A MORPHOLOGICAL AND MOLECULAR REAPPRAISAL OF SOOTY MOULDS

PUTARAK CHOMNUNTI

DOCTOR OF PHILOSOPHY
IN
BIOSCIENCES

SCHOOL OF SCIENCE
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Putarak Chomnunti

Dissertation Title	A Morphological and Molecular Reappraisal of Sooty Moulds
Author	Putarak Chomnunti
Degree	Doctor of Philosophy (Biosciences)
Advisor	Assoc. Prof. Dr. Kevin D. Hyde
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ABSTRACT

Sooty moulds are fungi growing on the surface of plants and produce dark mycelium that cover leaves and other structures, but do not generally infect the host. Plant pathologists regard these fungi as threats because their growth can block the amount of light absorbed by the leaves, reducing photosynthesis, and causing the products to be of lower quality. Furthermore, the taxonomy of these fungi has been confused. The lack of documentation on the morphological characters of existing species is the main reason for difficulties in identification. Sequence data in GenBank is also inadequate. In this study, I am investigating morphology and phylogeny of the sooty moulds on living leaves in northern Thailand. Specimens with dark mycelium on living leaves were collected in field work, and found to include sexual and asexual states of the fungi, sometimes in the same leaf. In present study I revisit sooty moulds collected in Thailand and assigned to the families *Capnodiaceae*, *Chaetothyriaceae*, *Trichomeriaceae* and other families known from the literature. For the latter I borrow and studied material kept in various herbaria and fungaria.

Selected genera in the *Capnodiaceae* are morphologically noted. Type specimens of the ascomycetous genera *Aithaloderma, Anopeltis, Callebaea, Capnodaria, Echinothecium, Phragmocapnias* and *Scorias* were re–examined, described and illustrated. *Leptoxyphium* is an asexual *Capnodiaceae*, together with *Polychaeton.* In fact, *Polychaeton* is a legitimate and earlier name for *Capnodium*, but in order to maintain nomenclatural stability I have proposed here that the name of the sexual stage should be considered for the approved lists of fungal names currently in preparation. Notes are provided on the ascomycetous genus *Scoriadopsis*. However, I was unable to locate and study the type of this monotypic genus during the time frame of this study, because in the original publication of the species, there is not mention of where the specimen is stored. The ascomycetous genera *Aithaloderma, Ceramoclasteropsis, Hyaloscolecostroma* and *Trichomerium* are excluded from *Capnodiaceae* on the basis of having ascostromata and trans-septate hyaline ascospores and should be accommodated in *Chaetothyriales*. Furthermore, *Callebaea* is excluded as the ascomata are thyriothecia and the genus is placed in *Micropeltidaceae*. *Echinothecium* is placed as synonym of *Sphaerellothecium* and thus transferred to *Mycosphaerellaceae*. The type specimen of *Capnophaeum* is lost and this should be considered as a doubtful genus. The coelomycetous *Microxiphium* is polyphyletic, while the status of *Fumiglobus*, *Polychaetella* and *Tripospermum* is unclear. Fourteen new collections of sooty moulds made in Thailand were isolated and sequenced. The nuclear large and small rDNA was partially sequenced and compared in a phylogeny used to build a more complete understanding of the relationships of genera in *Capnodiaceae.* Four new species (*Capnodium coartatum, Leptoxyphoum cacuminum, Phargmocapnias asiaticus* and *P. siamensis*) are described and illustrated, while *Phragmocapnias* and *Scorias* are epitypified with fresh collections.

Several taxa of epiphyllous ascomycota belonging to *Chaetothyriaceae* (*Chaetothyriales*, Eurotiomycetes) were collected in northern Thailand. This family is poorly understood both in current morphological and phylogenetic studies. Chaetothyriaceae is a family characterized by ascomata which form beneath mycelia

pellicle lying on the leaf surface, and which is attached to the upper part of ascomata, ascomata sub-globose to globose with or without setae. Asci bitunicate, clavate or pyriform, short pedicellate and ascospores are clavate, muriform hyaline, with or without mucilaginous sheath. This study deals with three new species, i.e. *Ceramothyrium thailandicum*, *Chaetothyrium brischofiacola* and *Phaeosaccardinula ficus* which are fully described and illustrated. A DNA sequence analyses of LSU and ITS rDNA genes shows that the new species cluster in the *Chaetothyriaceae*. In addition this research adds six sequences for *Chaetothyriaceae* to GenBank providing additional data for the family.

Trichomerium is a genus of foliar epiphytes with the appearance of sooty moulds, in the literature assigned to the *Capnodiales*. Species have ascostromata with setae and develop on a loosely interwoven mycelial mass of dark brown hyphae, while asci have a bitunicate appearance, containing hyaline ascospores. In this study, I made 16 collections of the genus from Thailand. All were isolated, and the LSU and ITS rDNA gene regions sequenced. Phylogenetic analysis indicated that the *Trichomerium* species form a monophyletic clade within *Chaetothyriales* and warrant the introduction of a new family *Trichomeriaceae* within this order. Bootstrap support for the *Chaetothyriales* is 100% and clearly separates *Trichomeriaceae* from *Capnodiales* which otherwise are morphologically very similar. A detailed account of *Trichomerium* is provided and I describe and illustrate three new species based on morphological and molecular data. It would be better if *T. foliicola* could be adopted as the generic type of *Trichomerium* because the holotypeit has been impossible to obtain the holotype specimen of *T. coffeicola* and also no molecular data exists in worldwide databases for this species or genus.

Keywords: *Capnodiaceae*/Classification/*Chaetothyriaceae*/Molecular/Sooty moulds

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CHAPTER 1

INTRODUCTION

1.1 The Sooty Moulds

The sooty moulds, numbering over 200 species, are a group of epifoliar fungi that live on plant surfaces. They produce superficial, brown to black colonies on leaves and smaller twigs which comprise a thin network of dense, dark hyphae (Hughes, 1976; Faull, Olejnik, Ingrouille & Reynolds, 2002). Sooty moulds are most common in tropical and subtropical regions worldwide. Most of the sooty moulds have hyphae with mucilaginous outer walls which readily absorb water and thus, consequently maintain moisture on leaf surfaces for a longer period (Batista & Ciferri, 1963b). Hyphae of sooty moulds of different taxa are often mixed together on the host surface. These include both asexual and sexual stages of the same or different species, and sometimes sooty moulds may or may not produce the sexual stage (Figure 1.1) (Hughes 1972; Hughes, 1976; Hughes & Seifert, 2012). As sooty moulds frequently grow mixed together and show a noticeable pleomorphy, this compounds the problem of species circumscription in the mixed colonies. Some species are thought to produce as many as three asexual states which have been allied to one sexual state (Hughes, 1976), however this information is based on association rather than cultural or molecular proof.

Historically, the term ‘sooty mould’ was first used by Berkeley & Desmazières (1849) in his detailed study of some members of the genus *Fumago*. He based his work on herbarium specimens from Sri Lanka, i.e. ‘the coffee leaves with a deep black colour of sooty mould related with coccus or bug’, and on ‘the serious sooty mould on citrus leaves in the Azores and Madeira’, and on *Citrus* species grown in greenhouses in France. The term was later popularized in the US plant pathology literature, starting with Weber’s paper in 1897 (Hughes, 1976; Faull et al., 2002).

It is generally understood that sooty moulds are fungi that grow on honeydew secreted by insects in the Order Homoptera, which includes aphids, whiteflies, soft scale, mealy bugs, leafhoppers and psyllids, on plant parts and other surfaces (Barr, 1987). Some sooty moulds form sponge-like masses of thick-walled hyphae superficially on living plants, including leaves, stems and flowers (Rikkinen, Dörfelt, Schmidt & Wunderlich, 2003) resulting from interactions between sap-feeding insects and non parasitic fungi (Hughes, 1976). Sooty moulds are fairly harmless saprobes of honeydew, the immature and adult stages of these insects however, cause harm by sucking the sap from plants and producing the honeydew.

Note. (a) *Sapotaceae*. (b) *Dracaenaceae*. (c) *Convallariaceae*. (d) *Euphorbiaceae*. (e) *Acanthaceae*. f) *Euphorbiaceae*. (g) *Asteraceae*. (h) *Rubiaceae*.

Figure 1.1 Sooty Moulds on Various Host

1.2 Historical background of sooty moulds

Persoon (1822) described the first sooty mould, *Fumago vagans* Pers., recognizing its black mycelium with small tubes covering the leaves of lime, elm, poplar, ash and willow. Spegazzini (1918) reorganized the sexual state of sooty moulds in the Perisporiales in seven families, including the *Capnodiaceae* and *Chaetothyriaceae* which contained species of sooty moulds with ostiolate perithecia. Sooty moulds, including asexual states were named as Deutocapnodieas; pycnidial forms were placed in Asbolisieas, while hyphomycetous forms were placed in Hypasbolisieas. Batista & Ciferri (1963a) transferred some of Spegazzini's anamorphic group Asbolisieas to Hypasbolisieas because of their elongated pycnidia or synnematous form. Saccardoan principles of differences in spore morphology and conidiomata anatomy were followed during the 19th century by the different authors for fungal classification. In Saccardo (1899) includes 121 species and varieties of sooty mould, organized into 28 genera.

The above concepts prevailed for much of the century until Hughes (1976) collected and monographed many sooty moulds from New Zealand. The sooty moulds form obvious elements in the mycota of every habit and habitat, viz. the branches and trunks of trees, stones and even the forest floor can support a thick carpet of these fungi. Reynolds (1998) provided some information on phylogeny of sooty moulds, the capnodiaceous sooty moulds constituting a monophyletic group, named the Capnodiales which are represented by both asexual and sexual states.

The sooty moulds are fungi included in the *Antennulariellaceae*, *Capnodiaceae*, *Chaetothyriaceae*, *Coccodiniaceae*, *Euantennariaceae*, *Metacapnodiaceae* and *Trichomeriaceae* (Reynolds, 1998; Winka, Eriksson & Bång, 1998; Chomnunti, Ko Ko et al., 2012; Hughes & Seifert, 2012). Recently, the *Schifferulaceae* has been described as a new sooty mould family for saprobic fungi from India (Hosagoudar & Riju, 2011). However, these cannot be cultured so their systematic arrangement cannot be ascertained with molecular techniques, and morphologically produce hyphopodia. This character is present in many species

referred to the *Meliolaceae* and *Asterinaceae*, so these fungi have not been included in this study.

The taxonomy of sooty moulds has been very unsatisfactory; they exist as community on plant surfaces, comprising many different asexual and sexual states, often linked on the basis on host association; the connections between these states are still unclear. Traditionally the name sooty mould was associated with the families *Capnodiaceae* Sacc. and *Chaetothyriaceae* Hann (McAlpine, 1896; Faull et al., 2002). Morphological characterization has been the traditional taxonomic method used to identify the fungi and still continues to be an important practice to recognize species. Their ascomata are black, ovoid to ellipsoidal or dome-shaped perithecia that may have setae. They contain a type of bitunicate ascus with eight hyaline to brown septate ascospores, and their asexual reproductive structures have been interpreted by some as synnemata.

In recent times, mycologists, in addition, use molecular phylogeny as a modern taxonomic method to confirm the identity of fungi, besides many biochemical characters (Webster & Weber, 2007). However, using molecular phylogeny still is problematic as most type materials are unavailable for DNA extraction, so in addition epitypification has been undertaken for some species using fresh material. Thus, the epitype helps to interpret the new features or attributes of specimens. For a definition of what is an epitype one should consult Article 9.8 of the latest edition of the *International Code of Nomenclature* for algae, fungi, and plants (Melbourne Code) (McNeill et al., 2012; see also: http://www.iapt-taxon.org/nomen/main.php). Thus, re-examination of type materials and establishment of epitype with living cultures is essential for progress. Multi gene analysis with distinct morphological characters are needed to develop a strong species-based taxonomic system (Phillips, Oudemans, Correia & Alves, 2006; Phillips, Crous & Alves 2007; Crous, Braun & Groenewald, 2007; Shenoy et al., 2007; Alves, Crous, Correia & Phillips, 2008).

During the most recent Nomenclature Section of the International Botanical Congress XVIII (IBC) held in Melbourne, Australia (July 2011), the participants approved the concept ‘one fungus = one name’ and this is now reflected in the new code. This change is directly linked to the advances in molecular phylogenetic analysis because a more natural systematic position of the fungus is revealed by

molecular evidence. The above began to have a significant impact on the taxonomy of fungi. Comparisons of DNA sequences made possible to reliably connect asexual morphs to their sexual states. It is now realized and hoped that when each fungus gets only one name it will solve the confusions arouse from dual naming so far prevailing in ascomycete fungal systematics (Hawksworth, 2011; McNeill & Turland, 2011; Rossman & Seifert, 2011; Wingfield et al., 2012). An example of this is the sooty mould *Fumago citri*. The species was introduced by Persoon in 1822, but because it was considered not to be described in a 'complete' way, it was transferred to the genus *Polychaeton* by the French mycologist Léveillé (1847). However, mycologists Berkeley & Desmazieres (1849) transferred all the species once known in the genus *Fumago*, and by default *F. citri*, to *Capnodium*, and after that, the species became known as *Capnodium citri*. This generic name is used for fungi showing ascus and ascospores, the sexual stage, which is not often morphologically observed. So, the name *Polychaeton* was still used when the sooty mould did not produce ascospores. With the advent of molecular techniques we can now show that both fungi are the same organism, therefore, there is no need to continue using different names. More information on the adoption of a single name for this fungus can be found in Art. 57 of the Melbourne Code (2012).

The present taxonomy of each of the sooty mould families is outlined and discussed below, list of genera of each family also presented in Table 1.1.

1.3 Families of Sooty Moulds

1.3.1 ***Antennulariellaceae*** Woron., is a poorly known family of sooty moulds which was described by Woronichin (1925) in Capnodiales, including six genera with 27 species (Kirk, Cannon, Minter & Stalpers, 2008) while Lumbsch & Huhndorf (2010) list only two genera. Species in the family have a widespread distribution, and are found in warm temperate to tropical locations, where they grow as black sooty molds on plants (Cannon & Kirk, 2007). Woronichin (1925) mentioned that *Antennulariellaceae*, based on *Antennulariella*, was the most representative family in *Capnodiales* along with *Capnodiaceae* and *Coccodiniaceae*, as the species have

ostiolate ascomata. This was later also mentioned by Hughes (1976). *Antennulariellaceae* differs from *Coccodiniaceae* in having completely closed ascomata with irregular hyphae, and in its conidial states (Woronichin, 1925). *Achaetobotrys*, a second genus with two species was soon added by Woronichin (1926) in this family, *Achaetobotrys affinis* (L.R. Fraser) Bat. & Cif. is presented in Figure 1.2 (Material examined: Australia, New South Wale, Mitchell river district, between Grafton an Glen Innes. On living leaf of *Rhodosphaeria rhodanthema*. January, 1935. L. Fraser). The asexual states of *Antennulariaceae* have been recorded as pycnidial in *Antennariella* Bat. & Cif., and described as small and dark brown, subglobose to obovoid in shape, and produced terminally on a short stalk, or intercalary, with a short neck and ostioles at maturity; the pycnidial wall is pseudoparenchymatous, smooth or roughened (Hughes, 1976). However, *Capnodendron* (Hughes, 2000) is hyphomycetous, presenting somewhat lateral conidiophores. Furthermore, Hughes (2003) recently included his new hyphomycetous sooty mould genus, *Capnofrasera* in the *Antennulariellaceae*; the genus is known from New Zealand, Chile, Venezuela, Brazil, Canada and USA. No DNA sequences of species in *Antennulariaceae* are available in GenBank and therefore molecular data is required to establish the ordinal placement of this family, and the relationships between the sexual and asexual states of the various genera.

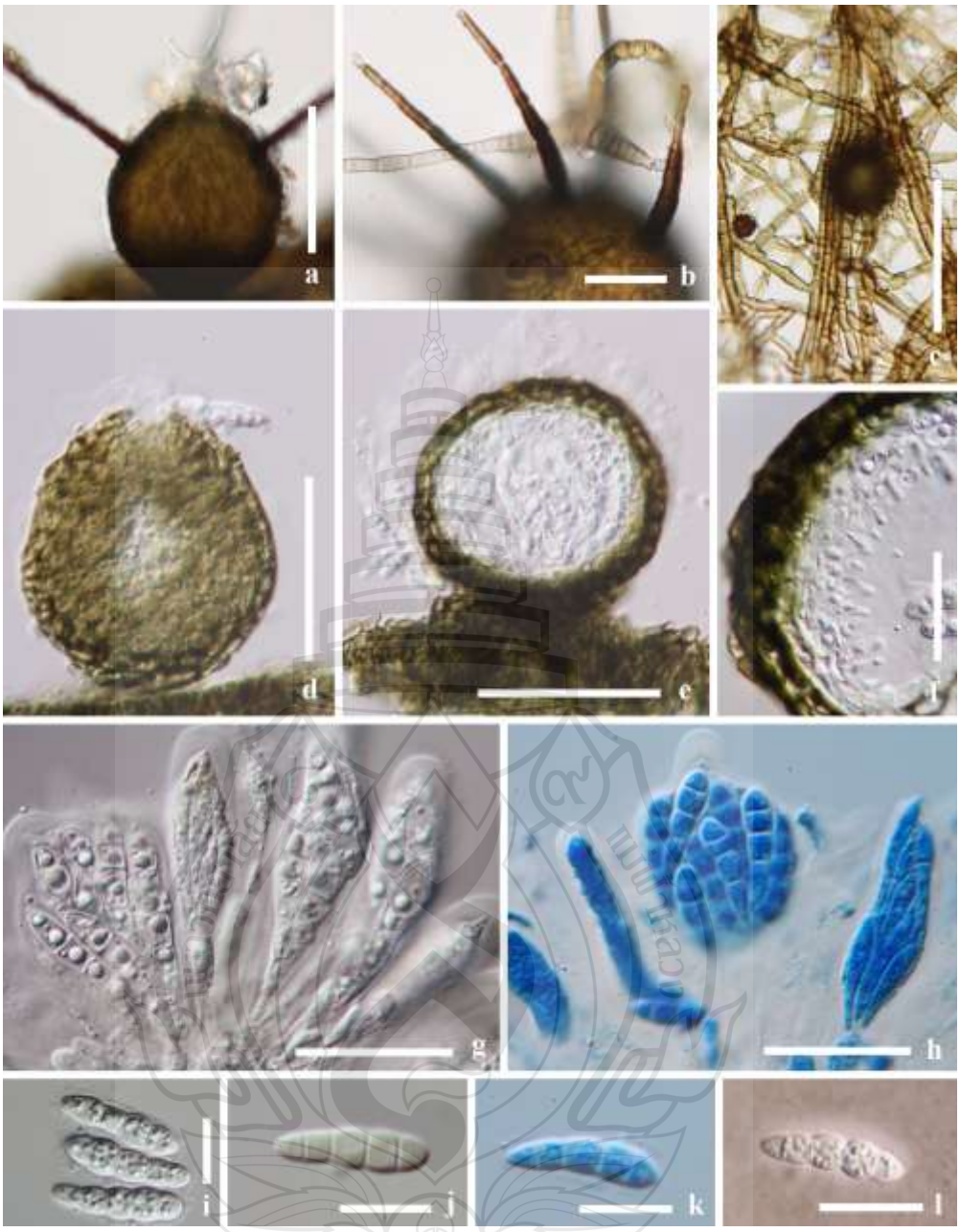
Note. (a). Sooty mould on host. (b) – (f). ascomata. (g). Ascomata develop from repeated divisions of hyphae. (h). Septate hyphae. (i) – (k). Asci. Scale bars: (c). = 50 µm, (b), (d) – (g). = 20 µm, (h) – (k). = 10 µm.

Figure 1.2 *Achaetobotrys affinis* (L.R. Fraser) Bat. & Cif.

1.3.2 ***Capnodiaceae*** Höhn. ex Theiss. is probably the most species rich and common family of sooty moulds. Kirk et al. (2008) stated that it includes 26 genera and 117 species, although Lumbsch & Huhndorf (2010) only accepted 13 genera. The family name was introduced by Höhnel (1910) and validated by Theissen (1916). Von Arx & Müller (1975) and Hughes (1976) circumscribed the family on the basis of ecological characters. Later, Reynolds (1982) defined sooty moulds as a dothideomycetous group mostly known by their pycnidial in asexual stages.

Chomnunti et al. (2011) revisited the *Capnodiaceae* and removed many genera to other families based on morphological characters of type specimens, thus only five genera are now accepted in *Capnodiaceae*. Species in the family have a widespread distribution, especially in tropical and subtropical regions worldwide. For example; *Capnodium* species are the most commonly found sooty moulds in gardens and landscapes (Laemmlen, 2011). The taxa of this family can be recognized by black mycelial growth spreading on the host surface which produces superficial colonies with septate, cylindrical, dark-brown hyphae and bitunicate asci. The asexual stages form elongated pycnidia that develop from a superficial mycelium on living plant surfaces. Pycnidia have short or long narrow necks with a conspicuous oval swelling which produces minute, unicellular and hyaline conidia from near the base, middle or apex (Chomnunti et al., 2011). The first major monographic review of capnodiaceous sooty moulds was by Fraser (1935) who based it on sexual and asexual species, and placed the species in the *Eucapnodiaieae*. Afterwards Batista & Ciferri (1963a) monographed the *Capnodiaceae* as Capnodiales. Hughes (1976) reviewed and re-classified *Capnodiaceae*, characterizing the species by the structure of the hyphae, the presence or absence of paraphyses and by deviating conidial states. Molecular techniques are now available to confirm their relationships in the *Capnodiaceae*. Crous, Schoch et al. (2009) used molecular methods to classify the members of the order *Capnodiales* and included three genera of *Capnodiaceae* in their study. They concluded that the order probably contained diverse lineages, and some of these might merit a new family, but more sequence data was needed to support this. Chomnunti et al. (2011) used a RAxML maximum likelihood tree based on combined LSU and SSU genes to show that *Capnodium, Leptoxyphium, Phragmocapnias* and *Scorias* are well defined genera in *Capnodiaceae*. The collection of sooty mould in Thailand

Phragmocapnias betle (Material examined: THAILAND, Chiang Rai. On living leaf of *Sapotaceae*. Saowanee wiki. 27 December 2011. MFLU11-1155), is presented in Figure 1.3.

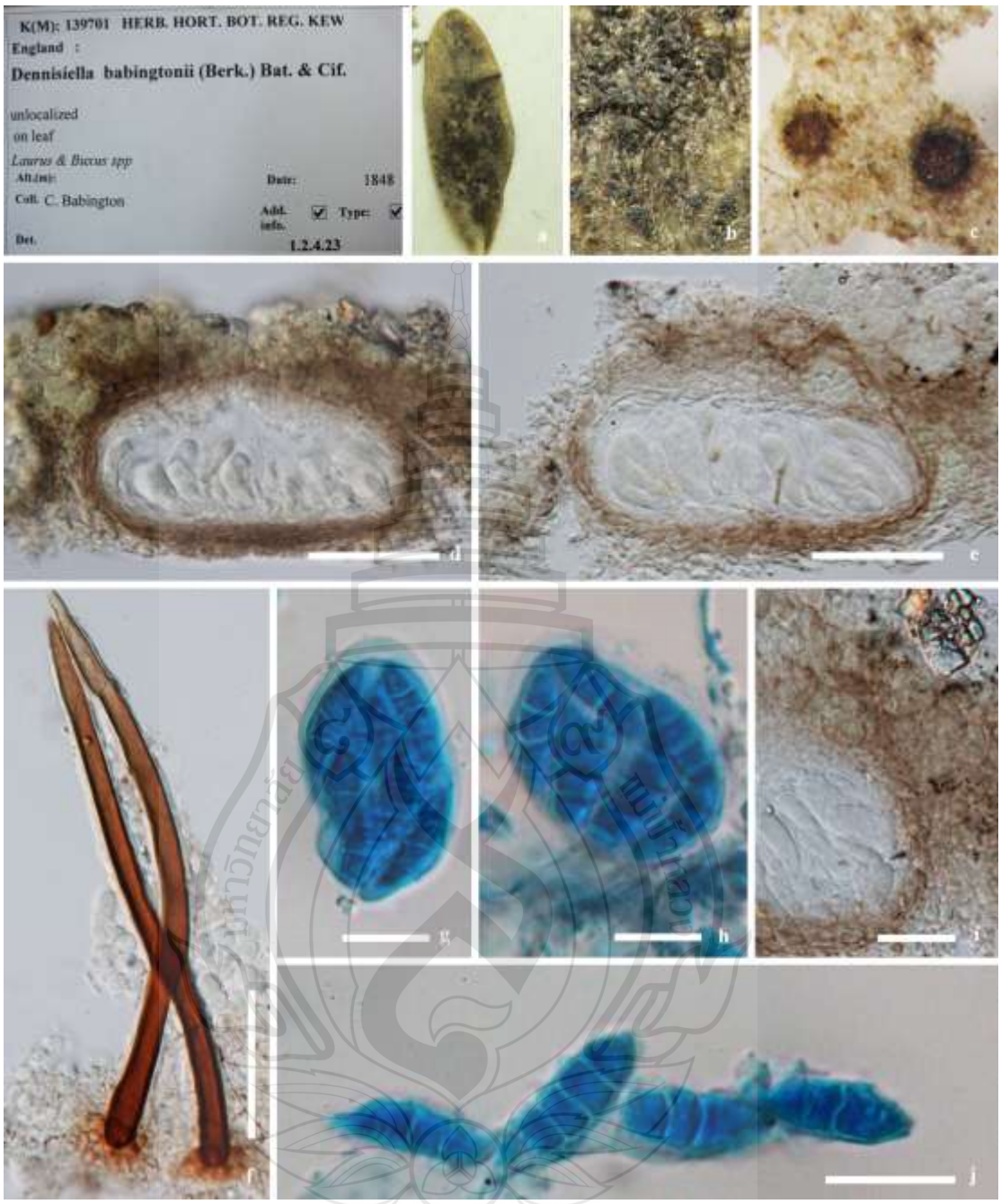


Note. (a), (d). ascomata superficial on host. (b). Setae. (c).Hyphae. (e). Vertical section through ascomata. (i). Peridium. g). Asci. (h). Asci strained with cotton blue. (i) Ascospores. (j). Ascospore stained with Melzer's reagent. (k). Ascospore stained with cotton blue. (l). Ascospore stained with Indian ink. Scale bar: (a), (c) – (f). = 50 µm, (b), (g) – (h). = 20 µm, (i) – (l). = 10 µm.

Figure 1.3 *Phragmocapnias betle*

1.3.3 ***Coccodiniaceae*** Höhn., ex O.E. Erikss. is a family of sooty moulds described by Eriksson (1981), which comprises three genera; the type genus *Coccodinium* A. Massal., *Dennisiella* Bat. & Cif. and *Limacinula* Höhn (Lumbsch & Huhndorf 2010), In this study, *Dennisiella babingtonii* (Berk) Bat. & Cif. (Material examined: Unlocalized, on leaves of *Laurus* & *Buxus* spp. 1848, C. Babington. K(M) 139701) were observed and presented in Figure 1.4. Kirk et al. (2008) recognize five genera which include the asexual genera *Bisbyopeltis* Bat. & A.F. Vital and *Microxiphium* (Harv. ex Berk. & Desm.). In his re-classification of sooty mould, Hughes (1976) did not include the *Coccodiniaceae*. The family is characterised by limacinuloid ascomata (dark brown collabent ascomata) on living leaves and sometimes other plant parts. The fungus develops on a scanty or well-developed subiculum or darkened hyphae usually surrounded in its immediate vicinity by a very loose arrangement of hyphae which appear light to whitish macroscopically and which are individually connected as a component of the lower portion of the fruit body wall ascomata sessile on a subiculum, globose to subglobose, brownish, uniloculate, think-walled, with ostiole periphysate. Asci are bitunicate, 8-spored, irregularly arranged ascospores, stalked, saccate. Ascospores are ellipsoidal or clavate, fusiform, transversely septate or muriform, constricted at septa, hyaline or rather dark brown. The hyphomycetous asexual states develop on rosettes of phialides, with mycelium forming a thin setose pellicle, of aseptate hyphae, which curve at the tips (Reynolds, 1971, Reynolds & Gilbert (2005) as in species of *Microxyphium* and *Bisbyopeltis*.

Winka et al. (1998) studied the morphology of *Coccodiniaceae* and found the asexual state resembled a *Capnodendron* species, which is a conidial stage of sooty moulds in the family *Antennulariellaceae*. Therefore, the asexual state of *Coccodiniaceae* may be related to the *Anntennularielaceae* according to its morphology. Phylogeny derived from molecular analysis indicated that some *Microxiphium* species are members of *Capnodiaceae*. This genus is however, polyphyletic as it has also been linked to *Dennisiella* in *Coccodiniaceae* (Schoch et al., 2006; Crous et al., 2007; Ruibal, Sakayaroj et al., 2009). Phylogenetic data of Chomnunti et al. (2011) indicate that *Microxyphium citri* is a member of *Capnodiaceae*).



Note. (a) – (c). Ascostromata and setae on host surface. (d) – (e). Vertical section of ascostromata. (f). Seatae. (g) – (h). Asci stained with cotton blue. i). Peridium connect with mycelium. (j). Ascospores stained with cotton blue. Scale bars: (d) – (f). = 50 µm, (i). = 20 µm, (g) – (h), (j). = 10 µm.

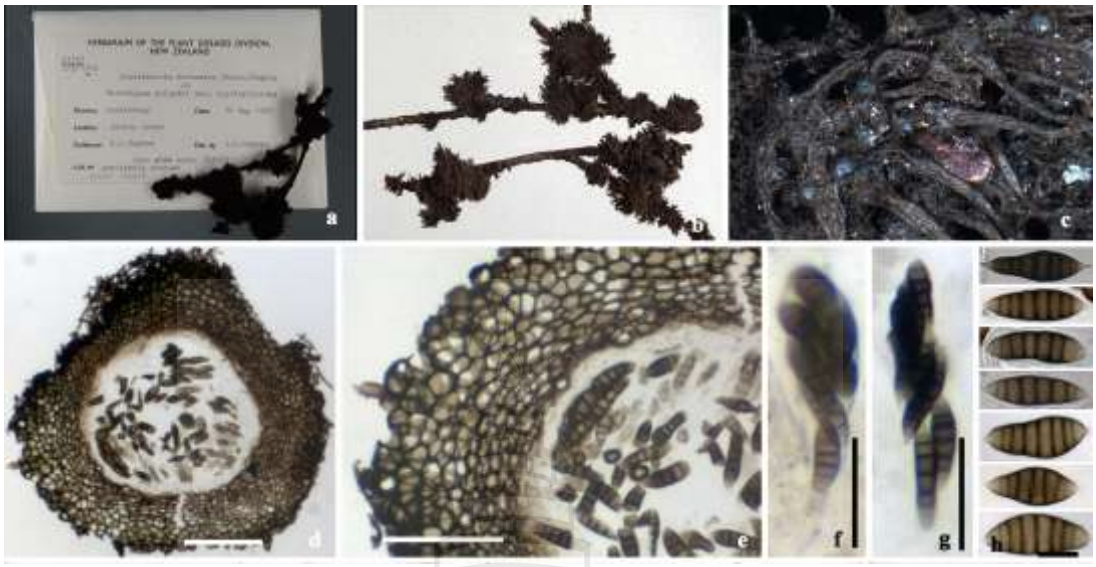
Figure 1.4 *Dennisiella babingtonii* (Berk) Bat. & Cif.

1.3.4 ***Euantennariaceae*** was introduced by Hughes & Corlett in 1972, currently includes nine genera and 28 species (Kirk et al., 2008). It is recognized by superficial, brown, cylindrical mycelium network, consisting of septate hyphae with smooth to coarsely roughened walls, lightly constricted at septa (Hughes, 1972). The ascostromata are subglobose, dark pigmented with thick walls, and ostiolate at maturity; they bear cylindrical hyphal appendages. Asci are bitunicate, usually 8-spored, and ellipsoidal. Ascospores are pale brown to dark brown, ellipsoidal to broadly ellipsoidal, and 3 to multiseptate. In this study, *Euantennaria mucronata* (Mont.) S. Hughes (Material examined: NEW ZEALAND, Canterbury, on *Nothofagus solandri* var. *cliffortioides*, 14 May 1963, S.J. Hughes, PDD 21317), were observed and presented in Figure 1.5 and 1.6.

The asexual state has two forms of conidia in *Euantennariaceae*, sometimes both present in the genus *Euantennaria* (Figure 1.6). Ameroconidia are also found in *Hormisciomyces* Bat. & Nascim. within conidiophores having terminal whorls of globose phialides, whereas phragmoconidial synanamorphs are found in *Antennatula* Fr., *Capnokyma* S. Hughes and *Trichothallus* F. Stevens. Furthermore, *Capnokyma* is recognized by its erect setae-like conidiophores, but not discrete conidiophores are found in *Antennatula*. The phragmoconidia are blastic and sessile, subhyaline to dark brown, 3-multiseptate, ellipsoidal to subcylindrical, tapered towards the end, straight or curved (Eriksson, 1981; Hughes, 1976; Hughes & Seifert, 2012).

Note. (a). Synnemata. (b) – (c). Synnema with head of conidia. (d) – (e). Hyphae from teased, mature synnema with conidia. (f) – (h). Conidiophores. (i). Anastomosed hyphae. (j). Hyphal appendages on ascostromata. (k) –(l). Hyphal forming. (m) – (n). Conidia. Scale bars: (e), (h) – (j), =20 µm, (k), (m) – (n). = 50 µm, (g). = 200 µm.

Figure 1.5 *Euantennaria mucronata* (Mont.) S. Hughes



Note. (a). Type collection packet. (b). Type specimen. (c). Perithecia present. (d), (e). Vertical section of ascostromata. (f), (g). Asci. (h). Ascospores. Scale bars: (f) – (g), (h) – (n) = 20 µm, (d), (e) = 100 µm.

Figure 1.6 *Euantennaria mucronata* (Mont.) S. Hughes.

1.3.5 ***Metacapnodiaceae*** was introduced by Hughes & Corlett in 1972, includes six genera and 19 species It is represented by *Metacapnodium* and species are widespread in tropical regions (Kirk et al., 2008). This sooty mould produce superficial mycelium, of spongy subiculum, composed of brown to dark brown, moniliform, i.e. constricted at the septa, and branched hyphae, with terminal cells usually tapered towards the apex (Hughes & Seifert, 2012). Ascomata immersed in the subiculum, broadly ellipsoidal, or globose surrounded by appendages. Peridium comprising cells of *textura angularis*. *Asci* bitunicate, 8–spored, ellipsoidal with pedicel. Ascospores ellipsoidal with somewhat conical end cells, 3–spetate, thick-walled, brown to dark brown, darker brown at the septa.

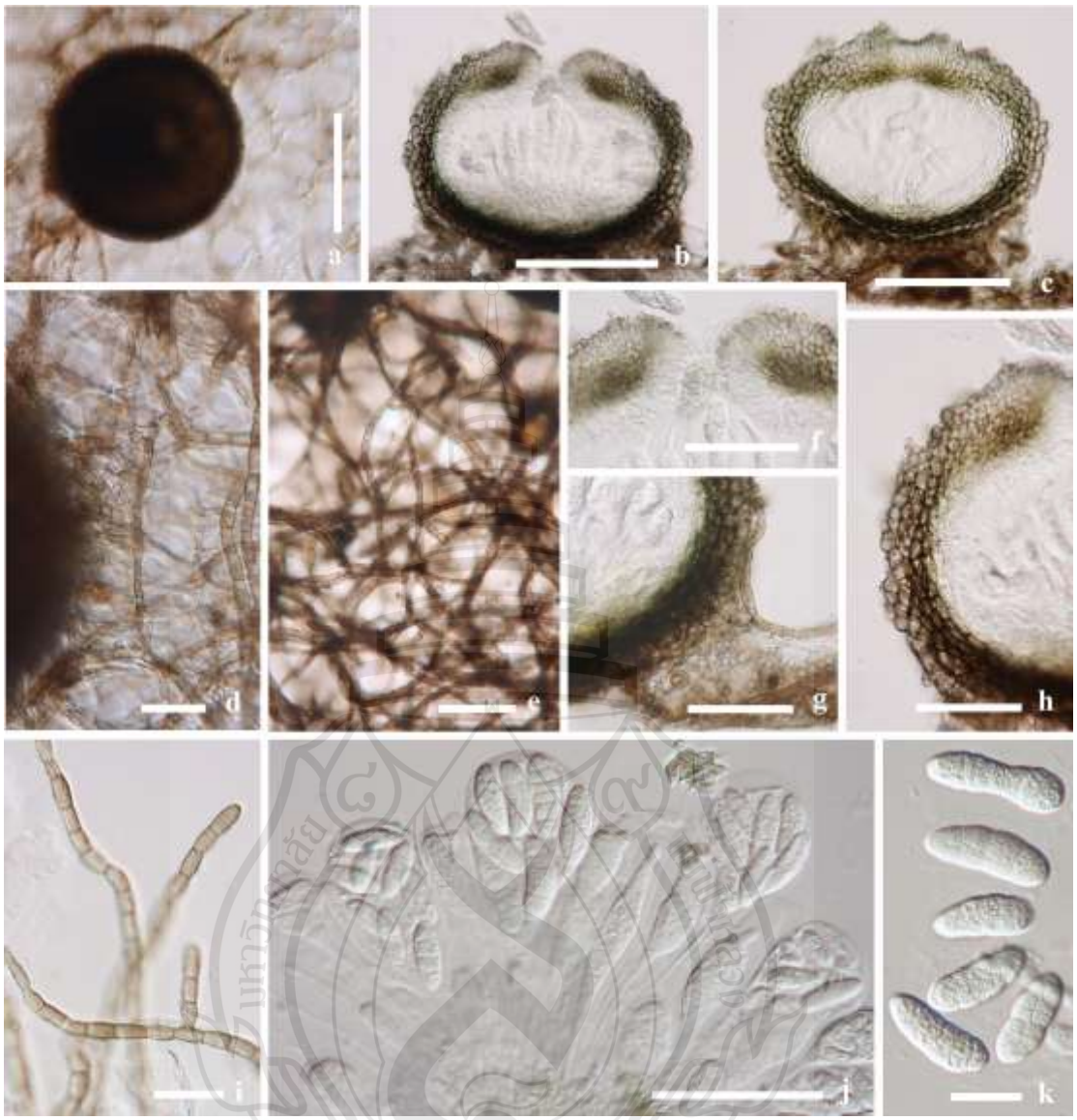
This sooty mould family has hyphomycetous conidial asexual states, which distinguishes it from *Capnodiaceae* (Hughes, 1972). This hyphomycetous state forms a thick, brown to black, dense subicula arranged in a pseudoparenchymatous cushion,

which can produce several syanamorphs. Asexual states have been reported in *Capnophialophora* and also *Capnocybe*, *Capnosporium*, *Hormiokrypsis* and *Hyphosoma*. All *Metacapnodiaceae* species share a *Capnophialophora* asexual state recognized by plump, ampulliform phialides, developing on the narrowing parts of the moniloid conidiophores, and producing small ameroconidia. Other synanamorphs include *Hormiokrypsis* with solitary dry stauroconidia, *Capnocybe* with slimy heads of phragmoconidia, and *Capnosporium* with solitary, dry phragmoconidia which produce phialides and microconidia (Batista & Nascimento, 1957; Hughes, 1966; Hughes & Seifert, 2012). However, as more than one sooty mould species will grow on a leaf, the relationships of these asexual states with *Metacapnodium* needs molecular confirmation or culture work (Chomnunti et al., 2011; Hughes & Seifert, 2012). The collection *Metacapnodium spongiosum* (Material examined: SPAIN, W from Jimena de la Frontera close to Las Cañillas, at the road 3331, on bark of *Erica arborea*, H. Voplmayr & W. Jakhtsch, 21 March 2011, MFLU12-0140), is presented in Figure 1.7

Note. (a). Thick woolly black mass of mycelium on *Erica arborea.* (b) – (c). Section through globose ascoma. (d). Peridium. (e). Hyphae. (f) – (g). *Capnophialophora* state. (h) – (j). Ascospores. (k) – (m). *Capnocybe* conidia. Scale bars: (a) = 100 μm. (c), (e). = 50 μm. (f) – (g). = 20 μm. (i) – (m). = 10 μm.

Figure 1.7 *Metacapnodium spongiosum*

1.3.6 ***Chaetothyriaceae*** Hann. Includes nine genera (Lumbsch & Huhndorf, 2010) while 13 genera and 98 species were reported by Kirk et al. (2008). It is unclear for many *Chaetothyriaceae* species, whether they are saprophic or biotrophic. Chaetothyriaceous species are often confused with capnodiaceous sooty moulds which are associated with insects and have similar characters on various hosts. (Hansford, 1946). Whether *Chaetothyriaceae* are associated with insect honeydew needs further clarification. Studies on *Chaetothyriaceae* have been mainly conducted by Hansford C.G, Hughes S.J. and Batista A.C., and colleagues, from 1940 to 1970, and there have been a few studies since. Characterized by ascomata which form beneath mycelia pellicle lying on the leaf surface, and which is attached to the upper part of ascomata, ascomata sub-globose to globose with or without setae. Asci bitunicate, clavate or pyriform, short pedicellate and ascospores are clavate, muriform hyaline, with or without mucilaginous sheath The family is poorly circumscribed and most previous work consists of brief descriptions with line drawings (Hansford, 1946, Batista & Ciferri, 1962). The basis for arrangement of genera is hard to understand and perhaps rather subjective being based on the importance placed by the individual authors on certain characters, such as spore septation, presence of ascomata setae and mycelium color (Batista & Ciferri, 1962; Hughes, 1976). *Chaetothyriaceae* are easily recognized form other sooty moulds on leaf surfaces by the shape of the ascomata. In *Capnodiaceae* ascomata are single, subglobose to globose, with or without setae, while in *Chaetothyriaceae* they form ascostromata, i.e. the ascomata are surrounded by a pellicle of superficial mycelium, and are often multilocular. In addition, phylogenetic analyses from LSU and ITS sequence data have clearly shown them to be unrelated, even placed in two separate classes: *Dothideomycetes* and *Eurotiomycetes* respectively (Schoch et al., 2006; Schoch et al., 2009; Geiser et al., 2006; Chomnunti, Ko Ko et al., 2012). The collection of sooty moulds *Phaeosaccardinula ficus* (Material examined: THAILAND, Chiang Mai. On living leaf of *Ixora* sp. Ratchadawan Cheewangkul. 12 October 2010. MFLU09-0643), from Thailand is presented in Figure 1.8



Note. (a). Black ascomata superficial on living leaves. (b), (c). Vertical section through ascomata with ostiole. (d), (e), (i). Hyphal network and septate hyphae. (f). Ostiole. (g). the subiculum–like structure of mycelia at the base of the ascomata. (h). Peridium. (j). Asci oboviod to clavate. (k). Muriform ascospores. Scale bar: (a) – (c). = 100 µm, (e). = 50 µm, (d), (f) – (j). = 20 µm, (k). = 5 µm.

Figure 1.8 *Phaeosaccardinula ficus* Chomnunti & KD Hyde

1.3.7 ***Trichomeriaceae*** Chomnunti & K.D. Hyde was introduced by Chomunti et al. (2012) in Chaetothyriales with *Trichomerium* as type family which is contains 23 species (Kirk et al. 2008). The fungus has ascostromata with a thin-walled peridium containing setae, developing on loosely interwoven mycelial masses of dark brown hyphae. Asci have a bitunicate appearance and ascospores are hyaline, fusoid, 3–septate, some with longitudinal septa, constricted at the septa, middle two cells wider and obliquely septate. Species are similar to those in *Capnodiaceae* and *Chaetothyriaceae* but phylogenetic analysis clearly show that they are unrelated (Chomnunti, Ko Ko et al., 2012). *Trichomerium* species can be distinguish from *Capnodiaceae* species by loosely mycelium, ascomata beneath from mycelium with abundance of setae surround ascomata, asci cylindrical to clavate with an apical ring, and ascospore fusoid with 3 transeptate or some with longitudinal septa. They can also be distinguish from *Chaetothyriaceae* species as *saprobes* on honey dew insect excretions, ascomata sessile peridium pale brown to brown or olivaceous, with asci an apical ring, cylindrical to clavate, and ascospores fusiform, middle two cells wider and round at ends with 3 transeptate. The collection of sooty moulds *Trichomerium foliicola* (Material examined: THAILAND, Chiang Rai. On living leaf of *Psidium* sp. Putarak Chomnunti, 20 October 2010. MFLU10-0002) from Thailand is presented in Figure 1.9.

Note. (a). ascostromata with setae. (b). setae. (c). hyphae on leaves of Guava. (d). ascostromata with peridium. (e), (f), (g). asci. (h). conidia. (i), (j) = ascospores. (k), (l) = spore germination. Scale bars: (c) – (d) = 100 µm, (h), (i) = 50 µm, (e) – (g), (j) = 20 µm, (k) = 10 µm.

Figure 1.9 *Trichomerium foliicola* Chomnunti & KD Hyde

Table 1.1 Families of sooty moulds with genera: Combined and Modified Data from Lumbsch & Hundorf (2010) & Kirk et al. (2008)

Antennulariellaceae	***Capnodiaceae***	***Chaetothyriaceae***	***Coccodiniaceae***	***Euantennariaceae***	***Metacapnodiaceae***	***Trichomeriaceae***
Achaetobotrys	*Capnodium*	*Actinocymbe*	*Coccodinium*	*Euantennaria*	*Metacapnodium*	*Trichomerium*
Antennulariella	*Capnophaeum*	*Ceramothyrium*	*Dennisiella*	*Rasutoria*		
Capnofrasera	*Leptoxyphium*	*Chaetothyriomyces*	*Limacinula*	*Strigopodia*		
	Phragmocapnia	*Chaetothyrium*		*Trichopeltheca*		
	Scorias	*Euceramia*				
		Microcallis				
		Phaeosaccardinula				
		Treubiomyces				
		Yatesula				

1.4 Life Cycle of Sooty Moulds

The disease cycle of sooty moulds starts when the spores are dispersed by wind or rain splash. It is not known if the insects themselves disperse the sooty mold spores (Nelson, 2008). Sap-feeding insects feed on plant foliage and excrete honeydew as a waste product. The honeydew drips on the foliage below and covers leaves, twigs and even plants, soil and rocks below with a sticky sugary coating. Spores of sooty moulds land on the leaf surface or other parts on which honeydew has been deposited. After this the sooty mould fungus grows on the host surface, using the honeydew as a food source and turning the host surface black (Figures 1.10-11) (Hughes, 1976; Reynolds, 1999; Nelson, 2008). Honeydew is mostly made of sugars up to 80% with smaller amounts of amino acids, proteins, minerals, vitamins and other organic compounds (Auclair, 1963). The association of sooty moulds and sap-feeding insects are prominent in ecosystems, for example, *Capnodium* is a saprobic association with sucking insect infestations on *Citrus* spp. and a wide variety of ornamental plants (Reynolds, 1999).

As the sooty mould colonies develop they grow across the leaf surface, often fusing with colonies of the same or other species, and in acute cases, will form a film on the entire plant or leaf. The first reproductive structures to appear are the asexual stages and as the colonies mature the sexual states may also develop. These asexual and sexual genera have often been linked as the same biological species by association in the same colony. However, these linkages need to be verified based on culture work or molecular data. It must also be considered if any of the asexual states are hyperparasites of the sooty moulds.

In three years of collecting sooty moulds in northern Thailand we found more than 40 collections of leaves by sooty molds, and found that 70% of the samlpes were colonized by only asexula states of sooty moulds. In fact in every visit to a small area of forest, orchard or gardens one will variably find numerous sooty mould infections. In northern Thailand most of the infections are caused by *Capnodiaceae*.

Figure 1.10 Sooty Moulds Cycle, Diagram: S. Nelson (2008)

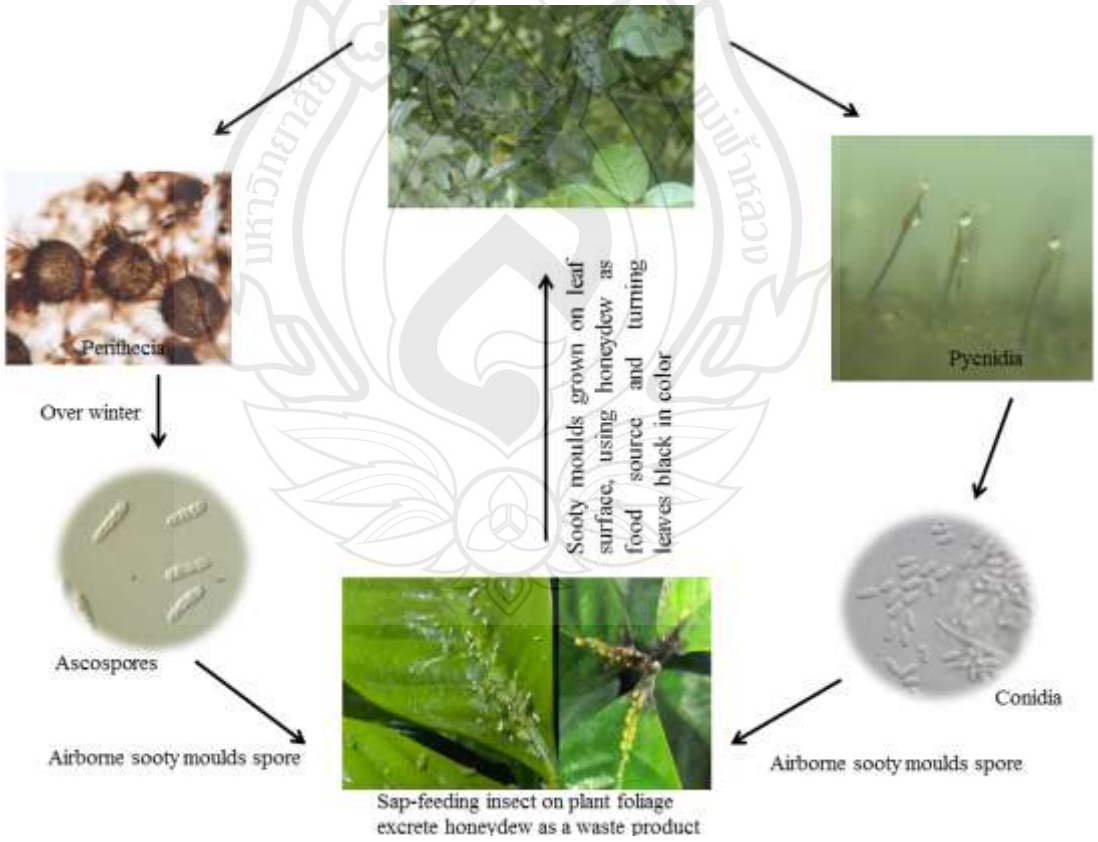


Figure 1.11 The Sooty Mould Life Cycle

1.5 Ecology, Distribution and Control

1.5.1 Ecology

Sooty moulds can grow under a wide range of environmental conditions. The temperature and moisture are fundamental. They are common in warm to tropical regions and there is a higher diversity of species in warmer climates (Jouraeva et al., 2006; Nelson, 2008). The frequency of sooty moulds appears to be greater after rainy season as during heavy rains the honeydew may be washed off leaves while during dry periods it will remain for long periods (Batista & Ciferri 1963a). In general, sooty moulds do not infect the plant tissues and their damage is cosmetic. The science of plant pathology however, treats them as plant disease agents because of their negative effects on photosynthesis: they block sunlight from reaching leaf chloroplasts where the plant harvests the sun's energy and converts them into biological energy for growth (Nelson, 2008; Laemmlen, 2011). Reduction of leaf photosynthesis by sooty moulds indirectly results in stunted plants and thereby reducing yield. Black sooty mould colonies on fruits or vegetables reduce their marketability since customers will not purchase these dirty, disease-like, infected products (Stover, 1975).

