Culture characters – Ascospores germinating on PDA at 25–28°C for 12 h with dark, hyphae germinating at each cells of the ascospores, septate, constricted at the septum, hyaline to brown at the beginning, and become black to greenish, darker at the margin. Colonies slow growing reaching 2 cm diam. after 7 days on PDA, colony superficial to erumpent, surface verrucose, velvety.

Material examined – Thailand, Chiang Rai, Tasud, Mae Fah Luang University, AD2 building, on leaves of *Plumeria* sp., 10 January 2015, C. Singhapop, PST1-2 (MFLU 15-3564, **holotype**); *ibid.* (**isotype** in KIB) – ex-type culture in MFLUCC 15-0205.

Notes – *Conidiocarpus plumeriae* is most typical of *C. imperspicua* (Sacc.) Cif. & Bat., but differs in having long and hyaline ascospores, which are 5-septate at maturity, while *C. imperspicua* has short and brownish ascospores, with 4 septa at maturity. *Conidiocarpus plumeriae* is also similar to *C. betle*. It however differs in having subcylindrical to obovoid asci, and 5-septate ascospores, while *C. betle* has broadly clavate asci, and 4-septate ascospores, surrounded by a hyaline sheath. Phylogenetic analyses based on LSU and ITS sequence data indicate that *C. plumeriae* is closely related to *C. betle*, but is morphologically distinct.

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