1.5.2 Distribution and control

Sooty moulds are found on almost any host, have a wide distribution and occur on both to cultivated and wild plants, including greenhouse plants (Zoff, 1879). The main factor in the distribution of sooty moulds are the insects. Hemipteran insects (chiefly scales and aphids) produce honeydew or excreta on the leaves which, as mentioned above, provide a base for the colonization of sooty moulds (Batista & Ciferri, 1963a). Laboratory experiments showed that pycnidiospores of *Ashbolisiaceae* and *Micropeltaceae* germinated well in the solutions of honeydews secreted by aphids and scale insects, as well as in some plant decoctions (Yamamoto, 1956; Batista, 1959).

A study of 270 collections of sooty moulds from major herbaria and fungaria by Olejnik, Ingrouille & Faull (1999) found that the samples came from 34 countries on all continents except for Antarctica. Fifty four per cent of them were from Borneo,

Cuba, India, Malaysia, and the Philippines, and a few specimens from other tropical or sub-tropical regions thus confirming that these groups of fungi are distributed from the Mediterranean to tropical regions.

As early as 1849, Gardner reported that coccus or bugs were associated with sooty moulds on coffee plants in Sri Lanka. His observations were in accordance with the appearance of scale insects and sooty moulds on leaves of exotic plants in England and the serious outbreaks in the orange plantations of the Azores and Madeira, and on leaves of *Arbutus* in British Columbia (Berkeley & Desmazières, 1849). *Limacinia fernandeziana*, is the sooty mould found on wild plant in the forests of Juan Fernandez (Johow, 1896). Sooty moulds are also common diseases in Europe and parts of North America, especially on Tilia, Salix and Ulmus trees (Hughes, 1976), and in Australia. Hughes (1966) described *Capnocybe* and *Capnophialophora* from New Zealand and mentioned that sooty moulds occur in abundance in this country, collecting over 500 specimens in the year 1963 alone. Later Hughes (1972) investigated the sooty moulds from New Zealand further and described two new families: *Euantennariaceae* and *Metacapnodiaceae*.

In cool-temperate climates, evergreen substrata provide suitable environments for the growth of sooty moulds, particularly ornamental shrubs such as species of *Rhododendron*, *Camellia*, and *Prunus laurocerasus* (Royal Botanic Gardens, Kew, UK National Collection of Dried Fungi, unpublished data). However, the *Capnodiaceae* are scarce during the winter. The number of sooty moulds in the UK is low, thirteen species have been recorded, the most common being *Capnodium salicinum* and *Denisiella babingtonii* (Cannon, Hawksworth & Sherwood-Pike, 1985; Royal Botanic Gardens, Kew, UK National Collection of Dried Fungi, unpublished data). On the other hand, the warm-temperate climates in Australia and the Mediterranean countries provide an abundance of perennial foliage on which the sooty moulds are able to establish themselves during the winter, and so persist from one season to the next (Fraser, 1935; Reynolds & Gilbert, 2005). Flessa, Peršoh & Gerhard (2012) looked at sooty patches on deciduous and evergreen leaves in Germany using spread plate culture techniques, and sequencing analysis of ITS rDNA gene, and found eight different fungi. Their study did not show host specificity

between the fungi involved. In the north of tropical Thailand we have found 11 species of sooty moulds, many being undescribed species.

Control of sooty moulds

The first step in the control of sooty molds is to rinse any sticky surfaces on the leaves with a jet of water and to wash off honeydew before the mould can grow. Sooty molds can be indirectly controlled by reducing populations of sucking insects that excrete honeydew. If one of the horticultural oils is used for control, it also has the advantage of helping to loosen sooty moulds from the plant surface. This hastens the weathering away of the sooty moulds. Horticultural oils formulated by many companies are available through garden centers, hardware stores, and similar establishments. (Lamborn, 2009).

Cultural practices to control sooty moulds include early detection and control of the insect pests, prevention of further insect and sooty mould infestation; over-pruning, over-watering and directly taking out insect pests from the plant. (Laemmlen, 2011).

Ant management is also an important consideration to halt development of sooty moulds. Ants are attracted to and use honeydew as a food source. In fact, ants probably farm the aphids, and harvest the honeydew before other predators and parasites feed on it. Presence of ants is a good indicator of honeydew being produced by insects (Nelson, 2008; Lamborn, 2009; Because sooty moulds are more common in warmer regions, the higher temperatures and increased drought stress brought on by a changing climate are expected to increase the prevalence of sooty moulds. During drought, aphid populations and their honeydew production typically increase on foliage undergoing moisture stress. Under dry conditions, less rain would be available to remove or dilute honeydew concentrations which otherwise are suitable for sooty mould growth on leaves and other surfaces. During the extended summer drought of 1988, sooty moulds were more prevalent throughout the Northeastern USA (Kessler, 1992), see also: http://www.na.fs.fed.us/spfo/pubs/howtos/ht_sooty/ht_sooty.htm

Pathogens of sooty moulds.

Remarkably sooty mould colonies are also hosts to several pathogens although their effect on the colonies in unclear. The genus *Trichothyriomyces* Bat. & H. Maia,

now transferred to the family *Microthyriaceae*, has been reported to be an hyperparasite of species of *Meliola*. This hyperparasite fungus produces its ascomata directly on the surface of the 'sooty moulds'. Such parasitism (as least in northern Thailand) appears to be uncommon as we found a single species, *Rhombostilbella rosea*, parasitising a collection of *Chaetothyrium*. This fungus has been known to parasitise *Chaetothyriaceae* and *Capnodiaceae* from Indonesia and southern-eastern United States (Pohlad, 1988).

Industrial potential of sooty moulds

Sooty moulds develop on sugary solutions excreted from insects. If malt extract agar is exposed to the air it will quickly be colonized by airborne fungi such as *Aspergillus* and *Pencillium*. Yet the sugary excretions on leaves are colonized by sooty moulds and common airborne fungi are absent. There are two probable reasons for the dominance of the sooty moulds. Firstly we suspect that the sooty moulds will produce antibiotic and antifungal agents which will prevent the common airborne fungi developing. If this is the case then the sooty moulds should be screened to establish which antimicrobial they produce and if they might have any medicinal importance. Secondly, the sugary excretions are likely to dry rapidly and therefore the fungi that can grow on this nutritious food resource are likely to be limited to those that can grow at low water activity. We therefore suspect that the sooty moulds are xerophilic. Thus if industry requires fungi that can perform functions at low water activity levels the sooty moulds may be appropriate candidates.

Some species of sooty moulds are commercially and agriculturally important, for example, *Caldariomyces fumago* is used to produce the enzyme chloroperoxidase for industrial purposes (Pickard, Kadima & Carmichael, 1991; Faull et al., 2002). Bussaban, Boontim & Lamyong (2011) reported on an edible gelatinized sooty mould species from Thailand; this is a first report of an edible sooty mould and generates income for the villagers. Interestingly, a tropical sooty mould (*Capnodium* sp.) is known to produce antibiotics such as tetramic acid, methiosetin and epicorazin A. (Herath et al., 2012).

1.6 Objective and Research Content

1.6.1 Research objectives

To document the morphology and taxonomy of the fungal families *Capnodiaceae*, *Chaetothyriaceae* and *Trichomeriaceae* and their anamorphs in Thailand.

To carry out phylogenetic relationships of selected, hitherto unresolved sooty moulds.

To carry out taxonomic revision of sooty moulds based on type specimens.

To examine morphology of generic types of selected genera of Dothideomycetes and contribute to the understanding of the natural classification of this group.

1.6.2 Research content

This thesis presents newer and additional knowledge on sooty moulds with emphases on *Capnodiaceae*, *Chaetothyriaceae* and *Trichomeriaceae* (all in the Dothideomycetes), based on morhophological characters. The identity of some of the wrongly diagnosed sooty mould fungi has been corrected. The taxonomic revision of selected genera is presented with detailed descriptions and illustrations. These are presented in the following chapters.

In Chapter 1 the subject matter is introduced with inputs on current knowledge on status of sooty moulds, historical background and the need of taxonomic revisions. It is well known that the phylogenetic analysis of most fungi and resultant pragmatic taxonomy is still incomplete.

In Chapter 2 details are given how the sooty moulds in *Capnodiaceae* were isolated and examined for morphological characters. Phylogenetic relationships of *Capnodiaceae* and related genera were examined through analysis of multi-genes sequences data. Results of morphological and phylogenetic analysis are correlated. The generic types of *Capnodiaceae* were reexamined, redescribed and illustrated in detail.

Chaetothyriaceae and *Trichomeriaceae* are often confused with capnodiaceous sooty moulds as they are normally growing together in the same host. The taxonomy of these fungi has been sorted out.

Chapter 3 details of fresh material of *Chaetothyriaceae* collected and examined are given. The fungi were isolated, cultured and studied. Taxonomic description and illustrations were provided for new species. Phylogenetic results of *Chaetothyriaceae* clearly confirm the group as a family, and the study contributes more sequences to support this. Generic types of this family were examined, re-described and illustrated were possible by obtaining the types on loan. When types were not available, literature references were studied and evaluated.

Chapter 4 *Trichomeriaceae* fam. nov. was introduced. Previously *Trichomerium* was placed within the *Capnodiaceae*. However, according to morphological characters and phylogenetic results showed that they are separated completely from *Capnodiaceae* and *Chaetothyriaceae.*

Chapter 5 Morphological descriptions and illustrations of generic types of 11 families in Dothideomycetes were presented.

Chapter 6 Include a general discussion and conclusion of this study. The thesis provides an exhaustive collection of literature on the subject.

This thesis embodies the results of a detailed taxonomic and floristic study of sooty mould fungi, collected from various localities in northern Thailand, which have been verified by studying type specimens borrowed from various international fungal reference collections. The work also reassessed the taxonomy of selected groups within the sooty mould in family *Antennulariellaceae, Metacapnodiaceae* and *Cocoodiniaceae*. This project aims to investigate the taxonomic status of sooty moulds especially in the Families of *Capnodiaceae, Chaetothyriaceae* and *Trichomeriaceae*. Details of their asexual stages were studied based on morphological characteristics and molecular analyses. Numerous collections were made in Thailand and a comparative study was carried out with those of the type strains identified from other countries (i.e., Europe, America and some parts of Asia). It was felt necessary and important to use these type specimens as references. The results from this study have shed light on taxonomic status of *Capnodiaceae, Chaetothyriaceae* and *Trichomeriaceae*. Besides, some important species were epitypified and used as

reference in the study. These documents will form references to future studies on sooty mould in Thailand. Phylogenetic relationships were constructed using DNA sequences (i.e. ITS, LSU and SSU) and new sequencing are deposited in GenBank. The taxonomy of selected sooty moulds is presented by combining traditional and molecular phylogenetic methods. Therefore the research in this thesis will make a major contribution to the classification of this group of fungi with a taxonomic revision on the sooty moulds (Capnodiales) of the Dothideomycetes, *Chaetothyriaceae* and *Trichomeriaceae* (Chaetothyriales).

CHAPTER 2

TAXONOMY AND PHYLOGENY CLASSIFICATION OF SOOTY MOULDS IN *CAPNODIACEAE*

2.1 Introduction

The family *Capnodiaceae* was introduced by Höhnel (1910) with the generic type *Capnodium* Montagne (1849) and presently includes 14 genera and 117 species (Kirk et al. 2008, Lumbsch & Huhndorf, 2010). *Capnodiaceae* are sooty moulds with bitunicate asci borne in ostiolate ascomata; the family however is based mostly on ecological characters (von Arx & Müller, 1975). The first complete monographic review of capnodiaceous sooty moulds, recognizing both sexual and asexual species, was in *Eucapnodiaceae* (Fraser, 1935). Batista and Ciferri (1963b) provided a monograph of *Capnodiaceae* in the order *Capnodiales*. Hughes (1972) reviewed and re–classified *Capnodiaceae* which was characterized by the structure of hyphae, presence or absence of pseudoparaphyses, and by deviating conidial states. Members of this family also had superficial ascomata with ovoid asci in fascicles and hyaline to dark, one to multiseptate ascospores (Hughes, 1976). More recently, *Capnodiaceae* have been redefined by the following features: superficial mycelium of interwoven, mucilaginous, brown, cylindrical or tapering hyphae mostly constricted at the septa, and occurring as leaf epiphytes associated with the honeydew of insects (Hughes, 1976; Andrew, 1982; Blakeman & Fokkema, 1982) the ascomata lack pseudoparaphyses and the asci are bitunicate. The fungi are known as sooty moulds, and tend to live in complex communities, often with multiple fungal parasites, inhabiting a common sooty mass (Faull et al., 2002; Hughes, 2003). These fungi are noted for the production of darkly pigmented hyphae, often of very characteristic morphology (Hughes, 1976; Reynolds, 1998). Anamorphs so far reported in

Capnodiaceae are the following: *Acanthorus*, *Apiosporium*, *Conidiocarpus*, *Conidioxyphium*, *Fumagospora*, *Fumiglobus*, *Leptoxyphium*, *Mycogelidium*, *Phaeoxyphiella*, *Polychaetella*, *Polychaeton*, *Scolecoxyphium*, and *Tripospermum* (Hyde, McKenzie & Ko Ko, 2011). The *Capnodiaceae* are often confused with *Chaetothyriaceae* which are also referred to as sooty moulds since they share the same ecological niche and similar appearance. The main differences are found in the characteristics of the ascomata, being single locules in *Capnodiaceae*, and ascostromata, often with more than one locule in *Chaetothyriaceae*. In addition, phylogenetic analyses have clearly shown them to be unrelated and, therefore, they were placed in two separate classes: *Dothideomycetes* and *Eurotiomycetes* respectively (Schoch et al., 2006; 2009; Geiser et al., 2006; Chomnunti, Ko Ko et al., 2012). The easiest character by which to distinguish these families on leaf surfaces is the form of the ascomata. In *Capnodiaceae* ascomata are subglobose to globose, with or without setae (von Arx & Müller, 1975), while in *Chaetothyriaceae* they are ascostromata surrounded by a pellicle of superficial mycelium (Chomnunti, Ko Ko et al., 2012). The purpose of this study is to revisit the family *Capnodiaceae* by examining available generic types which are described and illustrated in detail. Besides, fresh specimens were collected from Thailand and the fungi were isolated in culture and sequenced for the species. In all, 14 new taxa are incorporated based on molecular analysis and a more complete tree than has been presented before for *Capnodiales* is provided (Crous, Schoch et al., 2009).

2.2 Material and Methods

2.2.1 Observation of Generic Types, Isolation from Fresh Specimens and Studies on Morphology

Type specimens of *Capnodiaceae* were obtained from various herbaria (Table 2.1). The specimens, located using literature reviews and Mycologists Handbook by Hawksworth (1974) were obtained via MFLU Herbarium Centre. Macro-characters of type specimens were carefully recorded including photomicrographic details under stereo microscope. The herbarium specimens were rehydrated in 5% KOH for 10 min and free hand thin sections prepared looking under the stereo microscope. Using a fine needle, the contents from inside the ascomata were removed and mounted in water. The asci were stained by Melzer's reagent to search for a starch-like reaction. This is an iodine based solution used to test the amyloidity of the apical ring in the asci and can help distinguish bitunicate fungi from unitunicate fungi. The ascospores were stained in cotton blue which is an acid dye that stains the chitin present in cell wall of fungi, and in conjuction with 90% lactic acid provides a clear view of the tissues and can be used to prepare semipermanent slides; also for similar preservation one can use lactoglycerol instead (see 'Mounting Media' in Kirk et al. 2008, p. 440). Micro-characters were examined and observed under a compound microscope (Nikon 80i) fitted with DIC, and measurements made with Tarosoft (R) Image Frame Work. Details of the type specimens were documented morphologically and revised with new photo-plates. The type material of some species could not be located during the time frame of this study; nevertheless, they are discussed based on the original description and subsequent publications.

Table 2.1 Type Specimens of *Capnodiaceae* were Obtained from Various Herbaria

Genus	Type specimens	Herbaria*
Aithaloderma Syd. & P. Syd	*Aithaloderma clavatisporum* Syd. & P. Syd. (1913)	NY and IMI
Anopeltis Bat. & Peres.	*Anopeltis venezuelensis* Bat. & Peres (1960)	K and URM
Capnodium Mont.	*Fumago citri* Pers. (1822)	S
Capnodaria tiliae (Fuckel) Theiss. & Syd.	*Capnodaria tiliae* (Fuckel) Theiss. & Syd. (1918)	G
Callebaea Bat.	*Callebaea rutideae* (Hansf.) Bat. (1962)	K
Hyaloscolecostroma Bat. & J. Oliviera	*Hyaloscolecostroma rondoniense* Bat. & J. Oliveira (1967)	URM
Ceramoclasteropsis Bat. & Cavalc	*Ceramoclasteropsis coumae* Bat. & Cavalc. (1962)	URM
Phragmocapnias Theiss. & Syd.	*Phragmocapnias betle* (Syd., P. Syd. & E.J. Butler) Theiss. & Syd. (1918)	S
Echinothecium Zopf.	*Echinothecium reticulatum* Zopf (1898)	B
Scorias Fr.	*Scorias spongiosa* (Schwein.) Fr. (1832)	K
Trichomerium Speg.	*Trichomerium coffeicola* (Puttemans) Speg. (1918)	URM
Scoriadopsis Mend.	*Scoriadopsis miconiae* J.M. Mend. (1930)	BPI
Polychaeton (Pers.)Lev.	*Fumago* sect. *Polychaeton* Pers. (1822)	K

Note. *Abbreviations of fungal collection, full name of herbaria is provided in index.

Several sites were visited throughout the provinces of Chiang Mai and Chiang Rai in northern Thailand. Leaves of various plants with sooty mould–like colonization were collected. Sections of ascomata were made free–hand and mounted in lacto–glycerol. Melzer's reagent was used to test the amyloidity of the apical ring and Indian ink was used for demonstrating the presence of mucilaginous sheath (before adding the lactoglycerol). Pure cultures were obtained by single spore isolation. For that, a part of the hymenium containing ascospores was removed from 4–5 ascomata using a sterile needle, and placed in a drop of sterile water on a glass slide. The spore suspension was transferred with a sterile pipette onto the surface of a Petri dish with 2% Difco potato-dextrose agar (PDA) as medium. The plates were left overnight, for ascospores to germinate, and observed within 12 h. Germinating spores were individually transferred onto a fresh Petri dish. Isolates were grown on PDA at 28 C for 12 h of light/12 h of dark for routine maintenance. Colony colour and characteristics were assessed after 4 weeks, and this material was used for molecular study. Types of isolated new species are deposited at the Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand, and the cultures in the Culture Collection of the same institution (MFLUCC) and in BIOTEC Culture Collection (BCC).

2.2.2 DNA Isolation, Amplification and Sequencing

The analysis was performed with two markers: partial sequences from the small and large subunits of the nuclear ribosomal RNA genes (SSU, LSU). The individual genes were aligned with SATé (Liu, Raghavan, Nelesen, Linder & Warnow, 2009) using MAFFT (Katoh, Asimenos & Toh, 2009) as the external sequence alignment tool and RAxML(Randomized Accelerated Maximum Likelihood) as the tree estimator. MAFFT is an excellent online implementation for multiple sequence alignments (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/). RAxML is a Maximum Likelihood tree reconstruction program which is efficient for a large number of highly variable taxa (Stamatakis, 2006). Representative sequences from *Capnodiales* were downloaded from GenBank according to Table 2.2 *Dothidea insculpta* and *D. sambuci* were selected as outgroups.

Phylogenetic analyses of the single genes did not yield conflicts in clades with RAxML bootstrap above 70% (data not shown), therefore sequences were

concatenated in BioEdit (Hall, 1999). This resulted in 15.5% missing and gap characters out of a total set of 1982 characters (967 obtained from SSU and 1015 obtained from LSU). The final data matrix had 51 taxa including outgroups. A phylogenetic analysis was performed at the CIPRES webportal (Miller, Pfeiffer & Schwartz, 2010) using RAxML v. 7.2.8 as part of the "RAxML–HPC2 on TG" tool (Stamatakis, 2006; Stamatakis, Hoover & Rougemont, 2008). A general time reversible model (GTR) was applied with a discrete gamma distribution and four rate classes. Fifty thorough maximum likelihood (ML) tree searches were done in RAxML v. 7.2.7 under the same model, each one starting from a separate randomised tree and the best scoring tree selected with a final ln value of –7912.128405. One thousand non parametric bootstrap iterations were run with the GTR model and a discrete gamma distribution. The resulting replicates were plotted on to the best scoring tree obtained previously. The phylogram with bootstrap percentage values (BP) which is indicate of how frequently the isolates connected to that node clustered together during bootstrap replications is presented above the branches in Figure 2.1 by using graphical options available in TreeDyn v. 198.3 (Chevenet, Brun, Banuls, Jacq & Christen, 2006). We also analyzed the same data set mentioned above using the Bayesian method of Huelsenbeck, Ronquist, Nielsen & Bollback (2001) by implementing Markov Chain Monte Carlo (MCMC) sampling using the software Mr Bayes v.3.1.2 (Huelsenbeck & Ronquist, 2001) on the CIPRES webportal. Data were analysed with a GTR model with gamma–distributed rate variation across sites (invariance, partitioning across genes) were applied with four discrete gamma categories. The Bayesian prior distributions treated all trees as equally likely and two parallel runs were performed with four chains each. This was continued for 2000 000 generations and every 100th tree was saved. The two runs were verified for convergence and the first 50% of each run was discarded as burn in with the remaining two sets of 10 000 trees combined. The combined set of 20 000 trees were used to estimate the posterior probabilities (PP). Bayesian posterior probabilities correspond to the number of time a node is represented in the Bayesian tree sample. The best scoring RAxML tree was used as a template in Figure 2.1 and the support values (BP/PP) are indicated above the branches.

Table 2.2 Species and Sequences Database Accession Numbers Used in this Study

Taxa	Strain no./Voucher	Host	Country	GenBank Accession no.	
				SSU	LSU
Conidiocarpus asiana	MFLUCC10-0062	*Coffea arabica*	Thailand	JN832597	JN832612
C. siamense	MFLUCC10-0061	*Mangifera indica*	Thailand	JN832592	JN832607
C. siamense	MFLUCC10-0063	*Coffea arabica*	Thailand	JN832593	JN832608
C. siamense	MFLUCC10-0064	*Mangifera indica*	Thailand	JN832594	JN832609
C. siamense	MFLUCC10-0065	*Euphorbia* sp.	Thailand	JN832595	JN832610
C. siamense	MFLUCC10-0074	*Bischofia javanica*	Thailand	JN832596	JN832611
Leptoxyphium cacuminum	MFLUCC10-0049	*Mimusops elengi*	Thailand	JN832587	JN832602
L. cacuminum	MFLUCC10-0059	*Gossypium herbaceum*	Thailand	JN832588	JN832603

Table 2.2 (Continued)

Taxon	Strain no./Voucher	Host	Country	GenBank Accession no.	
				SSU	LSU
L. cacuminum	MFLUCC10-0086	*Ficus* sp.	Thailand	JN832589	JN832604
Phragmocapnias betle	MFLUCC10-0050	*Mimusops elengi*	Thailand	JN832590	JN832605
Ph. betle	MFLUCC10-0053	*Ixora* sp.	Thailand	JN832591	JN832606
Polychaeton coartatum	MFLUCC10-0066	*Euphorbia* sp.	Thailand	JN832598	JN832613
P. coartatum	MFLUCC10-0069	*Psidium* sp.	Thailand	JN832599	JN832614
P. coartatum	MFLUCC10-0070	*Alstonia scholaris*	Thailand	JN832600	JN832615
Scorias spongiosa	MFLUCC10-0084	*Entada* sp.	Thailand	JN832586	JN832601

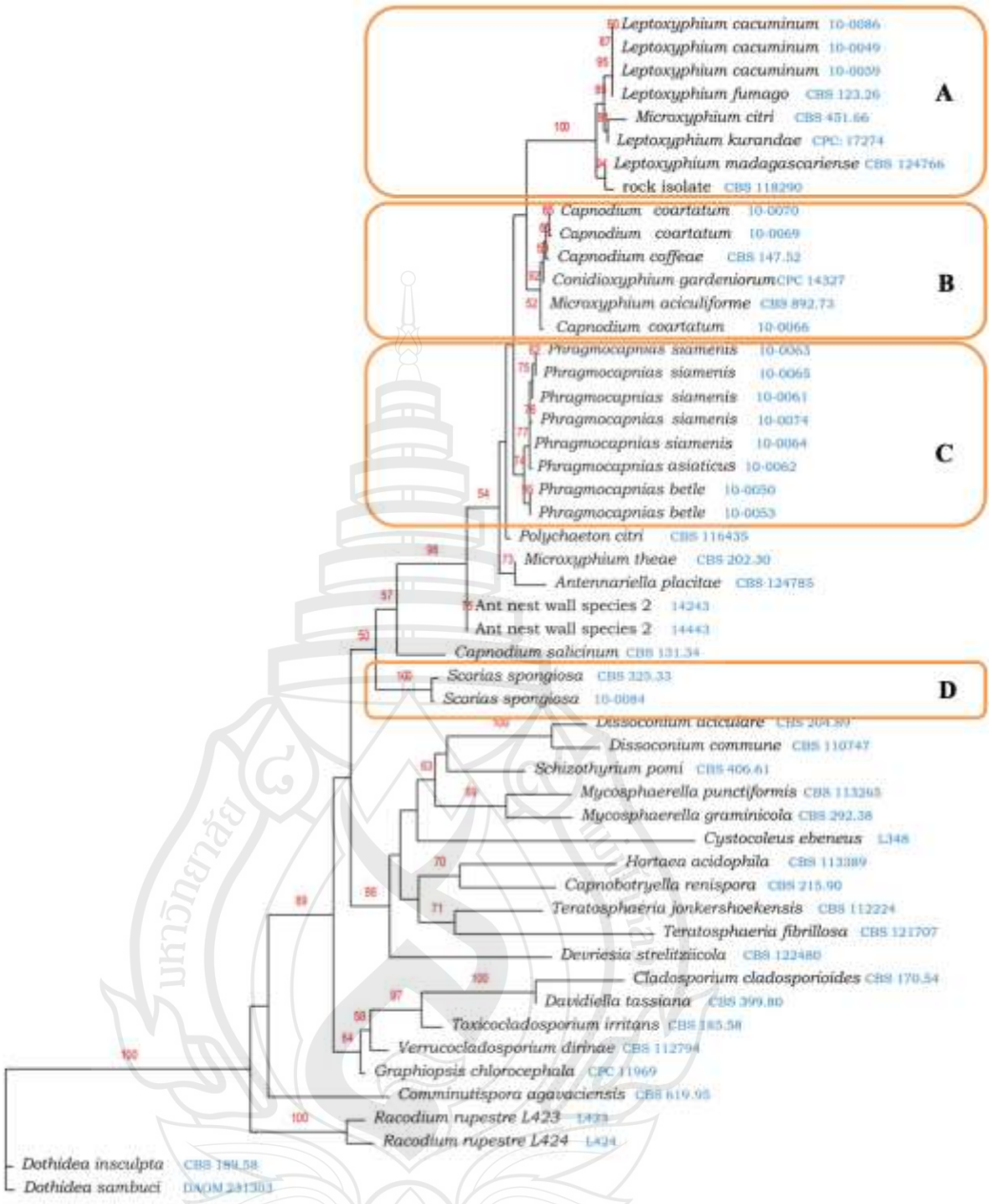
2.3 Results

2.3.1 Molecular Phylogeny

In the tree (Figure 2.1) we included representative sequences of *Capnodiaceae, Davidiellaceae, Dissoconiaceae, Mycosphaerellaceae, Schizothyriaceae, Teratosphaeriaceae* as well as uncertainly placed lineages in *Capnodiales* mainly following an earlier analysis by Crous, Schoch et al. (2009). All groups agreed with this analysis. The phylogeny presented includes the biggest sampling of *Capnodiaceae* to date and includes several samples from earlier studies (Crous, Schoch et al., 2009; Reynolds, 1998; Ruibal, Sakayaroj et al., 2009; Schoch et al., 2006). The 15 specimens of sooty moulds from Thailand including *Leptoxyphium cacuminum, Phargmocapnias betle, P. asiaticus, P. siamensis, Polychaeton coartatum* and *Scorias spongiosa* clustered within *Capnodiaceae* (Figure 2.1). The clade containing *Capnodiaceae* was only recovered in 50% of bootstrap *P* value (BP) tree using the RAxML analysis but received a 99% posterior probability (PP) in the Bayesian analysis. This clade is recovered with high frequency in other analyses including protein coding gene markers (Schoch et al, 2009) but not well supported in other studies using only ribosomal sequence comparisons (Crous, Schoch et al, 2009).

The *Capnodiaceae* comprises four clades (A–D). Clade A (100% BP and PP) comprises three *Leptoxyphium* sequences from GenBank and three new strains with characters typical of *Leptoxyphium sensu* Hughes (1976), Olejnik et al. (1999) and Cheewankoon et al. (2009) as well as *Microxiphium citri* and a rock isolate with 97% support. *Microxiphium citri* was isolated from citrus fruit in Spain and may be wrongly named. Clade B (52% BP, 92% PP 100%) comprises *Capnodium coffeae, Conidioxyphium gardeniorum*, *Microxiphium aciculiforme* and a new species of anamorphic *Capnodium* (= *Polychaeton*). *Conidioxyphium gardeniorum* is either wrongly named or is a synonym of *Capnodium*. Clade C are *Phragmocapnias* (= *Conidiocarpus*) species with one teleomorph species being identical to the generic type and is thus epitypified. The genus is represented by at least two morphological species which are described as new in this paper. Further collections and analyses are likely to reveal other taxa in this genus. Basal to the *Phragmocapnias* Clade C is

Polychaeton citri, a strain of *Antennariella placitae*, *Microxyphium theae* and two ant nest wall strains. *Polychaeton citri* was isolated from a leaf of *Citrus aurantium* together with *Pseudococcus citri* (Crous, Schoch et al., 2009). Because of difficulty in isolating *Capnodium* species and the fact that many species occur on the same leaf in close proximity we suspect this may be wrongly named and this will have to be verified. *Antennariella placitae* was described by Cheewangkoon et al. (2009) but it is not clear if this is correctly placed and more collections would be needed to establish its familial relationships. Sister to these taxa is a putative strain of *Capnodium salicinum* which also require verification. Finally, Clade D (100% bootstrap support) comprises two isolates of *Scorias spongiosa*. The new isolate from Thailand is designated as the epitype here. Three *Microxiphium* species are included in the analysis, which in the present study show that the genus is polyphyletic. Based on morphological examination of the type species, this genus may belong in *Coccodiniaceae*.



Note. A data set of 51 taxa including representatives of *Capnodiales*, focused on *Capnodiaceae*, comparing two genes (SSU, LSU). The first set of numbers above the nodes are bootstraps from 1000 pseudorepetitions and the second represent Bayesian posterior probabilities expressed as percentages. Only values above 50% are shown.

Figure 2.1 A RAxML Maximum Likelihood Tree

2.3.2 Generic Type Observation, Fresh Specimens Isolates and Morphology

Capnodiaceae (Sacc.) Höhn. ex Theiss. 1916.

Habit saprobic on honey dew excretions from insects, usually forming blackened sooty–like regions on green healthy leaves, stems, bark and even rocks (Figure 2.2), and often in association with numerous other species and fungicolous taxa. *Thallus* comprising of mycelium with black sooty growth on the host surface, producing superficial colonies with septate, cylindrical, brown hyphae. *Ascomata* arising from the mycelial mass, subglobose to globose, coriaceous, with or without setae, dark brown, with a central ostiole. *Peridium* brown, relatively thin, comprising cells of *textura angularis*. *Pseudoparaphyses* lacking. *Asci* bitunicate, saccate, with a short pedicel, lacking an ocular chamber. *Ascospores* multiseptate or muriform, hyaline to brown. Anamorph states pycnidial, mostly elongated, with short or long narrow necks, with or without a swelling where the conidia are produced. *Ostiole* prominent at the apex of the pycnidial neck. *Conidia* developing on typical capnodiaceous hyphae within the pycnidia, small, one–celled, ellipsoidal and hyaline.

Family type: *Capnodium* Mont. (Montagne, 1849) (= *Polychaeton* (Pers.) Lév.). The Melbourne Botanical Congress has recently in July 2011 approved large scale changes in the process of naming fungi, including the abolition of Article 59. Therefore, by applying the principle of priority *Polychaeton* would be the earlier and valid name for the species currently assigned to the genus *Capnodium*. However, this process is still being finalized and it was proposed that anamorph typified names should not be taken up to displace teleomorph typified names in use until each case has been reviewed by the Fungal Committee established at the Melbourne Congress (Hawksworth 2011). As the name *Capnodium* is taken up for the name of the ranks of family and order in this group of fungi, this is a prime example that retention of the name should be proposed for approval to the Committee.

The family *Capnodiaceae* was introduced by Höhnel (1909) and validated by Theissen in 1916 according to von Arx and Müller (1975) and Hughes (1976), and is circumscribed as dothideomycetous sooty moulds with mostly pycnidial anamorphs (Reynolds, 1982). The taxa of this family can be recognized by black mycelial growth spreading on the host surface which produces superficial colonies with septate,

cylindrical, dark–brown hyphae (Figures 2.3a, b). Ascomata arise from the mycelial mass and are subglobose to globose (Sivanesan, 1984; Barr, 1987; Figure 2.2, 2.3). Their anamorphic states, placed mostly in *Asbolisiaceae* by Batista & Cifferi (1963b) form elongated pycnidia, with short or long narrow necks, an apical ostiolar canal, and minute, one–celled and hyaline conidia (*sensu* Hughes, 1976).

In the past, some members of the family were known only from anamorphic stages, and these pycnidia had been assigned to one or other teleomorphic genera and families depending on whether the researchers gave more emphasis to Saccardoan generic concepts such as spore morphology and colour, or colony hyphal development, hyphal morphology and ascomatal and pycnidial formation (as with Hughes, 1976), but now the use of modern molecular techinques has enabled us to confirm their relationship in the *Capnodiaceae*.

Crous, Schoch et al. (2009) used molecular methods to evaluate the monophyly of the order *Capnodiales*, yet they did not discuss the family in detail, though concluding that it probably contained diverse lineages. In the present account we illustrate genera of the group and compare our morphological findings of some generic types examined with molecular results of taxa found in south eastern Asia.

Note. (a). Unidentified tree. (b). *Bischofia javanica*. (c). *Euphorbia* sp. (d). *Psidium guajava*. (e). *Cestrum diurnum*. (f) – (i). Representative pycnidia of *Capnidiaceae*. (f). *Phragmocapnias*. (g). *Capnodium*. (h). *Leptoxyphium*. (i). *Scorias*.

Figure 2.2 Sooty Moulds Growing on Host Leaves.

Key to genera accepted in *Capnodiaceae*

1. Pycnidia with a black stalk, and an upper brown swollen region producing onidia; ascospores hyaline……*Phragmocapnias*
1. Pycnidia without a black stalk or an upper brown swollen region producing conidia; ascospores brown or hyaline…….2
2. Pycnidia lacking a basal bulbous part; ascospores brown…. *Capnodium*
2. Pycnidia with basal bulbous part; ascospores other……3
3. Ascomycetous state unknown, pycnidia with narrow bulbous base, apex cup–like which produces conidia…….……*Leptoxyphium*
3. Ascomata present, gregarious on blacked mycelial mat; pycnidia with swollen bulbous base…….…….4
4. Ascospores mostly 3–septate……*Scorias*
4. Ascospores mostly 1–septate…….*Scoriadopsis*

Phragmocapnias Theiss. & Syd., Ann mycol. 15(6): 480 (1918) [1917]

= *Conidioxyphium* Bat. & Cif., Quad. Lab crittogam., Pavia 31: 72 (1963)

= *Podoxyphium* Speg., Physis. B. Aires 4: 294 (1918)

= *Conidiocarpus* Woron., Ann mycol. 24 (3/4): 250 (1926)

Saprobic on sugary exudates from insects, dark mycelium forming a soot–like coating on the upper surface of leaves. *Thallus* composed of black, pelliculose, reticulately branched, dense, cylindrical, radiating, septate hyphae. *Ascomata* scattered, subglobose to broadly ellipsoidal, barely stalked, firmly attached to the basal hyphae, dark brown, thick–walled, ostiolate, with setae. *Peridium* consisting of pale to dark brown cells forming a *textura angularis*. *Asci* bitunicate, 8–spored, broadly clavate, with short pedicle. *Ascospores* cylindric–clavate, hyaline, 4–septate and constricted at the septum. *Pycnidia* with a black stalk, with an upper brown swollen part which produces conidia, wall comprising cylindrical cells. *Ostiole* surrounded by hyaline hyphae. *Conidiogenous cells* form from inner cell surface of swollen part. *Conidia* small, ellipsoid, 1–celled, hyaline, smooth–walled.

Anamorph: *Conidiocarpus* Woron. (Hyde et al., 2011)

Type species

Phragmocapnias betle (Syd. P. Syd. & E.J. Butler) Theiss. & Syd., Ann mycol. 15(6): 480 (1918) [1917]

≡ *Capnodium betle* Syd. P. Syd. & E.J. Butler., Ann Mycol 9 (4): 384 (1911)

Description from holotype

Saprobic on sugary exudates from insects growing on the surface of living leaves (Figure 2.3a). *Thallus* thin, amphigenous, black, pelliculose, composed of reticulately branched, A dense, cylindrical to somewhat constricted, radiating, septate hyphae (Figture 2.3b). *Ascomata* up to 101–110 μm diam, 99–111 μm high ($\bar{x}$ = 106 × 105 μm, n = 5), scattered, subglobose to broadly ellipsoidal, with short stalks, firmly attached onto the radiating basal hyphae, dark brown, thick–walled, ostiole present in mature ascomata, setae with blunt apices scattered over the ascomata (Figure 2.3c). *Peridium* 15–19 μm wide ($\bar{x}$ = 17 μm, n = 10), composed of pale to dark brown, cells arranged in a *textura angularis* (Figure 2.3e). *Asci* 37–48 × 17–30 μm ($\bar{x}$ = 42 × 22 μm, n = 5), 8–spored, bitunicate, broadly clavate, with short pedicle (Figure 2.3f–h). *Ascospores* 18–27 × 5 μm ($\bar{x}$ = 21 × 5 μm, n = 5), fasciculate, cylindric–clavate, with rounded ends, upper cells slightly wider than the lower cells, hyaline, 4–septate, constricted at the septa, smooth–walled (Figure 2.3f–i).

Description from epitype

Saprobic on sugary exudates from insects growing on the upper surface of living leaves (Figure 2.4a). *Thallus* thin, amphigenous, black, pelliculose, composed of reticulately branched, dense, cylindrical to somewhat constricted, radiating, septate hyphae (Figure 2.4a). *Ascomata* 82–93 × 84–105 μm diam ($\bar{x}$ = 88 × 92 μm, n = 10), superficial, subglobose to broadly ellipsoidal, dark brown to black, shiny, coriaceous, ostiolate at maturity, with 3–5 setae around the ostiole, with setae 50–75 × 3–5 μm ($\bar{x}$ = 57 × 4 μm, n = 10), dark brown, septate and tapering with rounded ends (Figure 2.4a, b). *Peridium* thin, comprising cells *textura angularis*. *Asci* 43–53 × 13–33 μm ($\bar{x}$ = 24 × 21 μm, n = 10), 8–spored, bitunicate, fusiform to broadly clavate, with a short pedicel, with a short ocular chamber when young (Figure 2.4d, e). *Ascospores* 20–24 × 4.8–5.8 μm ($\bar{x}$ = 23 × 5.3 μm, n = 10), fasciculate, cylindric–clavate, with

rounded ends, with upper cells slightly wider than the lower cells, hyaline, 4-septate, constricted at all septa, guttulate, some surrounded by a mucilaginous sheath (Figure 2.4f–l).

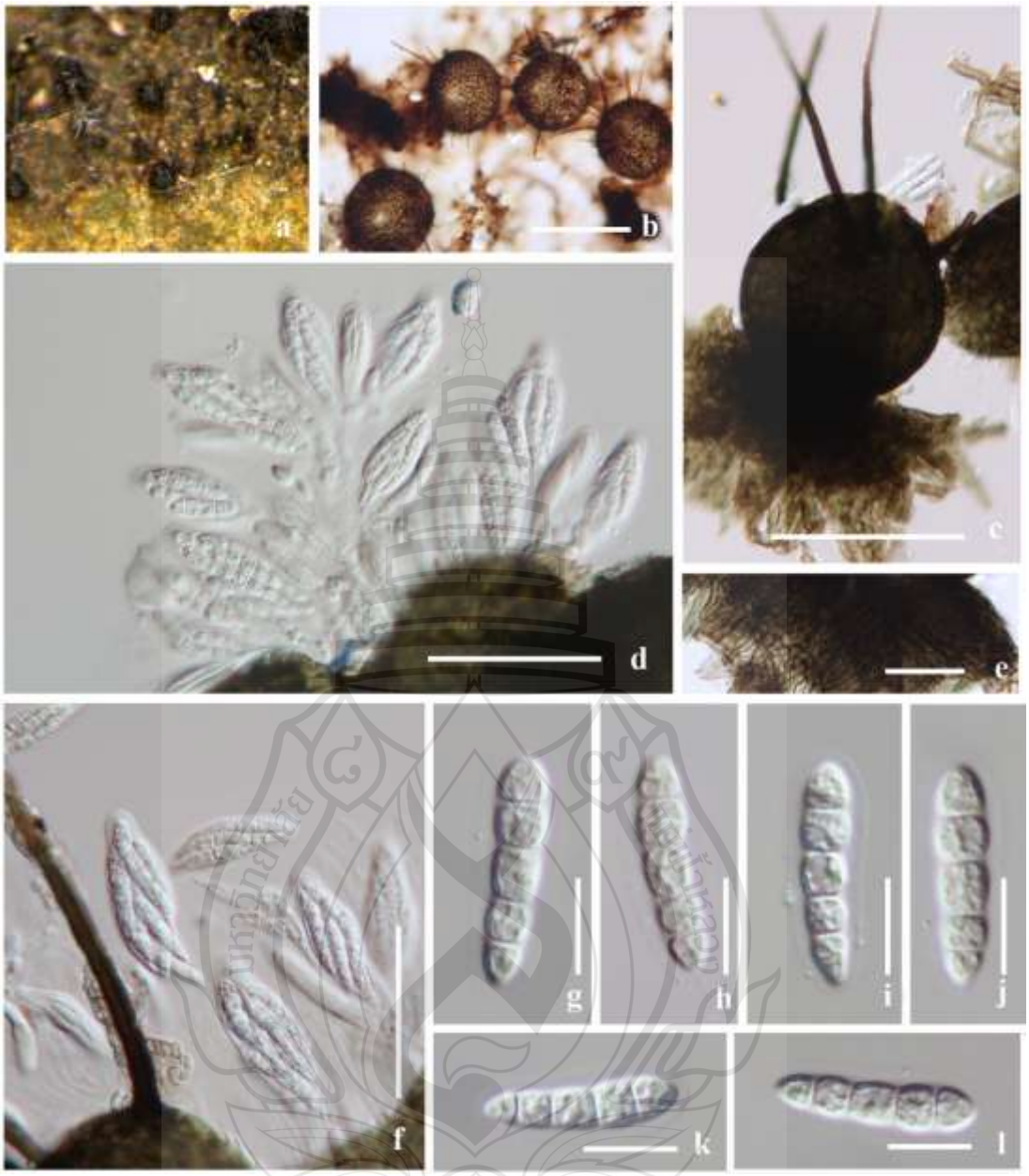
Material examined: BANGLADESH, Dhaka, on leaves of *Piper betle*, 5 April 1910, A.L. Som 1061 (S, holotype of *Capnodium betle*); THAILAND, Chiang Rai Province, Mae Fah Luang University, on living leaf of *Ixora* sp., 24 April 2009, Putarak Chomnunti, DPC 014 (MFLU09–0650, epitype designated here), extype living culture in MFLUCC10–0053; *Ibid.*, on living leaf of *Mimusops elengi* Linn., 14 July 2009, Putarak Chomnunti (MFLU 09–0647), living culture in MFLUCC10–0050.

Phragmocapnias betle has been reviewed by Reynolds (1979) who recognized this genus as having stalked ascomata with setae and hyaline ascospores with trans–septa. In MFLU 09–0647, the ascomata are slightly bigger than in the type: 87–137 × 80–125 µm diam. ($\bar{x}$ = 103.5 × 94.5 µm, n = 10), but the asci and ascospores fit the range given in the above descriptions: 42–50 × 10–15 µm ($\bar{x}$ = 41 × 13 µm, n = 10) and 20–25 × 5–8 µm ($\bar{x}$ = 22 × 5 µm, n = 20) respectively. *Phragmocapnias* has been discussed fairly extensively and there are eight names listed in *Index Fungorum* of which five are given as congeneric (see http://indexfungorum.org/Names/Names.asp). Reynolds (1978), however, concluded that there was only one species, *P. betle* and excluded the other species based on various reasons. The anamorph of *P. betle* was reported to be *Conidiocarpus* (Hughes 1976), but Reynolds (1979) concluded that the anamorph of *Scorias* and *Phragmocapnias* were uncertain. However, the phylogenetic data presented here (Figure 2.1) confirm that *Conidiocarpus* is the anamorph of *Phragmocapnias*.

In the combined gene phylogenetic analysis, the epitype and second collection of *Phragmocapnias betle* clustered in *Capnodiaceae* and are basal to two species (6 strains) of anamorphic *Phragmocapnias* (= *Conidiocarpus*) confirming the anamorph–teleomorph linkage. The eight strains of *Phragmocapnias* used in this study cluster together with 75% bootstrap support with the two *Phragmocapnias* species closest with 99% bootstrap support. *Phragmocapnias asiaticus* is closest to the *Phragmocapnias* species and the five strains of *Phragmocapnias siamensis* cluster above with 81% bootstrap support indicating that they may be more than one species.

Note. (a) – (b). Ascomata on leaves. (c), (e). Vertical section through stalked ascoma. (d). Ascomata exterior wall with setae. (f) – (h). Asci with short pedicel. (i). Cylindric–clavate ascospores. Scale bars: (e) = 100 µm, (c), (d) = 50 µm, (f) – (g) = 20 µm, (i) = 10 µm.

Figure 2.3 *Phragmocapnias betle* (holotype of *Capnodium betle*)



Note. (a). Ascomata on living leaf. (b) – (c). Ascomata which is subglobose with setae. (d), (f). Bitunicate asci. (e). Mycelium beneath ascomata. (g) – (l). Ascospores surrounded by a mucilaginous sheath. Scale bars: (b) – (c) = 100 µm, (d), (f) = 50 µm, (e). = 20 µm, (g) – (l) = 10 µm.

Figure 2.4 *Phragmocapnias betle* (epitype)

Conidiocarpus penzigii Woron. (*as penzigi*), Ann mycol. 24(3/4): 250 (1926).

Saprobic on sugary exudates from insects forming a sooty–like coating on the upper surface of living leaves, which crumbles away easily when dry. *Thallus* of blackish–gray, comprising superficial mycelia 5–6 µm wide, with cylindrical, pale brown cells, constricted at the septum, anastomosing.

The generic type of *Conidiocarpus* is *C. penzigii* Woron. which is saprobic on sugary exudates from insects forming a sooty–like coating on the upper surface of living leaves, which crumbles away easily when dry (Woronichin, 1926). Pycnidia are reported to be 420–500 µm high, arising from mycelial mass, olive–brown, stalk 270-335 µm long, 23-30 µm thick, blackish, swollen part 100 × 33 µm, comprising short, olive–brown, angular cells, neck 50–66 × 13 µm, with ostiole surround by hyaline hyphae up to 20 µm long. *Conidia* are 5–9 × 1.5 µm, oblong–ellipsoid, hyaline, unicellular (Woronichin 1926). We were unable to locate the type of *C. penzigii* which is described from Georgia. *Conidiocarpus* however, is a common sooty mould and we made several collections in Thailand. Presently only two species are listed in the genus, *C. penzigii* (Woronichin 1926) and *C. longicollus* Matsush (Matsushima, 2003). *Conidioxyphium* (2 species) and *Podoxyphium* (16 records) are considered as synonyms and the group needs further study. Below we introduce two new species of *Phragmocapnias* as anamorphic states with molecular data which differ from existing *Conidiocarpus* species in conidial size. We also transfer *C. penzigii* (Woronichin, 1926) and *C. longicollus* Matsush. to *Phragmocapnias*.

Phragmocapnias asiaticus Chomnunti & KD Hyde, sp. nov.

MycoBank: 563360

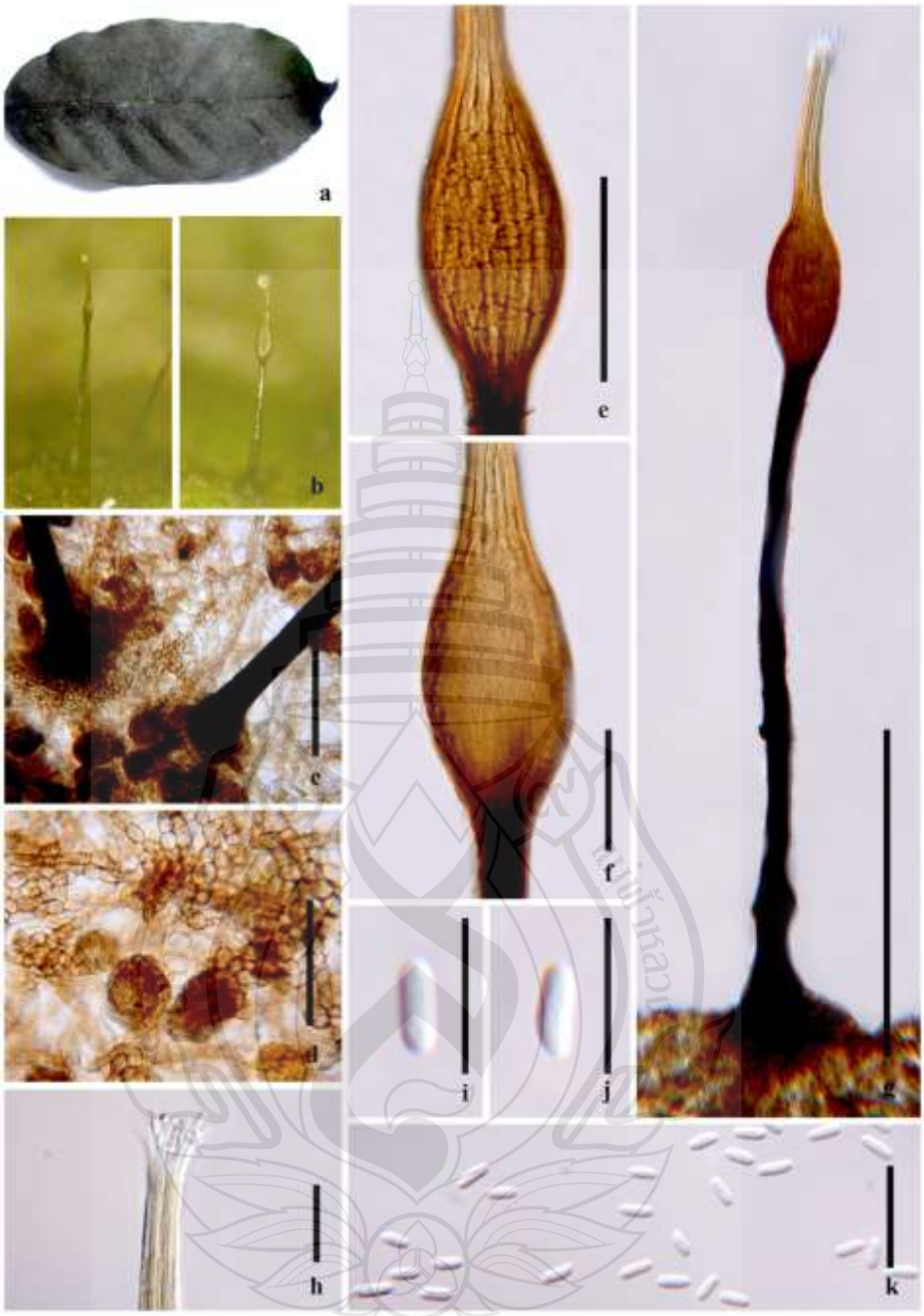
Conidiocarpus penzigii Woron. similis sed conidia 2.5–3.7 × 1–1.4 µm differt.

Etymology: '*asiaticus*' in reference to it origin in Asia.

Habit forming a soot–like coating on the upper surface of leaves (Figure 2.5a). *Thallus* superficial, consisting of a network of cylindrical and septate, 2.2–3.7 µm, thick ($\bar{x}$ = 3 µm, n = 30) hyphae, constricted at the septum, pale brown, but dark brown towards the edge (Figure 2.5c, d). *Pycnidia* 302–387(–471) µm high ($\bar{x}$ = 366 µm, n = 10), arising from capnodiaceous type hyphae, black at the base and stalk, 17–25 µm wide ($\bar{x}$ = 21 µm, n = 10), the conspicuous oval swelling which produce

conidia is 18–24 µm wide ($\bar{x}$ = 21 µm, n = 10) and brown, comprising of cylindrical septate cells (Fig 5e–g). *Ostiole* 4.9–6.5 ($\bar{x}$ = 5.6 µm, n = 10), surrounded by hyaline hyphae (Figure 2.5h). *Conidiogenous cells* formed in the inner cells of the oval part. *Conidia* 2.5–3.7 × 1–1.4 µm ($\bar{x}$ = 3.1 × 1.2 µm, n = 30), oblong to ellipsoid, 1–celled, hyaline, rounded ends (Figure 2.5i–k).

Material examined: THAILAND, Chiang Rai Province, Doi Tung, on living leaf of *Coffea arabica*, 15 August 2009, Jian Kui Liu, DPC 027 (MFLU09-0660, holotype), extype living culture in MFLUCC10-0062.



Note. (a). Black mycelium. (b). Pycnidium with long stalks. (c) – (d). Mycelial network. (e) – (f). Conical pycnidium and pycnidium wall. (g). Pycnidia. (h). Ostiole, (i) – (k). Conidia. Scale bars: (c) – (d) = 50 µm, (e) – (f) = 20 µm, (h), (k) = 10 µm, (i) – (j) = 5 µm.

Figure 2.5 *Phragmocapnias asiaticus* Chomnunti & KD Hyde (holotype).

Phragmocapnias longicollus (Matsush.) Chomnunti & KD Hyde, comb. nov.

≡ *Conidiocarpus longicollus* Matsush. Matsush. Mycol. Mem. 10: 85 (2003) [2001]

MycoBank: 374479

Phragmocapnias penzigii (Woron.) Chomnunti & KD Hyde, comb. nov.

≡ *Conidiocarpus penzigii* Woron. [as penzigi], Ann mycol. 24(3/4): 250 (1926).

MycoBank: 273921

Phragmocapnias siamensis Chomnunti & KD Hyde, sp. nov.

MycoBank: 563361

Conidiocarpus penzigii Woron. similis sed conidia 4.5–5.2 × 1.9–2.4 µm differt.

Etymology: from *siamensis* in reference to the Latin name of Thailand, where the species originated.

Saprobic on sugary exudates from insects growing on the upper surface of living leaves forming soot–like coating. *Thallus* of 3–5 µm thick ($\bar{x}$ = 4 µm, n = 20) hyphae, which is superficial, cylindrical, septate, constricted at the septum and pale brown (Figure 2.6c, d). *Pycnidia* brown, comprised of cylindrical septate cells, 378–458 µm high ($\bar{x}$ = 408 µm, n = 10), stalk black, 22–31 µm high ($\bar{x}$ = 27 µm, n = 10) at the base, the oval part produces conidia, ostiolate, 36–41 µm wide ($\bar{x}$ = 38 µm, n = 10) (Figure 6e, f). *Ostiole* 9–15 ($\bar{x}$ = 12 µm, n = 10), surrounded by hyaline hyphae. *Conidiogenous cells* formed on the inner cell walls of the oval part. *Conidia* 4.5–5.2 × 1.9–2.4 µm ($\bar{x}$ = 4.8 × 2.1 µm, n = 20), ellipsoid, aseptate, hyaline, smooth-walled, rounded at both ends (Figure 2.6g).

Material examined: THAILAND, Chiang Rai Province, Thasud, on living leaf of *Mangifera indica*, 20 August 2009, Rungtiwa Phokhomsak, DPC 029 (MFLU09–0662, holotype), extype living culture in MFLUCC10-0064; *Ibid.*, Bandoo, on living leaf of *Mangifera* sp., 7 June 2009, Putarak Chomnunti, DPC 026 (MFLU09-0656), living culture in MFLUCC10-006; *Ibid.*, Doi Tung, on living leaf of *Coffea arabica*, 15 August 2009, Jian Kui Liu, DPC 028 (MFLU09-0661), living culture in MFLUCC10-0063; *Ibid.*, on living leaf of *Euphorbia* sp., 15 August 2009, SC

Karunarathna, DPC 030 (MFLU09-0663), living culture in MFLUCC10-0065; *Ibid.*, Mae Fah Luang University, on living leaf of *Bischofia javanica*, 9 January 2010, Putarak Chomnunti, DPC 036 (MFLU10-0003), living culture in MFLUCC10-0074.

Note. (a). Black mycelium covering the leaf surface. (b). Pycnidia on host, (c) – (d). Mycelial network, (e) – (f). Conical pycnidia and pycnidia wall, (g). Conidia. Scal bars: (f) = 100 μm, (c) – (e) = 50 μm, (g) = 20 μm.

Figure 2.6 *Phragmocapnias siamensis* Chomnunti & KD Hyde (holotype).

Leptoxyphium Speg., Physis, B. Aires 4: 294 (1918)

= *Astragoxyphium* Bat., Nascim. & Cif., in Batista & Ciferri, Quad. Lab, crittogam., Pavia 31:45 (1963).

= *Caldariomyces* Woron., Ann mycol 24 (3/4): 261 (1926).

= *Megaloxyphium* Cif., Bat. & Nascim., Publcoes Inst. Micol. Recife 47:3 (1956).

Saprobic on sugary exudates from insects growing on the surface of living leaves. *Thallus* of superficial, grey brown to brown, septate, branched mycelium, constricted at the septa, forming an irregular network. *Pycnidia* superficial, gregarious, arising from aggregated hyphae, with bulbous base, comprising of parallel hyphae, straight to slightly flexuous, sometimes with helically twisting, comprised of cylindrical hyphae and expanded to become funnel–shaped, cupulate at the apex. *Conidia* ellipsoidal, hyaline, 1–celled guttulate (Woronichin 1926, Hughes 1976).

Type species

Leptoxyphium graminum (Pat.) Speg., Physis, B. Aires 4:294 (1918)

≡ *Capnodium graminum* Pat., J. Bot., Paris 11: 348 (1897)

Mycelium forms an irregular network which comprises cylindrical hyphae. *Pycnidia* arising from helically twisting hyphae or ropes of repent hyphae, stalk with a stout base, mostly unbranched, forming a fringe of sterile hairs at the apex. *Conidiogenous cells* formed on the inner cell walls of the swollen apex. *Conidia* usually broadly ellipsoidal, some 1–septate, and pigmented when forming a mass on the host surface (Saccardo 1899, Hughes 1976).

Leptoxyphium cacuminum Chomnunti & KD Hyde, sp. nov.

MycoBank 563359

Leptoxyphium graminum (Pat.) Speg. similis sed conidia 4.1–6.7 × 2.1–2.7μm differt.

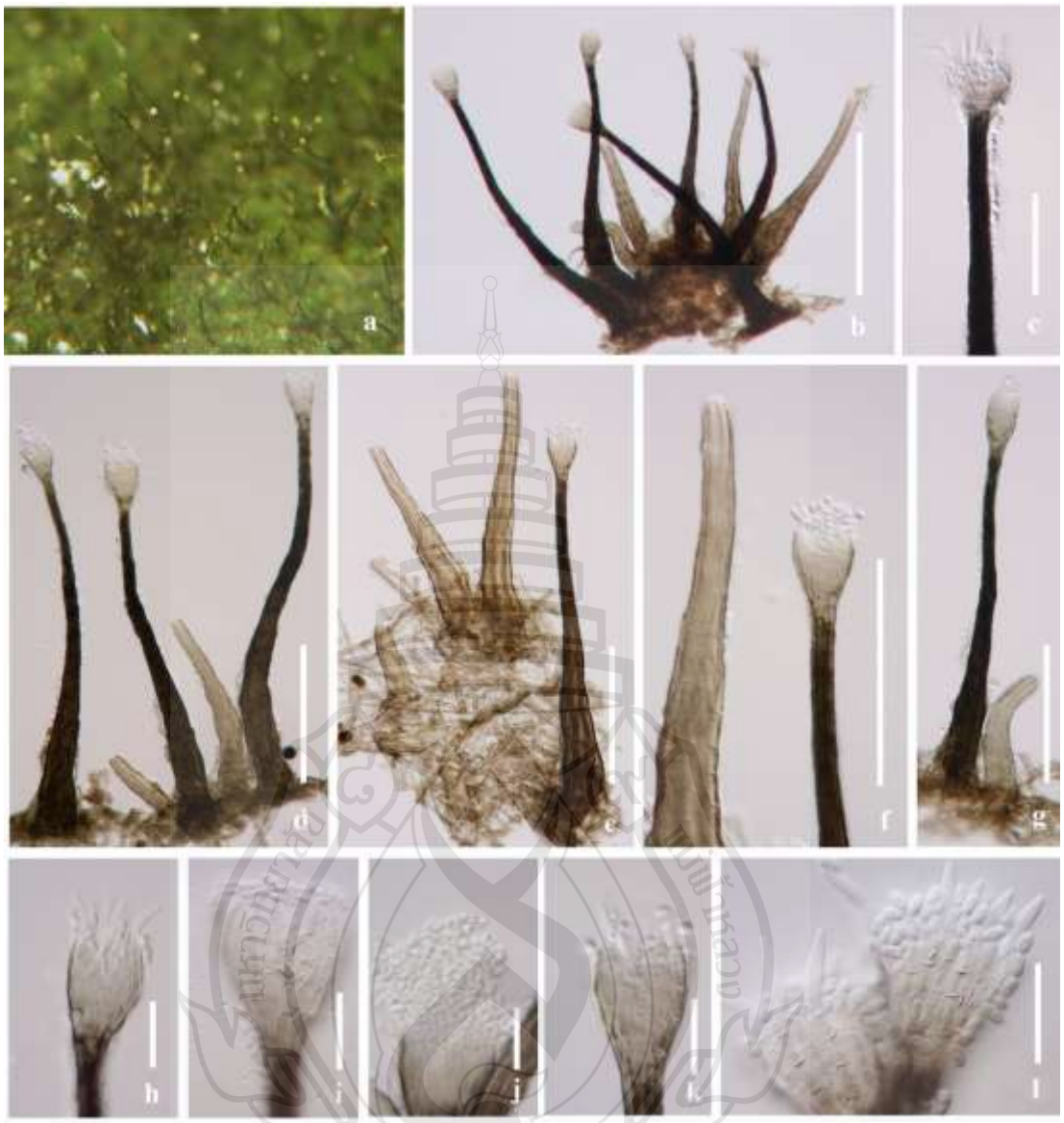
Etymology: from the Latin – *cacumin* meaning swollen, in relation to the pycnidia.

Saprobic on sugary exudates from insects growing on the surface of living leaves (Figure 2.7a). *Thallus* of 3.2–6.2 μm high ($\bar{x}$ = 4.4 μm, n = 20), grey brown to brown, septate, branched, superficial mycelium, constricted at the septa, forming an

irregular network. *Pycnidia* 341–446 µm high ($\bar{x}$ = 392 µm, n = 10), and 19–30 µm base ($\bar{x}$ = 26 µm, n = 10), superficial, gregarious, arising from aggregated hyphae, with bulbous base, comprising parallel hyphae, straight to slightly flexuous, sometime with helical twisting (Figure 7b–e), ostiolate. Stalked pycnidia comprising cylindrical hyphae expanding at the end into a funnel–shape, resembling a cup, 27–43 × 28–45 µm [$\bar{x}$ = 33 × 35 µm, n = 10] (Figure 2.7f–h). *Conidiogenous cells* arising from the inner cell wall of the cupulate apex. *Conidia* ellipsoidal, hyaline, aseptate and guttulate, 4.1–6.7 × 2.1–2.7µm ($\bar{x}$ = 5.2 × 2.4 µm, n = 20) (Figure 2.7i–l). On PDA, colonies reaching 5 cm diam. after 10 days growth. *Colonies* flat, irregular in the middle but radiating towards the edge, dull black, becoming olive–green towards the edge (Figure 2.8a–c). *Mycelium* composed of cylindrical and septate hyphae 3.6–4.8 µm ($\bar{x}$ = 4.2 µm, n = 20), becoming aerial, branched, pale brown to deeply pigmented at the septum (Figure 2.8f–h). *Pycnidia* stalked, arising from a basal cell and developing into a cupulate swelling towards the apex. Stalk olive–green, and deeply pigment at the base, but at the apex the hyphae are hyaline (Figure 2.8d, e). *Conidia* broadly ellipsoidal, unicellular and guttulate, hyaline, 4–4.9 × 3.5–3.7 µm ($\bar{x}$ = 4.4× 3.6 µm, n = 20) (Figure 2.8i–l).

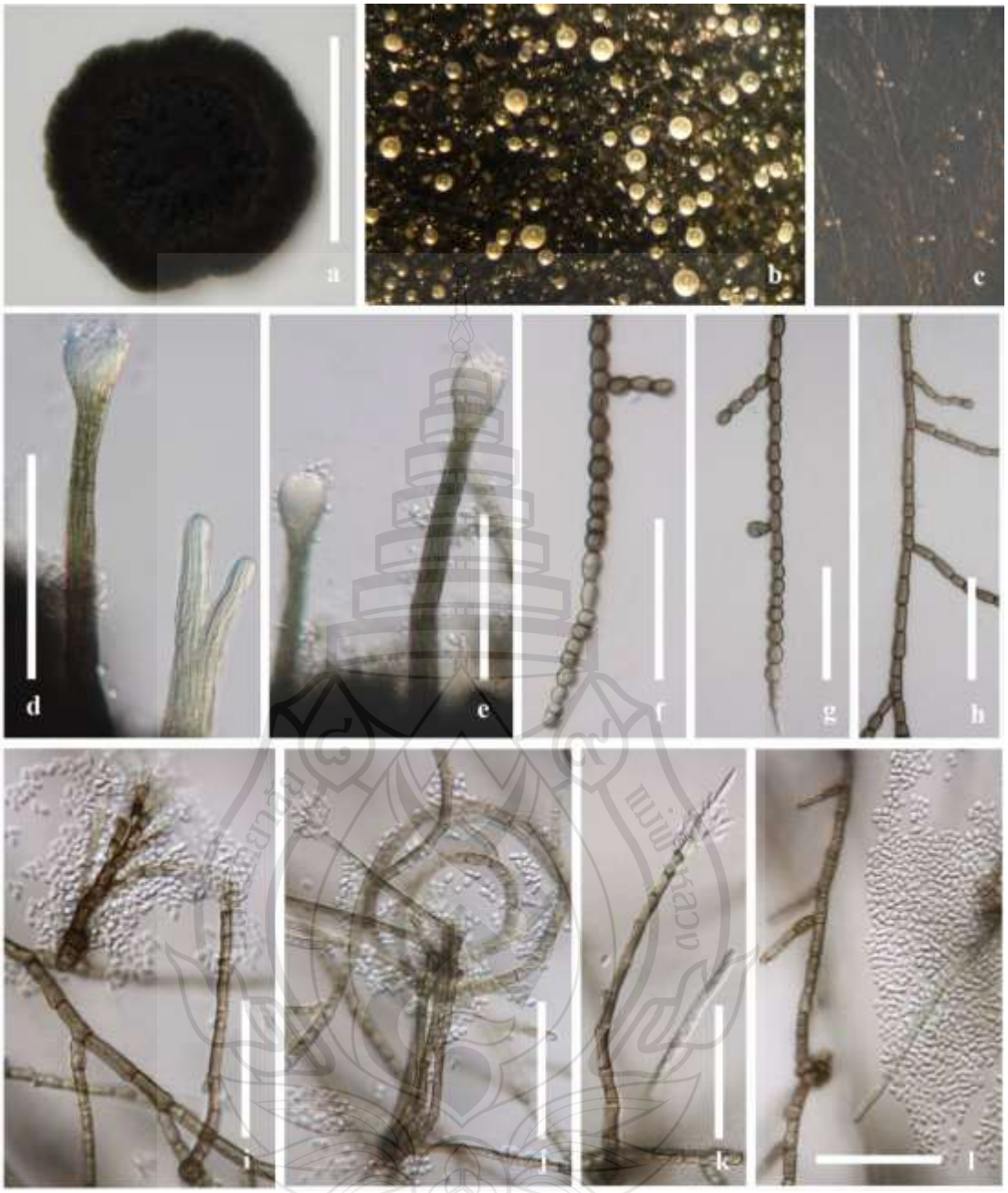
Material examined: THAILAND, Chiang Rai Province, Thasud, on living leaf of *Gossypium herbaceum*, 11 August 2009, SC Karunarathna, DPC 024 (MFLU09–0657, holotype), extype living culture in MFLUCC10–0059; *Ibid.*, Bandoo, on living leaf of *Ficus* sp., 4 January 2010, KD Hyde, DPC 050 (MFLU 10–0015), living culture in MFLUCC10–0086; *Ibid.*, Mae Fah Luang University, on living leaf of *Mimusops elengi*, 24 April 2009, Putarak Chomnunti, DPC 009 (MFLU09–0646), living culture in MFLUCC10–0049.

Sequences of *Leptoxyphium fumago, L. madagascariense* and *L. kurandea* are available in GenBank and *Leptoxyphium cacuminum* clearly clusters with them. Nevertheless, *Leptoxyphium cacuminum* differs from the other known species in the genus because of its hyaline conidia, never becoming septate or pigmented when matures.



Note. (a). Gregarious pycnidia on host surface. (b), (d), (g). Stalked pycnidia with wider base. (e). Formation of pycnidia from aggregated hyphae. (c), (f). Black stalked funnel cupulate apex. (h) – (l). Conidia, conidiogenous boundary with hyaline hyphae surrounding the ostiole. Scale bars: (b), (d) –(e), (g) = 200 µm, (c), (f) = 50 µm, (h) – (l) = 20 µm.

Figure 2.7 *Leptoxyphium cacuminum* Chomnunti & KD Hyde (holotype)



Note. In culture. (a). Colony on PDA. (b) – (c), (d). Conidial mass at the apex of conidia. (d) – (e). Olive–green stalked funnel shaped at apex. (f) – (h). Septate hyphae. (i) – (l). Conidia produced from the apex of conidiophores. Scale bars: (a). = 2 cm, (d). = 200 μm, (e) – (l). = 50 μm.

Figure 2.8 *Leptoxyphium cacuminum* Chomnunti & KD Hyde (holotype)

Capnodium Mont., Annls Sci. Nat., Bot., sér. 3 11: 233 (1849)

= *Polychaeton* (Pers.) Lév., in Orbigny, Dict. Univ. Hist. Nat. 8: 493 (1846)

= *Capnodaria* (Sacc.) Theiss. & Syd., Ann mycol. 15(6): 474 (1918) [1917]

= *Capnodium* sugen. *Capnodaria* Sacc., Syll. Fung. (Abellini) 1:74 (1882)

= *Capnodenia* (Sacc.) Theiss. & Syd. (1917)

= *Fumago* sect. *Polychaeton* Pers., Mycol. eur. (Erlanga) 1: 9 (1822)

= *Fumagospora* G. Arnaud, Annals d'École National d'Agric. de Montpellier, Série 2 10(4): 326 (1911)

= *Morfea* Roze, Bull. Soc. bot. Fr. 14: 21 (1867)

= *Morfea* (G. Arnaud) Cif. & Bat., in Batista & Ciferri, Saccardoa 2: 153 (1963)

= *Phaeoxyphiella* Bat. & Cif., Quad. Lab. crittogam., Pavia 31: 145 (1963)

= *Scolecoxyphium* Cif. & Bat. Publções Inst. Micol. Recife 47: 5 (1956)

Saprobic on sugary exudates from insects growing on the surface of leaves, fruits, stems and other non plant objects. *Thallus* a loose or dense network of pale brown, superficial hyphae or thick pseudoparenchymatous stromata, with sexual and asexual states often growing together. *Ascomata* superficial on mycelium of thallus, brown to dark brown or black, globose to ellipsoidal, short-stalked or sessile, ostiolate at maturity, scattered or in groups, without setae. *Peridium* comprising dark brown to pale brown, thick–walled cells forming *textura angularis*. *Asci* 8–spored, bitunicate, clavate, ovoid or saccate, aparaphysate, apedicellate. *Ascospores* brown, oblong or ovoid and some reniform, trans–septate with one or more vertical septa or without vertical septa. *Pycnidia* elongate, often with long stalks, dark brown. *Ostiole* at apex of pycnidia. hyphae continued upwards to the tapered neck, terminating in an ostiole which is surrounded by dull hyphal round ends. Conidia hyaline, 1–celled.

Anamorph: *Polychaeton* (Pers.) Lév. (Hyde et al., 2011).

Polychaeton was introduced by Léveillé (1847) based on Persoon's *Fumago* [subgenus] *Polychaeton*, and Hughes (1976) provided a detailed account of the choice of generic type including the reason why Spegazzini's lectotype *P. carolinense* (Berk. & Desm.) Speg. was inadmissible (not included by Léveillé at the time of

description). It is not clear whether Léveillé intended to include *F. citri* in the genus or whether it was meant to represent another genus. Of the five species in Persoon's subgenus *Polychaeton*, only *P. citri* or *P. quercinum* were considered suitable to be generic types by Hughes (1976), who chose *P. quercinum* (≡ *Fumago quercina*) as the lectotype species. However, and unlike Berkeley & Desmazieres (1849), he regarded this genus as the anamorphic stage of the genus *Scorias*, not *Capnodium*. This has been discussed by other authors, e.g. in Sutton (1977) as *Polychaeton*, see Index Fungorum as *Capnodium*, and as type unknown in MycoBank (see http://www.mycobank.org/MycoTaxo.aspx?Link=T&Rec=4305), or non designated as Index Nominum Genericoum In this study we accept *P. quercinum* as the generic type, but Persoon's original specimen was not available on loan. However, we have seen a possible type or authentic collection in herbarium K, which is part of M.J. Berkeley's herbarium.

Capnodium was introduced by Montagne (1849) based on *Fumago citri* Pers. and is the type genus of *Capnodiaceae* (Friend, 1965). Reynolds (1978) however, examined type material from L which only had anamorphic characters and this was similar for the supporting specimens listed in exsiccatae. Reynolds (1978) therefore chose a species where the ascus and the ascospores are known, the second species listed in the genus by Montagne (1849): *Capnodium salicinum* Mont. as the lectotype (Reynolds, 1999). however, lists this as a synonym of *C. citri* which we follow here. *Capnodium* is the teleomorph stage of *Polychaeton* (Figures 2.10–12), but it is the earlier name.As mentioned earlier that we advocate that*Capnodium* be considered for conservation under the "one fungus one name" concept that will be incorporated in the newly proposed Code for Algae, Fungi and Plants. In this study we provide nine sequences of 'polychaeton–like' *Capnodium* specimens and illustrate three species.

Type species

Capnodium citri Berk. & Desm., in Berkeley, J Rl Hort Soc 4: 11 (1849)

≡ *Fumago citri* Pers., Mycol. eur. (Erlanga) 1: 9 (1822).

≡ *Polychaeton citri* (Pers.) Lév., in Orbigny, Dict. Univ. Hist. Nat. 8: 493 (1846)

≡ *Microxiphium citri* (Berk. & Desm.) Speg., Boln Acad. nac. Cienc. Córdoba 26(2–4): 399 (1924) [1923]

= *Apiosporium citri* Briosi & Pass., Atti R.acad. Lincei, Trans., Sér. 3 7: 22 (1882).

= *Apiosporium salicis* Kunze, in Kunze & Schmidt, Mykologische Hefte (Leipzig) 1: 8 (1817)

= *Capnodium salicinum* Mont., *Annls Sci. Nat.*, Bot., sér. 3 11: 234 (1849)

= *Pleosphaeria salicina* (Mont.) G. Arnaud

= *Polychaeton salicinum* (Mont.) Kuntze, Revis. gen. pl. (Leipzig) 3: 1–576 (1891)

= *Teichospora salicina* (Mont.) Gäum.

= *Limacinia citri* (Briosi & Pass.) Sacc., Hedwigia 36: 20 (1897)

= *Meliola citri* (Briosi & Pass.) Sacc., Syll. fung. (Abellini) 1: 69 (1882)

Saprobic on sugary exudates from insects growing on the surface of leaves, fruits, stems and other non plant objects. *Thallus* comprising 4.2–6 μm wide, dark brown, superficial, cylindrical and septate hyphae, constricted at the septum (Figure 2.9). *Pycnidia* up to 345–391 long μm, 36–40 μm wide at the base, arising from dense mycelia, elongate, often long–stalked, dark brown (Figure 2.9C–E). *Conidiogenous cells* formed in the swollen base. *Ostiole* 13–15 μm. *Conidia* 6.5 × 5 μm, hyaline, ellipsoidal, 1–celled (Figure 2.9).

Material examined: France, unlocalised, ex herbarium Léveillé in herbarium Berkeley (as *Capnodium citri*) [K(M) 172364 – iconotype only?, specimen missing]. *Ibid.*, on *Olea* leaf, ex herbaria C. Montagne, PC, in herbarium Berkeley (as *Fumago citri* Pers. non Turpin) [K(M) 172363 – type?].

Note. (a). Superficial mycelium on host. (b), (f). Cylindrical, septate mycelium. (c) – (e). Elongate pycnidia. (g). Conidia. Scale bars: (b) – (c) = 200 μm, (d) – (e) = 100 μm, (f). = 50 μm, (g). = 20 μm.

Figure 2.9 *Capnodium citri* Berk. & Desm. (type?)

Capnodium coartatum Chomnunti & KD Hyde, sp. nov.

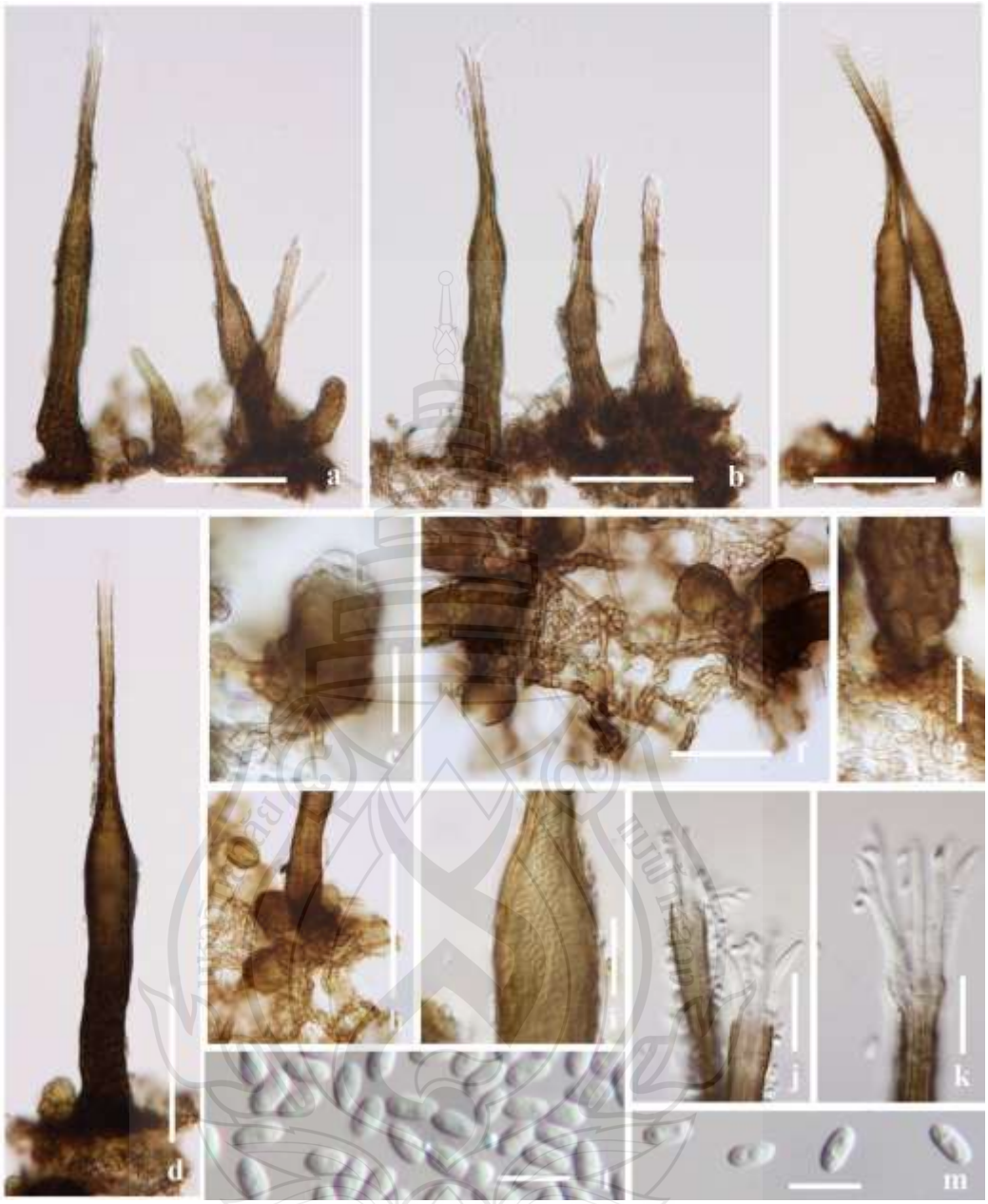
MycoBank 563362

Polychaeton citri (Pers.) Lév. similis sed conidia 4.2–4.6 × 1.9–2.4 μm differt.

Etymology: from the Latin *coartata* meaning narrow, in reference to pycnidia.

Saprobic on sugary exudates from insects growing on the surface of leaves, fruits, stems and other non plant objects. *Thallus* of dark brown mycelium growing over the surface of the plant with abundant pycnidia, produced on 3–5 μm wide ($\bar{x}$ = 4 μm., n = 20), irregularly branched, pale brown to brown, septate, sub–cylindrical hyphal cells, constricted at the septum (Figure 2.10f–h). *Pycnidia* 332–401 × 34–56 μm ($\bar{x}$ = 366 × 45 μm, n = 20), superficial, scattered or gregarious, blackish brown, synnematous in structure, ovoid to flask–shaped, elongated, somewhat branched, comprising mostly cylindrical cells, with slightly swollen or flattened base, base of pycnidium forming a pseudo–parenchymatous to prosenchymatous tissue, upper cylindric region tapering to apex, 7.6–11.5 μm wide ($\bar{x}$ = 9.8 μm, n = 10) (Figure 2.10a–d), ostiole surrounded by hyaline hyphae (Figure 2.10j, k). *Conidia* 4.2–4.6 × 1.9–2.4 μm ($\bar{x}$ = 4.4 × 2.1 μm, n = 20), produced within the swollen base, gathering in a terminal droplet, ellipsoidal, smooth, round ends, hyaline (Figure 2.10l, m).

Material examined: THAILAND, Chiang Rai Province, Baan Du, on living leaf of *Psidium guajava* (*Myrtaceae*), 24 September 2009, SC Karunarathna DPC 040 (MFLU10–0076, holotype), extype living culture in MFLUCC10–0005); *Ibid.,* Mae Fah Luang Unversity, on living leaf of *Alstonia scholaris*, 3 October 2009, P. Chomnunti DPC 041 (MFLU10–0077), living culture in MFLUCC10–0006.



Note. (a) – (d). Stalked pycnidia. (e). Immature pycnidia. (f). Mycelia. (g) – (h). Pycnidia rising from mycelium. (i). Abundant conidia at apex pycnidia and pycnidia wall. (j) – (k). Ostiole surround by hyaline hyphae. (l – (m = Conidia. Scale bars: (a) – (d) = 200 µm, (f) = 100 µm, (e), (g), (h), (i) – (k) = 50 µm, (l) – (m) = 20 µm.

Figure 2.10 *Capnodium coartatum* Chomnunti & KD Hyde (holotype)

Capnodium tiliae (Fuckel) Sacc.. Syll. fung. (Abellini) 1: 74 (1882)

≡ *Capnodaria tiliae* (Fuckel) Theiss. & Syd. Ann mycol.15(6): 474 (1918) (1917)

≡ *Fumago tiliae* Fuckel. Jb. Nassau. Ver. Naturk 23–24: 142 (1870) (1869–70)

Saprobic on sugary exudates from insects growing on bark (Figure 2.11a). *Thallus* of superficial *mycelium* comprising membranous, cylindrical, 4–6 µm ($\bar{x}$ = 5 µm, n = 20) wide, septate hyphae, constricted at the septa, dark brown at the septum and margin (Figure 2.11c). *Ascomata* 81–136 µm diam, 78–141 µm high ($\bar{x}$ = 96 × 102 µm, n = 10), superficial, globose, without setae, brown to dark brown (Figure 2.11b–d, f). *Peridium* 17–18 µm wide ($\bar{x}$ = 15 µm, n = 15), comprising dark brown cells of *textura angularis*, darker externally. *Hamathecium* comprising asci and 2.3–3.6 µm ($\bar{x}$ = 2.8 µm, n = 20) hyaline cells (Figure 2.11e). *Asci* 41–44 × 15–18 µm ($\bar{x}$ =42 × 17 µm, n = 5), 10–spored, bitunicate, clavate, apedicellate, ocular chamber not apparent (Figure 2.11g–i). *Ascospores* 13–17 × 5–7 µm ($\bar{x}$ = 15 × 6 µm, n = 20), oblong or ovoid and some reniform, 3–5 septate, constricted at the septa, rarely with longitudinal septa, ends narrowly round, brown, dark brown at septum and margin, wall verrucose (Figure 2.11j).

Material examined: GERMANY, Biebrich, on branch of *Tilia parviflora*, Herbier Fuckel no. 899 (1894), (G, Herbier Barbey Boissier).

Note. (a). Label data. (b). Vertical section through ascoma. (c). Dark brown mycelium. (d). Peridium. (e) – (f). Peridium with cells of *textura angularis*. (g) – (i). Cylindrical to cylindric–clavate asci. (j). Ascospores 3–4 septate. Scale bars: (f) = 200 μm, (b) – (d) = 100 μm, (e), (g) – (i) = 50 μm, (j) = 20 μm.

Figure 2.11 *Capnodium tiliae* Fuckel (exsicata of *Fumago tiliae*)

Scorias Fr. Syst. Orb. Veg. 1: 171 (1825)

= *Algorichtera* Kuntze, Revis. gen. pl. (Leipzig) 2: 637 (1891)

= *Antennella* Theiss. & Syd., Ann mycol. 15(6): 473 (1918) (1917)

= *Antennellina* J.M. Mend., in Stevens, Bulletin of the Bernice P. Bishop Museum, Honolulu, Hawaii 19: 55 (1925)

= *Hyalocapnias* Bat. & Cif., Saccardoa 2: 114 (1963)

= *Leptocapnodium* (G. Arnaud) Cif. & Bat., in Batista & Ciferri, Saccardoa 2: 121 (1963)

= *Paracapnodium* Speg., Anal. Mus. nac. B. Aires, Ser. 3 12: 325 (1909)

= *Scolecoxyphium* Cif. & Bat., Publções Inst. Micol. Recife 47: 5 (1956)

= *Xystozukalia* Theiss., Verh. zool. bot. Ges. Wien 66: 357, 358 (1916)

Saprobic on sugary exudates from insects growing on host. *Thallus* comprising black dense, septate mycelium with ascomata covering the surface of host. *Ascomata* dark brown to blackish, shiny, subglobose to broadly ellipsoidal, with a rounded apex, central ostiole present at maturity, with short stalk. *Peridium* composed of cells of *textura angularis*. *Asci* 8–spored, bitunicate, oblong to saccate. *Ascospores* hyaline, fusiform, with 3–4 trans–septa, the upper cells slightly wider than the lower cells. *Pycnidia* long stalked, flask–shaped, tapering to the apex, pycnidium wall helically twisting, synnemata–like, dark brown to black at the base, brown to pale brown towards the tapering apex. *Conidia* ellipsoidal, unicellular, hyaline.

Anamorphs: *Scolecoxyphium* Cif. & Bat. (Hyde et al., 2011).

Type species

Scorias spongiosa (Schwein.) Fr. Syst. mycol. (Lundae) 3(2): 291 (1832)

≡ *Botrytis spongiosa* Schwein., Schr. naturf. Ges. Leipzig 1: 127, (1822)

Description from type

Saprobic on sugary exudates from insects growing on host (Figure 2.12a). *Thallus* comprising 3.8–5.5 µm wide ($\bar{x}$ = 4.7 µm, n = 20) wide, black, dense, septate mycelium. *Ascomata* 72–88 µm diam, 89–132 µm high ($\bar{x}$ = 82 × 110 µm, n = 5), covering the surface of thallus, gregarious, dark brown to black, velvety, stalked, subglobose to broadly ellipsoidal, with a rounded apex, central ostiole present at maturity (Figure 2.12c–j). *Peridium* 14–25 µm ($\bar{x}$ = 18 µm, n = 20), composed of

cells of *textura angularis* (Figure 2.12k). *Asci* 8–spored, bitunicate, oblong to saccate, apedicellate, with a long ocular chamber (Figure 2.12L–N). *Ascospores* 13–15 × 2–4 µm ($\bar{x}$ = 13 × 3 µm, n = 20), hyaline, fusiform, 3–4 trans–septate, the upper cells slightly wider than the lower cells (Figure 2.12o, p).

Note. (a) – (b). Ascomata on hyphal mass on host. (c) – (d). Squash of ascomata. (e) – (j). Vertical section through ascomata. (k. Stalked ascomata. (l) – (n). Asci with thick wall. (o) – (p). Pale brown ascospores. Scale bars: (d), (j) = 100 μm, (c), (i) = 50 μm, (k) – (n) = 20 μm, (o), (p) = 10 μm.

Figure 2.12 *Scorias spongiosa* Fr. (syntype)

Description of Asexual stage from Thai Collection

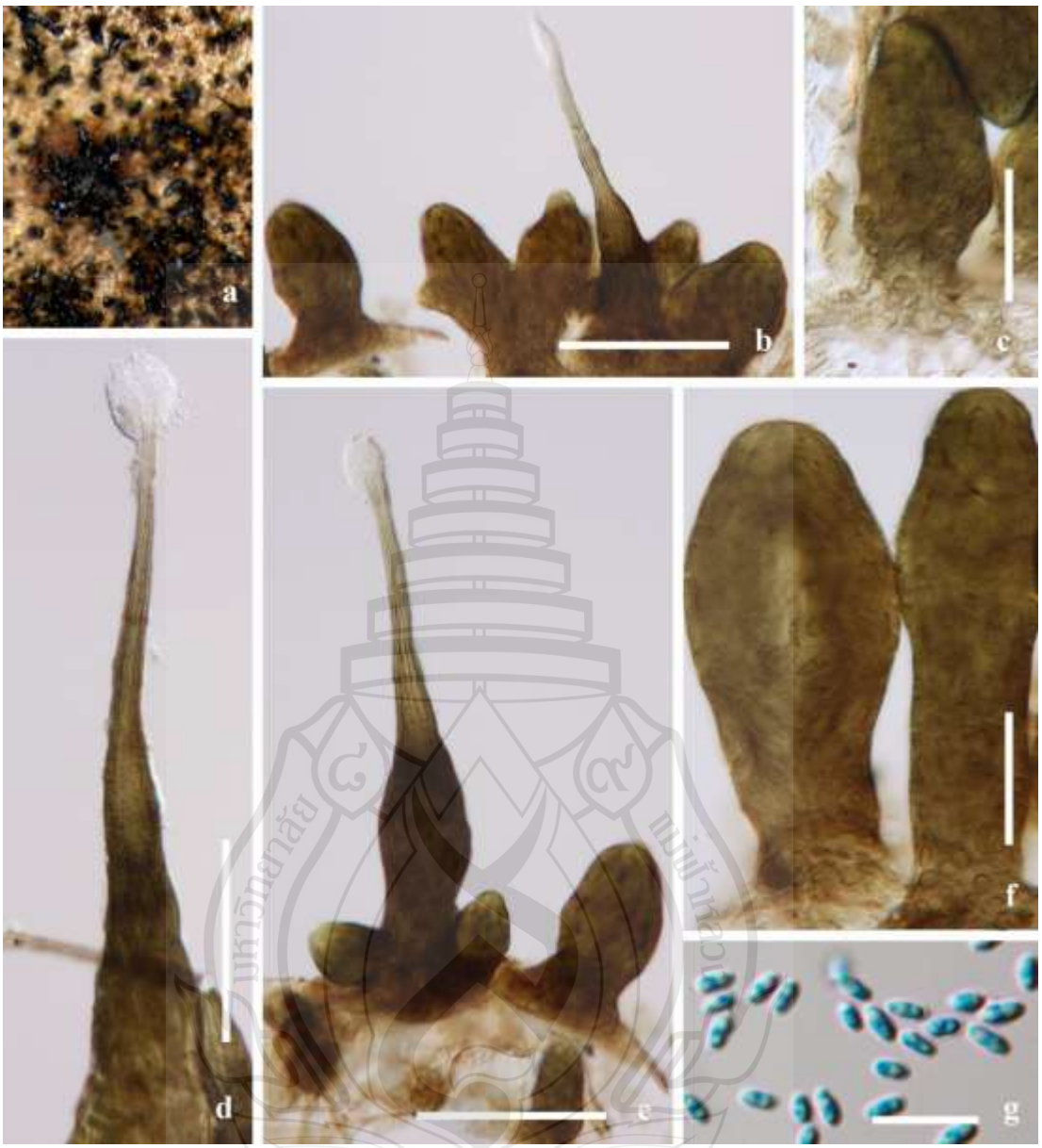
Saprobic on sugary exudates from insects, growing on the surface of living leaves (Figure 2.13a). *Thallus* mycelial, composed of 3.2–4.8 µm wide ($\bar{x}$ = 4 µm, n = 20), superficial, cylindrical, septate, pale brown hyphae (Figure 13b). *Pycnidia* 412–614 × 40–57 ($\bar{x}$ = 503 × 47 µm, n = 10), long stalked, flask–shaped, tapering to the apex, pycnidial wall helically twisting, synnemata–like, frequently with immature ascomata, dark brown to black at the base, brown to pale brown towards the tapering apex (Figure 2.13b–f). *Conidia* 3.1–4.2 × 1.6–2.4 ($\bar{x}$ = 3.7 × 2 µm, n = 20), ellipsoidal, unicellular, hyaline (Figure 2.13g). *Colonies* reaching up to 3 cm diam. after 10 days on PDA medium, flattened, spreading radialy towards the edge, dark–brown in older parts, olive–green towards the edge. *Mycelium* 2.6–4.4 µm ($\bar{x}$ = 3.5 µm, n = 20), consisting of cylindrical, branched, thick-walled, septate hyphae, mucilaginous in the outer layer, with numerous aerial hyphae (Figure 2.13a). *Pycnidia* stalked, flask–shaped, arising from the mycelium plate, comprised of hyaline hyphae helically twisting towards the apex and surrounding the ostiole. Stalk pale olive–green and hyaline at the apex, swollen at base (Figure 2.13b–g). *Conidia* 3.5–4.4 × 1.5–2.2 µm ($\bar{x}$ = 3.9 × 1.9 µm, n = 20), ellipsoid, unicellular, guttulate, hyaline (Figure 2.14i–k).

Material examined: Type specimen: ‘Car. N. 1311. *Podiosoma? epiphega*. Schwein.! *In litt*.’ (UPS Fries – lectotype). USA?, unlocalised, ex herbarium Schwein., in herbarium M.J. Berkeley [K(M) 171138 – syntype]; *ibid*. Ohio, on wood, March, H.W. Ravenel 145 (as *Scorias spongiosa*) [K(M) 171139 & ? IMI 30376–authentic]; *ibid*. South Carolina, *ad ramos Alni*, Dec. H.W. Ravenel 1384 (as *Scorias spongiosa*) [K(M) 171140 – authentic]; *ibid*. Ohio, on *Fagus* sp., ex herbarium M.J. Berkeley (as *Scorias spongiosa*) [K(M) 171141 – authentic]; *ibid*. Ohio, on leaf and bark of *Fagus* sp., ex herbarium M.J. Berkeley (as *Scorias spongiosa*) [K(M) 171142 – authentic]; THAILAND, Chiang Rai Province, Khunkorn, on living leaf of *Entada* sp. (*Fabaceae*), 18 December 2009, Putarak Chomnunti, DPC048 (MFLU10–0013, epitype designed here), living culture in MFLUCC10–0084.

The species has been sanctioned by Fries Fr., Syst. mycol. 3(2): 291 (1832) with a collection from Carolina number 1311. In the herbarium of M.J. Berkeley at K

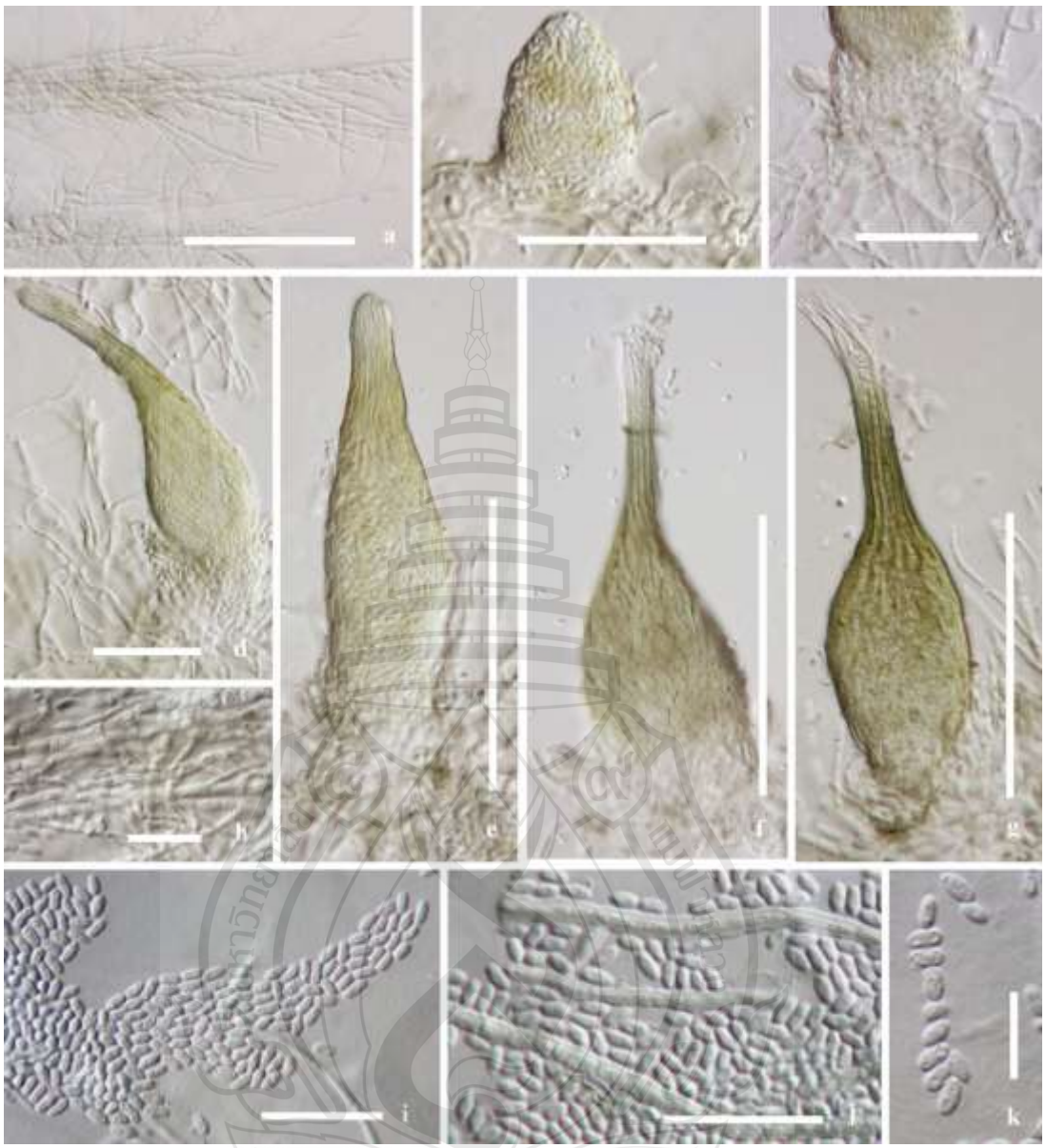
there are five collections labeled *Scorias spongiosa* from Ohio numbered 145 found on *Fagus* leaves and twigs, and one from South Carolina collected by Ravenel on *Alnus* without a number, and a representation of these have been examined by Reynolds (1975). All of those are authentic material of *Scorias spongiosa* according to Saccardo (Sylloge Fungorum I: 83, 1882). Nevertheless, there is a further collection in herbarium Berkeley, originally from herbarium of Schweinitz, which contains no further annotations, but may be part of Fries's listed 1311 specimen. From this collection we have chosen to make a slide, and the description is included above. Further to the above the IMI 230376 herbarium (now part of K herbarium's holdings) contains several slides labeled type from Berkeley's material, but it is not clear what specimen they used for preparing the collection.

The type illustrated contained only the teleomorph. The fresh collection from Thailand contained only the mature anamorph and immature ascomata. In the molecular analysis *Scorias spongiosa* (CBS 325.33) clustered with strain MFLU10–0084 with 100% bootstrap support and is clearly placed in *Capnodiaceae*. Nine species are currently recorded in the genus in Index Fungorum (http://www.indexfungorum.org/Names/Names.asp; accessed on 12 July 2011).



Note. (a). Ascomata and pycnidia on surface of leaf. (b), (d) – (f). Immature ascomata and pycnidia. (c). Pycnidia arising from mycelium. (g). Conida. Scale bars: (a) = 200 μm, (d) – (e) = 100 μm, (b), (f) = 50 μm, (g) = 20 μm.

Figure 2.13 *Scorias spongiosa* Fr. (epitype)



Note. (a). Mycelia. (b). Immature pycnidia. (c). Mycelium bearing pycnidium. (d) – (g). Mature pycnidia. (h). Septate hyphae. (i) – (k). Hyaline conidia. Scale bars: (a). = 200 μm, (e) – (g). = 100 μm, (b) – (d). = 50 μm, (h) – (j). = 20 μm, (k). = 10 μm.

Figure 2.14 *Scorias spongiosa* Fr. (epitype)

Genera of *Capnodicaceae incertae cedis*

Scoriadopsis J.M. Mend., in Stevens, Ann mycol. 28(5/6): 365 (1930)

Scoriadopsis miconiae J.M. Mend., in Stevens, Ann mycol 28(5/6): 365 (1930)

≡ *Rizalia miconiae* (J.M. Mend.) E. Müll., in Müller & von Arx, Beitr. Kryptfl. Schweiz 11 (no. 2): 597 (1962)

Colonies sooty black, on upper surface and closely associated with *Meliola*. *Thallus* perisporioid, slimy, loosely woven, weft–like, hyaline to straw–colored hyphae. *Ascomata* globose or ovoid, ostiolate, stalked, gelatinous, dark brown. *Asci* 40 × 14 μm, numerous, 8–spored, ovate, pseduoparaphyses lacking. *Ascospores* 16 × 4 μm, fusiform, 1–septate (Stevens, 1930).

We have not seen type material of this species from BPI but the specimens is not good condition. The taxon is similar to *Scorias*, but has 1–septate ascospores, while *Scorias* has 3–4 trans–septate ascospores which are pigmented when mature. We retained *Scoriadopsis* in *Capnodiaceae incertae sedis* as colonies appear like sooty moulds on the host and ascomata are globose, black, and ascospores turn brown when mature. However, the melioaceous habit would be unusual.

Genus transferred to *Mycosphaerellaceae*

Echinothecium Zopf, Nova Acta Acad. Caes. Leop. –Carol. German. Nat. Cur. 70: 250 (1898)

Thallus superficial, composed of thick brown often anastomosing hyphae. *Ascomata* globose, ostiolate, provided with stiff, simple, appendages. Asci 8–spored, sessile, lacking paraphyses. *Ascospores* 2 celled, hyaline, oval, the upper cell slightly wider than the lower cell.

Type species

Sphaerellothecium reticulatum (Zopf) Etayo, Cryptog. Mycol. 29(1): 87 (2008)

≡ *Echinothecium reticulatum* Zopf, Nova Acta Acad. Caes. Leop. –Carol. German. Nat. Cur. 70: 250 (1898)

Thallus comprising 4–6 μm thick ($\bar{x}$ = 5 μm, n = 20), superficial, dense, brown, often anstomosing, reticulate, branching, septate hyphae (Figure 2.15a, f, g). *Ascomata* 32–68 μm diam, 31–69 μm high ($\bar{x}$ = 50 × 49 μm, n = 5), spherical or

depressed–globular, dark brown, surrounded by 14–18 × 4–6 µm ($\bar{x}$ = 16 × 5 µm, n = 10) hyphal appendages (Figure 2.15b–e). *Asci* 23–36 × 10–13 µm ($\bar{x}$ = 27 × 11 µm, n = 10), 8–spored, bitunicate, ovoid, apedicellate, with an ocular chamber (Figure 2.15h–j). *Ascospores* 8–6 × 2.5–4 µm ($\bar{x}$ = 7 × 3 µm, n = 20), ovoid, hyaline, the upper cell slightly wider than the lower cell, 1–septate, constricted at the septum (Figure 2.15k, l).

Material examined: ITALY, South Tyrol, Fondo, Mendehof, on bark of spruce trees on *Parmelia sulcata*, 18 August 1897, Arnold exs. 1743 (K(M) 171135, 171136 syntypes!).

Echinothecium occurs on lichens and has been found in Europe and North America (Navarro–Rosinés & Gómez–Bolea 1989). *Lichenostigma cosmopolites* Hafellner & Calat has been most wrongly identified and confused with *Echinothecium reticulatum* which has ascomata with septate seta–like hyphae and seems to be restricted to *Parmelia* s. str. (Calatayud, Navarro–Rosinés & Hafellner, 2002). This is the only genus in the *Capnodiaceae* which has a lichenicolous habit. Etayo (2008) placed this species in the genus *Sphaerellothecium* and after critical study of the holotype of *Sphaerellothecium* we agree with his decision. This needs rewording. We have examined the holotype of *Sphaerellothecium* and it is similar to *Echinothecium* and thus considered a synonym. The generic type, *Sphaerellothecium araneosum* is described below.

Sphaerellothecium araneosum (Rehm) Zopf, Nova Acta Acad. Caes. Leop. – Carol. German. Nat. Cur.70: 178 (1897)

≡ *Sphaerella araneosa* Rehm, Ascomyceten Dign.: no. 133 (1872)

≡ *Discothecium araneosum* (Rehm) Vouaux, Bull. Soc. mycol. Fr. 29: 55 (1913)

= *Echinothecium glabrum* M.S. Christ., Alstrup & D. Hawksw., in Alstrup & Hawksworth, Meddr Grønland, Biosc. 31: 28 (1990)

≡ *Endococcus araneosus* (Rehm) H. Olivier, Bull. Acad. Intern. Géogr. Bot. 17: 127 (1907)

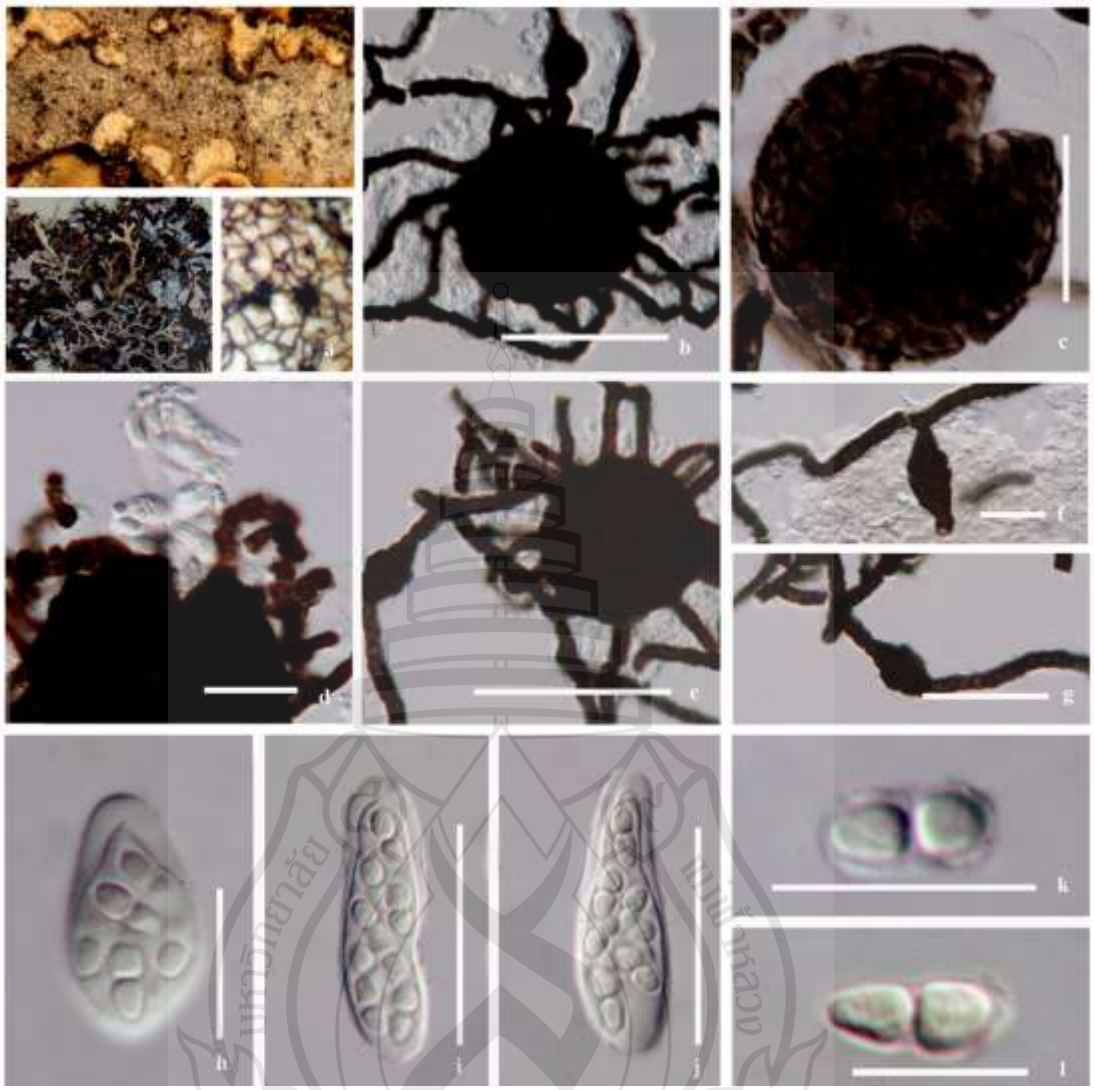
≡ *Epicymatia araneosa* (Rehm) Sacc., Syll. fung. (Abellini) 1: 572 (1882)

≡ *Mycosphaerella araneosa* (Rehm) Lindau, Hilfsb. Sammeln Ascomyc. (Berlin): 125 (1903)

≡ *Phaeosphaerella araneosa* (Rehm) Sacc. & D. Sacc., Syll. fung. (Abellini) 17: 676 (1905)

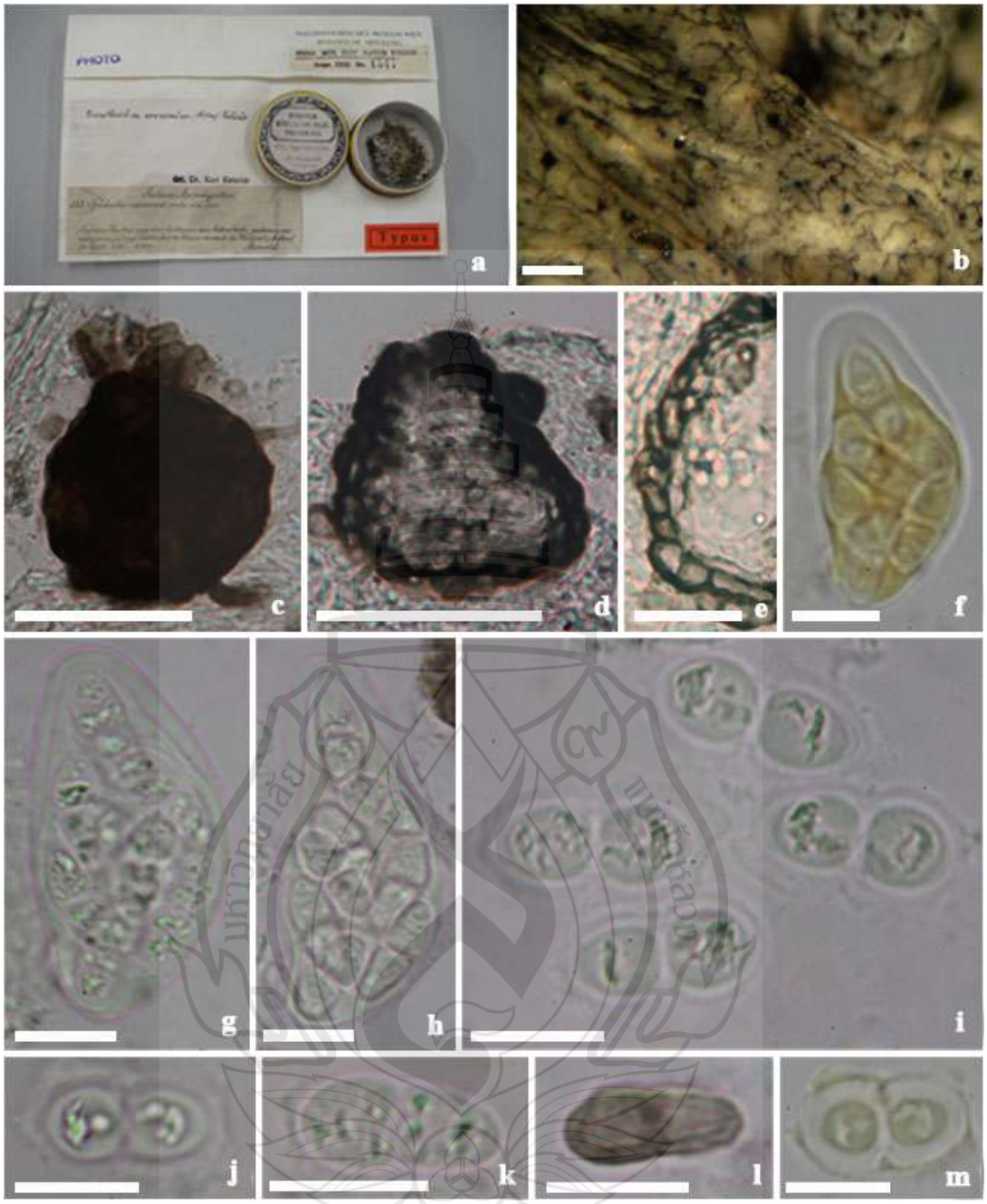
Thallus with superficial net of dark brown, septate, vegetative hyphae on host tissue (Figure 2.16a). *Ascomata* 43–72 μm diam, (42–) 45–79 μm high ($\bar{x}$ = 57.4 × 57.3 μm, n = 10), superficial on host surface, visible as black dots, uniloculate, individually globose to subglobose, scattered or gregarious, dark to dark brown (Figure 2.16a–c). *Peridium* 3–6 (–9.5) μm wide ($\bar{x}$ = 4.6 μm, n = 10), comprising 1–2 layers of dark to dark brown cells of *textura angularis* (Figure 2.16d). *Asci* (26–) 30–37 (–45) × 16–21.5(–25) μm ($\bar{x}$ = 35 × 19 μm, n = 10), 8–spored, bitunicate, saccate, ovoid or occasionally obclavate, non pedicellate, apically rounded with an ocular chamber (Figure 2.16e–g). *Ascospores* (11–)13–17.5 × 6–8.5 (–9.5) μm ($\bar{x}$ = 14.7× 6.8 μm, n = 20), irregularly seriate, fusiform to ellipsoidal, both ends obtuse, 1–septate, constricted at the central septum, upper cell larger than lower cell, thick–walled, initially hyaline, becoming brown when mature (Figure 2.16h–k).

Material examined: AUSTRIA, Tyrol, Materi am Brenner, Waldrast, on the thallus and apothecia of *Ochrolechia pallescens* var. *upsaliensis* (L.), Alt. 6700', August 1872, F. Arnold (M–0044221, holotype).



Note. (a). Ascomata on *Parmelia sulcata*. (b) – (e). Ascoma with eternal hyphal appendages. (f) – (g). Mature hyphae. (h) – (j). Bitunicate asci. (k) – (l). Ascospores. Scale bars: (a), (c), (d), (f) = 50 μm, (b), (e), (g) – (i) = 20 μm, (j) – (k) = 10 μm.

Figure 2.15 *Sphaerellothecium reticulatum* (syntype of *Echinothecium reticulatum*)



Note. (a). Fruiting bodies on host tissue. (b). Ascomata with hyphae. (c). Section through ascoma. (d). Section through peridium. (e) – (g). Ascus. (h) – (j). Ascospores. (k). Mature ascospore. Scale bars: (a) = 200 µm, (b) = 100 µm, (c) = 50 µm, (d) = 20 µm, (e) – (k) = 10 µm.

Figure 2.16 *Sphaerellothecium araneosum* (holotype of *Sphaerella araneosa*)

Genera transferred to *Chaetothyriaceae*

The following genera do not belong to *Capnodiaceae* and are placed in *Chaetothyriaceae*.

Aithaloderma Syd. & P. Syd. Ann mycol. 11: 256 (1913)

= *Blastocapnias* Cif. & Bat., Saccardoa 2: 67 (1963)

= *Chaetopotius* Bat., Mycopath. Mycol. appl. 5: 151 (1951)

= *Ciferrioxyphium* Bat. & H. Maia, in Batista & Ciferri, Quad. Lab. crittogam., Pavia 31:65 (1963)

= *Hypocapnodium* Speg., Physis, B. Aires 4: 287 (1918)

= *Phaeochaetia* Bat. & Cif., Beih. Sydowia 3: 62 (1962)

= *Vertixore* V.A.M. Mill. & Bonar, University of Calif. Publ. Bot. 19: 406 (1941)

Saprobic on sugary exudates from insects growing on leaves of various plants. *Thallus* comprising superficial, brown to black, septate, hyphae, constricted at the septa, mostly dense and anastomosing, covering the surface of leaves, forming a dark mycelial mat. *Ascostromata* superficial, brown to dark brown, in vertical section globose to subglobose, covered by subiculum or ascosomatal layer, consisting of brown, septate hyphae, with a circumferential space around the maturing ascomata, which results from expanding the expansion of the ascomata. Towards the upper part of the ascomata wall, the pellicles are very tightly packed, with 5–10 short black and dull setae which surrounded the ostiole. *Peridium* comprising of cells *textura angularis*, pale brown to hyaline; without interthecial filaments. *Asci* 8–spored, bitunicate, wide fusiform to saccate, short pedicellate, lacking an ocular chamber. *Ascospores* hyaline, trans–septate, some surrounded by a mucilaginous sheath.

Aithaloderma was assigned to *Capnodiaceae* by Sydow and Sydow (1913) and currently includes 15 species (Kirk et al., 2008). *Aithaloderma* was placed in *Chaetothyriaceae* by Spegazzini (1918) based on the dome–shaped or flattened ascostromata and pleomorphic pycnidia. Hughes (1976) suggested that *Aithaloderma* could be placed within *Capnodiaceae* because the mycelial hyphae are similar to those in *Phragmocapnias*. Recently, *Aithaloderma ferrugineum* Fraser has been reviewed by Reynolds and Gilbert (2005), who also included the species within the *Capnodiaceae* pending molecular data. The genus is characterized by globose

perithecium with setae, lageniform pycnidia which produce continuous, hyaline conidia and funnel to funnel–globulose ascostromata with short setae especially around the ostiole, lack paraphyses and asci contain 8–spored, transversely septate, clavate, hyaline ascospores (Yamamoto, 1954; von Arx & Müller, 1975; Hughes, 1976).

Leptoxyphium was reported to be the anamorph of *Aithaloderma* but in this study *Leptoxyphium* clusters in *Capnodiaceae*. Since there is no reported connection between *Leptoxyphium* and *Aithaloderma clavatisporum* (the generic type) we transfer *Aithaloderma* to *Chaetothryriaceae* while considering *Leptoxyphium* within *Capnodiaceae*.

Anamorphs: ?*Ciferrioxyphium* Bat. & H. Maia, *Leptoxyphium* Speg. (Hyde et al., 2011).

Type species

Aithaloderma clavatisporum Syd. & P. Syd. Annls. Mycol. 11: 256 (1913)

≡ *Chaetothyrium clavatisporum* (Syd. & P. Syd.) Hansf., Mycol. Pap. 15 (1946)

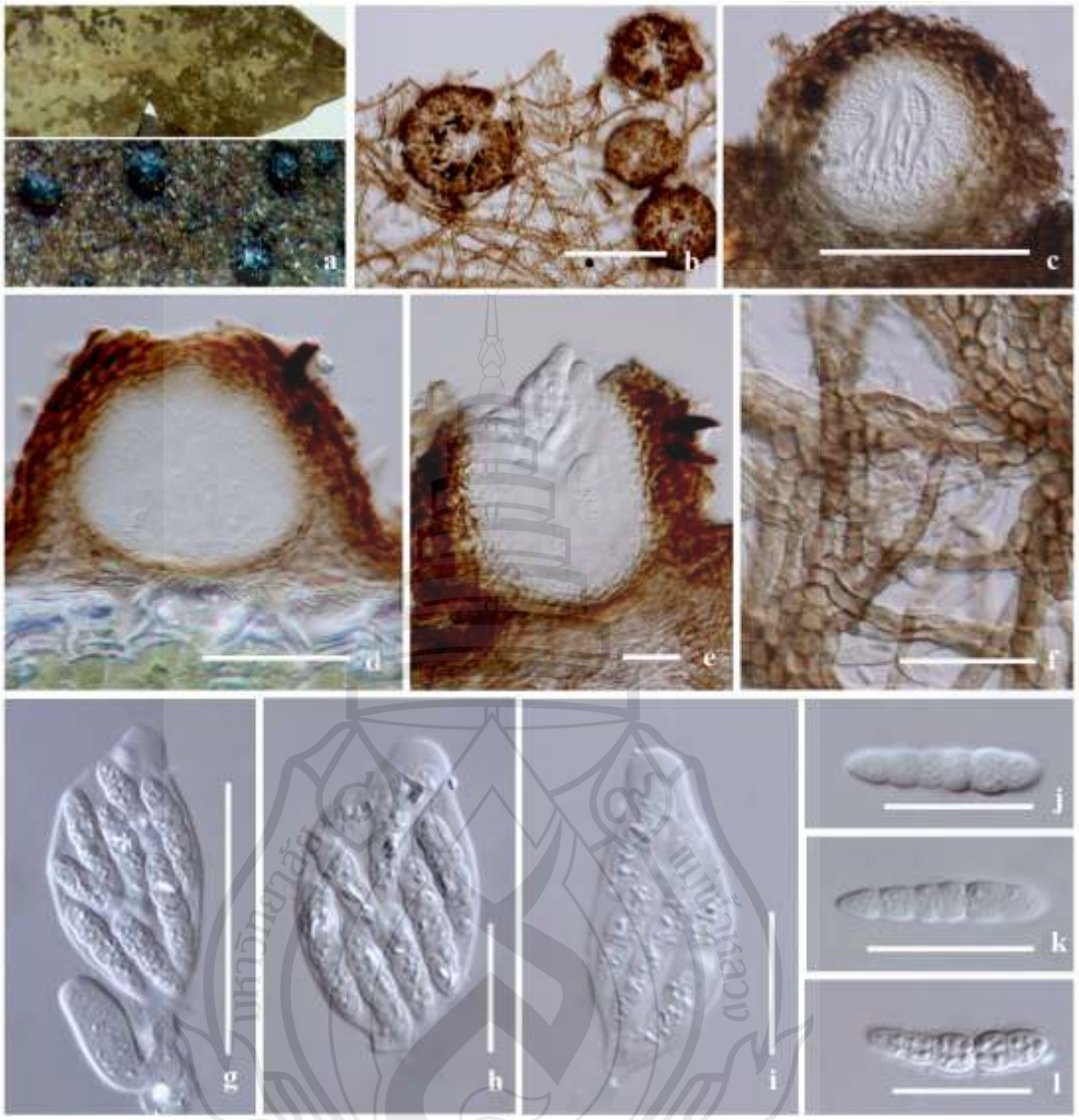
≡ *Phaeochaetia clavatispora* (Syd. & P. Syd.) Bat. & Cif. Beih. Sydowia 3: 67 (1962)

Saprobic on sugary exudates from insects growing on the surface of living leaves (Figure 2.17a) *Thallus* comprising 2.4–4.1 µm thick ($\bar{x}$ = 3.5 µm, n = 20), superficial, brown to black, septate, hyphae, constricted at the septa, mostly with 90º branching, mostly dense and anastomosing, covering the surface of leaves forming a dark mycelia mat (Figure 2.17b, f). *Ascostromata* 100–124 µm diam, 80–122 µm high ($\bar{x}$ = 122 × 100 µm, n = 5), superficial, brown to dark brown, initials arising from cells of mycelium, in vertical section globose to subglobose, covered by a subiculum or a layer of brown septate hyphae, with a circumferential space around the maturing ascomata, which results from the expansion of the ascomata. Towards the upper part of the ascomata wall, the pellicles are very tightly packed, with 5–10 short black and dull setae surrounding the ostiole (Figure 2.17b–e). *Peridium* 4.51–9 µm wide ($\bar{x}$ = 7 µm, n = 20), a single layer, comprising of cells forming a *textura angularis*, pale brown to hyaline. *Pseudoparaphyses* not observed. *Asci* 39–69 ×10–31 µm ($\bar{x}$ = 51 × 21 µm, n = 10), 8–spored, bitunicate, wide fusiform to saccate, short

pedicellate, lacking an ocular chamber (Figure 2.17h–j). *Ascospores* 12–25 × 3–6 µm ($\bar{x}$ = 19 × 4.5 µm, n = 20), overlapping triseriate, fusiform, hyaline, 4–5 septate, constricted at the septa, upper cells wider than lower cells, some surrounded by a mucilaginous sheath (Figure 2.17k–m).

Material examined: PHILIPPINES, Laguna Province, Los Baños, in living? leaves of *Voacanga globosa* (*Apocynaceae*), 1 February 1913, CF. Baker, Sydow, Fungi Exotici Exsiccata 174 (IMI 26048, isotype).

Some morphological characters of *Aithaloderma* are similar to those found in *Chaetothyriaceae* (Batista & Ciferri, 1962; Chomnunti, Ko Ko et al., 2012). These include the nature of the superficial ascostromata on a mycelial mat with a circumferential space around the subglobose maturing ascomata, with setae surrounding the ostiole, wide fusiform to saccate asci and clavate, hyaline, 5–septate ascospores. These characters indicate that *Aithaloderma* is better placed in *Chaetothyriaceae* and we follow this here.



Note. (a). Sooty mold and ascostromata on surface of host. (b). Ascostromata with short setae. (c) – (e). Section through ascostromata. (f). Network of hyphae. (g) – (i). Asci. (j) – (l). Ascospores. Scale bars: (b) – (e) = 100 μm, (f) – (g) = 50 μm, (h) – (l) = 20 μm.

Figure 2.17 *Aithaloderma clavatisporum* Syd. & P. Syd. (isotype)

Ceramoclasteropsis Bat. & Cavalc., in Batista, Perez & Bezerra, Brotéria, sér. Ci. Nat. 31(2): 101 (1962)

Ceramoclasteropsis coumae Bat. & Cavalc., in Batista, Perez & Bezerra, Brotéria, sér. Ci. Nat. 31(2): 101 (1962)

This species has superficial ascomata that form on superficial dark brown mycelium and appear to be ascostromata (Batista, Peres & Bezerra, 1962). Asci are bitunicate and apedicellate and ascospores are 7.5–14 × 2–4 µm, clavate, hyaline with 1–3 transverse septa. The taxon is reported to have paraphyses as in typical members of the *Chaetothyriaceae*. The ascospores appear to be typical of *Limacinula* and fresh collections are required to confirm if this is a distinct genus.

Hyaloscolecostroma Bat. & J. Oliveira, Atas Inst. Micol. Univ. Pernambuco 5: 448 (1967)

Hyaloscolecostroma rondoniense Bat. & J. Oliveira, Atas Inst. Micol. Univ. Recife 5: 449 (1967)

This genus was described from Brazil and is monotypic. This is a sooty mould with superficial ascomata, cylindrical unitunicate asci and trans–septate cylindric–fusiform ascospores (Batista & Silva, 1967). Apparently this fungus is associated with homopterean insects. The drawing provided in Batista and Silva (1967) is not a convincing taxon of *Capnodiaceae* especially as it shows a thin unitunicate ascus layer. Thus we place the genus in the *Chaetothyriaceae incertae cedis.*

Trichomerium Speg., Physis, B. Aires 4: 284 (1918)

Trichomerium coffeicola (Puttemans) Speg., Physis, B. Aires 4: 284 (1918)

≡ *Limacinia coffeicola* Puttemans, Cryptog. Mycol. 20: 163 (1904)

Trichomerium is included in *Capnodiaceae* in Index Fungorum (http://www.indexfungorum.org/Names/Names.asp), but not in Lumbsch and Huhndorf (2010). The genus was introduced by Spegazzini (1918) with type species *T. coffeicola* (Reynolds, 1982) and include 23 species, mostly folicolous fungi. The ascospores are similar to *Phragmocapnias* and *Scorias* in septation and in being hyaline, but *Trichomerium* has sessile ascomata. We have made several collections of *Trichomerium* in this study which cluster in *Chaetothyriaceae* in the phylogenetic analysis (data not shown) In this study we transfer *Trichomerium* to new family

Trichomeriaceae according to their morphology and phylogeny result of LSU, SSU and ITS

Anamorphs reported for genus: *Tripospermum* Speg. (Hyde et al. 2011).

Tripospermum Speg., *Physis*, B. Aires 4(no. 17): 295 (1918)

= *Pentaposporium* Bat., Revta Biol., Lisb. 1(2): 106 (1957)

Tripospermum acerinum P. Syd., Physis, B. Aires 4(no. 17): 295 (1918)

Tripospermum was introduced by Corda in 1837 for *T. elegans* and by Spegazzini in 1918 as segregate of *Tripospermum* with type species *T. acerinum* (Hughes 1951). As the anamorph of *Trichomerium* Speg. (Hyde et al 2011) it should be transferred to *Chaetothyriaceae*.

Genus in *Coccodiniaceae*

Microxiphium (Harv. ex Berk. & Desm.) Thüm., Physis, B. Aires 4(17): 293 (1879)

Microxiphium footii (Harv. ex Berk. & Desm.) Thüm., Physis, B. Aires 4(17): 293 (1879)

This genus is polyphyletic with species in *Coccodiniaceae* and *Capnodiaceae*. However the type of the genus is presently placed as synonym of *Dennisiella babingtonii* and thus included in the *Coccodiniaceae*. The specimen of *Microxyphium citri* used in the phylogenitical tree (see Figure 2.1) is likely to be a misidentification. *Microxyphium citri*, *M. aciculiform* and *M. theae* are also dispersed amongst the *Capnodiaceae* in the tree indicating its polyphyletic nature.

Genus transferred to *Micropeltidaceae*

Wu et al. (2011) discusses this family and the following genera are better placed therein.

Callebaea Bat. in Batista, Perez & Bezerra, Brotéria, sér. bot. 31: 100 (1962)

Mycelium superficial, irregularly scattered. *Thyriothecia* circular, scattered, superficial, membraneous, brownish, lower peridium poorly developed easily removed from the host surface, with a central irregular ostiole; in section lenticular. *Upper wall* comprising of an irregular meandering arrangement of compact hyphae. *Hamathecium* comprising asci; pseudoparaphyses not obvious. *Asci* immature and ascospores not observed.

Type species

Callebaea rutideae (Hansf.) Bat., In Batista, Perez & Bezerra, Brotéria, sér. bot. 31: 100 (1962)

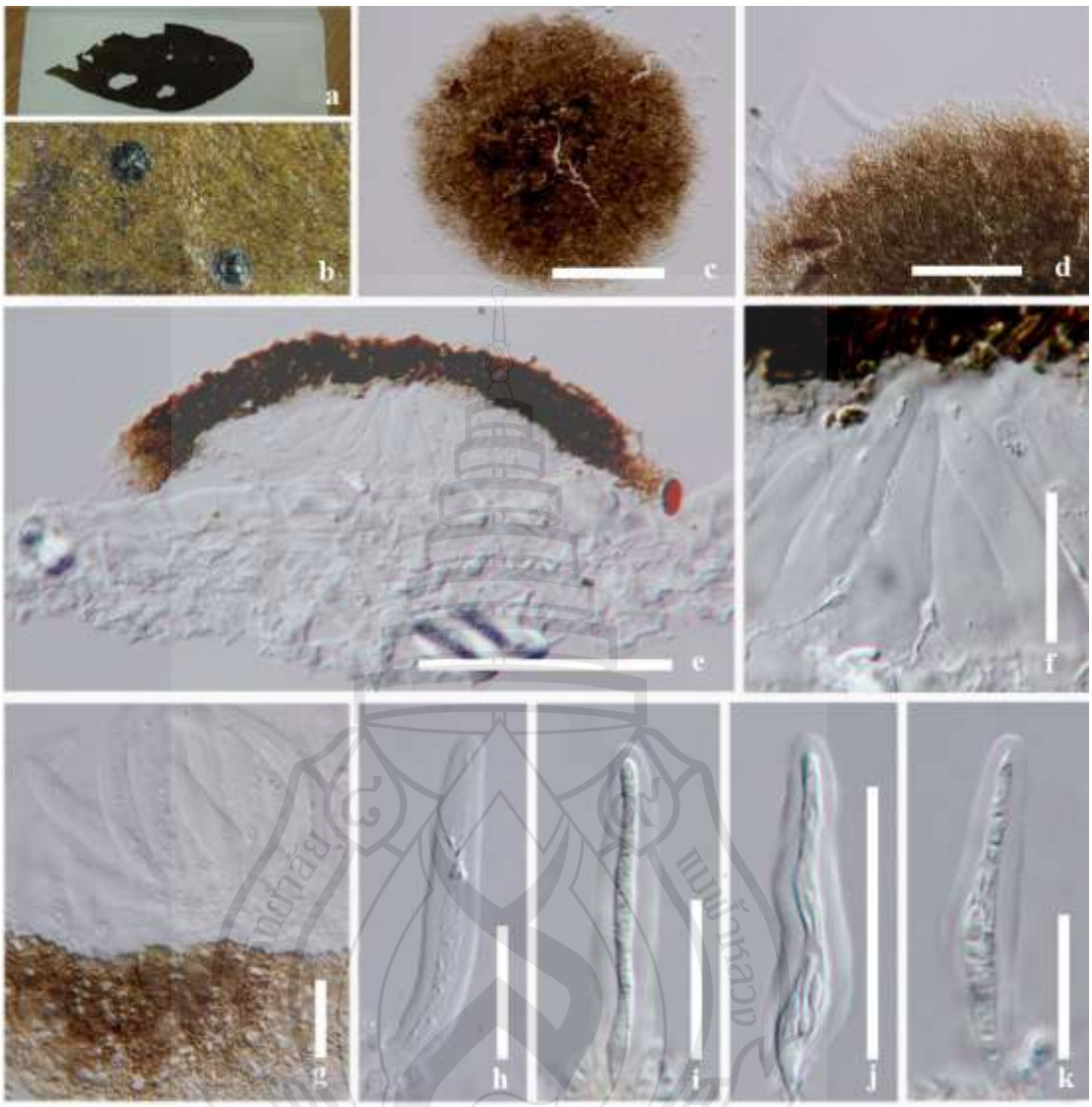
≡ *Microcallis rutideae* Hansf., Proc. Linn. Soc. London 158: 40 (1947)

≡ *Microcalliopsis rutideae* (Hansf.) Bat. & Cif., Bein. Sydowia 3: 58 (1962)

Foliar epiphyte on upper surface of leaves, lacking superficial mycelium (Figure 2.18a, b). *Thyriothecia* 83 (–196)–228 μm diam × 41–63(–85) μm high ($\bar{x}$ = 183 × 59 μm, n = 5), circular, scattered, superficial, pale brown to brown, basal peridium poorly developed, easily removable from the surface of leaves, lacking an ostiole; in section lenticular. (Figure 2.18e). *Upper wall* comprising an irregular meandering arrangement of compact hyphae, 2–3 μm wide ($\bar{x}$ = 2.5 μm, n = 10). (Figure 2.18c, d). *Hamathecium* comprising asci, pseudoparaphyses not obvious. *Asci* immature (Figure 2.18f–k) and ascospores not observed.

Material examined: UGANDA, Entebbe Road, in leaves of *Rutideae smithii* (*Rubiaceae*), August 1944, C.G. Hansford 3560 (K(M) 164029, holotype).

Hansford (1947) introduced *Microcallis rutidaea* as new species in *Chaetothyriaceae*. We examined the type material and found a few flattened ascomata on host surface and these lacked mature asci and ascospores. The thyriothecium however, indicated that *Microcallis rutideae* should be transferred to *Micropeltidaceae*. However the specimen is immature and must be treated as doubtful until a better specimen or fresh collections are found.



Note. (a) – (b). Ascomata on leaf surface. (c) – (d). Circular ascomata comprising meandering hyphae. (e). Vertical section of ascostomata, (f) – (k). Young asci. Scale bars: (c), (e) = 100 μm, (d), (j) = 50 μm, (f) – (i), (k) = 20 μm.

Figure 2.18 *Microcallis rutideae* (Hansf.) Bat. (holotype)

Doubtful genera

Anopeltis Bat. & Peres. Nova Hedwigia 2: 472 (1960)

Foliar epiphyte on leaves. *Thallus* comprising superficial, olive–brown, cylindrical, branching, irregularly reticulate, septate hyphae, lacking setae. *Ascostromata* semi–immersed, dark–brown to brown at the margin, flattened, round, scattered, surrounded with mycelium, the locule/asci produced under the stromata, hypostromata central or lateral. *Ascostromata* wall composed of angular brown cells. *Peridium* thin. *Conidia* clavate or oblong, 1–septate, deeply constricted at the septum, thick walled, wrinkled.

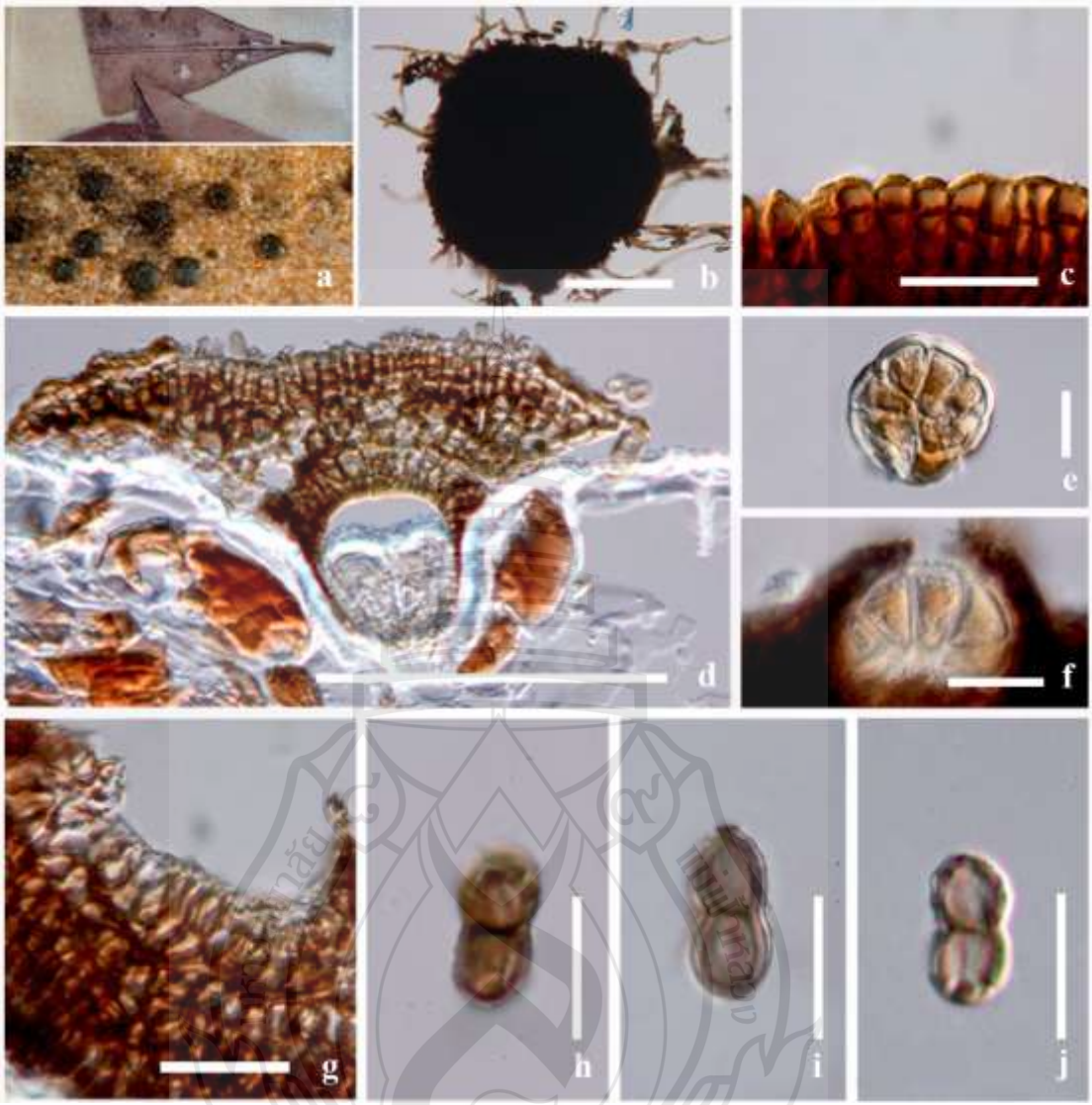
Type species

Anopeltis venezuelensis Bat. & Peres, Nova Hedwigia 2: 472 (1960)

Foliar epiphyte on leaves (Figure 2.19a). *Thallus* superficial, 2.7–5 μm wide ($\bar{x}$ = 3.7 μm, n = 20), comprising olive–brown, slender, branched, septate, cylindrical hyphae, irregularly reticulate, lacking setae. (Figure 2.19b). *Ascostromata* 145–208 μm diam, 74–93 μm high ($\bar{x}$ = 175 × 84, n = 5), uniloculate, scattered, semi–immersed, dark–brown to brown, surrounded by mycelium. The wall of ascostromata composed of angular brown cells (Figure 2.19e, f). *Peridium* thin, 2–3 μm wide ($\bar{x}$ = 2.5 μm, n = 10) (Figure 2.19d, g). Dark brown conidia cover and surround the ascostromata. *Conidia* 9–14 × 3–6 μm ($\bar{x}$ = 11 × 4, n = 15), clavate or oblong, 1–septate, deeply constricted at the septum, thick walled and wrinkled (Figure 2.19h–j).

Material examined: VENEZUELA, Miranda, Caracas, Los Palos Grandes (above), Mt Naiguata (S slope of), c. 1200m, in the leaves of unidentified plant, 13 July 1959, R.W.G. Dennis 2321 (K(M) 171577 – holotype).

Anopeltis venezuelensis should be placed in *Ascomycete incertae cedis* according to its semi–immersed ascomata, lack of mycelium and peridium of *textura angularis*. The nature of this taxon is not clear from the type specimen as asci or ascospores were not observed. The taxon needs to be recollected and examined from fresh material.



Note. (a). Appearance of leaves colonized by the fungus. (b). Ascostromata with mycelium and conidia. (c). Pale brown angular cells of ascostromata edge. (d). Section of ascostomata with angular cells. (e). Young locule/asci. (f). Vertical section of ascostromata with young asci, (g). Angular cells of ascostromata. (h) – (j). Brown conidia with 1–septa. Scale bars: (b) – (d) = 100 μm, (c), (e) – (g) = 20 μm, (h) – (j) = 10 μm.

Figure 2.19 *Anopeltis venezuelensis* Bat. & Peres (holotype)

Capnophaeum Speg. Physis, B. Aires 4: 287 (1918)

Capnophaeum indicum C. Bernard, Physis, Rev. Soc. Arg. Cienc. Nat. 4: 287 (1918)

We requested the type specimen from BO but were informed that it is lost and therefore the genus must be considered as doubtful.

Fumiglobus D.R. Reynolds & G.S. Gilbert., Cryptog. Mycol. 27(3): 252 (2006)

Fumiglobus ficina (Bat., Nascim. & Cif.) D.R. Reynolds & G.S. Gilbert., Cryptog. Mycol. 27(3): 253 (2006)

≡ *Asbolisia ficina* Bat. Nascim. & Cif. in Batista & Ciferri, Quad. Lab. crittogam. Pavia 31: 41 (1963)

Fumiglobus was introduced by Reynolds and Gilbert (2005) as new genus to accommodate *Asbolisia nomen confusum,* which included nine species; typified by somewhat aerial, membraceous, superficial mycelium, comprised of dark brown septate hyphae, constricted at the septum, with pycnidia borne from several hyphae. Conidia are globose to subglobose, hyaline, single–celled, bacillate to ellipsoidal, or cylindrical. Fresh collections are needed to establish if the genus is distinct.

Polychaetella Speg., Physis, B. Aires 4: 295 (1918)

Polychaetella schweinitzii (Berk. & Desm.) Speg., Physis, B. Aires 4: 295 (1918)

≡ *Capnodium schweinitzii* Berk. & Desm. (1849)

Polychaetella was classified in *Capnodium* section IV by Saccardo (1822) and were anamorphs of various *Capnodium* species (Sutton, 1977), and have elongated pycnidia producing dictyospetate, hyaline or chlorinous conidia (Hughes, 1976). Batista and Ciferri (1963b) added *Polychaetella* in *Asbolisiaceae* and accepted *P. araucariaceae* (Thüm) Speg. as type species. Hughes (1976) observed the DAOM syntype of *Capnodium araucariae* but could not find the hyaline dictyospetate conidia. Therefore the genus must be treated as uncertain.

2.4 Discussion

We examined nine generic types and sequenced 15 taxa of *Capnodiaceae.* Phylogenetic analysis showed *Capnodium, Leptoxyphium, Phragmocapnias* and *Scorias* to be well defined genera in *Capnodiaceae,* while *Aithaloderma, Anopeltis, Callebaea, Echinothecium* and *Trichomerium* are removed to other families or are doubtful. This study thus provides an expansion in the documentation of capnodiaceous sooty moulds. This group now embodies three easily recognized genera, i.e. *Phragmocapnias = Conidiocarpon, Capnodium = Polychateon* and *Scorias*, comprising of both sexual and asexual states, and the exclusively anamorphic *Leptoxyphium*. It is not clear whether the genera *Anopeltis, Capnophaeum* and *Scoriadopsis* however, are acceptable in *Capnodiaceae* yet.

The present study has shown that the general term "sooty moulds" encompasses a broad set of species within the families *Antennulariellaceae, Capnodiaceae, Chaetothyriaceae* and *Metacapnodiaceae* (Hughes, 1976; Reynolds, 1986; Reynolds, 1998; Chomnunti, Ko Ko et al., 2012) which often grow in close association on a single leaf. This had posed considerable challenges to taxonomists since the mid–19th century (Friend, 1965; Hughes, 1976; Reynolds, 1986; 1998; Chomnunti, Bhat et al., 2012). Pycnidiaceous sooty moulds were placed in the generally unaccepted family *Asbolisiaceae* (Batista & Ciferri, 1963b) and Hosagoudar and Riju (2011) recently treated them in a new family *Schifferulaceae,* as black mildews, which he termed "saprobic sooty moulds". The *Schifferulaceae* may well be synomymous with *Asterinaceae* but this can not be corroborated without DNA sequence comparions. DNA sequence data remains rare in other families too. Only one putative species of *Antennulariellaceae* (*Antennariella placitae*) has however, been sequenced and clustered in *Capnodiaceae* (Cheewangkoon et al., 2009).

This study and that of Chomnunti, Bhat et al. (2012) clearly shows that *Capnodiaceae* and *Chaetothyriaceae* consist of unrelated taxa belonging to different classes and yet they can hardly be distinguished by morphology and growth habit. It seems clear that the similar morphological characters in these groups evolved under selection pressures unique to their shared niche to utilize sugary insect exudates on leave surfaces.

Besides, the species associated with leaf surfaces and particularly with honey dew produced by insects, our analysis indicates other possible environmental niches in *Capnodiaceae*. One sequence in our analysis was obtained from a member of an ecological guild of rock–inhabiting fungi (Ruibal, Gueidan et al., 2009). Members of this, found interspersed throughout the classes *Dothideomycetes* and *Eurotiomycetes,* can tolerate extreme conditions found on rock surfaces. It would be interesting to establish if capnodiaceous species grew on proximity to honeydew on rocks surfaces. Another group of sequences were isolated from a species found in ant nest walls. This undescribed species act as mutualists by aiding in reinforcing the nest walls. They are cultivated by the ants which nourishes them with honeydew (Schlick–Steiner, et al.,. 2008). Finally, there are several pathogenic species which occur on *Capnodiaceae* and include species in the genera *Rhombostilbella* (Pohlad, 1988). It will be important that these taxa are considered carefully when studying sooty moulds to avoid confusion in future.

CHAPTER 3

TAXONOMY AND PHYLOGENY CLASSIFICATION OF SOOTY MOULD IN *CHAETOTHYRIACEAE*

3.1 Introduction

The *Chaetothyriaceae* are typical of capnodiaceous Dothideomycetes as they form on the surface of leaves and resemble the common sooty moulds (Batista & Ciferri, 1962). Species of *Chaetothyriaceae* are mostly epiphytes, colonizing the surface of living leaves with mycelium attached to the host cuticle without penetrating the host tissues (Batista & Ciferri, 1962; von Arx & Müller, 1975). The ascomata are surrounded by a very thin pellicle of superficial mycelium forming black sooty mould–like areas on leaves. They are easily detachable from the cuticle (Batista & Ciferri, 1962). However the ecology of many species of *Chaetothyriaceae* is poorly studied and it is unclear whether they are saprotrophic or biotrophic (Barr, 1987). Members of *Chaetothyriaceae* are often confused with capnodiaceous sooty moulds in view of their similar morphology and habitat preferences. However, these fungi are never associated with insects such as several *Capnodiaceae* (Hansford, 1946). Sooty moulds are a general taxonomic term for capnodiaceous and/or chaetothyriaceous fungi; common genera from both these groups are often or found growing together in sooty moulds complexes in plant exudates or the sugary honeydew secreted by insects, e.g. *Aithaloderma* (*Leptoxyphium*), *Aureobasidium, Capnodium, Cladosporium, Microxyphium, Podoxyphium, Scorias* and *Trichomerium* (*Tripospermum*) (Thaung 2006).

Studies on *Chaetothyriaceae* have been conducted mainly by Hansford (1946), Batista and Ciferri (1962), von Arx and Müller (1975), and Hughes (1976) and there have

been very few studies since then. The members of *Chaetothyriaceae* are primarily tropical species characterized by dark mycelium forming a loose net of hyphae over the substrate, and produce ascomata beneath a mycelial pellicle with or without setae (Batista & Ciferri, 1962; Hughes, 1976; Pereira, Dornelo-Silva, Inacio & Dianese, 2009). The family is poorly circumscribed and most previous work comprised brief descriptions with line drawings (e.g. Hansford, 1946; Batista & Ciferri, 1962). The arrangement of genera often seem rather subjective because individual authors emphasize certain characters, such as, spore septation, presence of ascomata setae and mycelium color (Batista & Ciferri, 1962; Hughes, 1976). Batista and Ciferri (1962) considered the family *Chaetothyriaceae* to be the type family in the Order Chaetothyriales. This group shares a number of centrum characters with members of the Dothideomycetes, such as the presence of bitunicate asci, periphysoids and periphysate ostioles. Eriksson (1982) placed the *Chaetothyriaceae*, together with Herpotrichiellaceae, in the Order Dothideales. Barr (1987) however, placed Chaetothyriaceae in the order Chaetothyriales and incorporated eight families. The majority of early systematic studies on *Chaetothyriaceae* were based on the morphological data, and their taxonomic placement remains unclear (Eriksson, 1999).

Without sequence data it is difficult to distinguish which taxa belong in *Capnodiaceae* or *Chaetothryriaceae*, unless species are identified to a genus and thus placed in appropriate orders. Consequently it becomes important to restudy these poorly known groups using modern molecular techniques (Eriksson, 2006). A number of studies (Spatafora, Mitchell & Vilgalys, 1995; Berbee, 1996; Winka et al., 1998; Haase, Sonntag, Melzer-Krick, & de Hoog, 1999; Untereiner, 2000; Badali, Gueidan & Najafzadeh, 2008; Gueidan et al., 2008) made an effort to provide some data relating to the phylogenetic position of this group. Based on the nucleotides data of 18s rDNA gene, some authors (Spatafora et al., 1995; Berbee, 1996; Winka et al., 1998; Haase et al., 1999) suggested that Chaetothyriales is more closely related to the Eurotiales than to Dothideales, Pleosporales or any other ascostromatic ascomycetes. Winka et al. (1998) transferred Chaetothyriales to the class Eurotiomycetes, and she assumed that the order comprised three families, *Chaetothyriaceae*, *Herpotrichiellaceae* and *Coccodiniaceae*. A combination of protein-coding gene and ribosomal operon data show the black yeasts of the Chaetothyriales align within a

subclass, the Chaetothyriomycetidae (Miadlikowska & Lutzoni 2004), which is sister to the Eurotiomycetidae (Eurotiomycetes) (Lutzoni et al., 2004; Reeb, Roux & Lutzoni, 2004). The most recent study of Schoch et al. (2006) confirmed removal of Chaetothyriales from Dothideomycetes and strongly support their placement in the Eurotiomycetes. Moreover, the information from the small subunit rDNA gene showed that *Ceramothyrium linnaeae* (*Chaetothyriaceae*) appeared to be more closely related to the *Herpotrichiellaceae* (Winka et al., 1998). The combined ITS rDNA, EF1 and TUB phylogeny showed that a chaetothyriaceous species *Ceramothyrium carniolicum* are closely related with the plant-associated *Cladophialophora* species (*Herpotricheillaceae*), however the human pathogenic group of *Cladophialophora* clustered with the remaining *Herpotrichiellaceae* (Badali et al,. 2008). In addition, *Ceramothyrium carniolicum* clustered together with rock-inhabiting *Capronia peltigerae* (*Herpotrichiellaceae*) following analysis of combined nucLSU-nucSSU-mitSSU-RPB1 sequences (Untereiner, Gueidan & Diederich, 2011). Nevertheless the phylogenetic position of *Chaetothyriaceae* and their relationships with other members of Chaetothyriales are still unclear (Untereiner, 2000; Badali et al., 2008; Gueidan et al., 2008) since many species of Chaetothyriales are poorly known and the data from molecular studies of these organisms are still rather limited.

At present, the order Chaetothyriales includes two families of non-lichenised ascomycetes−the Chaetothyriaceae and Herpotrichiellaceae (Geiser et al., 2006; Kirk et al., 2008). There are 13 genera included in Chaetothyriaceae (Kirk et al., 2008). Anamorphs are only known for two of these genera; *Merismella* Syd. is the anamorph of *Chaetothyrium* Speg. and *Stanhughesia* Constant. is the anamorph of *Ceramothyrium* Bat. & Maia (Hyde et al., 2011). Other chaetothyriaceous anamorphs without known teleomorphs are *Vonarxia* Bat. 1960 and *Cyphellophora* G.A. de Vries 1962.The purpose of this study is to represent the taxonomic placement of *Chaetothyriaceae* species from northern Thailand by using morphology and phylogeny and we hereby describe three species that are new to science.

3.2 Materials and Methods

3.2.1 Observation of Generic Types, Isolation from Fresh Specimens and Studies on Morphology

Collecting sites were visited throughout the provinces of Chiang Mai and Chiang Rai in northern Thailand and leaves of various plants with sooty mould-like fungal colonization were collected. The material was brought to the laboratory in plastic zip-lock bags. Technique to observed morphological characters and singles spore isolation see Material and methods in Chapter 2.

3.2.2 DNA Isolation, Amplification and Sequencing

DNA was extracted from the mycelium using Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) according to the manufacturer's protocol for maximum yield. Partial 28s rDNA regions were amplified using primers LR0R and LR6 (Vilgalys & Hester, 1990). Internal transcribed spacers (ITS) region including 5.8s of the nuclear ribosomal DNA were amplified using primers ITS4 and ITS5 (White, Bruns, Lee & Taylor, 1990). Amplification reaction mixtures contained 50 ng of template DNA, PCR Master Mix 1X, 0.5 μM of each primer in a 25 μL volume, 0.5 U of Taq DNA Polymerase, 400 μM of each dNTP, 3 mM of $MgCl_2$. Amplification was performed in a GeneAmp PCR System under the following conditions: initial denaturation at 94 ºC for 5 min, followed by 35 cycles each consisting of 1 min denaturation at 94 ºC, annealing for 30 s at 55 ºC and extension at 72 ºC for 1 min and a final extension at 72 ºC for 5 min. To check homogeneity and size of amplicons, PCR products were run on 1.5% agarose gels (BIO–RAD Molecular Biology Agarose) containing ethidium bromide as the staining agent. DNA sequencing reaction was performed by using BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA). Electrophoresis of purified sequencing product was performed on ABI-PRISM3730 DNA Analyzer (Applied Biosystems).

3.2.3 Sequence Alignments and Phylogenetic Analysis

DNA sequences were aligned using BioEdit (Hall, 1999) and Clustal X 1.83 (Thompson, Gibson, Plewniak, Jeanmougin & Higgins, 1997) with available sequences of the Capnodiaceae, Chaetothyriaceae, Herpotrichiellaceae and Verrrucariaceae from GenBank. Details of used the sequences are presented in Table 3.1 *Teratosphaeria suberosa* was selected as outgroup. Phylogenetic analysis were performed by using PAUP* v. 4.0b10 (Swofford, 2002) for each gene separately. Maximum parsimony (MP) analyses were performed with the heuristic search option on 1000 random taxa addition with a stepwise starting tree, tree bisection and reconnection (TBR) as the branch-swapping algorithm. Gaps were treated as missing data and the ambiguously aligned regions were excluded from analyses. Clade stability was estimated in a bootstrap (BT) analysis with 1000 replicates (Hillis & Bull, 1993). Other measures used were consistency index (CI), retention index (RI) and homoplasy index (HI).

Bayesian analyses employing a Markov Chain Monte Carlo method were performed in MrBayes v.3.0b4 (Ronquist & Huelsenbeck, 2003) for combined gene dataset. The most suitable substitution models for the respectively data sets were selected using MrModeltest 2.2 (Nylander, 2004). The Bayesian analyses were conducted with the Markov chains run for 1 000 000 generations and trees were sampled every 100^{th} generations (Cai, Jeewon & Hyde, 2006). Trees were viewed in Treeview (Page, 1996). Sequences generated in this study have been deposited in GenBank (HQ895835, HQ895836 and HQ895837) and alignments in TreeBASE (TB2:S11868).

Table 3.1 LSU and ITS rDNA Sequences in this Analysis

Taxa	Strain	Host	Country	GenBank Accession no.	
				LSU	ITS
Ceramothyrium thailandicum	MFLUCC 10–0079	*Lagerstroemia* sp.	Thailand	HQ895835	HQ895838
Chaetothyrium brischofiacola	MFLUCC 10–0083	*Brischofia javanica*	Thailand	HQ895836	HQ895839
Phaeosaccardinula ficus	MFLUCC 10–0080	*Ficus* sp.	Thailand	HQ895837	HQ895840

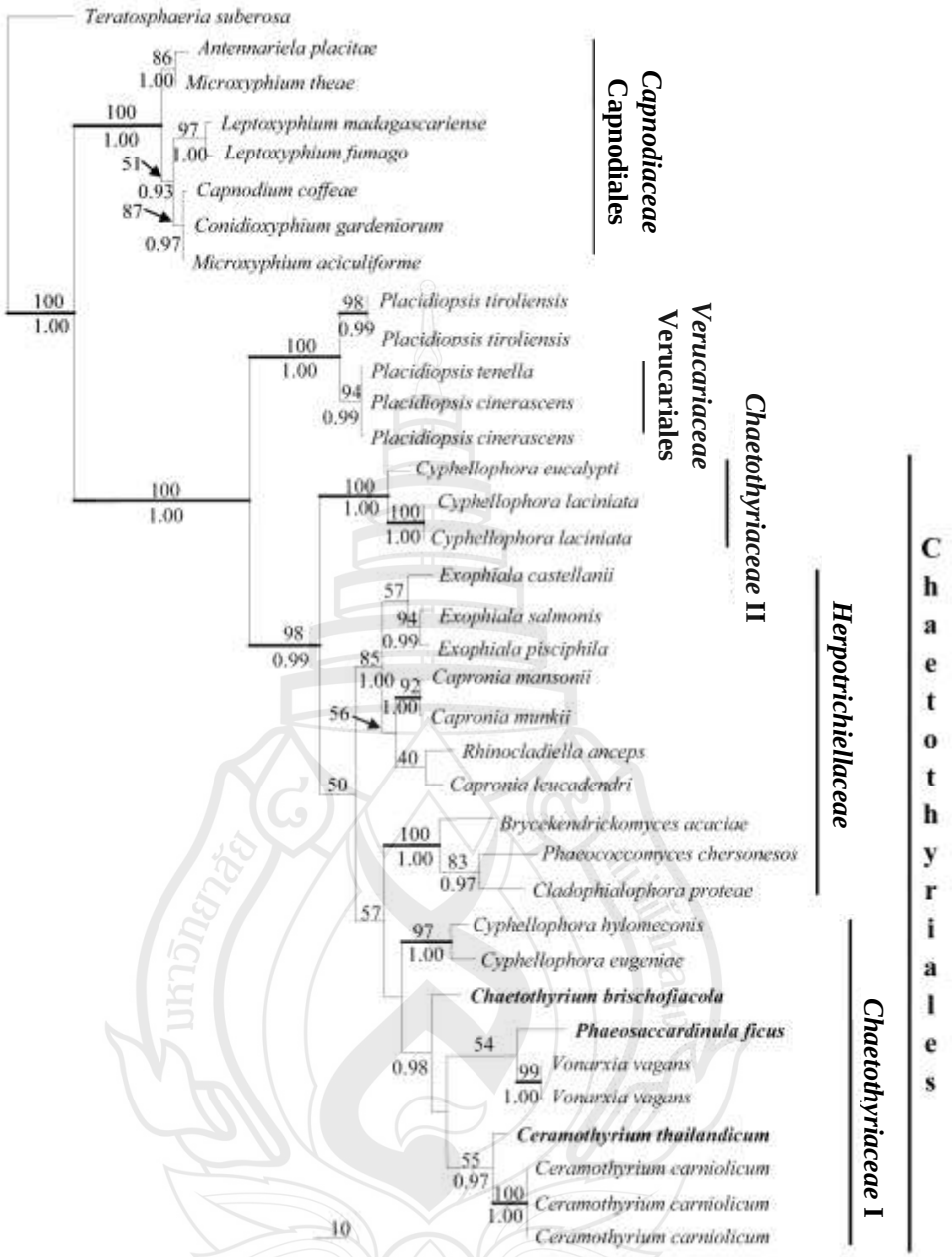
3.3 Results

3.3.1 Molecular Phylogeny

The aligned LSU rDNA sequences of approximately 860 bases were obtained from isolates listed in Table 3.1 We excluded 18 base pairs that did not align well from the analysis. Of the remaining 834 characters used in analysis, 592 characters were constant, 35 were variable and parsimony–uninformative and 207 characters were parsimony–informative. A heuristic search found 100 equally parsimonious trees with the length (TL) of 603 steps (CI = 0.574, RI = 0.844, RC = 0.485 and HI = 0.426). The overall topology of the 50 % majority–rule consensus tree of 10 000 trees sampled during the Bayesian analysis was similar to the MP tree. The MP tree is presented in Figure 3.1 with bootstrap support above the branches and Bayesian values marked below branches.

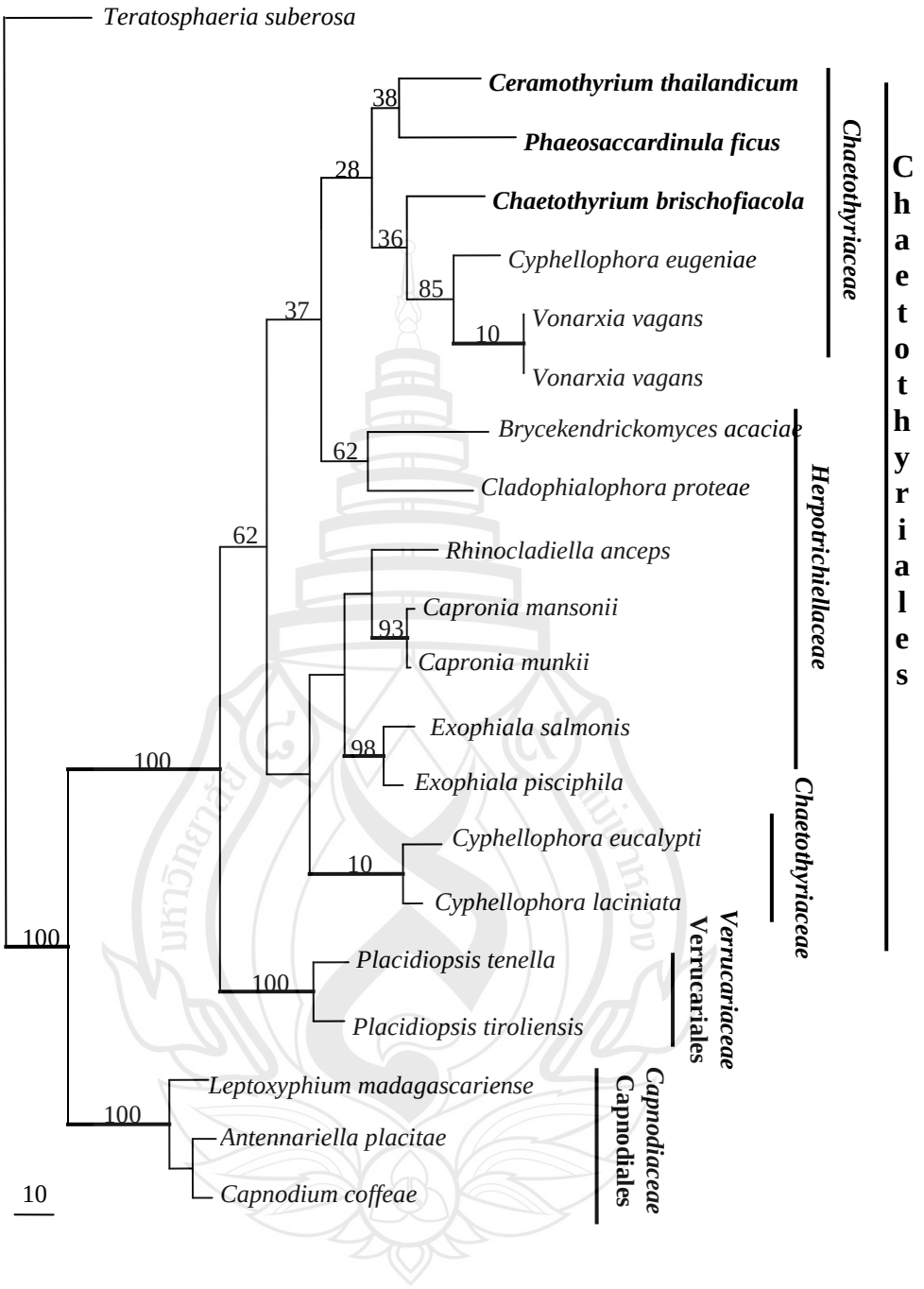
The phylogenetic hypothesis (Figure 3.1) highly supports three monophyletic groups *Capnodiaceae* (Capnodiales), *Verrucariaceae* (Verrucariales) and *Herpotrichiellaceae*/*Chaetothyriaceae* (Chaetothyriales). The sequences of the three new species described here cluster within the *Chaetothyriaceae* clade which includes *Ceramothyrium carniolicum*, *Cyphellophora eugeniae*, *Cyphellophora hylomeconis* and *Vonarxia vagans*. And they are unrelated to sequences of species of Capnodiaceae (Capnodiales) (Figure 3.1). The placement of *Ceramothyrium thailandicum* and *Phaeosaccardinula ficus* in this lineage received bootstrap support of 68% and 82%, and Bayesian posterior probabilities of 0.96 and 1.00, while *C. thailandicum* clusters in a subclade with *C. carniolicum* with significant support of 0.96 Bayesian posterior probabilities. The aligned ITS rDNA sequences of approximately 600 bases were obtained from isolates listed in Table 3.1 as well. We aligned ITS rDNA sequences to confirm the results of three new specimens were closely related with *Chaetothyriaceae*. We excluded 196 base pairs that did not align well from the analysis. Of the remaining 410 characters used in analysis, 216 characters were constant, 34 were variable and parsimony–uninformative and 160 characters were parsimony–informative. A heuristic search found 100 equally

parsimonious trees with the length (TL) of 527 steps (CI = 0.583, RI = 0.655, RC = 0.382 and HI = 0.417). The overall topology of the 50 % majority–rule consensus tree of 10 000 trees sampled during the Bayesian analysis was similar to the MP tree. The MP tree is presented in Figure 3.2 with bootstrap support above the branches. The phylogenetic hypothesis (Figure 3.2) the sequences of the three new species described here cluster in *Chaetothyriaceae.*



Note. Phylogenetic relationships among 7, 5 and 23 members of Capnodiales, Verrucariales and Chaetothyriales respectively, inferred by Maximum Parsimony and Bayesian analyses of LSU rDNA sequences. MP bootstrap values ≥ 50 % and Bayesian values ≥ 0.95 are shown above and below the branches respectively. The specimens isolated in this study are in bold.

Figure 3.1 Phylogenetic Tree Generated from MP Analysis of LSU rDNA Sequences



Note. Phylogenetic relationships among Capnodiales, Verrucariales and Chaetothyriales respectively, inferred by Maximum Parsimony of ITS rDNA sequences. MP bootstrap values ≥ 50 % are shown above. The specimens isolated in this study are in bold.

Figure 3.2 Phylogenetic Tree Generated from MP Analysis of ITS rDNA Sequences

3.3.2 Generic Type Observation, Fresh Specimens Isolates and Morphology

Ceramothyrium thailandicum Chomnunti & K.D. Hyde, sp. nov.

MycoBank: MB519683

Etymology: in reference to its occurrence in Thailand.

Ascomata 200–255 μm diam. × 100–160 μm alta, superficialia, uno vel dispersi, brunnea ad pallida brunnea et tenuiter viridula. *Peridium* 12–19 μm latum, hyalinum, brunneum ad atra brunneum in basi *texturae angularis*, cum compressa *texturae prismatica* in latus. *Asci* 70–96 × 39–53 μm, 8-spori bitunicati, clavati vel pyriformes, denique pedicellati, masime evanescens. *Ascosporae* 24.7–35.5 × 5.7–8.7 μm, 3–5-seriata, hyalinae, cylindric-clavatae, 7–9 transversus septatae, constricta in septatae, circum mucilagina vaginae.

Habit on living leaves of *Lagerstroemia* sp., covering the upper leaf surface with dark mycelium without penetrating host tissues, leaves remaining green and healthy (Figure 3.3a). Mycelial mat subiculum-like, comprising hyphae which are mostly narrow, 3.1–4.6 μm wide ($\bar{x}$ = 3.7 μm, n = 20); brown or brownish green at margin, slightly constricted at the septa, dense, radiating outwardly, anastomosing at their tips with cells of the hyphal network. *Ascomata* 200–255 μm diam., 100–160 μm high ($\bar{x}$ = 255 × 128 μm, n = 5); superficial, solitary or scattered, pale brown to greenish brown, setae absent, in vertical section globose to subglobose, somewhat flattened when dry, covered by a subiculum or layer, composed of a few hyaline to subhyaline, septate hyphae, with a circumferential space around the maturing ascomata, which results from rising of the ascomata. Towards the upper part of the ascomata wall, the pellicle is very tightly packed (Figure 3.3e). *Peridium* 12–19 μm wide ($\bar{x}$ = 15.3 μm, n = 20); hyaline, cells brown to dark brown at the base of *textura angularis*, with compressed *textura prismatica* at the sides (Figure 3.3d). *Hamathecium* of cellular, hyaline, pseudoparaphysate. *Asci* 70–96 × 39–53 μm ($\bar{x}$ = 82 × 44 μm, n = 15), 8-spored, bitunicate, clavate or pyriform, short pedicellate, mostly evanescent (Figure 3.3f-g). *Ascospores* 24.7–35.5 × 5.7–8.7 μm ($\bar{x}$ = 30.3 × 6.8 μm, n = 30), overlapping 3–5-seriate, hyaline, cylindro–clavate, with 7–9

transverse septa, constricted at the septa, smooth-walled; surrounded by a 3–5 μm wide mucilaginous sheath (Figure 3.3h-k); germinating mostly from apex and base of ascospores, a few also germinating from the central cells (Figure 3.3l).

Culture characteristics: Colonies slow growing reaching 2 cm diam. after 20 days on PDA, colony erumpent, surface dull, velvety with a folded, radiating, wavy margin, shiny, brownish gray, and olivaceous at margin, grayish sparse aerial mycelium outer region, reverse dark, sunken to the surface of media. No anamorph was produced in PDA after 60 days incubation (Figure 3.6d).

Material examined: THAILAND, Chiang Mai Province, Doi Suthep, medicinal garden, on living leaves of *Lagerstroemia* sp. (Lythraceae), 5 Nov 2009, Putarak Chomnunti, DPC043, (HOLOTYPE, MFLU10–0008) extype living culture MFLU (CC) 10–0079, and BCC same locality and host plant; same locality and host plant, 18 Sep 2010, Putarak Chomnunti (PARATYPE, MFLU11–0005); same locality and host plant, 10 October 2010, Liu Jian Kui, (PARATYPE, MFLU11–0006).

Notes: Batista and Maia (1957), and Hughes (1976) described several species of *Ceramothyrium* that lack setae. Species of *Ceramothyrium* are characterized by ascomata that are covered by a subiculum or mycelial layer, with a circumferential space around the maturing ascomata. In species of *Phaeosaccardinula* ascomata are formed beneath a layer which attaches the ascomata to the host surface. Ascospores of *Phaeosaccardinula* are also muriform but this is never the case in *Ceramothyrium*. The genus presently includes 20 species (Kirk et al., 2008). *Ceramothyrium thailandicum* is distinct in by having mostly 8–septate, clavate ascospores with a distinct mucilaginous sheath and the number of septa (Hansford, 1946; Batista & Ciferri, 1962). Species with clavate ascospores include *C. boedijnii*, *C. europaeum* and *C. calycanthi*. *Ceramothyrium thailandicum* differs from *C. boedijnii* and *C. europaeum* in the size of ascomata, asci and ascospores, the former having larger ascomata, longer asci and ascospores with 7–9 septa and the latter two have 3 and 2 septa, respectively. *Ceramothyrium thailandicum* differs from *C. calycanthi* by smaller asci and ascomata with thicker peridium (35–40 versus 12–19 μm) composed of subglobose and polygonal texture while *C. thailandicum* has a *textura prismatica*.

Note. (a). Habit of ascomata on the host surface. (b). Ascomata covered by a subiculum or layer. (c). Margin of ascoma. (d) – (e). Vertical section through ascomata. (f), (h). Bitunicate asci. (g). Ascospores in Indian ink demonstrating the mucilaginous sheath. (i–(k). Ascospores which are cylindro-clavate with 7–9 septa. (l). Germinating ascospores with germ tubes growing from apex and base and occasionally center. Scale bars: (a), (d) = 100 μm, (b) – (c), (e), (k) = 50 μm, (f) – (j) = 20 μm.

Figure 3.3 *Ceramothyrium thailandicum* Chomnunti & K.D. Hyde (holotype)

Chaetothyrium brischoficola Chomnunti & K.D. Hyde, sp. nov.

MycoBank: MB 519828

Etymology: from the host *Brischofia* and Latin *cola*, meaning "dwelling on", in reference to the occurrence on the host.

Ascomata 130–145 μm diam. × 89–101 μm alta, cupulata in siccitate, subglobosa, brunnea, cum setae. *Asci* 58–74 × 21–45 μm, 8-spori, bitunicati, clavati vel ellipsoidei, breve pedicellati. *Ascosporae* 17–27 × 5.3–9.7 μm, 3–4–seriatae, oboviodae, hyalinae, 4–6 transversus septatae, 2–4 longitudinalis septatae.

Fungus appearing as black dots scattered on the upper surface of *Brischofia javanica* leaves and also dark mycelium cover the surface of leaves which become blackened, mycelium superficial, pale brown to hyaline, hyphae reticulate-branched, constricted, pale brown to hyaline 3.4–5.5 μm, thick ($\bar{x}$ = 4.5 μm, n = 20) (Figure 3.4a-b). *Ascomata* developing beneath a brown layer attaching it to the leaf surface, cupulate on drying; ostiole or papilla not apparent and method of spore release not established, in vertical section subglobose, 130–145 μm diam., 89–101 μm high ($\bar{x}$ = 140 × 97 μm, n = 5), brown to dark brown, scattered. *Ascomatal setae* 10–12, scattered, dark brown at base, brownish at the tip, erect, rigid 47–68 × 5–6 μm ($\bar{x}$ = 60 × 5μm, n = 20) (Figure 3.4c, e-f). *Peridium* membranous, 10–14 μm thick ($\bar{x}$ = 12 μm, n = 20) pseudoparenchymatous, composed of *textura angularis* and *texura prismatica* in the outer region, becoming light brown to hyaline and flattened in the inner region (Figure 3.4d). *Asci* 58–74 × 21–45 μm ($\bar{x}$ = 65 × 31 μm, n = 10) 8-spored, bitunicate, clavate to ellipsoid, short pedicellate or sessile with J-apical ring 4.9–7.1 × 2.3–3.6 μm ($\bar{x}$ = 5.8 × 3 μm, n = 10) (Figure 3.4g-i). *Ascospores* 17–27 × 5.3–9.7 μm ($\bar{x}$ = 6.6 × 20 μm, n = 10), 3–4-seriate, obovoid, hyaline, with 4–6 transverse septa, some with 2–4 longitudinal septa, constricted at the septum germinating from apical and central cells (Figure 3.4j-l).

Culture characteristics: Colonies on PDA growing to 15 cm diam. after 20 days, flattened and surface brown to pale brown, spreading smooth margins with sparse aerial mycelium, olivaceous–green at margin, reverse iron–gray to brown. No anamorph was produced in PDA after 60 days incubation (Figure 3.6a-b).

Material examined: THAILAND, Chiang Rai Province, Khunkorn water fall, on living leaf of *Brischofia javanica* (Phyllanthaceae), 18 December 2009., Putarak Chomnunti, DPC047, (HOLOTYPE, MFLU10−0012) extype living culture MFLU(CC)10−0083 and BCC same locality and host plant species; same locality and host plant species, 30 Aug 2010, Liu Jian Kui (PARATYPE, MFLU11−0009); same locality and host plant species, 21 Sep 2010, Putarak Chomnunti (PARATYPE, MFLU11−0010).

Notes: The genus *Chaetothyrium* Speg. is characterized by having setae on the ascomata as well as scattered on the host around the ascomata (Batista & Ciferri, 1962). The genus includes 26 species (Kirk et al., 2008). *C. brischoficola* differs from other species by having obovoid ascospores with transverse septa and occasionally longitudinal septa. In this respect the species might be better placed in *Treubiomyces* which has cylindric−oblong, muriform ascospores (Batista & Ciferri, 1962; Hughes, 1976). Many of the ascospores in our species, however, lacked longitudinal septa and are obovoid. Since this species might be border-line between *Chaetothyrium* and *Treubiomyces* we place it in the former genus, the type of the family. *C. brischoficola* differs from other species in the genus in having a distinct apical ring and ascospores which occasionally have longitudinal septa. We examined type material of specimens of *Chaetothyrium* at K (i.e. *C. boedijnii* Hansf., *C. capense* (Doidge) Hansf., *C. concinnum* Syd., *C. fusisporum* L.R. Fraser., *C. mangiferae* Bat. and I.H. Lima., *C. permixtum* Syd., *C. roseum* Hansf., *C. sorysorensis* Hansf. and Thirum. and *C. ugandense* Hansf.) and none of these species has an apical ring in the ascus. We do not however, opine that these characters warrant the introduction of a new genus at this time, so this taxon is included in *Chaetothyrium*. Future studies should include more species of *Chaetothyrium*, to verify whether the genus is a monophyletic group or if the species with longitudinal septa and an apical ring are phylogenetically distant.

Note. (a). Appearance of leaves colonized by the fungus. (b). Cupulate ascomata on living leaves of *Brischofia javanica.* (c). Globose ascoma with black setae. (d). Section of peridium. (e). Ascomata removed from leaf. (f). Vertical section of ascoma. (g) − (i). Subglobose asci with apical ring. (j) − (k). Ascospores. (l). Germinating ascospore. Germ tubes grow from apical and central cells. Scale bars: (c) – (f) = 50 μm, (g) – (l) = 20 μm.

Figure 3.4 *Chaetothyrium brischoficola* Chomnunti & K.D. Hyde (holotype)

Phaeosaccardinula ficus Chomnunti & K.D. Hyde, sp. nov.

MycoBank: MB 519829

Etymology: in reference to its occurrence on *Ficus* sp.

Ascomata scattered, cupulata in siccitate, 188–226 μm diam. × 116–193 μm alta. superficialia, globosa, nigra, setae nulla, cum medius ostiolum 30–36 × 25–31 μm. *Asci* 120–185 × 49–64 μm, bitunicati, puriformes ad clavati et paene oboviodei ad ellipsoidae, sessile vel denique pedicellati. *Ascosporae* 33–56 × 10–17 μm, 2–4-seriatae, hyalinae, oblonga–ellipsoidae, muriformes, 7–8 transversus septatae and 6–8 longitudinalis septatae, constricta in septae.

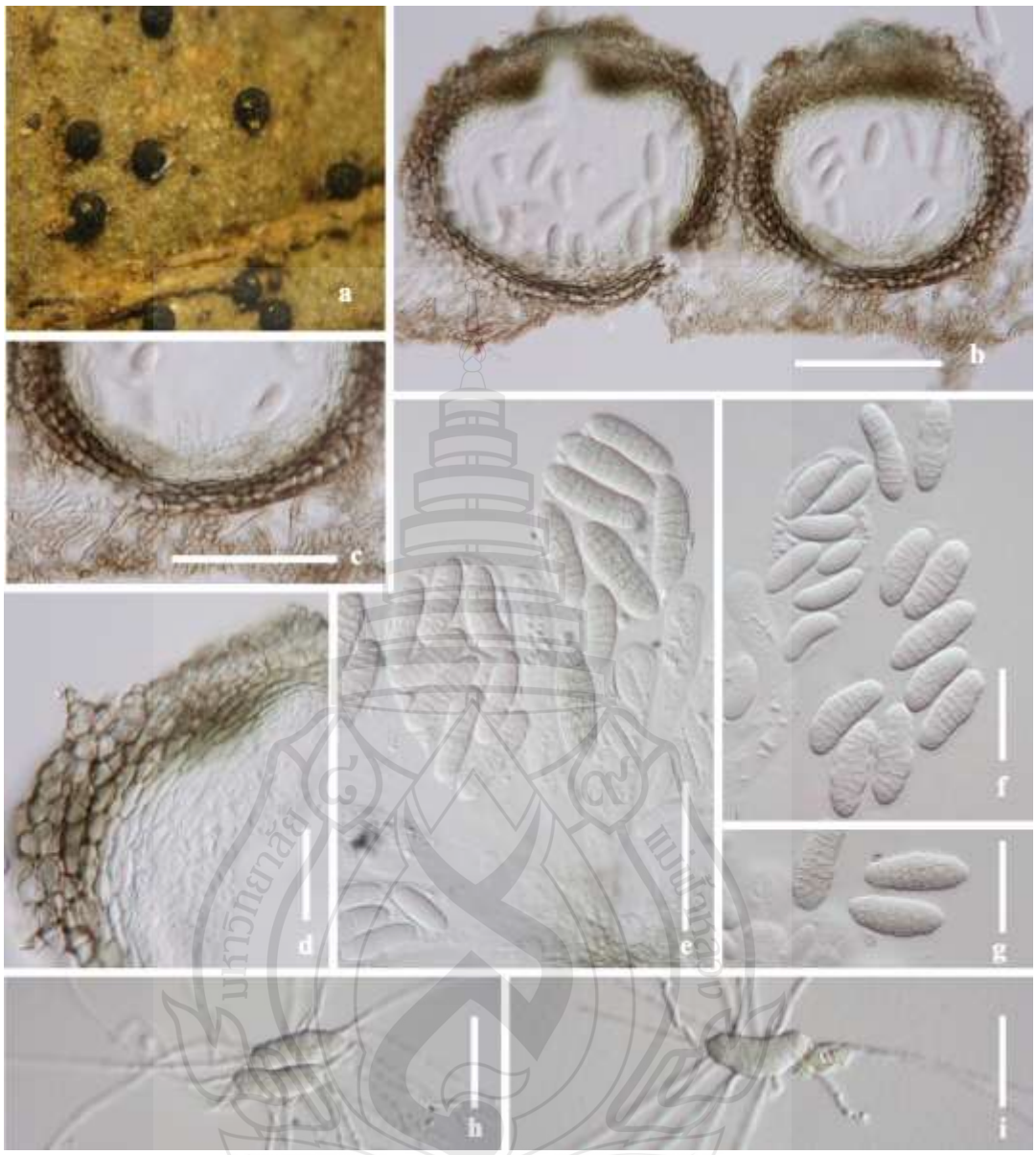
Mycelium forming sparse covering on host surface, hyphae brownish and pale brown, constricted at the septa, 3.7–5.4 μm wide ($\bar{x}$ = 4.4 μm, n = 20). *Ascomata* scattered on upper surface of living leaves of *Ficus* sp., cupulate when dry, 188–226 μm diam., 116–193 μm high ($\bar{x}$ = 209 × 174 μm, n = 10), superficial, globose, dull black, lacking setae, with a central ostiole that has a white center, forming beneath a layer which attaches the ascomata to the host surface. Ostiole 30–36 × 25–31 μm ($\bar{x}$ = 33 × 28 μm, n = 5); present in mature ascomata (Figure 3.5a-c). *Peridium* 20–32 μm wide ($\bar{x}$ = 27 μm, n = 30), with 2–3 strata with the spine on the surface, glabrous, comprising *textura globulosa* at the outside to *textura angularis* and flattened inwardly hyaline of *textura prismatica* (Figure 3.5d). Pseudoparaphyses hyaline, non septate. *Asci* 120–185 × 49–64 μm ($\bar{x}$ = 148 × 55 μm, n = 10), 8–spored, bitunicate, pyriform to clavate and almost oboviod to ellipsoid, sessile or short pedicellate, early evanescent, lacking an ocular chamber (Figure 3.5e). *Ascospores* 33–56 × 10–17 μm ($\bar{x}$ = 41 × 13 μm, n = 50), overlapping 2–4–seriate, hyaline, olivaceous–green at the septa of mature ascospores, oblong–ellipsoid, muriform, with 7–8 transversal septa and 6–8 longitudinal septa, constricted at the septum, with mucilaginous sheath (absent in some mature ascospores), with germ tubes developing from numerous cells (Figure 3.5f-i).

Culture characteristics: Colonies on PDA erumpent, growing slowly, 2 cm diam. after 20 days, spreading, with folded, velvety, wavy margin and water droplets forming on the surface, with dark mycelium, olivaceous–green at margin, white to

grayish sparse aerial mycelium covering colony, reverse dark, sunken. No anamorph was produced in PDA after 60 days incubation (Figure 3.6c).

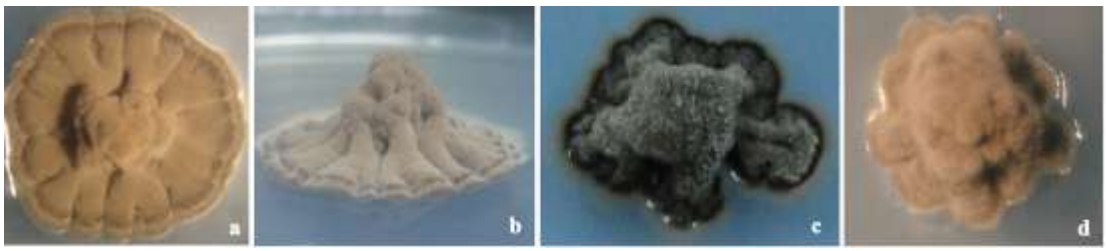
Material examined: THAILAND, Chiang Rai Province, Baan Du, on living leaf of *Ficus* sp. (Moraceae), 6 Nov 2009, K.D. Hyde, DPC044, (HOLOTYPE, MFLU10−0009) extype living culture in MFLU(CC)10−0080 and BCC same locality and host plant; same locality and host plant, 25 Jan 2011, Putarak Chomnunti (PARATYPE, MFLU11−0007, MFLU11−0008).

Notes: The genus *Phaeosaccardinula* contains 14 species (Kirk et al., 2008) and is characterized by the presence of superficial, globose or flattened−globose, dark, non−setulose ascomata, and muriform, hyaline to brownish ascospores. The specimen found on *Ficus* sp. is most similar to *P. dictyosporum* and *P. javanica*. *P. javanica* can be distinguished from *P. ficus* by the larger ascomata (300 × 250 μm) and smaller ascospores (30–35 × 8–15 μm). *P. dictyosporum* is different from *P. ficus* due to a tessellate scutellum (750 μm) with a clypeus–like margin (Eriksson & Jingzhu, 1985), and relatively longer and wider ascospores (35–61 × 16–24 μm). Mature ascospores of *P. ficus* are olivaceous−green at the septa and have a mucilaginous sheath, while ascospores of *P. javanica* are ellipsoid to fusoid with rounded ends, without a mucilaginous sheath (Hansford, 1946).



Note. (a). Black ascomata scattered on living leaves of *Ficus* sp. (b). Vertical section through ascomata showing ostiole. (c). Hyphal network of subiculum at base of ascoma. (d). Peridium (e). Asci oboviod to clavate. (f) – (g). Muriform ascospores. (h) – (i). Germinating ascospores. Germ tubes develop from numerous cells of ascospores. Scale bars: (b) – (c) = 100 µm, (e) = 50 µm, (d) – (i) = 20 µm.

Figure 3.5 *Phaeosaccardinula ficus* Chomnunti & K.D. Hyde (holotype)



Note. (a) – (b). *Chaetothyrium brischofiacola*. (c). *Phaeosaccardinula ficus*. (d). *Ceramothyrium thailandicum*.

Figure 3.6 Colony on PDA after 4 Weeks at 27 °C in Dark.

3.4 Discussion

In the present study, we provided detailed descriptions of three new species of Chaetothyriaceae illustrated with photomicrographs, and discussed their taxonomic placement as inferred from LSU sequence analysis. *Ceramothyrium thailandicum*, *Chaetothyrium brischoficola* and *Phaeosaccardinula ficus* are chaetothyriaceous 'sooty moulds' characterized by ascomata which form beneath a mycelial pellicle lying on the leaf surface, and which is attached to the upper part of ascomata (Batista & Ciferri, 1962). *Ceramothyrium thailandicum* was placed in *Ceramothyrium* because the ascomata and mycelium do not have setae and ascospores are clavate with a distinct mucilaginous sheath and numbers of septa differ from existing species (Hughes, 1976; Constantinescu, Holm, K. & Holm, L., 1989). *Phaeosaccardinula ficus* is accommodated in *Phaeosaccardinula* characterized by ascomata and mycelium which lack setae and muriform ascospores (Batista & Ciferri, 1962; Hughes, 1976; Eriksson & Jingzhu, 1985). Both *Chaetothyrium* and *Treubiomycetes* are characterized by ascomata with setae (Hughes, 1976), however in the former genus species have obovioid ascospores with transverse septa only, while in the latter genus species have cylindric–oblong, muriform ascospores (Batista & Ciferri, 1962; Hughes, 1976). Many of the ascospores in our species are obovoid and may have or

lack longitudinal septa. We, therefore, prefer to place our species in *Chaetothyrium*. A new genus *Chaetothyriomycetes* from Brazil was recently introduced by Pereira et al. (2009) and this differs as it is characterized by asci with 16 ascospores.

Few rDNA sequences of *Chaetothyriaceae* are available in GenBank. SSU rDNA sequences were provided for *Ceramothyrium linnaeae* (Dearn.) S. Hughes by Winka et al. (1998) who showed them to be closely related to *Chaetothyrium* Speg., the type genus of *Chaetothyriaceae*. Phylogenetic analyses of LSU as well as ITS sequences revealed that the three new species here cluster within the Chaetothyriales (*Chaetothyriaceae* and *Herpotrichiellaceae*) and are remote from the Capnodiales (sooty moulds) (Figure 3.1, Figure 3.2). Verrucariales was also shown to be sister to Chaetothyriales (Figure 3.1) thus confirming the results of previous morphology and molecular studies (Lutzoni, Pagel & Reeb, 2001).

The three new taxa described in the present study have phylogenetic similarity with a putative member of the *Chaetothyriaceae* (*Ceramothyrium carniolicum*) and anamorphic taxa (*Vonarxia vagans*, *Cyphellophora eugeniae* and *Cyphellophora hylomeconis*) (*Chaetothyriaceae*; Figure 3.1). Type derived sequences of members of *Chaetothyriaceae* have not been used in previous phylogenetic analysis since no such sequences were available. Therefore further studies with more taxa sampling are needed to obtain a better understanding of the *Chaetothyriales* complex (Badali et al., 2008; Gueidan et al., 2008; Untereiner et al., 2011). As such, the present study contributes six more chaetothyriaceous sequences including the first LSU and ITS rDNA sequence for the type genus *Chaetothyrium* and this should provide better data for future work on the sooty mould complex.

CHAPTER 4

MOLECULAR PHYLOGENY OF *TRICHOMERIACEAE* FAM. NOV.

4.1 Introduction

The taxonomy of genera of foliar epiphytes is poorly known as they have not been well-studied. No molecular data is available for most genera and therefore an understanding of the higher level classification of these fungi is rather inadequate. We have, therefore, initiated a research program to collect and study these important taxa using morphology and phylogeny. Our initial study (Chomnunti, Ko Ko et al., 2012) resulted in the transfer of the genus *Trichomerium*, previously placed in *Capnodiaceae* to *Chaetothyriaceae* in *Chaetothyriales*. We have also provided an account of *Microthyriaceae* (Wu et al., 2011) and are presently studying other genera of foliar epiphytes. Examples of foliar epiphyte genera with a sooty mould-like appearance are *Aithaloderma*, *Capnodaria*, *Phragmocapnias* and *Scorias*. Chomnunti et al. (2011), gave an account of the genera in *Capnodiaceae*, while Chomnunti, Ko Ko et al. (2012) dealt with species of *Chaetothyriaceae.* The genus *Trichomerium* was placed in *Chaetothyriaceae* but no further data was provided (Chomnunti et al., 2011). Hughes and Seifert (2012) provided notes on the taxonomy and nomenclature of sooty mould names, but further work is required to resolve their interrelationship, especially at the molecular level.

Trichomerium was introduced by Spegazzini (1918) based on *Trichomerium coffeicola* (Puttemans) Speg. (1918) and it is estimated that the genus now includes 23 species (Kirk et al., 2008). Thirty-one names are listed in Index Fungorum. *Trichomerium* species are all foliar epiphytes, with superficial, setiferous, uniloculate

ascostromata surrounded by loosely interwoven mycelium, with bitunicate asci and hyaline, septate ascospores (Spegazzini, 1918). Batista & Ciferri (1963a), Hughes (1976), Reynolds (1982), Reynolds & Gilbert (2005), Kwee (1988), Thang (2006) and Chomnunti et al. (2011) have also commented on this genus.

Batista & Ciferri (1963a) provided a key, descriptions and illustrations for several *Trichomerium* species and placed them in *Capnodiaceae*. They characterized the fruiting body (perithecium) as globose, long and sessile with scattered setae, aparaphysate and with 8-spored asci. Unfortunately, the key is hard to follow and the illustrations are sketchy and therefore it is very hard to understand Batista and Ciferri's concept for the genus and its species. Hughes (1976) later transferred *Trichomerium* to *Triposporiopsidaceae*, but subsequent workers did not follow this arrangement. *Triposporiopsidaceae* is based on the genus *Triposporiopsis* which is now considered to be a species of *Phragmocapnias* and thus a synonym of *Capnodiaceae*. Reynolds (1982) re-examined all available collections and literature on *Trichomerium* and placed all species names as synonym under *T. grandisporum* (Ellis & G. Martin) Bat. & Cif., thus treating the genus as monotypic. The generic type, *T. coffeicola* was included as a synonym although it is not clear if Reynolds (1982) had examined the type material which has smaller ascospores than *T. grandisporum*. Recently, Chomnunti et al. (2011) transferred *Trichomerium* from *Capnodiaceae* to *Chaetothyriaceae* based on the morphology of the ascostromata and possession of trans-septate hyaline ascospores.

In this study, we re-describe the genus *Trichomerium* based on six specimens collected and examined from northern Thailand, including combined LSU and ITS rDNA sequence analysis. We have also examined type material of *T. coffeicola* var. *macrosporum* and describe three new species.

4.2 Materials and Methods

4.2.1 Observation of Generic Types, Isolation from Fresh Specimens and Studies on Morphology

Specimens of *Trichomerium* sp. on living leaves were collected from various localities and plants in northern Thailand Technique to observed morphological characters and singles spore isolation see Material and methods in Chapter 2. DNA isolation, amplification and sequencing see Material and methods in Chapter 3.

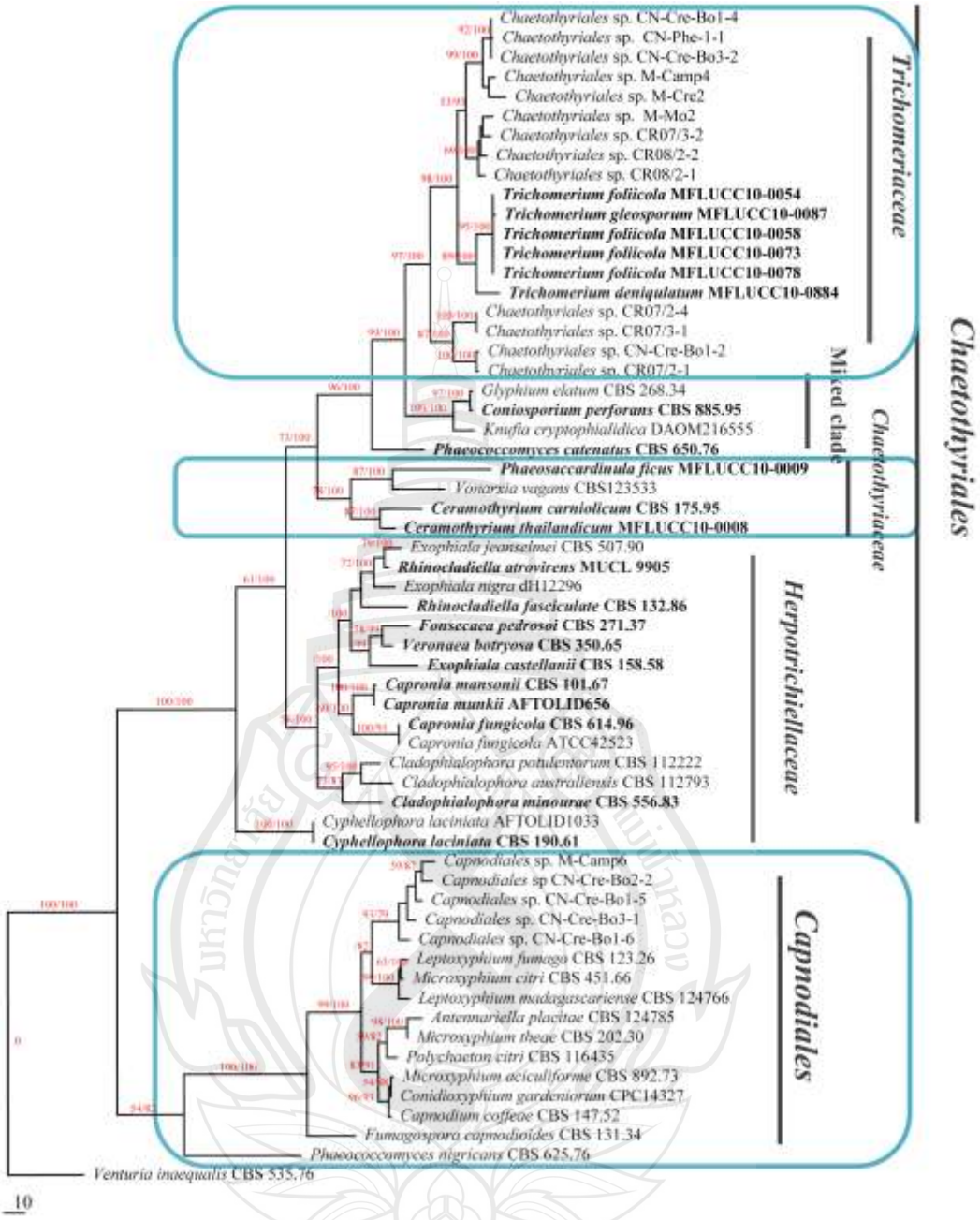
4.2.2 Sequence Alignments and Phylogenetic Analysis

DNA sequences were analyzed with available sequences of the *Capnodiaceae, Chaetothyriaceae* and *Herpotrichiellaceae* obtained from GenBank. Sequences were aligned using BioEdit (Hall, 1999) and Clustal X v.1.83 (Thompson et al., 1997) and phylogenetic analysis were performed using PAUP* v. 4.0b10 (Swofford, 2002). Ambiguous regions in the alignments were excluded from the phylogenetic analyses, gaps were treated as missing data. Maximum parsimony (MP) was performed with the heuristic search option on and addition of sequence using 1000 random with a stepwise starting tree, tree bisection and reconnection (TBR) as the branch-swapping algorithm. The parsimony scores including tree length (TL), consistency index (CI), retention index (RI) and homoplasy index (HI) were calculated. Clade stability was estimated in bootstrap (BT) analysis with 1000 replicates (Hillis & Bull, 1993). Model of substitution used for Bayesian analyses was using MrModeltest 2.2 (Nylander, 2004). The Bayesian analyses were performed in MrBayes 3.04b (Huelsenbeck & Ronquist, 2001). Bayesian analyses were conducted with the Markov chains run from random starting tree for 1 000 000 generations and trees were sampled every 100 generations. The Markov Chain Monte Carlo (MCMC) algorithm was used to estimate posterior probabilities (PP) and obtained for each clade. Trees were visualized in TreeView (Page, 1996). Details of sequences used are presented in Table 1. The LSU and ITS region were used in the phylogenetic analysis to determine generic or family placements. *Venturia inaequalis* was selected as outgroup. Sequences data are deposited in GenBank.

4.3 Results

4.3.1 Molecular Phylogeny

The phylogenetic analysis includes representative sequences of *Capnodiaceae* and *Chaetothyriaceae*; the alignment of combined partial LSU and ITS rDNA comprised 73 taxa and 199 base pairs were excluded, the remaining 1131 included characters used in analysis, 767 characters were constant, 56 were variable characters are parsimony-uninformative and 308 were parsimony informative. A heuristic search found 100 equally parsimonious tree with the length (TL) of 1191 steps (CI = 0.472, RI = 0.846, RC = 0.399, HI = 0.528). All trees were similar in topology and not significantly different. A best scoring Maximum Parsimony tree is shown in Figure 4.1. The phylogenetic tree obtained from Bayesian and maximum likelihood analyses is in agreement with a previous study based on MP analysis (Voglmayr et al., 2010). The six new *Trichomerium* strains formed a monophyletic group and clustered with nine strains of *Chaetothyriales* sp. associated with ants with 98% of bootstrap support, but received a 100% posterior probability (PP) in the Bayesian analysis (Clade A). The *Trichomerium* strains included the new species *T. deniquelatum, T. foliicola* and *T. gloeosporum* which clustered with high bootstrap support (89% bootstrap support and 100% PP). Other sequences of *Chaetothyriales* spp. associated with ants (CR08/2-2, CR08/2-1, CR073-2, M-Mo2) also grouped within clade A with 97% of bootstrap support, but received a 100% PP. GenBank sequences for *Coniosporium perforans, Glyphium elatum* and *Knufia cryptophialidica* clustered in Clade B with strong support (100% bootstrap support and 100% PP). Species in Clade C are mostly teleomorphic genera of *Chaetothyriales* and form a monophyletic cluster (78% bootstrap support and 100% PP). Taxa in Clade D are members of *Herpotrichiellaceae* (anamorphic *Chaetothyriales*), some being human pathogens or rock inhabiting fungi. Clade E comprised 15 taxa of *Capnodiaceae* with five strains of *Capnodiales* isolated from ants (Voglmayr et al., 2010) and cluster together with strong support (100% bootstrap support and 100% PP). Phylogenetic data clearly shows that *Trichomerium* belongs in *Chaetothyriales* and incorporates a strongly supported new family, *Trichomeriaceae* which is introduced below.



Note. A data set of 73 taxa including *Chaetothyriales* and *Capnodiales*, comprising LSU, ITS rDNA genes. The first set of numbers above the nodes are bootstrap values over 50% and the second represents Bayesian posterior probabilities of more than 90% and expressed as percentages. New sequences and types are in bold.

Figure 4.1 Maximum Parsimonay Tree of LSU and ITS rDNA Sequences

Table 4.1 LSU and ITS rDNA Sequences Generated from this Study.

Species	Strain no.	Host	Country	Collector(s)	GenBank Accession no.	
					LSU	ITS
Trichomerium foliicola	MFLUCC10–0078	*Murraya paniculata*	Thailand	Putarak Chomnunti	JX313661	JX313655
T. foliicola	MFLUCC10–0054	*Mangifera indica*	Thailand	Putarak Chomnunti	JX313657	JX313651
T. foliicola	MFLUCC10–0073	*Psidium guajava*	Thailand	Putarak Chomnunti	JX313658	JX313652
T. foliicola	MFLUCC10–0058	*Phoenix dactylifera*	Thailand	Samantha C. Karunarathna	JX313659	JX313653
T.gloeosporum	MFLUCC10–0087	*Ficus* sp.	Thailand	KD. Hyde	JX313662	JX313656

Table 4.2 Synopsis of Characters of *Trichomerium* Species Mentioned in this Study

Taxa	Setae	Ascomata	Ascospores	Host in protologue	References
T. coffeicola Speg.	Dark-sooty setae, simple, continuous, long and tapered	Ascostromata, conoid, neck truncate	15–18 × 5–6 μm hyaline, subfusoid, 3 guttulate, 2 septate	*Coffea arabica*	Spegazzini (1918)
T. deniqulatum Chomnunti & K.D. Hyde	(28–)32–54 × 3.7–6 μm, setae sparse, indistinct, aseptate to septate, brown	154–175 × 163–180 μm	18-25 × 6-8 μm, fusoid, 3-septate, constricted at the septa, middle two cells wider and obliquely septate	*Psidium guajava*	This paper
T. didymopanacis Bat. & Cif.	50–130 × 5–8 μm, numerous erect, straight or curved, septate, brown	105–150 μm.	16–24.5 × 5–7.5 μm, fusoid, with rounded ends, 1–3 septate, hyaline	*Didymopanax morototoni*	Batista & Ciferri (1963)
T. foliicola Chomnunti & K.D. Hyde	(44–) 61–118 × 4–7 μm, abundant, clearly septate and continuous, brown	133–179 × 140–181 μm	19–22 × 6–7 μm, 2–3 septate, not constricted, fusiod	*Murraya paniculata*	This paper

Table 4.2 (Continue)

Taxa	Setae	Ascomata	Ascospores	Host in protologue	References
T. hirtellum Bat.	56–65 × 5–7.5 μm, setose, septate to continuous all around the ascostromata, black	90–150 diam. μm, globose or pyriform	14.5–19.5 × 6–8.5 μm, fusoid, 2 septate, hyaline	*Persea*	Batista (1951)
T. ornatum Bat. & Cif.	58–120 × 5–9 μm, setae erect, simple, straight or curved, light brown or olivaceous, septate, obtuse, dull brown apex	125–250 diam. μm, globose with central ostiole	22-27 × 6-10 μm, cylindric-fusoid at first 1-septate then 3-septate, not constricted, hyaline	*Ocotea* sp.	Batista & Ciferri (1963)
T. pelliculosum (Berk. & Ravenel) Cif. & Bat.	54–95 × 5–10 μm, erect setae, setae apex rounded to narrowed, brownish	80–145 μm diam.	15-22 × 4-7 μm, fusiod, 1-3 septate, hyaline, not constricted, polystichous	*Prunus, Magnolia*	Batista & Ciferri (1963)
T. portoricense Speg.	Numerous	250 μm diam.	30 × 10 μm, fusiform, 3-septate, hyaline and olive when mature	*Psidium guajava*	Spegazzini (1924)

4.3.2 Generic Type Observation, Fresh Specimens Isolates and Morphology

Trichomeriaceae Chomnunti & K.D. Hyde, fam. nov.

MycoBank 800935

Epiphytes on living trees or *saprobes* on honey dew insect excretions. The colonies often mix together with capnodiaceous taxa. *Thallus* comprised of mycelium on host surface with septate, brown hyphae. *Ascostromata* arise from mycelium mass, which is a subiculum, and are sessile, spherical, brown, uniloculate, ostiolate, surrounded by setae. *Setae* brown to dark brown or olivaceous, erect, straight or curved, septate or continuous. *Peridium* pale brown to brown or olivaceous, comprising several layers of cells of *textura angularis*. *Asci* apparently bitunicate, with an apical ring, cylindrical to clavate, with overlapping ascospores and an apical ring. *Ascospores* hyaline, septate, fusiform, round at ends, with or without a mucilaginous sheath.

Family type: *Trichomerium* Speg.

Trichomerium Speg. Physis, B. Aires 4: 284 (1918).

MycoBank 5560

Foliar epiphytes on living leaves or *saprobes* on honey dew insect excretions. *Colonies* often mixed with capnodiaceous taxa. *Thallus* arising from the compacted mycelium on the host surface, composed of septate, cylindrical, pale brown to brown hyphae. *Ascostromata* arise from the mycelial mass, which is a subiculum, and are sessile, globose to subglobose, brown, uniloculate, with a central ostiole, with setae surrounding the upper part. *Setae* brown to dark brown or olivaceous, erect, straight or curved, septate or continuous. *Peridium* pale brown to brown or olivaceous, comprising 2-3 layers of cells of *textura angularis*. *Pseudoparaphyses* indistinct. *Asci* bitunicate, with an apical ring, cylindrical to clavate, with overlapping ascospores. *Ascospores* hyaline, septate, fusiform, narrowly rounded at both ends, widest in the centre, with or without a mucilaginous sheath.

Anamorph: possibly *Tripospermum* Speg. (1918) (Kirk et al. 2008).

Typification details:

Trichomerium coffeicolum (Puttemans) Speg., Physis, B. Aires 4: 284 (1918).

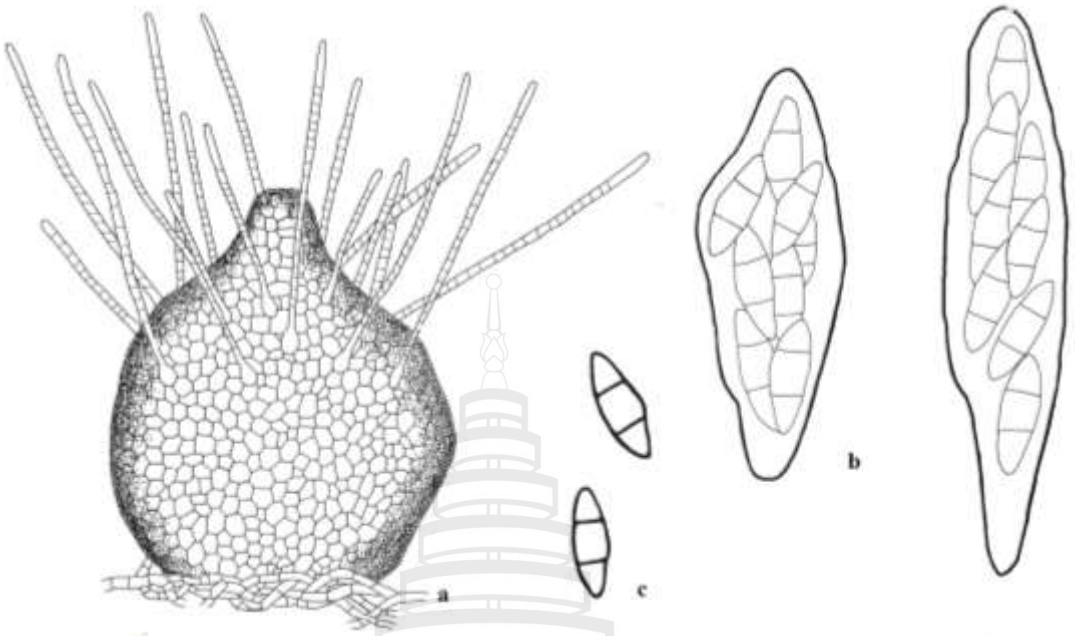
≡ *Limacinia coffeicola* Puttemans, Cryptog. Mycol. 20: 163 (1904).

Trichomerium coffeicolum (≡ *Limacinia coffeicola*) was described by Puttemans (1904) from living leaves of *Coffea arabica*. A translation of the species diagnosis reads "*Perithecium* black, ovoid, truncate at the neck, covered by dark-sooty, simple, continuous, long and tapered setae, aparaphysate. *Asci* diversified, often elongated, 50–70 × 15–20 μm, with hyaline, sub-fusoid, 2 septate, 3 guttulate, 15–18 × 5–6 μm, irregularly arranged ascospores" (Figure 4.2).

Puttemans (1904) added, "…this species was common on the surface of the leaves. The mycelium is developed and surrounded with conidia and this seems identical to the mycelium and conidial forms of *Capnodium*. The genera lacked *Triposporium* and *Limacinia* forms. I still cannot confirm the relationship with *Capnodium*".

We have tried to locate the type material of this species from URM and P but were not successful. Reynolds (1982) studied many specimens of *Trichomerium* Speg., including the type and concluded that the genus comprised a single species *T. grandisporum* and *T. coffeicola* was listed as a synonym. The description and drawings provided by Puttemans (1904) are informative and the generic concept for *Trichomerium* based on these illustrations of *T. coffeicola* is clear. In the protologue of Puttemans (1904) the ascospores are also smaller than those reported for *T. grandisporum* (15-18 × 5-6 versus 18-32 × 5-10 μm) and we doubt these taxa are the same species.

Here we therefore treat *Trichomerium* in the sense of *T. coffeicolum* based only on the protologue description and drawing. In this treatment *T. deniquelatum, T. foliicola* and *T. gloeosporum* are typical of *Trichomerium* and we suggest that it would be pragmatic to list *T. foliicola* as type of the genus in the Lists of Accepted Names to be developed by the subcommittee dealing with Dothideomycete names to be approved by the General Committee on Nomenclature (GCN) (Hawksworth, 2012). This will ensure stability in application of the generic name. *Trichomerium foliicola* is supported by both herbaria and living material and, in addition, molecular sequence data. Of course if fresh collections of *Trichomerium coffeicolum* were found and sequenced this would not be necessary.



Note. (a). Ascostromata with apical setae. (b). Asci. (c). 2–septate ascospores. (Redrawn from M.A. Puttemans 1904).

Figure 4.2 *Trichomerium coffeicola* Puttemans

Trichomerium deniqulatum Chomnunti & K.D. Hyde, sp. nov.

MycoBank 800933

Etymology: from the Latin 'denique' meaning short, referring to short setae

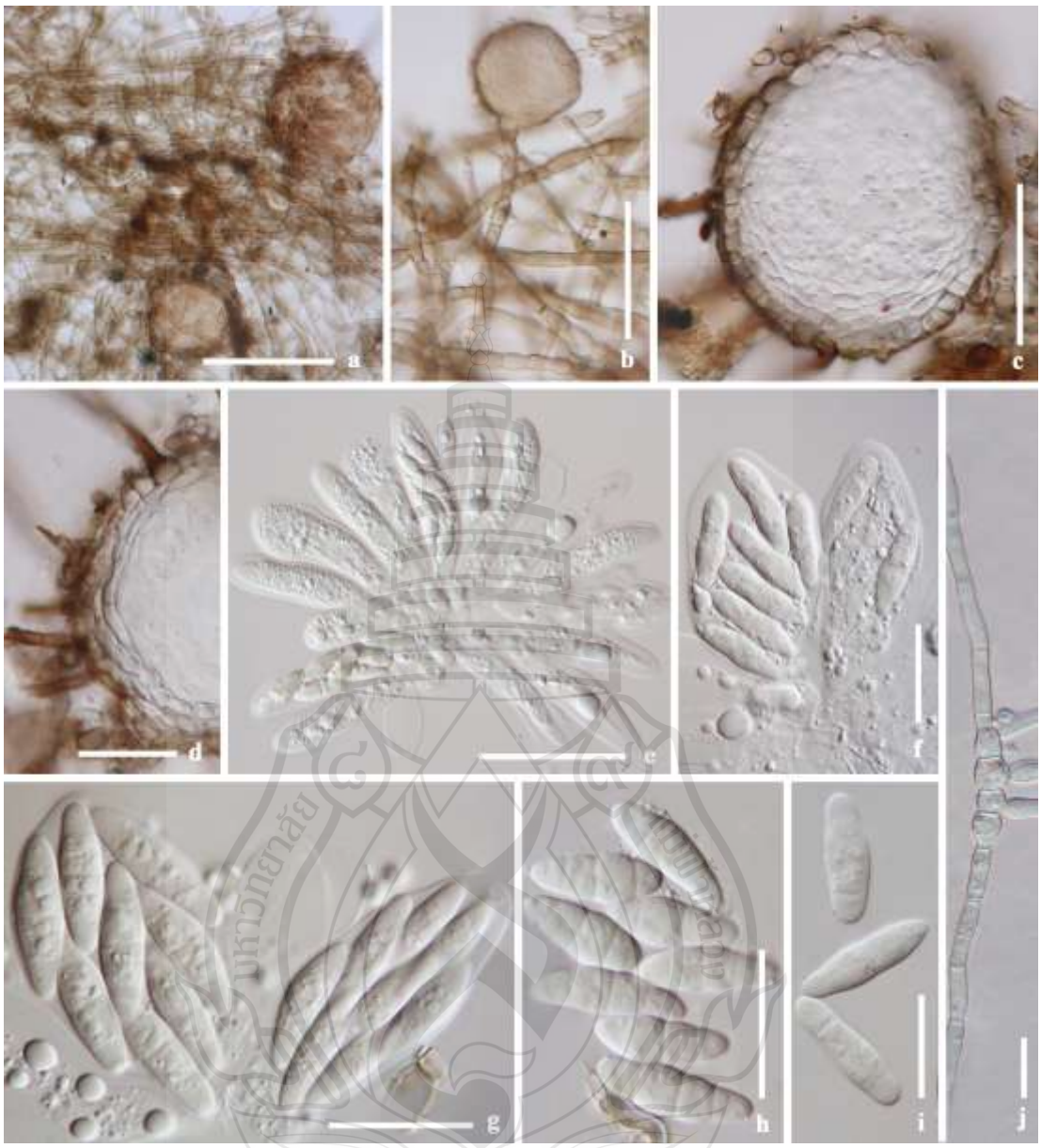
Black sheets of mycelia cover the leaves of the host which produce dark brown superficial, gregarious ascostromata. *Mycelium* superficial, septate, cylindrical, pale brown to brown hyphae, constricted at the septa; 5–7 μm wide ($\bar{x}$ = 6 μm, n = 20) (Figure 4.3a-b). *Ascostromata* initials arise from a small irregular group of cells formed by the repeated division of a few hyphal cells young ascomata are brown and mixed with the hyphae. *Ascostromata* 154–175 μm diam, 163–180 μm high ($\bar{x}$ = 165 × 168 μm, n = 10), subglobose to globose, sessile, brown, with up to 5 setae around the upper half of the perithecium; setae sparse, indistinct, aseptate to septate, brown, straight, with cylindrical cells, (28–)32–54 × 3.7–6 μm ($\bar{x}$ = 43 × 5 μm, n = 20),

aparaphysate (Figure 4.3c). *Ascostroma wall* 12-15 µm wide ($\bar{x}$ = 13 µm, n = 20) thick-walled, inwardly hyaline, brown towards the outside, comprised 2–3 layers of *textura angularis* (Figure 4.3d). *Asci* 47–60 × 22–31 µm ($\bar{x}$ = 54 × 25 µm, n = 10), 8-spored, ellipsoid to clavate, some subglobose, apparently bitunicate with an apical ring, aparaphysate (Figure 4.3e-g). *Ascospores* 18–25 × 6–8 µm ($\bar{x}$ = 22 × 7 µm, n = 20), tri-seriate, hyaline, fusoid, 3–septate, some with longitudinal septa, constricted at the septa, middle two cells wider and obliquely septate (Figure 4.3h-j).

Culture characteristics: Ascospores germinating on PDA within 12 h and germ tubes arise from both end cells (Figure 4.3j). Colonies growing slowly on PDA, reaching a diam of 4 cm after 14 days at 28ºC, velvety, radiating towards the edge. Mycelium initially black and dark green at the margin.

Material examined: THAILAND, Chiang Rai Province, Mae Fah Luang University, on living leaf of *Psidium guajava*. 12 September 2009, Putarak Chomnunti, DPC 039 (MFLU11–1150, holotype), ex-type living culture in MFLUCC10–0084 = BCC40712; *Ibid*., 28 June 2010, Putarak Chomnunti (MFLU11–1152); *Ibid.,* 11 October 2010, Putarak Chomnunti (MFLU11–1153)

Notes: Batista and Ciferii (1963a) described several species of *Trichomerium* with sparse setae mostly on the upper part of ascomstromata including *T. coffeicola, T. crotonis* Bat., *T. plumieriae* Bat. & Cif., *T. stuhlmannianum* (Henn.) Bat. & Cif. *and T. stuhlmannianum* var. *biseptatum* Bat. & Cif. *Trichomerium deniqulatum* has few setae on the ascostromata and differs from others in size of setae and ascospores, the number of septa on the ascospores. *T. deniqulatum* differs from *T. coffeicola* in having shorter setae (43 × 5 versus 100 × 3–4 µm). In *T. crotonis* ascospores are 3–5 septate, while in *T. deniqulatum* ascospores are 2-3 septate. In *T. plumieriae* ascospores are slightly smaller (20 µm long versus 22 µm long) and in *T. stuhlmannianum* var. *biseptatum* ascospores are not more than 2–septate, while in *T. deniqulatum*, ascospores are 2–3 septate and with or without longitudinal septa. We considered short setae and ascospores with longitudinal septa as major characters to recognize the new species.



Note. (a) – (b). Mycelium with immature ascostromata. (c). Vertical section through ascostromata. (d). Peridium. (e) – (g). Asci with apical ring in g (right ascus). (h) – (i). Ascospores. (j). Germination of ascospore. Scale bars: (a) =100 µm. (b), (c), (e) = 50 µm, (d), (f), (g), (h) = 20 µm, (i), (j) = 10 µm.

Figure 4.3 *Trichomerium deniqulatum* Chomnunti & K.D. Hyde (holotype)

Trichomerium foliicola Chomnunti & K.D. Hyde, sp. nov.

MycoBank 801117

Etymology: from the Latin foliicola meaning on living leaf, referring to various living host.

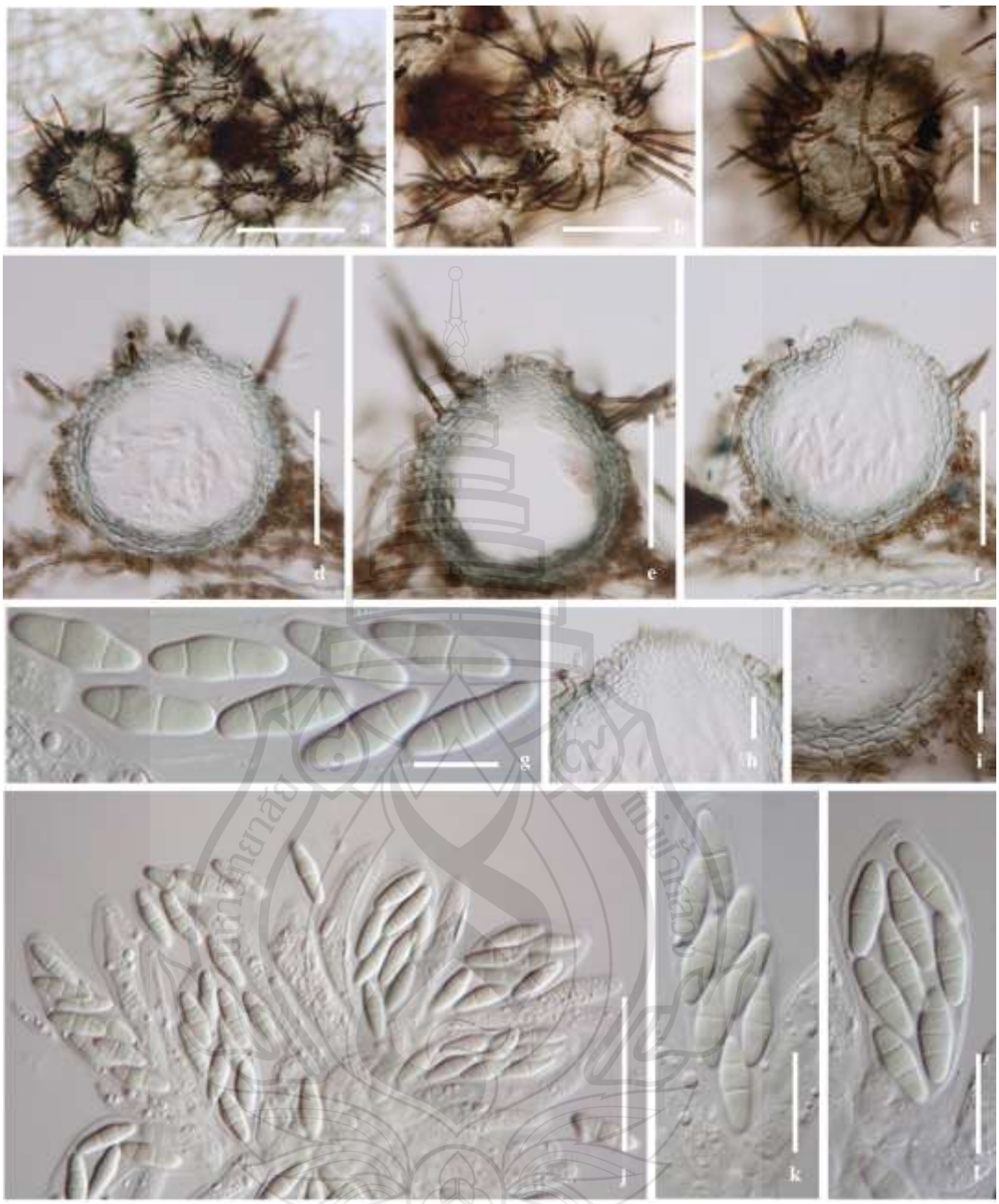
Black sheets of mycelia cover the leaves of the host and support dark brown superficial, gregarious ascostromata. The hyphae are septate, cylindrical, pale brown to brown with constrictions at the septa, mostly narrow; 3.5–7 µm wide ($\bar{x}$ = 5.5 µm, n = 20). *Ascostromata* initials arising from a small irregular group of cells formed by the repeated division of a few hyphal cells, usually at a hyphal branches and becoming dense. *Ascostromata* 133–179 µm diam, 140–181 µm high ($\bar{x}$ = 158 × 162 µm, n = 20), subglobose to globose, brown, with abundant straight, aseptate to septate, dark brown to brown setae, up to 15 setae, mostly on the upper half of the ascostroma, (44–) 61–118 × 4–7 µm ($\bar{x}$ = 83 × 6 µm, n = 20) present on a single ascostromata. Ostiole central, ostiolar canal 32 µm wide at the base, 39 µm high, with an apical ring, with periphyses (Figure 4.4a-f, h). Ascostroma wall 17–24 µm wide ($\bar{x}$ = 20 µm, n = 20), thick-walled, inwardly hyaline, pale brown and brown towards the outside, comprised of 2–3 layers of *textura angularis* (Figure 4.4i). *Asci* (47–) 63–70 × (14–) 20–26 µm ($\bar{x}$ = 65 × 22 µm, n = 10), 8-spored, ellipsoid to clavate, some obovoid, apparently bitunicate, with apical ring, aparaphysate (Figure 4.4j-l). *Ascospores* 19–22 × 6–7 µm ($\bar{x}$ = 21× 7 µm, n = 20) tri-seriate, hyaline with 2–3 septa, fusoid, with narrowly rounded ends (Figure 4.4g).

Culture characteristics: *Ascospores* germinating on PDA within 12 h and germ tubes produce from both end cells and from the middle cell. Colonies growing slowly on PDA, reaching a diam of 2.5 cm. after 14 days at 28ºC. Mycelium initially black with dark green margin visible from both sides of the dish, colony on PDA velvety, radial towards the edge.

Material examined: THAILAND, Chiang Rai Province, Mae Ka Jan, on living leaf of *Murraya paniculata*, 11 October 2009, Putarak Chomnunti, DPC 042 (MFLU10–0007, holotype), ex–type living culture in MFLUCC10–0078 = BCC40643; *Ibid*., 16 December 2009, Putarak Chomnunti (MFLU10-0683); *Ibid.,* 7 March 2010, Putarak Chomnunti (MFLU10–0987). *Ibid*., Baan Du, on living leaf of

Mangifera indica. 8 July 2009, Putarak Chomnunti, DPC 015 (MFLU09–0651), living culture in MFLUCC10-0054 = BCC38853; *Ibid.*, 2 September 2009, Putarak Chomnunti (MFLU09–0684); *Ibid.*, 24 May 2011, Putarak Chomnunti (MFLU11–1151); *Ibid*., on living leaf of *Psidium guajava*, 25 October 2009, Putarak Chomnunti, DPC 020 (MFLU10-0002); living culture in MFLUCC10–0073 = BCC41091; *Ibid.*, 22 April 2010, Putarak Chomnunti (MFLU10–0988); *Ibid*., on living leaf of *Phoenix dactylifera*, 11 August 2009, Samantha Chandranath Karunarathna, DPC 023 (MFLU09–0656); living culture in MFLUCC10–0058 = BCC39630; *Ibid*., 4 May 2010, Putarak Chomnunti (MFLU10–0989); *Ibid.*,20 August 2010, Putarak Chomnunti (MFLU10–0990); BRAZIL, Recife, Pernambuco, on leaves of *Didymopanax morotoni*, 4 February 1956, Severino José da Silva, URM 5302 (holoype); CUBA, Santiago de las Vesgas, in fragments of leaves of an unidentified host, 7 May 1913, F.L. Stevens (URM 13335); *Ibid*., on leaves of *Sanchezia nobilis* Hook., 19 January 1922, Charles and Ballon (URM 13483).

Notes: *Trichomerium foliicola* is similar to *T. didymopanacis* described from *Didymopanax morototoni* by Batista and Ciferri (1963a) and referred to *Capnodiales*. *Trichomerium didymopanacis* was described as having epiphyllous colonies with superficial, black, septate mycelium, with non-setose, narrowed, globose, membranous ascostromata, and with a central ostiole. Ascostromata have numerous setae which are erect, straight or curved, brown, and with obtuse tips. Asci are ellipsoid, sessile, and aparaphysate. Ascospores are fusoid, with rounded ends, 1–3 septate and hyaline (Batista & Ciferri, 1963a). The drawing provided in the protologue however, is not detailed. We examined the type of *T. didymopanacis* (URM 5302), however the material was not in good condition and we could not find ascostromata. Although, the ascostroma, asci and ascospores in the description of *T. didymopanacis* provided in Batista & Ciferri (1963a) overlap with those of *T. foliicola* (Table 4.2), it is not equivocal that these are the same species. We prefer to introduce a new species to avoid any confusion.



Note. (a) – (c). Ascostromata with ostiole and setae. (d) – (f). = Vertical section through ascostromata. (g). Ascospores. (h). Ostiolar canal. (i). Peridium. (j) – (l). Asci and ascospores. Scale bars: (a) – (f)=100 µm. (k), (l) = 50 µm, (h) – (i) = 20 µm, (g) = 10 µm.

Figure 4.4 *Trichomerium foliicola* Chomnunti & K.D. Hyde (holotype)

Trichomerium gloeosporum Chomnunti & K.D. Hyde, sp. nov.

MycoBank 800934

Etymology: from the Latin gloeoid meaning slimy, referring to ascospore outer sheath.

Black sheets of superficial of mycelia cover the surface of leaves of the host (Figure 4.5a). The hyphae are septate, cylindrical, pale brown to brown, constricted at the septa, 4–7 µm wide ($\bar{x}$ = 5 µm, n = 20). *Ascostromata* initials arise from a small group of irregular cells formed by repeated division of a few hyphal cells, when young brown and mixed with hyphae. *Ascostromata* 116–140 µm diam, 113–150 µm high ($\bar{x}$ = 128 × 138 µm, n = 10), subglobose to globose, sessile, brown, with abundant setae, up to 10 setae around the upper half of the ascostromata; setae septate, brownish to dark brown or olivaceous, straight, with cylindrical cells, 71–121 × 4–7 µm ($\bar{x}$ = 100 × 6 µm, n = 20) (Figure 4.5b-c, e). *Ascostroma wall* 2–3 layered, 15-21 µm wide ($\bar{x}$ = 18 µm, n = 20), thick walled, hyaline at the inner layers, brown at the outer layers, comprised of cells *textura angularis* (Figure 4.5d). *Asci* 62–86 × 18–23 µm ($\bar{x}$ = 75 × 21 µm, n = 20), 8–spored, ellipsoidal to cylindrical, with short pedicel, apparently bitunicate, with an apical ring, aparaphysate (Figure 4.5g-i). *Ascospores* 17–26 × 5–7 µm ($\bar{x}$ = 22 × 6 µm, n = 25), bi–seriate, hyaline, fusoid, 2–3 septate, not constricted at the septa, narrowly rounded at the ends, with a conspicuous mucilaginous sheath (Figure 4.5j-m).

Culture characteristics: Ascospores germinating on PDA within 12 h and germ tubes produced from both end cells (Figure 4.5j). Colonies growing slowly on PDA, reaching a diam of 3 cm after 14 days at 28ºC. Mycelium initially black with dark green margins, colony on PDA velvety, radial toward to the edge.

Material examined: THAILAND, Chiang Rai Province, Muang District, near Baan Du, Ban Kua Krae, *Ficus* sp. tree in rice field, on living leaf, 4 October 2010, K.D. Hyde, DPC 051 (MFLU10–0016, holotype), ex–type living culture in MFLUCC10–0087; *Ibid*., 30 January 2011, Putarak Chomnunti (MFLU11–1154).

Notes: *Trichomerium gloeosporum* is distinct from hitherto described species in the genus in having ascospores with a distinct mucilaginous sheath. Conidia of a *Trichomerium* sp**.** were associated with the sooty mould, but we could not establish

that they were related to the sexual morph. We collected material of a *Trichomerium* sp. from *Phoenix dactylifera* but unfortunately the specimen had too few of ascostromata to derive any valid morphological description. However, we derived a pure culture of the fungus from the specimen and carried out phylogenetic analysis. The culture did not produce any reproductive structures and therefore, at this stage, only the culture and sequence data are available. The sequence data is 95% similar to *T. gloeosporum* and hence accommodated under this species. This taxon is also very close to *T. didymopenacius* (95% bootstrap support, 100% PP) but further collections are needed to establish its identity.

Note. (a). Sooty mould on living leaf of *Ficus* sp. (b). Ostiole. (c). Vertical section through ascostromata. (d). Peridium. (e). Setae. (f). Conidia. (g) – (i). Asci. (j) – (m). Ascospores. Scale bars: (c) – (d), (g) = 50 µm, (b), (e) – (f), (h) – (i) = 20 µm, (j) – (m) = 10 µm.

Figure 4.5 *Trichomerium gloeosporum* Chomnunti & K.D. Hyde (holotype)

4.4 Discussion

In this study, we report on the morphology and sequence data for six freshly collected strains of *Trichomerium* isolated from Thailand. The strains are described as the new species *T. foliicola* (4 strains), *T. deniqulatum* (1 strain) and *T. gloeosporum* (1 strain), based on phylogenetic and morphological data. The phylogenetic data show them to belong in *Chaetothyriales* and cluster with *Chaetothyriales* spp. from ant nests chambers (Voglmayr et al., 2010). They are not closely related to *Chaetothyriaceae*, *Herpotrichiellaceae* or *Capnodiaceae.* Consequently, a new family, *Trichomeriaceae,* typified by sessile, setiferous ascostromata, with ostiolate, aparaphysate ascostromata, bitunicate asci with an apical ring and 2-3–septate to trans-septate, hyaline, ascospores with or without a sheath, is introduced. Ascostromata with a mycelial cover, asci with an apical ring and trans-septate and sheathed ascospores are not known in *Capnodiaceae*. We treat *Trichomerium* in the sense of *T. foliicola* as the holotype specimen of *T. coffeicola,* the generic type, is unavailable and also no molecular data exists for this species.

Reynolds (1982) examined several species of *Trichomerium*, clumped them under *T. grandisporum* and considered the genus to be monotypic. We have not followed Reynolds (1982) approach, as we believe the concept for *Trichomerium* as based on the generic type of *Limacinia coffeicola* Puttemans (1904) is clear. Furthermore, we accept four species in the present study and we believe that further studies of types and fresh collections will show the genus to be more speciose. Reynolds and Gilbert (2005) assigned the genus *Trichomerium* based on his concept of *Trichomerium grandisporum* (Ellis & G. Martin) Bat. & Cif. to *Capnodiaceae* using molecular sequence data (unpublished), which otherwise clustered with a black yeast clade (Babee, 1996). Recently, Chomnunti et al. (2011) excluded *Trichomerium* from *Capnodiaceae* on the basis of ascostromata and trans-septate hyaline ascospores and transferred the genus to *Chaetothyriaceae*. In this study, we diagnose *Trichomerium* based on morphological characteristics and DNA sequence data and accommodate the genus in a new family *Trichomeriaceae* in Chaetothyriales.

CHAPTER 5

REVISION OF MORPHOLOGICAL STUDIES ON THE GENERIC TYPES OF SOME SELECTED DOTHIDEOMYCETES FAMILIES

5.1 Introduction

Dothideomycetes is an important and largest Class of fungi in Phylum Ascomycota and the members include more than 19000 species with a broad range of ecological roles. Members of the Dothideomycetes are often found in varied relationships with plants, not only as pathogens, endophytes or epiphytes of living plants but also as saprobes degrading cellulose and other complex carbohydrates in dead or partially digested plant matter, or in leaf litter or dung. These fungi are not limited to associations with plants as several species are lichens while others occur on fungi as well as members of the kingdoms Animalia and Insecta. They are an important group of fungi economically, occurring on a wide range of substrata and a large number remains to be described; they have a huge potential in pharmaceutical area (Schoch et al., 2006; Hyde, 2011). Recently, many exiting publications on phylogenetic analyses based on single and multigene analysis of rDNA sequence data have appeared and it is good process to clearify their evolution and connectivity between sexual and asexual morphs. (Lumbsch, Lindemuth & Schmitt, 2000; Schoch et al., 2007; 2009; Kruys & Wedin, 2009; Kruys, Eriksson & Wedin, 2006; Mugambi & Huhndorf, 2009). However, more morphological studies on Dothideomycetes are required to desipher their classification and taxonomy which remained confused. In this study, we revisit the taxonomy of 10 type families from various herbaria (Table 5.1).

Study of history of fungi is important, not only to identify but it will help to understand biodiversity and supporting nature conservation. Fungal herbarium or fungorium is a collection of dried fungi and preserved in herbaria. They are source of historical information necessary for systematist defining and understanding morphological variations within particular species. They also serve as physical reference collection which is impotant for identification and monographic studies (Kirk et al., 2008; Mueller, 1999). In addition, herbarium specimens are source material for chemicals of DNA for molecular analyses, pharmaceutical compounds, ecological signals, and other studies (Arugete, Aldstadt & Mueller, 1998; Mueller, 1999). In order to understand and clearifiy major characters and systematic positions of some interesting Dothideomycete families full descriptions, illustrations and appropriate taxonomic notes are presented in this study.

5.2 Materials and Methods

5.2.1 Observation

Type specimens of the generic type (Table 5.1) were obtained from various herbaria and the collections are studied morphologically. The generic type of some family could not be located during the time frame of this study. However, they are discussed based on the original description and subsequent publications. The herbarium specimens were rehydrated in 5% KOH for 10 minutes and free hand sections prepared under the stereo microscope, mounted in water and later preserved in lactic acid. Micro-morphological characters were examined under a compound microscope (Nikon 80i) fitted with DIC unit, and measurements made with the Tarosoft (R) Image Frame Work.

Table 5.1 Generic Type of Selected Family of Dothideomycetes Obtained from Various Herbaria

Family	Family type	Herbaria
Anttenulariaceae Woron.	*Antennularia* Woron.	LEP
Coccodiniaceae HÖhn. ex O. E. Erikss.	*Coccodinium* A. Massal	BPI & K
Corynesporascaceae Sivan. 1996	*Corynesporasca* Sivan.	IMI
Davidiellaceae Schoch, Spatafora	*Davidiella* Crous & U. Braun	S
Hypsostromataceae Huhndorf 1994	*Hypsostroma* Huhndorf	NY
Mesnieraceae Arx & E. Müll. 1975	*Mesniera* Sacc. & P. Syd	FH
Metacapnodiaceae Hughes & Corlett	*Metacapnodium* Speg.	K
Mycoporaceae Zahlbr. 1903	*Mycoporum* Flot. ex Nyl.	FH
Piedraiaceae Viégas ex Cif., Bat. & S. Camposa	*Piedraia* Fonseca & Leão	K
Teratosphaeriaceae Crous & Braun.	*Teratosphaeria* Syd. & P. Syd.	BPI
Triposporiopsidaceae S. Hughes	*Triposporiopsis* W. Yamam.	FH

5.3 Results and Discussions

Antennulariellaceae Woron., Annls mycol. 23 (1/2):178(1925)

MycoBank: MB 80461

Foliar epiphytes as sooty moulds living on insect exudates. *Subiculum* dark brown to black, effuse, densely velutinous, somewhat dense or velvety, forming on the surface of leaves and twigs. *Hyphae* deeply pigmented at the margin, cylindrical to moniliform or regular, septate, hyphae with smooth or roughened wall. Erect hyphae dark, broad and irregularly anastomosing into a network, forming brown to black turf, dense or scanty. *Ascomata* develop from repeated divisions of hyphae (meristogenous), brown to dark brown, subglobose, obovoid to broadly ellipsoidal or ovoid, sessile, or with a robust stalk, with or without appendages, ostiolate at maturity, arising from terminal or intercalary cells on aerial hyphae. *Peridium* thin-walled, pseudoparenchymatous. *Asci* 8-spored, bitunicate, fissitunicate, small, pyriform to ellipsoidal or clavate. *Ascospores* minute, not numerous, irregularly arranged in the asci, ovoid, more or less oblong, hyaline, 1-3 septate, slightly constricted at the septum, with the upper cell slightly shorter and broader than the lower one, rounded at both ends. *Asexual state*: coelomycetous or hyphomycetous (see under notes).

Notes: *Antennulariellaceae*, a family of sooty moulds, was described by Woronichin (1925) in *Capnodiales*, including six genera with 27 species (Kirk et al., 2008), while Lumbsch and Huhndorf (2010) listed only two genera. Species in the family have a widespread distribution, and are found in warm temperate to tropical locations, where they grow as black sooty molds on plants (Cannon & Kirk, 2007). The asexual states of *Antennulariaceae* have been recorded as pycnidial (*Antennariella* Bat. & Cif., 1963) and hyphomycetous (*Capnodendron* Hughes, 1976; Hughes 2000). In *Antennariella* pycnidia are reported to be small and dark brown, with a smooth or roughened pseudoparenchymatous wall, subglobose to obovoid, terminal on a short stalk or intercalary, somewhat lateral on conidiophores, with a short neck and ostioles at maturity. Conidiogenous cells are rare and not reported by Hughes (2000). Conidia are minute, hyaline, more or less ellipsoidal and 1-celled

(Hughes, 1976). In *Capnodendron* hyphae are dark brown, cylindrical or irregular, septate, smooth or roughened. *Conidiophores* are scattered or gregarious and velutinous, straight or irregularly bent, barely different from aerial hyphae, arising as upright branches or repent hyphae or as modified upturned ends of hyphae and very variable in length, brown to dark brown, more or less cylindrical, smooth to coarsely roughened, with lateral branches terminating in a characteristic conidiogenous cell (Hughes, 1976; Hugehes, 2000). The terminal conidiogenous cell is very characteristic and more or less ovoid with a flat terminal scar left by the fallen conidium, sessile. *Conidia* are brown to dark brown, somewhat pale brown to brown, holoblastic, narrowly clavate to ellipsoidal or fusiform, straight or curved or irregularly curved, slightly constricted at the septa, smooth or coarsely roughened, sometimes with longitudinal striations, gently or abruptly tapered at the base to a flattened or denticulate scar, rounded at the apex or scarred at both ends of conidia in chains. Conidia and conidiophores can anastomose with each other; the asexual state has been found attached to ascostromata, with flexuous hyphal appendages in *Antennulariella.* (Hughes, 1976; Hughes, 2000).

Woronichin (1925) referred this family along with *Capnodiaceae* and *Coccodiniaceae* to *Capnodiales*. *Antennulariellaceae* may differ from these two families in having completely closed ascomata with irregular hyphae and in its conidial states. Woronichin (1925) mention that *Antennulariellaceae* was the most representative family in the order, the two families (*Capnodiaceae* and *Coccodiniaceae*) with ostiolate ascomata, being the most advanced. Hughes (1976) however, reported that the ascomata of *Antennulariella* are ostiolate at maturity. Woronichin (1926) added *Achaetobotrys* as second genus in the *Antennulariellaceae* with two species, both referred to *Limacinia* by Barr (1961) and to *Euantennaria* by Hughes (1972). Hughes (2003) introduced the hyphomycetous sooty mould genus, *Capnofrasera* from New Zealand, Chile, Venezuela, Brazil, Canada and USA in the *Antennulariellaceae*, and compared the genus with *Tomenticola*, *Paratomenticola*, *Sporidesmiopsis* and *Capnondendron*. The family needs a detailed study of both sexual and asexual states to establish the relationships among the various genera. We tentatively include all names mentioned in *Antennulariellaceae* pending further studies.

Type: *Antennulariella* Woron. Trudy Byuro po Prikladnoj Botanik. 8:769-807 (1915).

MycoBank: MB 221.

= *Antennariella* Bat. & Cif., Quad. Lab. crittogam., Pavia 31:22 (1963)

= *Capnodendron* S. Hughes, Mycologia 68(4): 750 (1976)

= *Heteroconium* Petr., Sydowia 3(1-6): 264 (1949)

Foliar epiphytes as sooty moulds living on insect exudates. *Mycelium* brown to dark brown, thin to dense, cylindrical, irregular, septate, slightly constricted at the septa, smooth-walled. *Ascomata* develop from repeated divisions of hyphae (meristogenous), dark brown, sessile, with a pseudoparenchymatous wall, subglobose to globose with a robust stalk, with long cylindrical hyphal appendages, wide at the base tapering to the apex, ostiolate at maturity, terminally or laterally on hyphae. *Peridium* thin-walled, brown to pale brown with *textura angularis*. *Hamathecium* aparaphysate. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical, clavate to ellipsoidal. *Ascospores* overlapping, hyaline, 1-septate, with the upper cell slightly shorter and broader than the lower one, rounded end at both apices. *Asexual state*: coelomycetous, *Antennariella* Bat. & Cif., pycnidial, terminal on long stalk, or lateral or intercalary, dark brown with pseudoparenchymatous walls, subglobose to ovoid, with ostioles. *Conidia* ellipsoidal, hyaline, one-celled. Hyphomycetous asexual state; *Capnodendron* Hughes, mycelium superficial, scattered to densely, pale brown to dark brown, smooth-walled, cylindrical, branched, upright hyphae straight or branched, septate, long, tapering to the apex. Some upright hyphae and their branches may become conidiogenous cells. *Conidia* in simple chains, subglobose to ellipsoidal to cylindrical, brown to dark brown, with or without septa, slightly constricted at the septa, various lengths in multiseptate conidia, with a rounded or slightly scar at one or both ends.

Antennulariella was introduced by Woronichin (1915) and now includes five species (Kirk et al., 2008, Index Fungorum). Hughes (1976) has seen many collections which have characteristics similar with those of the *Type species* as illustrated by Woronichin (1915). Phylogenetic estimates of *Capnodiaceae* and distribution of characters and classifications using character compatibility analysis show that *Antennulariella* and *Acrogenotheca* form one group, this group produce

ascospores which have only one septum at maturity in the ascus, the spore dispersal strategy incorporates delayed stages of hyphal initial germination and growth so that septation and pigmentation develop in steps, usually outside the ascus (Reynolds, 1986).

Antennulariella batistae Hughes (2000) was described as a new species with a hyphomycetous synanamorph *Capnodendron trichomericola*, which is the type of the genus *Capnodendron*. We therefore synonymize *Capnodendron* under *Antennulariella* above. *Antennulariella batistae* is a sooty mould which occurs alongside *Metacapnodium moniliforme* (Fraser) Hughes (1976), *Euantennaria mucronata* (Mont.) Hughes (1972) and *Trichopeltheca asiatica* Batista, Costa & Ciferri (1958) on many hosts and has been reported on more than 80 hosts, including ferns, conifers, monocotyledons and dicotyledons. *Capnodendron trichomericola* is also a synonym of *Antennulariella batistae.*

Antennariella unedonis is regarded as a synonym of *Capnodium=Polychaeton* (*Capnodiaceae*), while *Microxiphium footie* var. *ciliolatum* Sacc. is also listed as a synonym of *Capnodium = Polychaeton* (Species Fungorum 2013). Hughes (2000) lists *Capnodendron* and *Antennariella* as synanamorphs of *A. batistae* while Hughes (1976) links *Achaetobotrys* with *Antennariella*. *Antennulariella concinnata* (Fraser) Hughes (1976) is neotypified, illustrated and described by Hughes (2007) and mentions that *Heteroconium* is a synananmorph. It is therefore synonymized above. There is however considerable confusion in such linkages, as many different sooty mold species may occur on a single host. Linkage by association alone is not good enough evidence and in future studies individual spores should be isolated and subjected to sequence analysis to establish which sexual and asexual states are the same biological species.

Type species: *Antennulariella fuliginosa* Woron., Trudy Byuro Prikl. Bot. 8:769-807 (1915)

MycoBank: MB 224166

Material examined: RUSSIA, Sochi, Kraevskij Mountain. On living leaf of Ilex. 14 May 1913, Woronichin, (Figure 5.1).

Other genera included

Achaetobotrys Bat. & Cif., Saccardoa 2:49 (1963)

Type species: *Achaetobotrys affinis* (L.R. Fraser) Bat. & Cif., Saccardoa 2:49 (1963).

Capnofrasera S. Hughes, N.Z. Jl Bot. 41(1):139 (2003)

Type species: *Capnofrasera dendryphioides* S. Hughes, N.Z. Jl Bot. 41(1):141 (2003).

Key to genera of *Antennulariellaceae*

1. Ascostromata subglobose to globose, ...
   with hyphal appendages ..... *Antennulariella*
1. Ascostromata obovoid to broadly ellipsoidal, ...
   lacking hyphal appendages ..... *Achaetobotrys*

Note. (a). Herbarium specimen. (b) – (d), (h). Hyaphe with ascomata meristogenous. (e) – (f). Ascomata with long chylindrical appendage. (g). Hyphae. (i). Vertical section through ascomata. (j). Ascomata with ostiole. (k). Ascomata with a robust stalk. l). Asci and ascospores (redrawn from Woronichin, 1915). Scale bars: (b) – (d) = 50 μm, (e) – (f), (h) – (i), (k) = 20 μm, (g), (j) = 10 μm.

Figure 5.1 *Antennulariella fuliginosa* Woron. (holotype)

Coccodiniaceae Höhn., ex O.E. Erikss., Op. bot. Soc. bot. Lund. 60: 42 (1981)

MycoBank: MB 80615

Epiphytic or *biotrophic* sooty moulds on leaves and stems, dark globose hyphae covering the surface of the host, cells of ascostromata growing into the host tissue, with or without setae, surrounded by dark septate hyphae, constricted at the septum. *Subiculum* well-developed, especially at the ascomata base, surrounded by very loosely entangled dark hyphae, and superficial, blackish brown, effuse, subglobose or globose brown-walled cells, individual hyphae attached to the ascomata wall. *Ascostromata* developing on the surface of a mycelial pellicle which forms on a leaf or less commonly a stem or twig, scattered or gregarious, sessile on the subiculum or somewhat immersed, globose to subglobose, frequently cupulate when dry, brownish, uniloculate. *Ostiole* is centred in a slightly umbonate portion of the depressed area, well-devevloped with numerous periphyses. *Hamathecium* well-developed with numerous periphyses at the apex and pseudoparaphyses apparent in mature ascoma. *Peridium* thick-walled, inner cells flattened, hyaline to pale brown, outer cells angular, dark, outermost cells globose resembling subiculum cells. *Asci* 8-spored, bitunicate, fissitunicate, saccate, pedicellate, lacking a distinct ocular chamber, thick-walled. *Ascospores* 2-3-seriate, fusiform, ellipsoidal or clavate, with up to six transverse septa or muriform, hyaline when immature and brownish at maturity, slightly constricted at the septa, lacking a mucilaginous sheath. *Asexual state* hyphomycetous (Crous et al., 2007). *Hyphal strands* consisting of brown, globose cells, giving rise to indistinct phialidic loci, producing 1-3 conidia. *Conidia* fusoid-ellipsoidal to clavate, 3-5 septate, constricted at the transverse septa, apex obtuse, base subtruncate.

Coccodiniaceae is a family of sooty moulds. The honey-dew secreted by insects serves as nourishment for the hyphal mats. The family, described by Eriksson (1981), comprises three genera; *Coccodinium* A. Massal., as type species, *Dennisiella* Bat. & Cif. and *Limacinula* Höhn (Lumbsch & Huhndorf, 2010), while Kirk et al. (2008) recognize five genera which include the asexual genera *Bisbyopeltis* Bat. & A.F. Vital and *Microxiphium* (Harv. ex Berk. & Desm.) Thüm. Hughes (1976) referred the sooty moulds to a number of families (i.e. *Antennulariellaceae*,

Capnodiaceae, *Chaetothyriaceae*, *Euantennariaceae*, *Metacapnodiaceae* and *Triposporiopsidaceae*), but did not include the *Coccodiniaceae*.

Eriksson (1981) provided a Latin diagnosis for *Coccodiniaceae* thus validating the family which had been introduced as Coccodiniaceen by Höhnel (1918). Eriksson (1981) also described the type species *Coccodinium* and distinguished it from *Naetrocymbe* which previous authors had incorrectly placed in this genus. Based on *Coccodinium bartschii* A. Massal., the family comprises taxa that grow on dark superficial hyphal mats that grow on honey-dew on plants (i.e. sooty molds).

According to Reynolds (1971) all true *Coccodinium*, *Dennisiella* and *Limacinula* species are characterized by limacinuloid ascomata. A limacinuloid ascomata is defined by Reynolds (1971) as "a dark brown collabent ascomata on living leaves and sometimes other plant parts, developing on a scanty or well-developed subiculum or darkened hyphae usually surrounded in its immediate vicinity by a very loose arrangement of hyphae which appear light to whitish macroscopically and which are individually connected as a component of the lower portion of the fruit body wall". This type of subiculum and ascomata characterizes the family along with the hamathecium of pseudoparaphyses, bitunicate asci which open by a long rostrum, ascospores which are mainly transsepta and possibly the J+ reaction of the hymenium. Eriksson (1981) suggested that a restudy of the species are needed to establish if they are congeneric. The asexual states form on rosettes of phialidic conidiogenous cells from ascospores (Hughes, 1976) or are hyphomycetous and described as *Microxiphium* (Barr, 1987). Schoch et al. (2006), Crous et al. (2007) and Ruibal, Sakayaroj et al. (2009) showed species in *Microxiphium* to be members of *Capnodiaceae* in a phylogeny derived from molecular analysis indicating that *Microxiphium* is polyphyletic as it has already been linked to *Dennisiella* in *Coccodiniaceae*. *Strigopodia* was tentatively included in this family by Barr (1987), but is now accommodated in *Euantennariaceae* (Lumbsch & Huhndorf, 2010).

Winka et al. (1998) observed a culture of *Coccodinium bartschii* which is the type species of the genus, derived from a collection by S. Hughes (CANADA, Ontario, Ottowa, Central Experimental Farm), on branches of *Quercus macrocarpa* Michx., on the ground near the Carling Building, 19 July 1994 (UME30232). DNA was extracted from the culture and herbarium material and the 18S rDNA gene

sequenced. In a phylogenetic tree, *C. bartschii* clustered with the *Dothideaceae* in the MP and NJ tree (<50% and 72%, respectively). In ML tree *Coccodinium* sequences are basal to the *Patellariales* and *Pleosporales*, while *Dothideaceae* is paraphyletic to these orders. Winka et al. (1998) concluded that *C. bartichii* is a member of *Coccodiniaceae* and not related to *Chaetothyriales*, and best accommodated in *Dothideales*. This implies that the pseudoparaphyses in *Coccodiniaceae* are not necessarily homologous with those in *Chaetothyriaceae* and *Herpotrichiellaceae*. In fact, similar structures occur in other unrelated groups of ascomycetes, for instance *Verucariaceae* and *Hypocreaceae*. Crous et al. (2007) sequenced fresh material of *C. bartschii* from Canada, and based on parsimony analysis of the LSU region, *C. bartschii* was distinguished from *Teratosphaeriaceae* and clustered with 100% bootstrap support with *Herpotrichiellaceae* (*Chaetothyriales*). This result was supported by Liu and Hall (2004) based on *RPB2* protein sequences analysis, with *C. bartschii* clustering in the *Chaetothyriales*, which are typified by the presence of short apical sterile hyphal paraphyses. Winka et al. (1998) studied the morphology of *Coccodiniaceae* and found the asexual state resembled a *Capnodendron* species, which is a conidial state of sooty moulds in the family *Antennulariellaceae*. Therefore, the asexual morph of *Coccodiniaceae* may be related to the *Anntennularielaceae* according to its morphology. Colonies of *Coccodinium bartschii* on MEA are slow growing and form erumpent round, black colonies with sparse hyphal growth (Crous et al., 2007). Further colletions and sequence data are needed to sort out this confusion.

Possible asexual states which are hyphomycetous, with mycelium forming a setose pellicle, with aseptate hyphae, which are single celled with curved hyphal tips were noted by Reynolds (1971) and Reynolds and Gilbert (2005) as *Microxyphium* sp. and *Bisbyopeltis* sp. However, as with all sooty moulds one has to take great care in assigning asexual states based on their presence with the sexual state, as numerous species may occur in one sooty mould growth (Faull et al., 2002; Hughes, 2003). *Microxyphium* sp. is characterised by the rosette of phialidic cells found in the mycelium or often as a cortex of hyphae surrounding the mycelial setae. Phylogenetic data indicate that *Microxyphium citri* is a member of *Capnodiaceae* (Chomnunti et al., 2011). *Bisbyopeltis* sp. is characterised by subpellicular areas producing triradiate,

septate conidia, erect setae on hyphae encircled by a cortex of hyphae bearing terminal rosettes of subglobose, phialides which produce an abundance of hyaline conidia in a mucilaginous head (Reynolds, 1971; Reynolds & Gilbert, 2005). Furthermore, conidia of *C. bartschii* on MEA arise from indistinct phialidic loci on globose hyphal cells. Conidia are fusoid-ellipsoidal to clavate, 3–5-septate, constricted at the septa, apex obtuse, smooth, and widest in the upper third of the conidium (Crous et al., 2007). We do not include the asexual states in the key nor do we synonymise them as the connections are not proven.

Type: *Coccodinium* A. Massal., Atti Inst. Veneto Sci. lett., ed Arti, Sér. 2 5: 336 (1860)

MycoBank: MB 1140

Epiphytic or *biotrophic* sooty moulds on the host surface and related with sap feeding insects using the secreted honey dew as a food source. Dark hyphal mats thick, superficial on surface of host. *Ascomata* sessile on a subiculum, or somewhat immersed, globose to subglobose, collapsed when dry and swelling after water is added, brownish, uniloculate, thick-walled, sometimes setose, with periphysate ostioles. *Peridium* thick-walled, inner cells small, flattened, hyaline to pale brown, outer cells angular, somewhat flattened, dark, outermost cells globose, dark, resembling the cells of the subiculum. *Hamathecium* of well-developed and numerous periphyses. *Asci* 8-spored, bitunicate, fissitunicate, saccate, pedicellate, lacking a distinct ocular chamber, thick-walled. *Ascospores* irregularly arranged, ellipsoidal or clavate, fusiform, transversely septate or muriform, with 2-4 transverse septa, central segments with 1-2 longitudinal septa, end segments with 2 angular septa or sometimes with a longitudinal septum, somewhat constricted at septa, hyaline or dark brown, lacking a mucilaginous sheath. *Asexual state* hyphomycetous (Crous et al., 2007). *Hyphal strands* consisting of brown, globose cells, giving rise to indistinct phialidic loci, producing 1-3 conidia. Conidia fusoid-ellipsoidal to clavate, 3-5–septate, constricted at the transverse septa, apex obtuse, base subtruncate, guttulate, smooth, widest in the upper third of the conidium.

Coccodinium and *Naetrocymbe* Körb. have been merged in earlier studies by many mycologists (Rabenhorst, 1863; Arnold, 1858; Stizenberger, 1862; Körber, 1865) who accepted *Naetrocymbe* which had priority, but a few still used

Coccodinium. Höhnel (1918) realized that *Coccodinium* (1860) was validly published earlier than *Naetrocymbe* (1865). *Coccodinium bartschii* was transfered to *Cucurbitaria* by Batista and Ciferri (1957). Morphological characters of *Limacinula* and *Dennisiella* are similar to *Coccodinium*, as all have a hamathecium consisting of periphyses. *Limaciniula* however has hyaline to pale brown ascospores, while in *Coccodinium* the ascospores are dark brown. This taxonomic arrangement needs testing at the molecular level. *Denisiella* differs from *Limacinula* in having only transvers septate ascospores and setose hyphae. This genus may also be congeneric with *Limacinula* and *Dennisiella* (Eriksson, 1981). In the key below we maintain all three genera in *Coccodiniaceae* with reservations, based on the above discusssion.

Type species: *Coccodinium bartschii* A. Massal., Atti Inst. Veneto Sci. lett., ed Arti, Sér. 2 5: 336 (1860)

MycoBank: MB 153614

Material examined: CANADA, Ontario, Ottawa, Central Experimental Farm, near Carling Building. 45º23’ N, 75º42’ W. Alt. c. 60 m. On dead corticated branches of *Quercus macrocarpa* Michx., on the ground. 31 August 1999. S. J. Hughes (no. DAOM 226257) [BPI 858347, ex herb IMI 370066, K(M) 176016], (Figure 5.2).

Other genera included

Denisiella Bat. & Cif., Beih. Sydowia 3: 37 (1962)

Type species: *Denisiella babingtonii* (Berk.) Bat. & Cif., Beih. Sydowia 3: 38 (1962)

Limacinula Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 116: 101 (1907)

Type species: *Limacinula samoënsis* Höhn., Sber. Akad. Wiss, Wien, Math.naturw. Kl., Abt. 1 118: 1200 [44 repr.] (1909)

Key to genera of *Coccodiniaceae*

1. Hyphae with setae, ascospores hyaline……
   with transverse septa only……..*Dennisiella*
1. Hyphae without setae, ascospores mostly coloured, muriform 2
2. Ascospores hyaline to pale brown ..... *Limacinula*
2. Ascospores dark brown ..... *Coccodinium*

Note. (a). Label of herbarium. (b). Ascomata on bark of *Quercus macrocarpa*. (c), (d). Vertical section through ascomata with periphysate. (e). Peridium. (f) – (h). Asci. (i) – (n). Ascospores. Scale bars: (c), (d) =100 μm, (e) – (f) = 50 μm, (g) – (j) = 20 μm, (k) – (n) = 10 μm.

Figure 5.2 *Coccodinium bartschii* A. Massal (Type)

Cladosporiaceae Nann., Repert. mic. uomo: 404 (1934)

MycoBank: MB 80600

= *Davidiellaceae* C.L. Schoch, Spatafora, Crous & Shoemaker, in Schoch, Shoemaker, Seifert, Hambleton, Spatafora & Crous, Mycologia 98(6): 1048 (2007) [2006], MycoBank: MB 504453

Saprobic or *phytopathogenic*. *Ascomata* pseudothecial, gregarious or scattered, inconspicuous, black to red-brown, globose to subglobose, uniloculate, inconspicuous and immersed beneath stromata to superfcial, situated on a reduced stroma, with 1(–3) short, periphysate ostiolar necks; periphysoids frequently growing down into cavity; wall consisting of 3–6 layers of textura angularis. *Ostiole* necks, with numerous periphysoids. *Peridium* thick-walled, composed of many layers of dark brown cells of *textura globosa* or *angularis*. *Hamathecium* of hyaline, septate, subcylindrical pseudoparaphyses, present in mature ascomata. *Asci* 8-spored, bitunicate, fissituicate, subsessile, obovoid to broadly ellipsoid or subcylindrical, straight to slightly curved, with or without short pedicel, with an ocular chamber. *Ascospores* bi to multiseriate, or overlapping fasciculate, hyaline, obovoid to ellipsoid-fusiform, with irregular luminar inclusions, mostly thick-walled, straight to slightly curved; frequently becoming brown and verruculose in asci; thick-walled, 1-septate, constricted at the septum, at times covered by a mucilaginous sheath. Asexual state: Hyphomycetous. *Conidiophores* branched, brown, (genticulata). *Conidiogeous cells* Sympodial orsynchronous, (tretic), dark convex scars, terminal and intercalary in conidiophore, brown. *Conidia* Amero or didymo or phragmo, brown, branched or unbranched acropetal chains, ramoconidia, dry, schzo (Schoch et al., 2006; Seifert, Morgan-Jones, Gams &Kendrick, 2011).

Schoch et al. (2006) introduced a new famiy *Davidiellaceae* to accommodate *Davidiella* with its *Cladosporium* asexual morphs (type species *Davidiella tassiana*, anamorph *Cladosporium herbarum*). Previosuly, Ellis (1971) and Sivanesan (1984) had reported *Cladosporium* as *Mycosphaerella* asexual stages and placed the genus under *Mycosphaerellaceae*. Braun, Crous, Dugan, Groenewald and de Hoog (2003) clearly showed that *Mycosphaerella s.str.* and *Cladosporium herbarum*, the generic type of *Cladosporium* has different phylogenetic lineages. At the same time they showed several *Mycosphaerella*-like sexual stages grouped with *C. herbarum*

possessing ascomata which were very similar to *Mycosphaerella* sect. *tassiana*, hence they introduced a new genus *Davidiella.* This was confirmed by Schoch et al. (2006) who also introduced *Davidiellaceae.* Braun et al. (2003) treated *Cladosporium s.str* along *Davidiella*, and moved some other *Cladosporium*-like species to separate genera, as an example *C. malorum* was moved to *Alternaria* (however Crous, Summerella et al. (2009) moved this species to *Chalastospora*). Hawksworth (2012) proposed to use *Cladosporium* as it is the oldest and most widely used name. Crous, Summerella et al. (2009) also used *Cladosporium* names and used old family name *Cladosporiaceae* instead of *Davidiellaceae.* We also suggest using *Cladosporiaceae* hereafter, as it is older than *Davidiellaceae.* The family comprises only *Cladosporium* hyphomycetous asexual species and its sexual "Davidiella" state.

Type: *Cladosporium* Link, Ges. Naturf. Freunde Berlin Mag. Neuesten Entdeck. Gesammten Naturk. 7: 37. 1816, MycoBank: MB 7681

= *Davidiella* Crous & U. Braun, in Braun, Crous, Dugan & Hoog, Mycol. Progr. 2(1): 8 (2003)

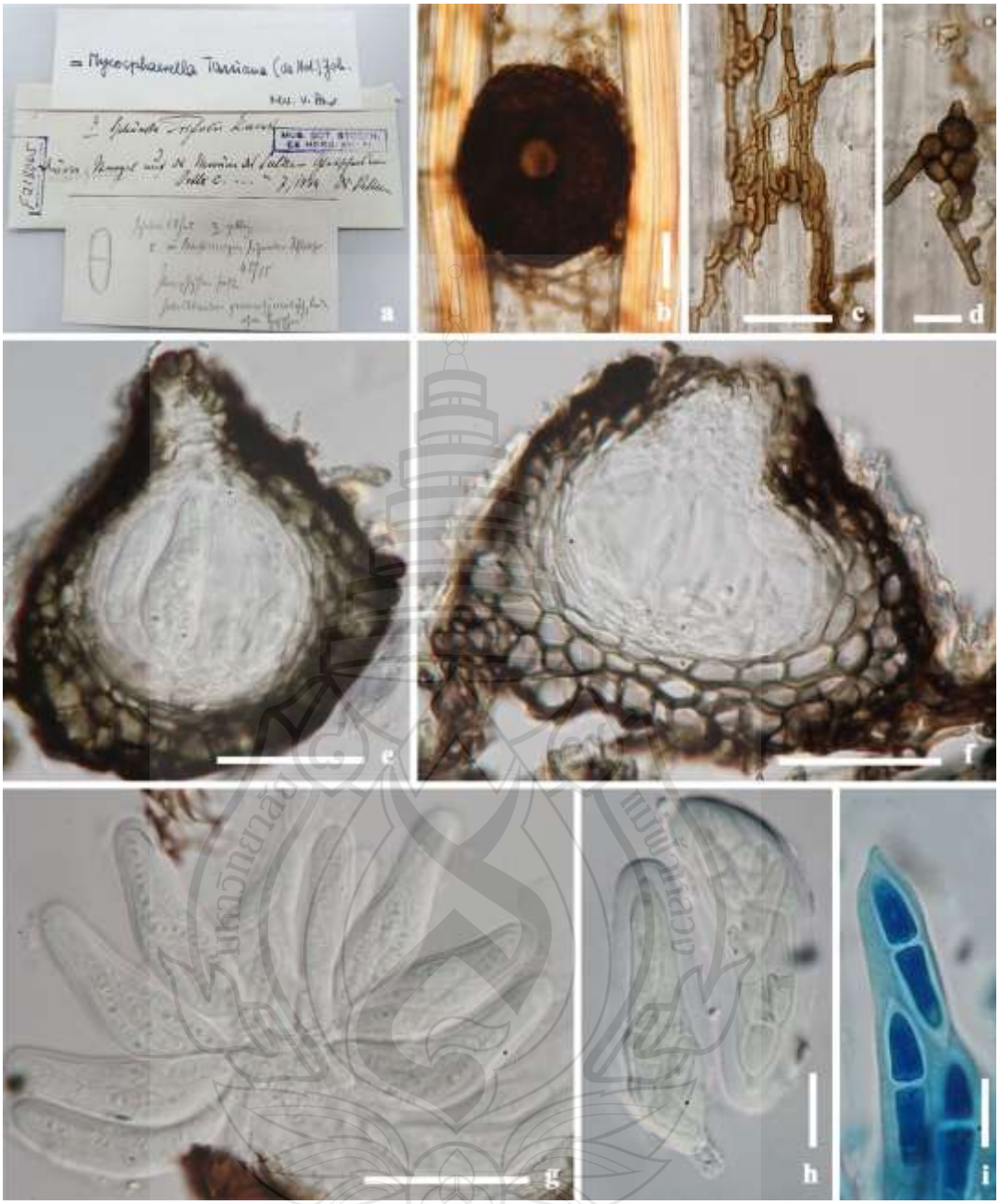
Mycelium superfcial, loosely branched, septate, sometimes constricted at septa, hyaline, subhyaline to pale brown, smooth or almost so to verruculose or irregularly roughwalled,sometimes appearing irregular in outline due to small swellings and constrictions, walls unthickened to somewhat thickened. *Conidiophores* both macro- and micronematous, arising laterally from plagiotropous hyphae or terminally from ascending hyphae. *Macronematous conidiophores* erect, straight to fexuous, somewhat geniculate-sinuous, nodulose or not, unbranched or occasionally branched, pluriseptate, pale to medium brown, older ones almost dark brown, walls thickened, sometimes even two-layered. *Conidiogenous cells* integrated, terminal or intercalary, mono- to usually polyblastic, nodulose to nodose or not, proliferation sympodial, with several conidiogenous loci, mostly situated on small lateral shoulders, more or less protuberant, characteristically coronate (SEM), i.e. with a convex central dome surrounded by a low to distinctly raised rim, appearing to be thickened and somewhat darkened-refractive. *Micronematous conidiophores* hardly distinguishable from hyphae, sometimes only as short lateral outgrowth with a single apical scar, short, conical to almost fliform or narrowly cylindrical, pluriseptate, usually short, subhyaline to pale brown, almost smooth to minutely verruculose or

irregularly rough-walled, 0–3-septate. *Conidiogenous cells* integrated, terminal or conidiophores reduced to conidiogenous cells, narrowly cylindrical or fliform, with a single or two loci. *Conidia* solitary (in *Heterosporium*-like species) to usually catenate, in unbranched or loosely branched chains, straight to slightly curved; small terminal conidia without distal hilum, obovoid to ellipsoid to subcylindrical, aseptate, subhyaline to pale brown; intercalary conidia with a single or sometimes up to three distal hila, limoniform, ellipsoid to subcylindrical, 0–1-septate; secondary ramoconidia with up to four distal hila, ellipsoid to cylindrical-oblong, 0–1(–2)-septate, pale greyish brown or brown to medium brown, smooth to minutely verruculose to verrucose, walls slightly to distinctly thickened, apex obtuse or slightly truncate, towards the base sometimes distinctly attenuated with hila situated on short stalk-like prolongations, hila slightly to distinctly protuberant, coronate structure as in conidiogenous loci, somewhat thickened and darkened-refractive; microcyclic conidiogenesis occurring; primary ramoconidia similar to secondary ramoconidia, except base truncate, uniform with conidiogenous cell, and more subcylindrical in shape (Crous, Summerella et al., 2009).

Type: *Cladosporium herbarum* (Pers.: Fr.) Link, Ges. Naturf. Freunde Berlin Mag. Neuesten Entdeck. Gesammten Naturk. 7: 37. 1816, MycoBank: MB 231458

= *Davidiella tassiana* (De Not.) Crous & U. Braun, in Braun, Crous, Dugan & de Hoog, Mycol. Progr. 2(1): 8 (2003)

Material examined: ITALY, Trentino-Alto Adige, Bolazano On Dürrern Stengel. July 1884, Rehm, F218065, (Figure 5.3).



Note. (a). Herbarium information. (b). Ascomata with ostiole. (c) – (d). Hyphae. (e) – (f). Vertical section through the ascomata and peridium. (g) – (h). Asci. (i). Ascospores strained by cotton blue. Scale bars: (b), (e) – (f) = 100 μm, (c) – (d), (g) = 50 μm, (h) = 20 μm, (i) = 10 μm.

Figure 5.3 *Davidiella* tassiana (De Not.) Crous & U. Braun

Corynesporascaceae Sivan., Mycol. Res. 100(7): 786 (1996)

MycoBank: MB 81981

Pathogenic and *saprobic* on leaves. *Ascomata* cleistothecial with conidiophores arising form cleistothecial walls, solitary to aggregated, sphaerical, superficial on mycelia, or immersed when grown in agar. *Peridium* thin, composed of pale brown, compressed cells forming a *texura globosa* or *angularis*. *Hamathecium* of paraphysoids, with branched, septate, apically free, cylindrical hyphae which break up when asci mature. *Asci* 8-spored, bitunicate, obovoid, deliquescent, short-stalked. *Ascospores* 2-3-seriate, oblong, pale to dark brown, 1-euseptate at the centre and indistinctly 1-distoseptate in the upper and lower cell, with lenticular to globose, granular lumena. *Asexual state*: *Conidiophores* formed on the superficial floccose mycelium and cleistothecial wall, macronematous, mononematous, single or caespitose, erect, simple, straight or flexuous, subhyaline to pale brown, none to few septate, smooth, cylindrical. *Conidiogenous cells* monotretric, integrated, terminal, percurrently proliferating. *Conidia* solitary, obclavate, subhyaline to pale brown, multi-distospetate (Sivanesan, 1996).

Corynesporascaceae was introduced by Sivanesan (1996) with *Corynesporasca caryotae* as the type species, isolated from a decaying leaf of *Caryota urens* collected in Sri Lanka. Sivanesan (1996) linked the sexual (*Corynesporasca caryotae*) and asexual (*Corynespora*) state in culture. *Corynesporascaceae* differs from *Testudinaceae* by its cephalothecioid peridium and hamathecium with cellular pseudoparaphyses (Hawksworth, 1979; Hawksworth & Booth, 1974). *Didymosphaeria* resembles *Corynesporasca* in having brown, 1-septate ascospores, but are rather small, smooth to echinulate and uni-seriately arranged inside the persistent, usually cylindrical asci which are provided with a narrow ocular chamber. The ascomata are immersed and clypeate unlike those in *Corynesporasca*. *Corynesporasca* has asci with wide ocular chamber and lacking a refractive ring. Immature ascospores are 1-septate almost in the middle and appear to be faintly distoseptate in each cell. The ascospore cells have lenticular to globose lumena. The ascospores lack a mucilaginous sheath. While *Massariaceae* have asci with a wide ocular chamber but often surrounded by refractive, non-amyloid ring; the ascospores

are usually distoseptate in some stage of their development, often with lenticular to globose lumena and surrounded by a mucilaginous sheath.

Pyrenulaceae and *Requienellaceae* are similar with *Corynesporasca* in having distoseptate and euseptate ascospores with lumena, but are indistinguishable from *Corynesporasca* by their lichenized habit and peridium comprised of *textura intricata* in *Pyrenulaceae*, or cellular pseudoparaphyses in *Requienellaceae* (Aptroot, 1991). *Typetheliaceae* differs from *Corynesporasca* in having lichenized ascomata which may be aggregated in a pseudostroma, peridium with *textura intricata* and pycnidial asexual state (Aptroot, 1991). *Tubeufiaceae* differs from *Corynesporasca* in having a hamathecium with cellular pseudoparaphyses and submedianly 1-septate hyaline to yellowish brown sheathed ascospores (Rodrigues & Samuels, 1994).

Type: *Corynesporasca* Sivan. Mycol. Res. 100(7): 786 (1996)

MycoBank: MB 27579

Pathogenic and *saprobic* on leaves. *Ascomata* cleistothecial with conidiophores arising form cleistothecial walls, sphaerical, solitary to aggregated, superficial on mycelia, or immersed when grown in agar. *Peridium* thin, composed of pale brown, compressed cells forming a *texura globosa* or *angularis* covered by mycelia and conidiophores. *Hamathecium* comprising paraphysoids, with branched, septate, apically free, cylindrical hyphae which break up when asci mature. *Asci* 8-spored, thick-walled, bitunicate, obovoid, deliquescent, short-stalked, fasciculate and arising to form a hymenium among deliquescent paraphysoids. *Ascospores* 2-3-seriate, oblong, pale to dark brown, 1-euseptate at the centre and indistinctly 1-distoseptate in the upper and lower cell, constricted at the median septum, smooth, often inequitable with a somewhat slightly longer basal cell which possesses an indistinct hyaline area at or near its centre, with three lenticular to globose, granular lumena, the central lumen occupying both the central cells with an indistinct transverse. *Asexual state*: *Conidiophores* formed on the superficial floccose mycelium and cleistothecial wall, macronematous, mononematous, single or caespitose, erect, simple, straight or flexuous, subhyaline to pale brown, none to few septate, smooth, cylindrical. *Conidiogenous cells* monotretric, integrated, terminal, percurrently proliferating, subhyaline to pale brown, smooth, cylindrical, becoming spathulate towards the apex. *Conidia* solitary, obclavate, wide at the truncate base, tapering

towards the apex, subhyaline to pale brown, acrogenous, unbranched, smooth, straight to curved, multi-distospetate. Conidia germinating from the apical end of the terminal cell by means of 1-4 germ tubes (Sivanesan 1996).

Corynesporasca is a monotypic genus with a possible *Corynespora* asexual state described by Sivanesan (1996). *Corynespora* includes 89 species and the genus has a widespread distribution (Kirk et al., 2008), or 173 species epithets (http://www.indexfungorum.org /names/Names.asp) and are saprobes, pathogens, and endophytes on woody and herbaceous plants, other fungi, nematodes, and human skin (Dixon, Schlub, Pernezny & Datnoff, 2009). The generic type of *Corynespora* is *Corynespora mazei* Güssow (current name *Corynespora cassiicola* (Berk. & M.A. Curtis) C.T. Wei). *Corynespora cassiicola* is an important fungus causing target-spot on a wide host range in tropical and subtropical countries, especially *Hevea brasiliensis* (rubber) in Sri Lanka and other countries (Liyanage de, Jayasinghe, Liyanage & Jayaratne, 1986). In Schoch et al. (2009, Figure 2A) *C. cassiicola* and *C. smithii* (Berk. & Broome) M.B. Ellis clustered in Clade K in *Pleosporales* and is basal to *Morosphaeriaceae*, while *C. olivacea* (Wallr.) M.B. Ellis clustered with *Massarinaceae*. *Corynespora* is obviously polyphyletic and requires detailed molecular analysis. We cannot therefore synonymize *Corynesporasca* under *Corynespora* (the older name) at this time, as the types of the two genera may be unrelated. Gemination of ascospores occurs with germ tube arising from the end of the basal cell. A single germ tube may emerge from the base, but single or multiple germ tubes may be produced from any surface area of the upper cell (Sivanesan, 1996).

Type speices: *Corynesporasca caryotae* Sivan., Mycol. Res. 100(7): 786 (1996).

MycoBank: MB 415571

Material examined: SRI LANKA, Kandy, isolate from living leaf of *Caryota urens*, 18 March 1994, J.M. Waller, 4058d, IMI362840a, (Figure 5.4).

Note. (a). Dried culture of herbarium specimen. (b) – (d). Vertical section through cleistothecium. (e). Surface view of cleistothecium wall. (f). Wall with conidiophores. (g) – (h). Peridium. (i) – (j) Asci. (k) – (m). Ascospores. Scale bars: (b) – (c) = 100 μm, (d) – (e), (h) = 50 μm, (f) – (g), (j) = 20 μm, (i), (k) – (m) = 10 μm.

Figure 5.4 *Corynesporasca caryotae* Sivan. (holotype)

Hypsostromataceae S.M. Huhndorf, Mycologia 86: 266 (1994),

MycoBank: MB 81962

Saprobic on wood and bamboo, in tropical terrestrial habitats. *Ascomata* superficial, large, surface roughened or bearing hyphae. *Peridium* coriaceous, multi-layered, pseudoparenchymatous or of small cells *textura globosa*. *Hamathecium* comprising numerous, trabeculate pseudoparaphyses, arising from a central, cellular, columnar structure. *Asci* 8-spored, bitunicate, elongate clavate or cylindrical, long pedicellate, apically rounded, with an ocular chamber fluorescing in Calcofluor, arranged basally. *Ascospores* uniseriate, oblong to ellipsoid, 1-3-septate, brownish-yellow to brown, wall smooth or roughened, with or without appendages or sheath. *Asexual state*: coelomycetous with pycnidia that are "Pleurophomopsis"-like and only known in *Hypsostroma saxicola*.

The family *Hypsostromataceae* was introduced by Huhndorf (1994) for taxa with large superficial ascomata, trabeculate pseudoparaphyses, cylindrical to clavate asci and septate pale brown to brown ascospores. Two tropical genera, *Hypsostroma* and *Manglicola*, were included in the family. *Manglicola* (type species *M. guatemalensis*) was collected from dead roots of *Rhizophora mangle* in Guatemala (Kohlmeyer & Kohlmeyer, 1971). Subsequently collections were made on intertidal prop roots of *Rhizophora apiculata* at Kapong Danay, Brunei (Hyde, 1996), and on the palm *Nypa fruticans* in Thailand (Suetrong, Sakayaroj, Phongpaichit & Jones, 2010). Kohlmeyer and Kohlmeyer (1971) noted a close relationship of *M. guatemalensis* and the *Pleosporales* or *Venturiaceae*. A second species *M. samuelsii* was described by Huhndorf (1994) from Guyana and collected on bamboo culms. *Hypsostroma* was introduced by Huhndorf (1992) for two neotropical wood-inhabiting species *H. saxicola* (type species) and *H. caimitalensis*, collected in Dominican Republic and Venezuela, respectively. "Pleurophompsis"-like pycnidia have been observed in *H. saxixola*.

Manglicola and *Hypsostroma* share the following features, superficial, large, elongate ascomata, short to long stalks, a soft-textured pseudoparenchymatous wall, trabeculate pseudoparaphyses, asci with an ocular chamber and fluorescing ring and pedicellate, basally arranged and fusiform, septate ascospores. Neither genus could be ascribed to a known family/order in the *Dothideomycetes*, although Huhndorf (1992)

opined an affinity to the *Melanommatales* (= *Pleosporales*). Subsequently, Huhndorf (1994) referred both genera to the *Hypsostromataceae*. Subsequently, Suetrong et al. (2010) introduced a new family, *Manglicolaceae*, to accommodate *M. guatemalensis* in the *Jahnulales*, as it did not group in the *Pleosporales* (Suetrong et al., 2010). The position of *Manglicola samuelsii* remains unresolved as no sequence data is available to determine its higher taxonomic rank. Therefore only two *Hypsostroma* species can be assigned to the family *Hyposostromataceae* at the present time while the *Manglicolaceae* (*Jahnulales*) is accepted for the marine species *Manglicola guatemalensis*.

Type: *Hypsostroma* Huhndorf, Mycologia 84: 750 (1992),

MycoBank MB 25538

Saprobic on wood. *Ascomata* clustered on a tormentose subiculum, superficial, obpyriform, stalked, hyaline to pale brown, surface roughened or hairy, with a papillate ostiole. *Peridium* coriaceous, three-layered, of *textura globosa*. *Hamathecium* comprising numerous, narrow, anastomosing, trabeculate pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 4-8-spored, bitunicate, dehiscence unknown, numerous, elongate clavate, very long pedicellate, basal on a columnar structure, apically rounded with an ocular chamber with fluoresces in Calcofluor. *Ascospores* biseriate, oblong to narrowly fusiform, pale brown, 3-septate, disarticulating, with straight germ slit in each part-spore, with no appendages or sheath. *Asexual state*: "Pleurophomopsis"-like, *pycnidia* obpyriform to ampulliformm roughened tuberculate with white crust-like exudate, ostiolate, with prominent broad papilla. *Conidiogenous cells* phialidic, cylindrical to elongate, hyaline, collarettes minute. *Conidia* ovoid, minute, aseptate, hyaline and guttulate.

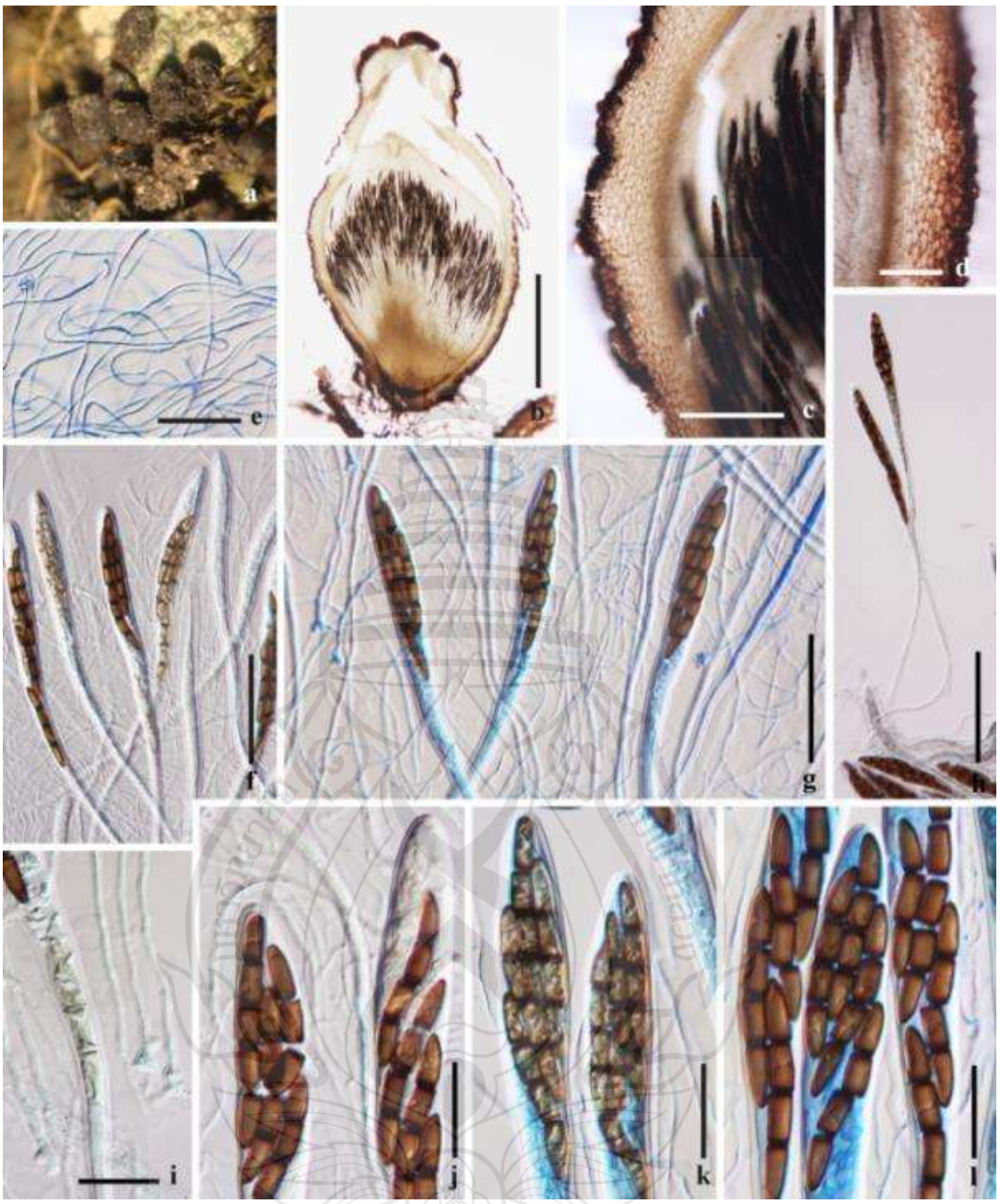
Hypsostroma saxicola and *H. caimitalensis* grouped together in a strongly supported clade (*Hyposostromataceae*) within *Pleosporales*, distinct from other families in the order (Mugambi & Huhndorf, 2009). However, in a more recent phylogenetic study, they group as a weakly supported sister clade to the *Aigialaceae*, *Pleosporales* (Suetrong et al., 2011). Collections made in Kenya (*H. caimitalensis*) and Costa Rica (*H. saxicola*) extend the tropical distribution of these species (Mugambi & Huhndorf, 2009). The distinctive feature of the genus is the obpyriform stalked ascomata, the extremely long pedicels of the asci, the central columnar

structure bearing asci and disarticulating ascospores with germ slits. *Manglicola samuelsii* differs from *Hyposostroma* species in processing longer ascomatal stalks, asci not borne on a central columnar structure, more cylindrical asci, and ascospores that lack germ slits (Huhndorf, 1994). The asexual state has only been reported for *H. saxicola.*

Type species: *Hypsostroma saxicola* Huhndorf, Mycologia 84: 750(1992)

MycoBank MB 360174.

Material examined: DOMINICAN REPUBLIC, Prov. Puerto Plata: Loma Isabel de Torres, Cordillera Septentrional, 19º46’N, 70º42’W. 700 m, humid broadleaf forest just below summit, 20 January 1991, Richard C. Harris, 26462, (Figure 5.4).



Note. (a). Ascoma on rock. (b). Section through Ascoma. (c) – (d). Peridium. (e). Pseudoparaphyses. (f) – (h). Asci. (i). Long stalked with pedicle. (j) – (k). Asci with ocular chamber. (l). Ascospores. Scale bars; (b) = 500 μm, (c) = 200 μm, (h) = 100 μm, (d), (f) – (g) = 50 μm, (e), (i) – (l) = 20 μm.

Figure 5.5 *Hypsostroma saxicola* Huhndorf (holotype)

Metacapnodiaceae S. Hughes & Corlett, in Hughes, N.Z. Jl Bot. 10: 239 (1972)

MycoBank: MB 81649

Foliar epiphytes on plant leaves and stems. *Mycelium* superficial, subiculum spongy, friable, composed of brown to dark brown, moniliform, anastomosing, septate, smooth or coarsely roughened, branched hyphae, sometimes lobed or in the form of hemispherical lumps, hyphae deeply constricted at the septa, thick-walled. *Ascomata* basally immersed in the subiculum, broadly ellipsoidal, or globose, with numerous, hypha–like, septate, appendages. *Peridium* comprising cells of *textura angularis*, at the outer peridium cells brown to dark brown. *Asci* 8–spored, bitunicate, ellipsoidal with pedicel, lacking an obvious ocular chamber. *Ascospores* bi or tri–seriate, ellipsoidal with somewhat conical end cells, 3–spetate, occasionally slightly constricted at the septa, thick-walled, brown to dark brown, rather dark brown at the septa. *Asexual states*: see notes below.

The distinctive hyphae of the *Metacapnodiaceae* are "broad, with dark brown walls, and composed of few or more cells with constrictions at the septa, giving the impression of a monilioid chain, the terminal cells are usually tapered towards the apex" (Hughes & Seifert, 2012). This sooty mould family has a hyphomycetous conidial state, which distinguishes it from *Capnodiaceae* (Hughes, 1972). The family is not well documented, the best accounts are those of Hughes (1972) and Hughes and Seifert (2012). These are classic sooty molds with relatively small (less than 250 um diam.) ascomata and a hyphomycetous asexual state forming on thick, brown to black, dense subicula with a basal pseudoparenchymatous cushion and producing several syanamorphs. The fungus probably gains its nutrition from insect or plant exudates. The hamathecium comprises pseudoparaphyses and asci are numerous, 8–spored, bitunicate, oblong to saccate, short pedicellate and lack an ocular chamber. *Ascospores* are ellipsoidal, brown, 3–septate with thick septal bands, with somewhat conical ends.

The asexual state also occurs on the subiculum and comprise hyphae with broad, dark brown cells, and chains of globose monilioid–like cells with strong constrictions at the septa. The terminal cells are often conspicuously tapered near the apex and in some species schizolytically disarticulating cells can acts as propagules

(Hughes & Seifert, 2012). *Hyphosoma* may be the appropriate name for such propagules according to Hughes and Seifert (2012). Asexual states have been reported in *Capnophialophora* and also *Capnocybe*, *Capnosporium*, *Hormiokrypsis* and *Hyphosoma*. All *Metacapnodiaceae* species have a *Capnophialophora* asexual state which have plump, ampulliform phialides on the narrowing parts of the monilioid conidiophores and produce small ameroconidia. Synanamorphs include *Hormiokrypsis* with solitary dry stauroconidia, *Capnocybe* with slimy heads of phragmoconidia, and *Capnosporium* with solitary, dry phragmoconidia which is produce phialides and microconidia (Batista & Nascimento 1957; Hughes, 1966; Hughes & Seifert, 2012). However, it is likely that more than one species will grow on the subiculum as with other sooty molds and care must be taken in interpretation (Chomnunti et al., 2011; Hughes & Seifert, 2012).

Type: *Metacapnodium* Speg., Physis, B. Aires 4: 288 (1918)

MycoBank: MB 3137

Foliar epiphytes as typical "sooty moulds" comprising black mycelium masses covering the twigs as subicula. *Mycelium* comprising dense, brown to dark brown, friable, spongy, comprising septate hyphae, which are deeply constricted at the septa, thick–walled, moniliform, and anastomosing. *Ascomata* relatively small, less than 200 μm diam, basically immersed in the subiculum, broadly ellipsoidal or globose, with numerous, septate, hypha–like appendages. *Peridium* comprising 3-4 layers of cells forming a *textura angularis*, inner cells hyaline to pale brown, outer layer cells brown to dark brown especially at the outside. *Asci* 8–spored, bitunicate, ellipsoidal, short pedicellate, ocular chamber not observed. *Ascospores* bi or tri–seriate, ellipsoidal with somewhat conical end cells, 3–septate, some slightly constricted at septa, thick–walled, brown to dark brown, rather dark brown at the septa, smooth–walled. *Asexual states* named as "Capnophialophora": *Phialides* develop on ascospores and monilioid hyphae, which is more or less subsphaerical, ellipsoidal to cylindrical, tapered or slightly flared, pale brown to brown. Phialides occurring singly or in pairs on hyphae or on apex of ascospores, globose, pale brown to brown, with collarette, hyaline; phialoconidia not observed.

The asexual states of *Metacapnodium* comprise conidiogenous cells as phialides on moniliform hyphae, on ascostroma initials, on ascospores or on germ

tubes. (Sivanesan, 1984). All species of *Metacapnodium* produce *Capnophialophora* phialides (Hughes & Seifert, 2012), some species may produce other asexual states which have variously been named as symopodioconidia (*Capnobotrys*), as those with or without poroconidia (*Capnosporium*), or sympodioconidia (*Capnocybe*) (Hughes 1966). Seven New Zealand taxa of *Metacapnodium* which produce a *Capnobotrys* asexual state were illustrated and briefly described by Hughes (1966). Most species have *Capnophialophora* (Hughes, 1966) asexual states and a few also produce *Capnosporium* states (Hughes, 1976). Sooty moulds normally grow mixed together and caution must be taken when interpreting data. Microscopic examination by using collodion technique has so far been used to clarify relationships between morphs. No *Metacapnodiaceae* species have been obtained in culture and no sequence data is deposited in GenBank (Hughes & Seifert, 2012). There are eleven epithets known for *Metacapnodium* (Kirk et al., 2008) such as *Metacapnodium crassum* (Pat.) S. Hughes, *M. dennisii* S. Hughes, *M. dingleyae* S. Hughes, *M. fraserae* (S. Hughes) S. Hughes, *M. guava* (Cooke) S. Hughes, *M. juniper* (W. Phillips & Plowr.) Speg., *M. moniliforme* (Fraser) S. Hughes, *M. quinqueseptatum* (Barr) S. Hughes, *M. smilacinum* (Mendoza) S. Hughes, *M. spongiosum* S. Hughes with *Capnocybe spongiosa* (Hoerl.) S. Hughes as asexual state and *M. succinum* (Dörfelt, A.R. Schmidt & J. Wunderl.) Rikkinen.

Type species: *Metacapnodium juniperi* (W. Phillips & Plowr.) Speg., Physis, B. Aires 4: 288 (1918)

MycoBank: MB 212907

≡ *Capnodium juniperi* W. Phillips & Plowr., Grevillea 13(no. 67): 75 (1885)

Material examined: SCOTLAND, Moray coast, Forres, on bark of Juniper twigs, 2 June 1882, Rev. Dr. Keith ex herb. C.B. Plowright, K(M)164026, (Figure 5.6, Figure 5.7).

Other genera included

Capnophialophora S. Hughes, N Z J Bot 4: 52 (1966)

Type species: *Capnophialophora fraserae* S. Hughes, N.Z. Jl Bot. 4: 352 (1966)

Capnocybe S. Hughes, N Z J Bot 4: 35 (1966),

Type species: *Capnocybe fraserae* S. Hughes, N.Z. Jl Bot. 4: 336 (1966)

Capnobotrys S. Hughes N Z J Bot 8: 05 (1970), (Figure 5.9).

Type species: *Capnobotrys neesii* S. Hughes, N.Z. Jl Bot. 8(2): 205 (1970)

Capnosporium S. Hughes, Mycologia 68: 52 (1976),

Type species: *Capnosporium moniliforme* S. Hughes, Mycologia 68(4): 752 (1976)

Hormiokrypsis Bat. & Nascim. Anais Soc. Biol. Pernambuco 15: 45 (1957),

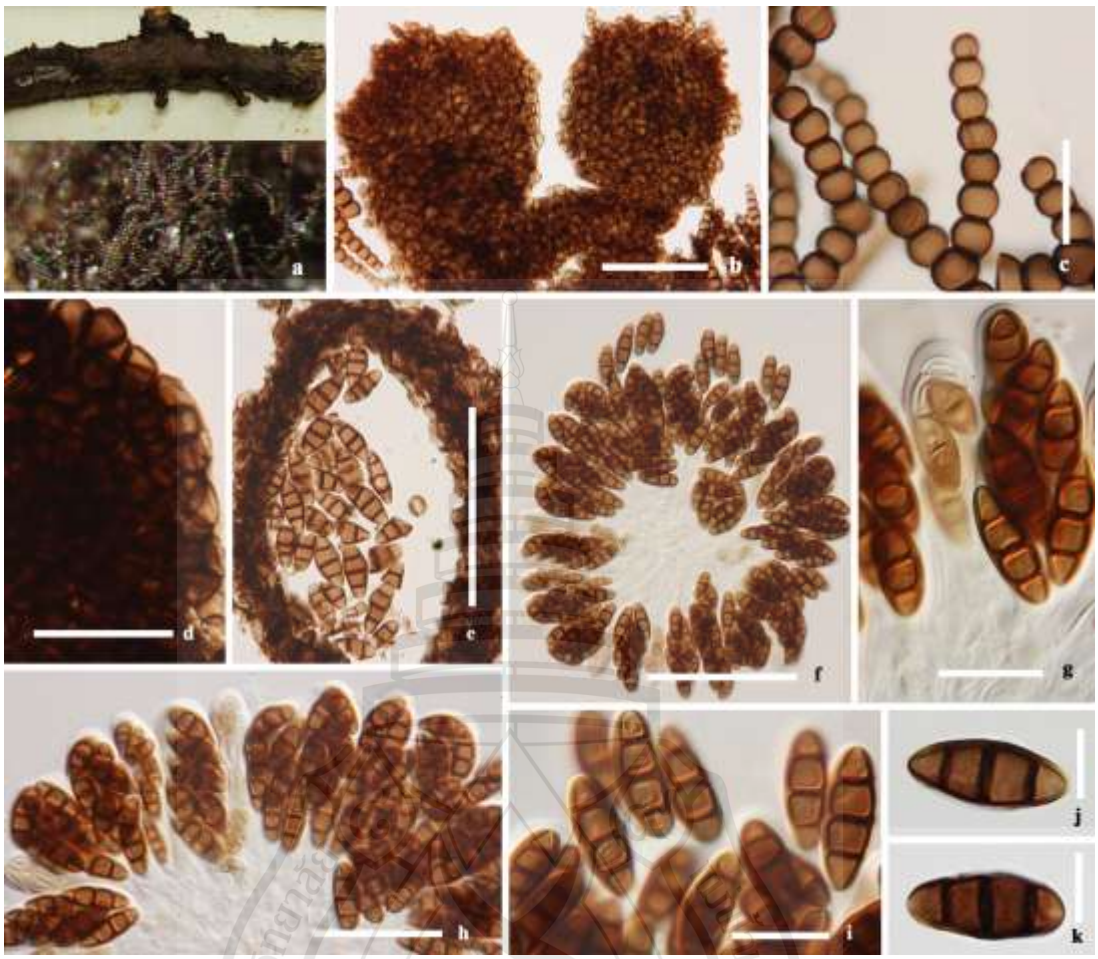
Type species: *Hormiokrypsis libocedri* Bat. & Nascim., Anais Soc. Biol. Pernambuco 15(2): 346 (1957), (Figure 5.8).

Hyphosoma Syd., Annls mycol 22: 15 (1924)

Type species: *Hyphosoma hypoxyloides* Syd., Annls mycol. 22(3/6): 315 (1924)

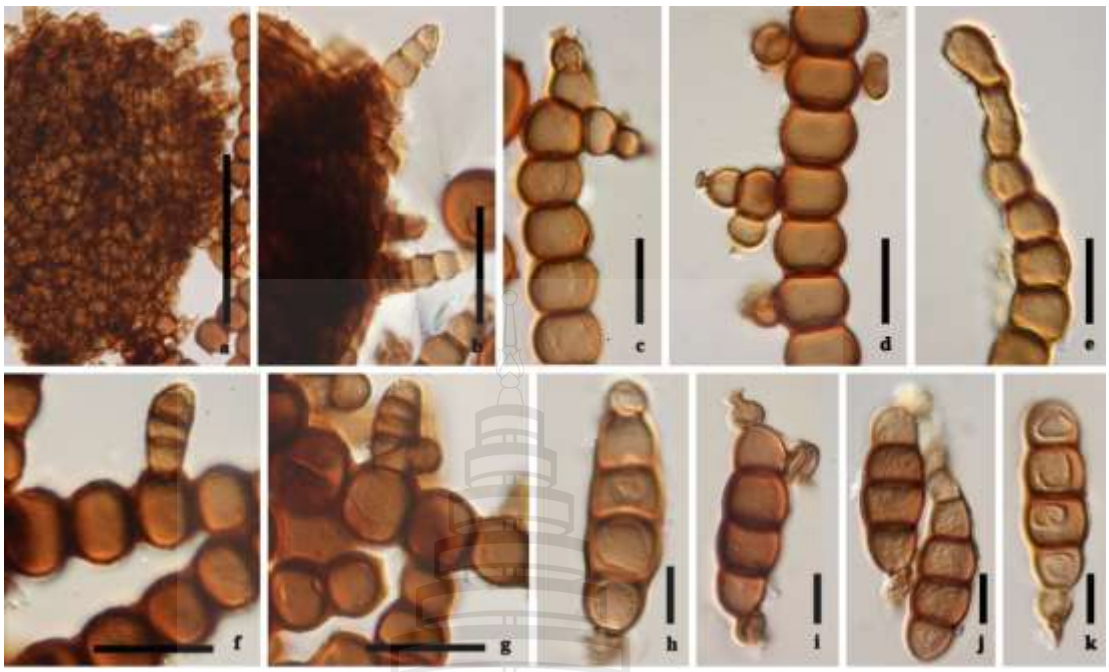
Key to genera of *Metacapnodiaceae*:

1. Conidia born on synnemata, sympodioconidia.....*Capnocybe*
1. Conidia not born on synnemata 2
2. Conidiogenous cells phialides.....3
2. Conidiogenous cells not phialides 4
3. Conidiogenous cells phialides, conidia hyaline.....*Metacapnodium*
3. Conidiogenous cells phialides, conidia pale brown ..*Capnophialophora*
4. Conidia phragmospores 5
4. Conidiogenous cells tretic, conidia staurospores, brown..*Hormiokrypsis*
5. Conidiogenous cells monoblastic with poroid scars, ...
   conidia phragmospores, brown.....*Capnosporium*
5. Acrogenous moniliform conidia, brown..... *Hyphosoma*



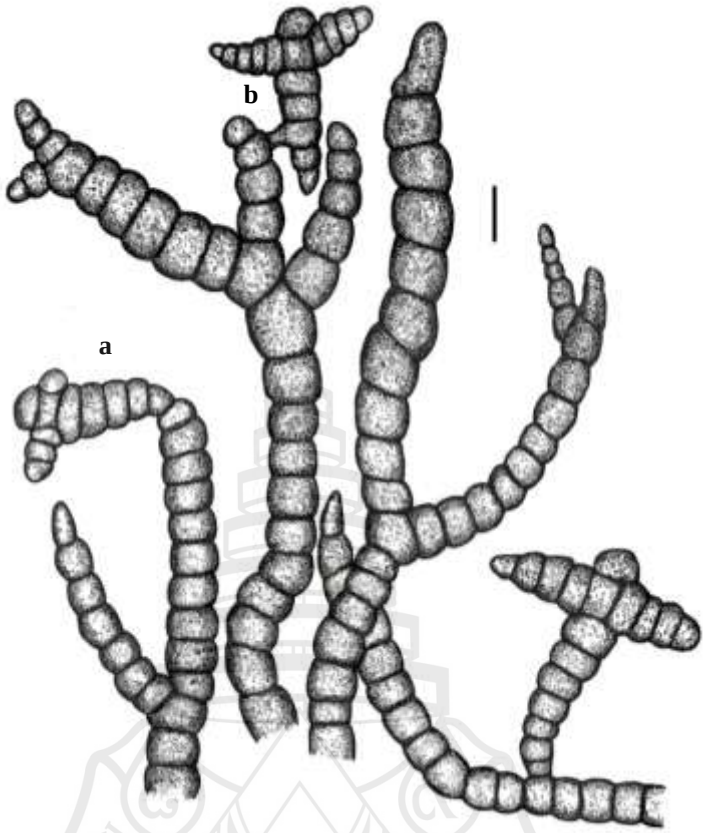
Note. (a). Sooty mould-like appearance on bark of *Juniperus communis*. (b). Ascomata. (c). Monilioid hyphae. (d). Ascoma wall. e Section through ascoma. (f) – (h). Asci. (i) – (k). Ascospores. Scale bars: (b), (e), (f) =100 μm, (c), (d) =50 μm, (h), (i) = 20 μm, (j), (k) =10 μm.

Figure 5.6 *Metacapnodium juniperi* (W. Phillips & Plowr.) Speg. (holotype)



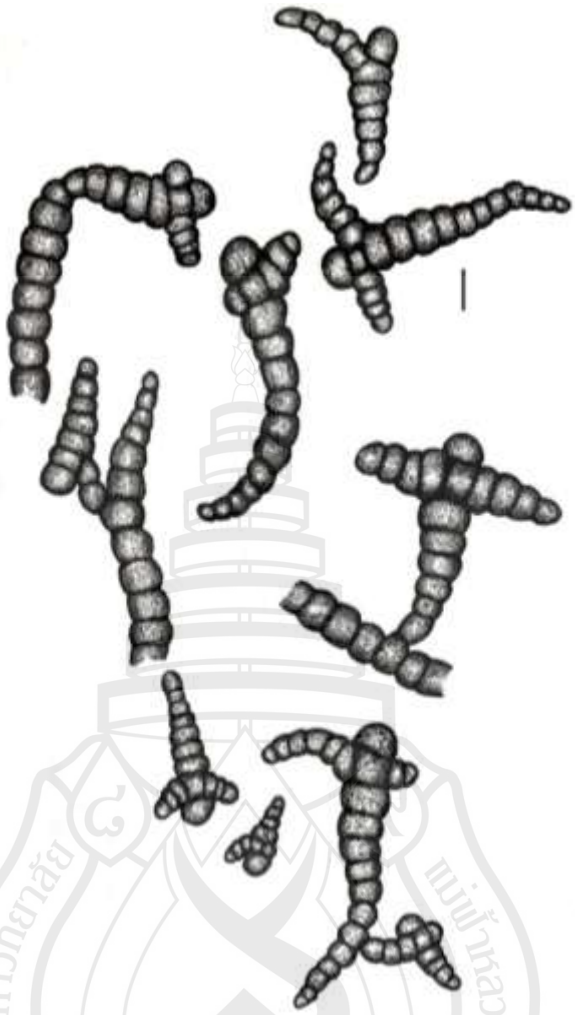
Note. (a), (b). Ascoma wall with hypha–like appendages. (c) – (e) *Capnophialophora* state, phialides with collarette developing on hyphae. (g) Conidiophores. (h) – (k) Phialides develop on ascospores. Scale bars: (a) =100 μm, (b) – (g) =20 μm, (h)–(k) = 10 μm.

Figure 5.7 *Metacapnodium juniperi* (W. Phillips & Plowr.) Speg. (holotype)



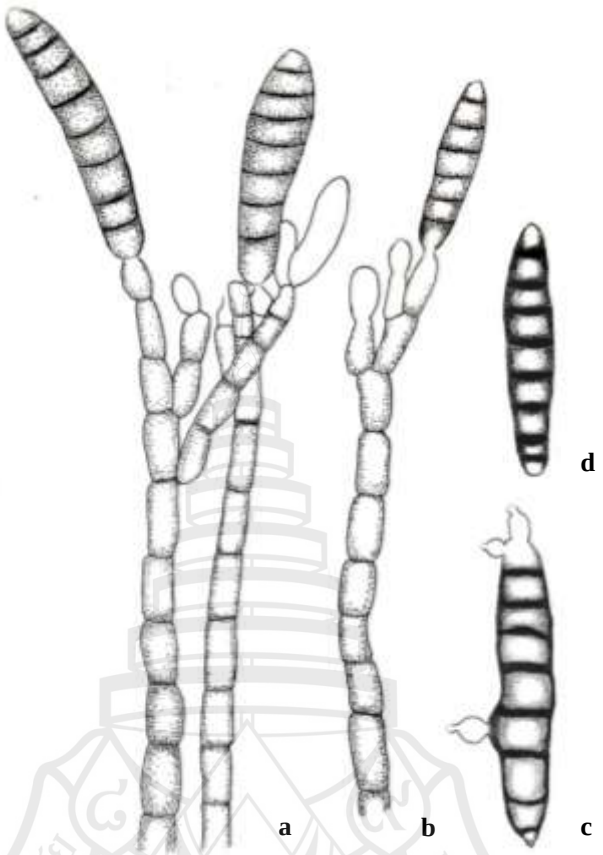
Note. Conidia and hyphae. a lateral. b origin. Scale bar: 20 μm. (Redrawn from Batista and Nascimento, 1957).

Figure 5.8 *Hormiokrypsis libocedri* Bat. & Nasc



Note. Free conidia of *Hormiokrypsis libocedri* Scale bar: 20 μm (Redrawn from Batista AC.and Nascimento ML., 1957).

Figure 5.9 *Hormiokrypsis libocedri* Bat. & Nasc



Note. (a), (b). Branched ends of synnematous hyphae with sypmodulae and sympodioconidia. (c). Sympodiconidium baring *Capnophialophora* phialides. (d). Sympodioconidia. (Redrawn from Hughes, 1966).

Figure 5.10 *Capnocybe fraserae* S. Hughes

Mesnieraceae Arx & Müller, Stud. Mycol. 9:94 (1975)

MycoBank: MB 80998

Parasitic on leaves, causing necrotic patches, or saprobic. *Mycelium* hyaline, developing within host tissue. *Ascomata* densely gregarious, immersed, spherical or flattened or globose to subglobose, yellowish green (brightlu coloured?), uniloculate with numerous asci and pseudoparaphyses. *Ostiole* present or lacking, usually seen apically opening with a pore, or widened and large hole in mature ascomata, erumpent through the upper epidermis, somewhat comprising elongated columnar cells. *Peridium* thin-walled, hyaline, composed of many layers of flattened *textura angularis* or comprising a few layers of elongate cells. *Hamathecium* comprising numerous, hypha-like, hyaline, filamentous, septate pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 4–24 spored, bitunicate, cylindrical to clavate or elongate ellipsoidal, short pedicel, pedicellate, inner membrane thickened in the upper part, apex rounded with or without ocular chamber. *Ascospores* dark brown to brown, or dark reddish-brown, ellipsoidal, aseptate or 1–septate constricted at the septum, thick–walled, with or without a mucilaginous sheath. *Asexua*l state unknown (Saccardo & Sydow, 1902; Eriksson, 1981; Hyde, 1996).

The family was introduced by Arx and Müller (1975) with *Mesniera rottlerae* (Racib.) Sacc. & P. Syd., as the type species. *Mesnieraceae* includes the genera *Bondiella*, *Mesniera* and *Stegasphaeria* (Kirk et al., 2008) while Lumbsch and Huhndorf (2010) include four genera with the addition of *Helochora*. *Bondiella* was observed, redescribed and illustrated by Hyde (1996) and is considered a good member in *Mesnieraceae* (Pirozynski, 1972; Eriksson 1981). *Helochora* was isolated from *Puya* sp. in Chile, and described as a new genus by Sherwood (1979) and was accommodated in *Polystigmataceae*, and has ornamented spores which are uniseriate in the asci. We observed the type specimen of *Helochora hypertropha* Sherwood and considered that *Helochora* does not belong to *Mesnieraceae* and transfer it to *Didymosphaeriaceae*.

Type: *Mesniera* Sacc. & Syd. Syll. Fung. 16: 440 (1902)

MycoBank: MB 3134

Parasitic on leaves, causing necrotic patches, or saprobic. *Ascomata* densely gregarious, immersed in yellowish-green necrotic patches, with radial arrangement, ring or spirals with progressively younger ascomata towards periphery, apically opening with a pore, or widened and large hole in mature ascomata. *Peridium* thin-walled, hyaline, composed of flattened *textura angularis*. *Hamathecium* comprising numerous, hypha-like, hyaline, filamentous, septate pseudoparaphyses. *Asci* 16-spored, bitunicate, cylindrical to clavate, short pedicellate, lacking an ocular chamber or apical ring. *Ascospores* ovoid to ellipsoidal, dark brown, 1–celled, without a mucilaginous sheath. (von Arx & Müller, 1975; Eriksson, 1981).

Type species: *Mesniera rottlerae* (Racib.) Sacc. & P. Syd. [as 'rotlerae'] Syll. fung. (Abellini) 16: 441 (1902)

MycoBank: MB 210494

≡ *Anthostomella rottlerae* Racib. Parasit. Alg. Pilze Java's (Jakarta) 2: 11 (1900)

Eriksson (1981) examined several collections of *Mesniera* and all had concentric rings of ascomata in necrotic patches, wide pores usually containing mature asci with ascospores, and mature ascomata present on both of the upper and lower side of the leaves. Unfortunately, we could not obtain a specimen of the type species of *Mesniera rottlerae* (≡ *Anthostomella rottlerae*), which may be lost, but we have observed *Bondiella palmicola* Piroz., *Stegasphaeria pavonina* Syd. from K and S respectively, and illustrate these here.

Other genera included

Bondiella Piroz., Mycol. Pap. 129: 6 (1972), MycoBank: MB 625

Type species: *Bondiella palmicola* Piroz., Mycol. Pap. 129: 6 (1972)

MycoBank: MB 309834

Material examined: TANZANIA, Kigoma, Kakombe, on fallen fronds of *Elaeis guineensis*, 19 December 1963, K.A. Pirozynski M26c, IMI 105789c, (Figure 5.11).

Ascomata solitary, immersed, subglobose, brown to dark brown, neck erumpent thought the upper epidermis. *Ostiole* present, canal contains hyaline

periphysis. *Peridium* thin-walled, comprising cells of *textuara angularis*, fusing with host at the outside. *Hamathecium* comprising numerous, cylindrical, septate pseudoparaphyses. *Asci* 24–spored, multi-seriate, bitunicate, cylindrical to clavate, short pedicellate, with ocular chamber. *Ascospore* 2–celled, constricted at septum, ellipsoidal, reddish-brown, with mucilaginous sheath.

Stegasphaeria Syd. Annls mycol. 14(5): 362 (1916)

Type species: *Stegasphaeria pavonina* Syd. Annls mycol. 14(5): 362 (1916),

MycoBank: MB 139864

Material examined: PHILIPPINES, Laguna, Mount Maquiling, near Los Baños, on living leaf of *Macaranga* sp. 7 March 1914, C.F. Baker no.4032, (Figure 5.12).

Spots distinctly and mumerous, spiral or radial partern on upper and lower side of leaf, yellow or yellowish-brown or greyish; stromata epiphyllous, black, blight. *Ascomata* densely gregarious, immersed, spherical, flattened-globose, erumpent through the upper epidermis, yellowish-brown, *Peridium* thin-walled, hyaline, composed of many layers of flattened with *textuara angularis*. *Ostiole* present, thick neck or usually seen apically opening with a pore. *Asci* not seen, but Sydow and Sydow (1916) report that the asci 4–8 spored, uniseriate, generally cylindrical, apex thickened and base rounded, short pedicelate with hyaline pseudoparaphyses. *Ascospores* 2-celled, consticted at septum, broadly ellipsoidal, immature ascospores yellow or yellowish become blackish-brown when mature.

Key to genera of *Mesnieraceae*:

1. Ascospores 1-celled, asci 16-spored ..... *Mesniera*
1. Ascospores 2-celled 2
2. Asci 4-8 spored .....*Stegasphaeria*
2. Asci 24-spored .....*Bondiella*

Note. (a). Ascomata semi immerges on host. (b). Ostiole canal with hyaline hamathecium. (c). Vertical section of ascomata. (d). Peridium. (e) – (h). Bitunicate asci. (i) – (j). Ascospores. (k). Ascospores strained with Indian ink reagent. Scale bars: (c) = 50 μm. (b) = 50 μm. (d) – (j) = 20 μm. (k) = 10 μm.

Figure 5.11 *Bondiella palmicola* Piroz. (holotype)

Note. (a). Peridium. (b). Ostiolate ascomata on host. (c). Vertical section of ascomata. (d), (e). Peridium. (f), (g). Ascospores 2 cells. Scale bars: (c) = 100 μm. (d) – (e) = 20 μm a, (f) – (g) = 10 μm.

Figure 5.12 *Stegasphaeria pavonina* Syd. (holotype)

Mycoporaceae Zahlbr., in Engler & Prantl, Nat. Pflanzenfam., Teil. I (Leipzig) 1*: 77 (1903)

MycoBank: MB 81042

Lichenized or rarely not lichenized, symbiotic with algae, growing on bark of trees or on stones, lichen thallus when present thin and often not clearly visible with mainly *Trentepohlia* algal components. *Hyphae* thick, almost hydnoid-corticioid, yellowish, consisting of loose, branched, with finely verrucose, cells born at tips of hyphae resembling blastospores. *Ascostromata* composed of pale mycelium, loose, copiously branched. *Locules* scattered, aggregated, botryose or immersed in a ascostroma, sphaerical or conical, erumpent or superficial, black, globose to subglobose or irregular, multiloculate, locules without wall of their own, containing numerous asci. *Ostiole* forming an apical pore or an elongated channel. *Asci* 8–spored, bitunicate, fissitunicate, cylindrical, obclavate or thickest in the middle, with long tapering ocular chamber, with short–pedicel. *Ascospores* irregularly arranged in asci, ellipsoidal, fusiform to clavate with upper hemispore broader, muriform, not constricted at the septa.

Mycoporaceae is of uncertain taxonomic placement in Dothideomycetes, it is monotypic taxon and contains a single genus *Mycoporum* with a widespread distribution, and is saprobic on woody tissue; several species are apparently non–lichenized (Cannon & Kirk, 2007; Kirk et al., 2008). Von Arx & Müller (1975) mention that the typical characters of this family are unclear. It is difficult to distinguish the genus from *Pleosporaceae* as some genera are intermediate as they have a lichen habit which is difficult to recognize. Lumbsch (1999) studied the structure and development of ascomata in *Mycoporum elabens*, and placed this family in *Dothideales sensu stricto*.

Type species: *Mycoporum* Flot. ex Nyl., Mém. Soc. Sci. nat. Cherbourg 3: 186 (1855)

MycoBank: MB 3337

Lichenized or rarely not lichenized, symbiotic with algae, growing on bark of trees or on stones, lichen thallus when present thin and often not clearly visible with mainly *Trentepohlia* algal components. *Ascostromata* composed of pale mycelium, loose, copiously branched. *Hyphae* thick, almost hydnoid-corticioid, yellowish,

consisting of loose, branched, with finely verrucose, cells born at tips of hyphae resembling blastospores. *Locules* scattered, aggregated, botryose or immersed in a ascostroma, sphaerical or conical, erumpent or superficial, black, globose to subglobose or irregular, mutiloculate, locules without wall of their own, containing numerous asci. *Ostiole* forming an apical pore or an elongated channel. *Peridium* of pseudoparenchymatous cells, thin-walled, pale brown to brown, hyaline, hymenium often gelatinous, comprising of *textura globosa*. *Pseudoparaphyses* filiform, hyaline, often septate. *Asci* 8–spored, bitunicate, fissitunicate, cylindrical, obclavate or thickest in the middle, ectotunica thin; endotunica very thick in upper half of ascus, thin in lower half without ring structures, with long tapering ocular chamber, with short–pedicel. *Ascospores* ellipsoidal, fusiform to clavate with upper hemispore broader, variably septate; transverse septa and sometimes longitudinal septa with divided by 5–7 transsepte and one longiseptum in two or all segments, not constricted at the septa, immature hyaline with thin gelatinous sheath asci firmly enclosed in strongly reticulate becoming brownish at maturity, irregularly arranged in asci. *Asexual state*: Unknown.

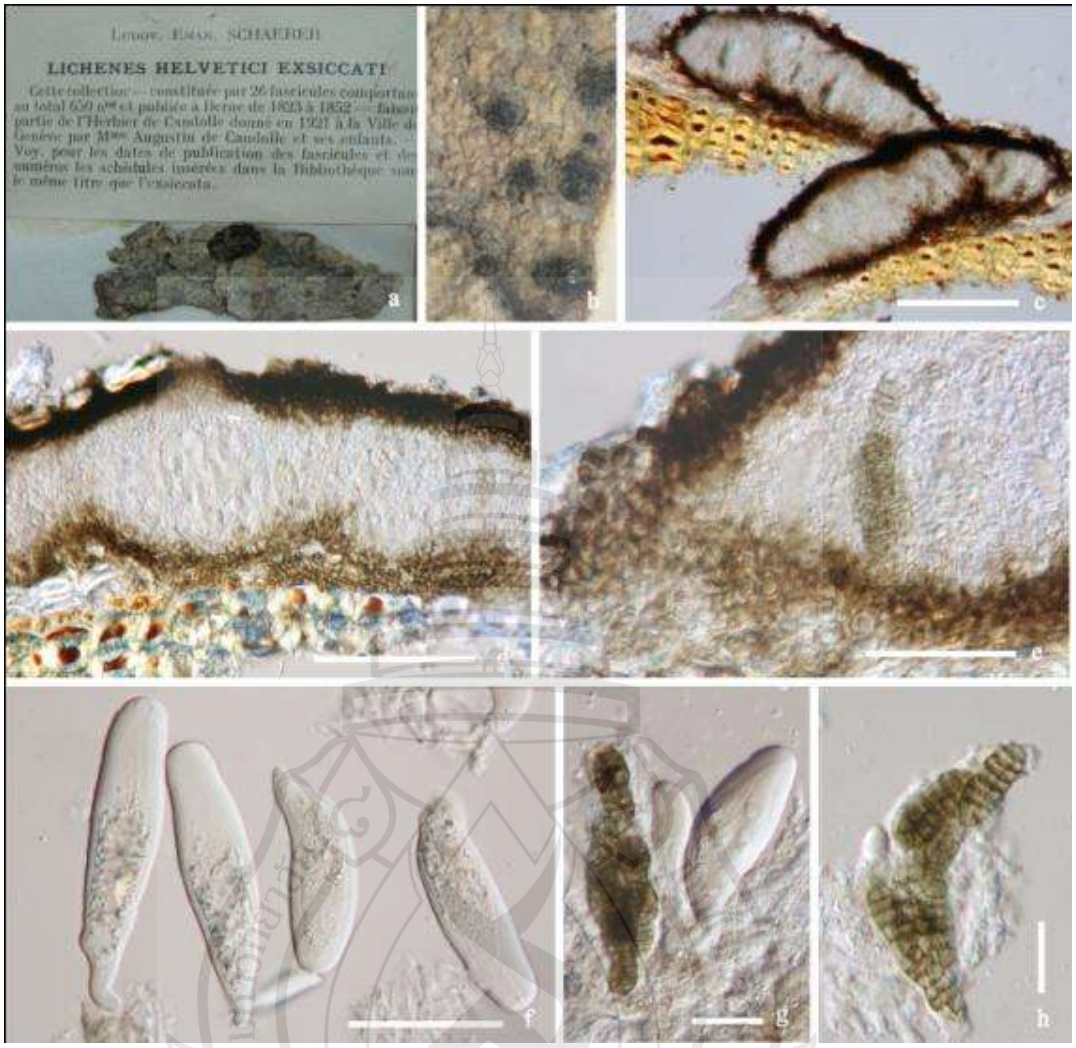
The systematic position of *Mycoporum* has been unclear and a discussion is provided in Eriksson (1981). Lumbsch (1999) interpreted this genus as being non–lichenized with algal cells found in the surroundings of the ascomata; they did not have close contact with the fungal hyphae. Lumbsch (1999) supported Harris (1973) and Poelt (1969) who had observed *M. elabens* and considered it as non–lichenized, while Eriksson (1981) had seen old material and unable to find any algal cells near ascomata or in surrounding the mycelium. Eriksson (1981) accepted *Mycoporaceae* with two species in *Mycoporum*; *M. elabens* and *M. pycnocarpum* Nyl.

Type species: *Mycoporum elabens* (Schaer.) Flot. ex Nyl. [as '*elabeus*'], *Act. Soc. linn. Bordeaux*, Trois. sér. **21**: 417 (1856)

MycoBank: MB 395757

≡ *Lecidea elabens* Schaer., sect. 4–5: 199 (1833)

Material examined: SWITZERLAND, Wachseldorn, on bark, Fries no. 232. G00110803, (Figure 5.13).



Note. (a). Herbarium specimen. (b). Superficial ascomata on host. (c) – (e). Vertical section through ascomata and peridium. (f) – (h). Immature asci and ascospores. Scale bars: (c) = 200 μm, (d) = 100 μm, (e) – (f) = 50 μm, (g) – (h) = 20 μm.

Figure 5.13 *Lecidea elabens* Schaer

Teratosphaeriaceae Crous & U. Braun, Stud. Mycol. 58: 8 (2007)

MycoBank: MB 504465

Saprobic or pathogenic on leaves and stems of various hosts. *Ascomata* pseudothecial, immersed to superficial, frequently situated in a stroma of brown pseudoparenchymatal tissue, globose, unilocular, papillate, ostiolate. *Ostiolar canal* periphysate. *Peridium* comprising several layers of brown-walled cells of *textura angularis*, inner layer comprising flattened, hyaline cells. *Hamathecium* mostly absent, when present consisting of subcylindrical, branched, septate, anstomosing pseudoparaphyses. *Asci* 8–spored, bitunicate, frequently with multi-layered endotunica, sessile, obclavate to globose or saccate, narrowing to a rounded and slightly thickened apex, pedicellate, ocular chamber mostly well defined. *Ascospores* ellipsoid-fusoid to obovoid, 1-septate, hyaline, but becoming pale brown and verruculose, frequently covered in mucoid sheath. *Asexual states*: Hyphae submerged to superficial, disarticulating or not into arthroconidia, or forming endoconidia, pale to dark brown, thick-walled, sphaerical or cylindrical, smooth to verruculose. *Conidiomata* pycnidial to acervular, well–developed, enclosed by host tissue or erumpent, brown to black, fasciculate to sporodochial or solitary. *Conidiophores* absent or short, brown, subcylindrical, solitary, with or without penicillate, branched or unbranched conidiogenous apparatus, fasciculate to synnematous or sporodochial. *Conidiogenuous cells* integrated in hyphae or in the distal ends of hyphae, holoblastic-thalloblastic, unilocal, with conspicuous annellations or periclinal thickening. *Conidia* in chains, holoblastic, solitary, on indistinct to well defined phialides on hyphae, thick-walled, smooth, brown, with or without hyaline basal appendages, aseptate or transversely or uniformly septate, conidia with subtruncate ends, smooth to verruculose, ellipsoidal to subglobose or globose to subcylindrical, with dehiscence scars on conidial body (Crous, Braun & Groenewald, 2007; Crous, Summerella, Carnegie, Wingfield & Groenewald, 2009).

Teratosphaeriaceae was introduced by Crous and Braun (Crous, Braun et al., 2007) based on ascospores morphology, culture characteristics, associated asexual states and its phylogenetic position within the *Capnodiales*, with some genera causing important diseases (Crous, Braun et al., 2007). *Teratosphaeriaceae* is presently too broadly defined and comprises several unresolved lineages (Crous, Summerella,

Carnegie, Wingfield & Groenewald, 2009). This family contains saprobes (*Catenulostroma* spp.), and halophilic, hyperhydrotic or lipophilic species that have been reported from humans (*Piedraia, Hortaea, Penidiella, Stenella*) (de Hoog et al., 2007; Bonifaz, Badali, Hoog, Cruz & Araizam, 2008; Plemenitaš, Vaupotič, Lenassi, Kogej & Gunde-Cimerman, 2008), with the most derived clades tending to contain plant pathogens (*Parapenidiella, Readeriella, Teratosphaeria*) (Crous, Braun et al., 2009). Crous, Summerell, Carnegie, Wingfield, Hunter et al. (2009) accepted nine genera in *Teratosphaeriaceae,* namely *Baudoinia, Capnobotryella, Catenulostroma, Devriesia, Penidiella, Phaeothecoidea, Readeriella, Staninwardia* and *Teratosphaeria* based on DNA sequence data derived from the LSU gene. Crous, Summerella, Carnegie, Wingfield and Groenewald (2009) described many new species in *Teratosphaeriaceae* based on multi-genes sequence data and morphology of asexual states, including *Penidiella pseudotasmaniensis, P. tenuiramis, Phaeothecoidea intermedia, P. minutispora, Readeriella angustia, R. eucalyptigena, R. menaiensis, R. pseudocallista, R. tasmanica, Teratosphaeria alboconidia, T. complicata, T. majorizuluensis, T. miniata, T. profusa* on Eucalyptus from Australia, and *Teratosphaeria xenocryptica* from Chile. The genera and asexual states in this family are polyphyletic, further studies are required to resolve its status (Binder et al., in prep.).

Type: *Teratosphaeria* Syd. & P. Syd. Annls mycol. 10(1): 39 (1912)

MycoBank: MB 5377

= *Kirramyces* J. Walker, B. Sutton & Pascoe, Mycol. Res. 96(11): 919 (1992)

= *Colletogloeopsis* Crous & M.J. Wingf., Can. J. Bot. 75(4): 668 (1997)

Plant pathogenic on leaves. *Stromata* consisting of brown, septate hyphae, superficial or immersed in host tissue, at times linking ascomata together. *Ascomata* separate or immersed in densely branched fibrils, arranged under a brown, flattended, root-like stroma in the leaf surface, subglobose to globose, black, uniloculate, thick–walled, with well developed ostiolar periphyses, somewhat pseudoparaphysoidal in immature ascomata. *Peridium* thick–walled, comprised 2–3 brown cell layers of texture angularis; inner cells of flattened, hyaline cells. *Hamathecium* when present consisting of subcylindrical, branched, septate pseudoparaphysys, or reduced to

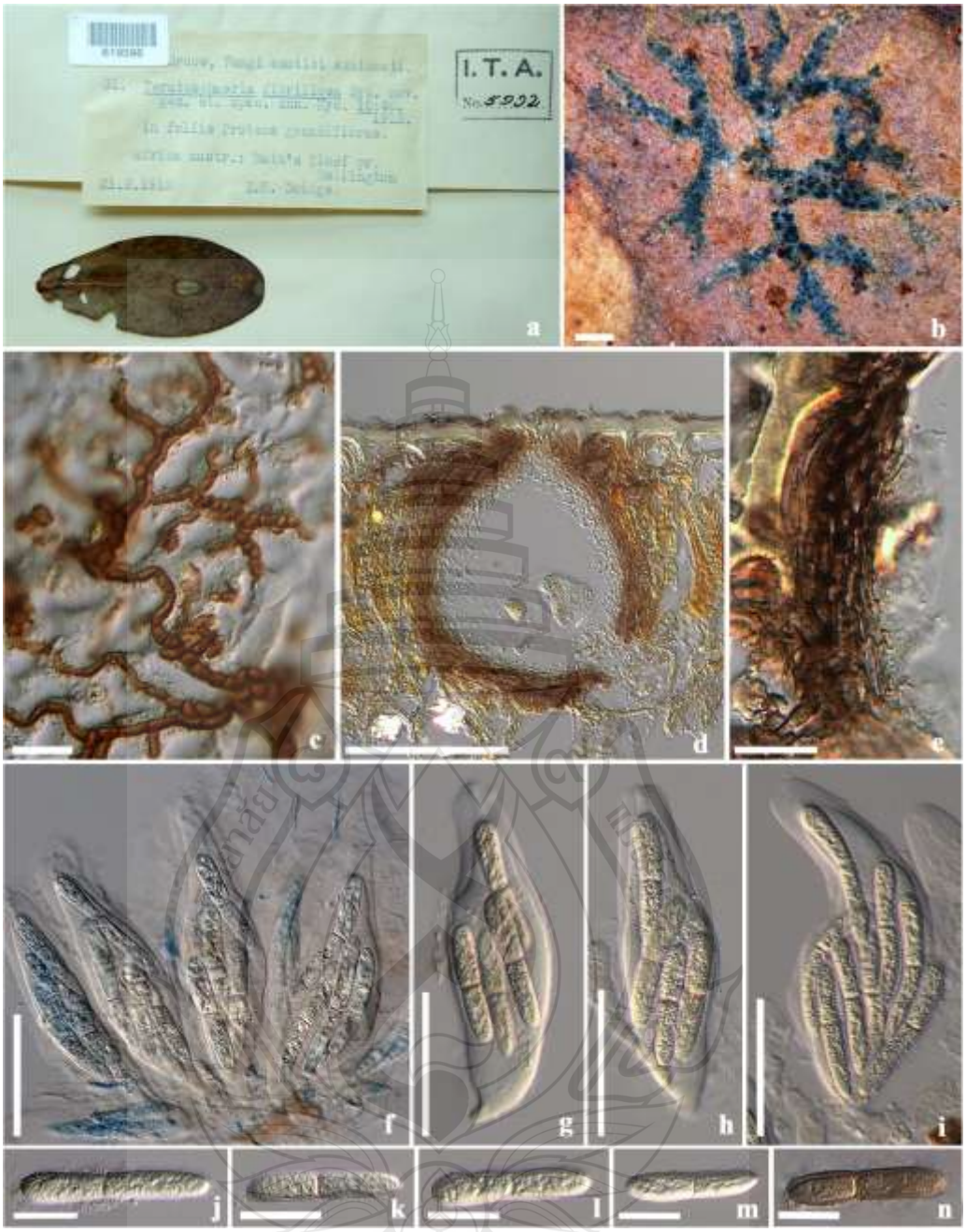
hyaline periphysoids lining the ostiole. *Asci* 8–spored, bitunicate with endotunica, sessile, clavate, apex rounded or tapered, short pedicellate with ocular chamber. *Ascospores* overlapping, fusiform to cylindrical, 1-septate, slightly constricted at the septum or not, often curved, rounded on both sides and upper cell slightly obtuse, rough and thick–walled, hyaline to brown. *Asexual state*: hyphae dark brown, septate, branched, mostly immersed. *Conidiomata* acervular to sporodochial, well–developed or reduced, solitary, brown, with pseudoparenchymatal. *Conidiogenuous cells* integrated, terminal or reduced to conidiogenous cells, proliferating percurrently and sympodially. *Conidia* verrucose, thin to thick walled, brown, with dehiscence scars on conidial body (Crous, Summerella, Carnegie, Wingfield & Groenewald, 2009).

Teratosphaeria was introduced by Sydow and Sydow (Sydow & Sydow, 1912) with *T. fibrillosa* as type species which is associated with leaves of *Protea grandiflora* (= *P. nitida*) (Taylor, Groenewald & Crous, 2003). *Teratosphaeria* can be distinguished from *Mycosphaerella* species based on its asci with multi-layered endotunica, ascospores that darken in older asci and frequently upon germination, and associated asexual genera. Taylor et al. (2003) reduced *Teratosphaeria* to synonymy with *Mycosphaerella* based on ITS DNA sequence data, while LSU sequence data generated by Crous, Braun et al. (2007) provided the first evidence that *Mycosphaerella* was polyphyletic, and split this complex into several families and numerous genera. Cortinas, Crous, Wingfield and Wingfield (2006) treated the species associated with stem cankers of *Eucalyptus*. Numerous species are associated with stem cankers and defoliation of *Eucalyptus* plantations worldwide, as recently reported from Uruguay (Pérez, Wingfield., Altier & Blanchette, 2009), Australia (Carnegie, Pegg, White & Burgess, 2011) and Brazil (Teodoro et al., 2012).

Type species: *Teratosphaeria fibrillosa* Syd. & P. Syd. Annls mycol. 10(1): 39 (1912)

MycoBank: MB 245140

Material examined: SOUTH AFRICA, near Wellington, Bains Kloof, on living leaves of *Protea grandiflora*, 21 February 1912, E.M. Doidge, BPI 619596, (Figure 5.14).



Note. (a). Herbarium specimen. b. Ascomata arranged under a brown flattended, root–like stroma on the leaf surface. (c). hyphae. (d). Vertical section through ascoma. (e). Peridium. (f) – (i). Asci. (j) – (n). Ascospores. Scale bars: (e) = 200 μm, (d) = 100 μm, (f) – (j) = 50 μm, (c), (k) – (n) = 20 μm.

Figure 5.14 *Teratosphaeria fibrillosa* (holotype)

Piedraiaceae Viégas ex Cif., Bat. & S. Camposa, Publções Inst. Micol. Recife 45(1-6): 7 (1956)

MycoBank: MB 82066

Habitat hair disease in human. *Stromata* variable in shape and size, parenchymatic, irregular roughened, elongated, dull to dark, densely, nodular flattened. *Ascostromata* black knots on primate hairs, multiloculate, containing numerous ascostromata irregularly through all the stroma, each locule being an erumpent, visible as small, containing a single ascus, pseudoparencymatous, globose to ellipsoidal stroma or irregular shape, enclosing underlying part of the hair, *Pseudostiolate* locules, without hamathecium, pseudoparaphyses and paraphysoid. *Asci* 8-spored, bitunicate, subglobose to broadly ellipsoidal. *Ascospores* 1-celled, hyaline, rarely light yellowish or greenish, without septa, thin-walled, fusiform, curved, more or less straight to falcate, tapering toward both ends to form the typical tip-like appendages or without appendage, with tapering gelatinous sheath. Germination by several germ tubes. (Ciferri, Batista & Campos, 1956; Arx & Muller, 1975; Eriksson, 1981; Liu, 2011) Colonies are slow growing, small, folded, velvety, dark brown to black in color; reverse is black, may produce a reddish brown diffusible pigment and remain glabrous or covered with short aerial hyphae. Hyphae are spetate, darkly pigmented, with intercalary chlamydoconidium-like cells. (Ciferri et al., 1956; Liu 2011)

Asexual state: Trichosporon sp. pseudohyphae and hyphae abundant and well-developed Blastoconidia unicellular, irregular shape, or arthroconidia unicellular, usually cubical, barrel or elongate in shape, appresoria and sarcinae, budding yeast cells, lightly pigmented, white to light-grown, loosely attached hair-shaft nodules with a soft texture on the salp hair, pubic hair, azillary hair, beards, moustraches, eyebrows and eyelashs, disperse in temperate and semitropical climates. Colonies are yeast-like, rapid growing, smooth, wrinkled, raised, and folded, glabrous to velvety, dull, brittle, waxy, white, or yellowish white to cream colored. (Collier, Balows & Sussman, 1998; Larone, 1995; Sutton, Fothergill & Rinaldi, 1998; Liu, 2011)

Piedraiaceae established by Viégas, and first described in Ciferri et al. (1956) in order *Myriangiales*. This family contains *Piedraia* Fonseca & Leão which is widespread mostly in tropical regions and consist two species (Kirk et al., 2008); *P.*

hortae as type species and *P. quintailhae*. *P. hortae* is a kerationlytic fungus, causing black piedra in humans, generate sexual spores in its parasitic phase while *P. quintailhae* is unknown pathogenicity in humans and differ from *P. hortae* in its ascospores without appendages. Piedra is a superficial fungal infection of hairs that forms hard nodes on the hair shaft. Two types of piedra; black piedra *Piedraia hortae* shows pseudoparenchymatous ascospores and white piedra *Trichosporon cutaneum* is softer and contains hyphae and is yeast that forms a skin when cultured on a liquid, both can be cultured on Saouraud agar. *Piedraia* is a dematiaceous mold, filamentous fungus commonly present in soil as well as stagnant water and crops in tropical regions. It is more importance disease, seen in South America, Asia and some Pacific islands. They are using plant oil on hair facilitates and produce asymptomatic, brown to black small firm nodules, almost exclusively the scalp is in volved nodules. The nodules are composed of ascostromata containing asci and aseptate ascospores (Braun-Falco, Plewig, Wolff & Burgdorf, 2000; Liu, 2011). Schoch et al. (2006) using multigene phylogeny analysis of overall phylogenetic classification of Dothideomycetes and placement *Piedraiaceae* in *Capnodiales*; *Piedraia hortae* allied with *Capnodiales* not *Myriangialeas* as Lindemuth, Wirtz & Lumbsch, (2001) report earlier. Phylogeny result from multigene analysis shown *Piedraia hortae* with low parsimony bootstrap support with Trimmatostroma abietis, a meristematic asexual state isolated from conifer needles and rock surface but this species was shown to be closely related to Mycosphaerella which is support the result from Selbmann et al., (2008).

Type: *Piedraia* Fonseca & Leão, Mem. Inst. Oswaldo Cruz 4(Suppl.): 125 (1928)

MycoBank: MB 4098

Habitat hair disease in human. *Stromata* irregular shape, parenchymatic, irregular roughened, elongated, dull to dark, densely, nodular flattened. *Ascostromata* black knots on primate hairs, multiloculate, containing numerous ascostromata, irregularly through all the stroma, each locule being an erumpent, pseudoparencymatous, globose to ellipsoidal stroma or irregular shape, enclosing underlying part of the hair, *Pseudostiolate* each locules, hamathecium, pseudoparaphyses and paraphysoid not present. *Asci* 8-spored, bitunicate, subglobose

to broadly ellipsoidal, short pedicel. *Ascospores* 1-celled, hyaline, rarely light yellowish or greenish, without septa, thin-walled, fusiform, curved or slightly curved, more or less straight to falcate, tapering toward both ends to form the typical tip-like appendages, with tapering gelatinous sheath. Germination by several germ tubes. (Ciferri et al., 1956; von Arx & Muller, 1975; Eriksson, 1981; Liu 2011)

Type species: *Piedraia hortae* (Brumpt) Fonseca & Leão, Memórias do Instituto Oswaldo Cruz, Supplemento 4 (Suppl.): 124 (1928), (Figure 5.15)

MycoBank: MB 267365

Figure 5.15 *Piedraia hortae* (redrawn from Shoemaker & Egger, 1982)

Triposporiopsidaceae S. Hughes, Mycologia 68(4): 712 (1976)

MycoBank: MB 81600

Sooty moulds on leaf and stem surface. Thallus comprised of septate mycelium on host surface. *Hyphae* superficial, cylindrical, septate, may or may not be constricted at the septum, or relatively inconspicuous septa, brownish to brown, smooth or slightly roughened, loose extensive network or somewhat dense stratum. *Ascostromata* arise from mycelial mass, subiculum, soft, sessile, brown, uniloculate, central ostiolate, bearing numerous setose hyaphal appendages, brown to dark brown, erect, straight or curved, septate or lacking. *Pseudoparaphyses* not present. *Peridium* thin-walled, pale brown to brown, comprising several layers of cells of *textura angularis*. *Asci* apparently bitunicate, cylindrical to clavate with an apical ring. *Ascospores* overlqapping, 2-3 septate, slightly constricted or not constricted at the septum, hyaline, fusiform or ellipsoidal, round at ends, without a mucilaginous sheath. *Asexual state*: *Tripospermum* sp., mycelium forms brown, effused, superficial colony composed of branched and anastomosing brown hyphae, smooth, septate, slightly constricted at the septum. *Conodiogenous cells* as phialides, composed of scattered, relatively long and subhyaline to pale brown producing stauroconidia. *Mature conidia* are typically tetraradiate with a short basal stalk cell, cylindrical with a rounded base, brown, hyaline to pale brown at apex, smooth or roughened, up to 6-septate, slightly constricted at the septa, wide at the base and tapering to a rounded apex, various sizes. Occasionally three of four arms are short. Germinating conidia, hyphae and ascostromata initials frequently bear more or less subulate or narrowly flask-shaped phialides or phialide-like structures erect on the repent hyphae and often crowded one to each cell, along considerable lengths of particular hyphae. Phialides produce hyaline, ellipsoidal to broadly ellipsoidal phialoconidia (Hughes, 1976).

Triposporiopsidaceae was described by Hughes (1976) based on the genus *Tripospoiopsis* linked to the asexual state *Tripospermum.* Von Arx and Müller (1975) classified *Tripospoiopsis* in *Capnodiaceae* and this was confirmed by Chomnunti, Bhat et al. (2012) based on molecuar evidence. Colonies of sooty moulds are often mixtures from different families and it is difficult to isolate and identify them. Hughes and Seifert (2012) has summarized hyphal morophologies and synanamorphic patterns of sooty moulds into three families *Metacapnodiaceae*, *Euantennariaceae*

and *Triposporiopsidaceae.* Hyphae of *Triposporiopsidaceae* are cylindrical, slightly constricted, medium brown, while *Metacapnodiaceae* has monilioid hyphae, deeply constricted, dark brown, while in *Euantennariaceae* hyphae brick-like, slightly constricted and brown or dark brown.

Type: *Triposporiopsis* W. Yamam., Pap. Dedic. Tochinai & Fukushi Commem. 60th Birthdays: 52-56 (1955)

MycoBank: MB 5609

Habit: Sooty moulds on living leaf. *Thallus* comprised of dark brown mycelium on host surface. *Hyphae* superficial, cylindrical, septate, slightly constricted at the septum, or relatively inconspicuous septa,brownish to brown, smooth or somewhat slightly roughened, loose, extensive network. *Ascostromata* borne on a mycelium mass, scattered, subiculum, soft, sessile, brown, uniloculate, central ostiole, bearing numerous setose hyaphal appendages, brown to dark brown, erect, straight or curved, septate or continuous, tapering to apex, rounded end. *Pseudoparaphyses* not present. *Peridium* thin-walled, pale brown to brown comprising several layers of cells of *textura angularis*. *Asci* 8-spored, bitunicate, with an apical ring, cylindrical to clavate. *Ascospores* overlapping, 3-septate, slightly constricted at the septum, hyaline, fusiform or ellipsoidal, narrowly rounded at ends, without a mucilaginous sheath.

Hughes (1976) observed many type collections of sooty moulds (*Paropodia* Cif. & Bat., *Vitalia* Cif. & Bat., *Trichomerium coccolobae* Bat. & Cif. and *Trichomerium stuhlmanianum* (P. Henn.) Bat. & Cif. var. *biseptatum* Bat. & Cif.) and found to be typical of *Triposporiopsis* and bearing *Tripospermum* conidia and the characteristic phialides, thus they are should be belong to *Triposporiopsidaceae.* Hyphae of *Aithaloderma* and *Triposporipsis* are similar in being septate, cylindrical and slightly constricted at the septum, but both can distinguished by *Tripospermum* conidia are present on typical *Triposporiopsis* hyphae which bear phialides (Hughes, 1976). Hyde et al. (2011) report that *Triporpermum* sp. is asexual state of *Trichomerium* sp. In the following year Chomnunti, Bhat et al. (2012) described and illustrated on *Trichomerium* and *Trichomeriaceae* was introduced and belong to Chaetothyriales which is proved by molecular analysis and morphology matching, asexual state refer to *Triporpermum* sp. as well but no any morphology and molecular analysis report. Ruibal, Gueidan et al. (2009) state that *Devriesia strelitziae,*

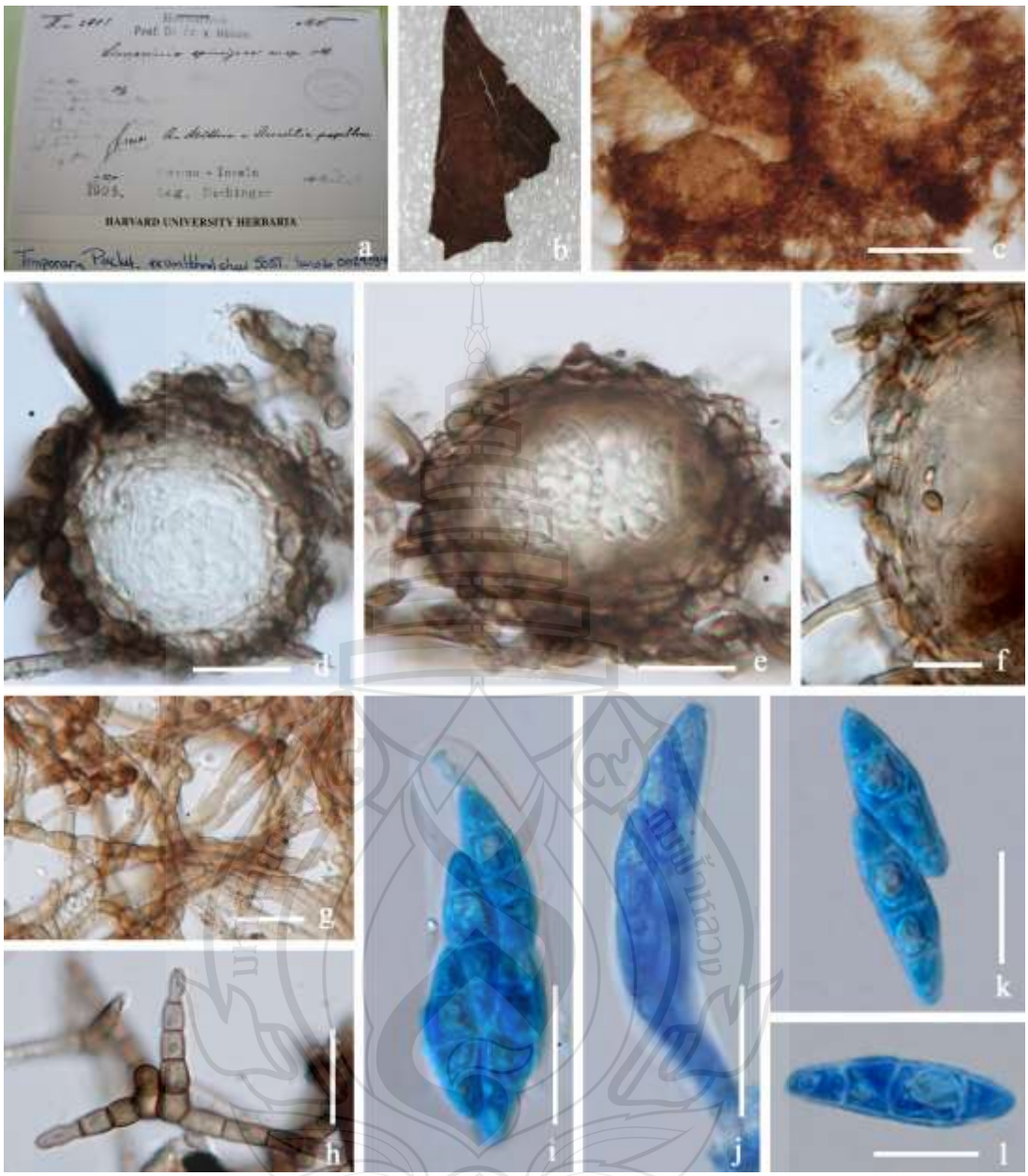
Mycosphaerella eurypotami and *Tripospermum myrti* are leaf-colonising species, a Maximum Likelihood analysis of the combined nucLSU, nucSSU and mtSSU show that they are clustered within *Teratosphaeriaceae* as well as unknown species of Capnodiales, the lichen species *Cystocoleus ebeneus* and 20 undescribed rock inhabiting strains, is supported as sister to the family *Mycosphaerellaceae.* Therefore, the sexual and asexual state of *Triposporiopsidaceae* are unclear thus molecular analysis is required.

Type species: *Triposporiopsis spinigera* (Höhn.) W. Yamam., Pap. Dedic. Tochinai & Fukushi Commem. 60th Birthdays: 52-56 (1955)

MycoBank: MB 3077143

≡ *Limacinia spinigera* Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 116: 100 (1907)

Material examined: SAMOA, on living leaf of *Sterculia populnea*, 1905, K. Rechinger FH 00290340, (Figure 5.16).



Note. (a). Label of herbarium. (b). Sooty mould on host. (c. Ascostromata. (d) – (e). Vertical section of ascostromata (f). Peridium. (g). Hyaphe. (h). Conidia. (i), (j). Bitunicate asci. (k) – (l). Ascospores. Scale bars: (c) = 100 µm**,** (d) – (j) = 20 µm**,** (k) – (l) = 10 µm.

Figure 5.16 *Limacinia spinigera* Höhn

CHAPTER 6

GENERAL CONCLUSION AND CONCLUSION

Sooty moulds are a complex of epifoliar fungi, usually forming sooty patches on leaves of most plant types and usually comprise multi-species associations, with different sexual and asexual states. The connections between these morphs are however often based on occurrence and thus unclear (Hughes, 1976; Reynolds, & Gilbert, 2005; Flessa et al., 2012). The phylogenetic and subsequent taxonomic relationships between sexual and asexual morphs of sooty moulds are incomplete and also largely confused (Faull, Olejnik, Ingrouille & Reynolds, 2002). In the present study, emphasis was made on systematics of sooty moulds found in Thailand, based on morphological characters using the type species as references and molecular analysis of DNA sequence data. The type species of *Capnodiaceae* were carefully examined, re-described and illustrated. They were also used as references to identify the freshly collected specimens from Thailand. Results from DNA sequencing analysis were used to confirm the evolution of *Chaetothyriaceae* whose species are often found mixed with *Capnodiaceae* on the host surface. Three new species of *Chaetothyriaceae* are introduced from this study. The genus *Trichomerium* was transferred from *Capnodiaceae* to a new family *Trichomeriaceae* which is introduced here based on morphological characters and DNA sequence analyses. The revision of some important families of the Dothideomycetes including sooty moulds are presented as part of this thesis. An overview of current status of molecular phylogeny, pleomorphy and taxonomy of sooty moulds is presented as a prelude to the study.

6.1 Occurrence of Sooty Moulds in Thailand

The sooty moulds are a group of tropical and subtropical fungi. However, so far only a few reports listed sooty moulds from Thailand. Sianglew (1989) encountered six families of foliar epiphytes in Thailand including *Asterinaceae, Capnodiaceae, Meliolaceae, Parmulariaceae, Parodiellinaceae* and *Tripos*poriopsidaceae. He reported five genera as hyperparasitic on sooty mould including *Dimerium*, *Domingoella*, *Philonectria* and *Spiropes*. In present study, we collected, studied and identified sooty moulds from various plants (e.g. *Bischofia javanica, Coffea arabica, Mangifera indica, Psidium guajava*). Many specimens of sooty moulds, associated with sap feeding insects such as cottony cushion scale, mealy bug and whitefly, were encountered. Normally several species were seen growing together in apparent harmony and often found forming characteristic black films on branches, leaves and twigs. More than 50 new collections of sooty moulds were made in this study, while 14 collections in *Capnodiaceae* comprised new species: viz. *Capnodium coartatum, Leptoxyphium cacuminum, Phragmocapnias asiaticus, P. siamensis,* on *Psidium guajava, Gossypium herbaceum, Coffea arabica* and *Mangifera indica* respectively. *Phragmocapnias betle* and *Scorias spongiosa* are epitypified. All collections were isolated and sequenced. In all, 30 sequences of SSU rDNA and LSU rDNA were deposited in GenBank and this data is pivotal for future phylogenetic analysis and systematics of sooty moulds in *Capnodiaceae*. The morphology and taxonomy of type specimens of the genera *Aithaloderma, Anopeltis, Callebaea, Capnodaria, Echinothecium, Phragmocapnias* and *Scorias* in *Capnodiaceae* were revisited. In this study, we have provided a clear understanding of morphological characters of genera of *Capnodiaceae*, provide photomicrographs and/or illustrations and taxonomic descriptions of type specimens of each genus; this information will be very useful as a reference source for the future study of sooty moulds in *Capnodiaceae*. *Chaetothyriaceae* in *Chaetothyriales,* also the sooty moulds, and often occur together with *Capnodiaceae* on hosts. In this study, we often found *Chaetothyriaceae* growing together with the sooty mould complex and phylogenetic analyses were used to classify them. As a result we describe three new

species of *Chaetothyriaceae* from Thailand, viz. *Ceramothyrium thailandicum*, *Chaetothyrium brischofiacola* and *Phaeosaccardinula ficus* on *Lagerstroemia* sp., *Brischofia javanica* and *Ficus* sp. respectively. Data for LSU and ITS rDNA sequences of three new species are now available in GenBank. Unfortunately, the members of *Chaetothyriaceae* are slow growing in culture and the asexual state was not produced on agar media. Further studies are needed to induce the asexual state and to elucidate the relationships between the sexual and asexual morphs. *Trichomerium* is a genus of sooty moulds and no sequence data was previously available. In this study, details of 16 collections of *Trichomerium* from various hosts (e.g. *Murraya paniculata Mangifera indica Psidium guajava Phoenix dactylifera* and *Ficus* sp.) are presented and taxonomically classified using morphology and molecular analysis; two new species, *Trichomerium foliicola* and *T. gloeosporum* are introduced from Thailand.

6.2 Re-evaluation of taxonomy of Sooty moulds

The sexual state of sooty moulds in Perisporiales including *Capnodiaceae* and *Chaetothyriaceae* which contained species of sooty moulds with ostiolate perithecia; the asexual state belonging to Asbolisiales (Spegazzini, 1918). Batista and Ciferri (1963b) transferred some of asexual Asbolisiales to Hypasbolisielas using the criteria such as elongated pycnidia or synnematous form. However, it was difficult to define sooty moulds solely from a biological or taxonomic view point (Batista & Ciferri, 1963a), until Hughes (1976) expanded the scope of sooty moulds as saprobic fungi, with dark colored hyphae producing brown to black colonies superficially on living plants; this dispensation was valuable for many subsequence researchers. Hughes, since 1963, examined the sooty moulds occurring in abundance in New Zealand, especially those in *Metacapnodiaceae* and their asexual morphs. However, his problem in studying sooty moulds was the morphological complexes, as many species were found to grow together in apparent harmony to form what appears to the naked eye as a growth of a single species; this made the establishment of species delimitation difficult. The difficulties of circumscribing the species increased because

some sooty moulds commonly produced two, three and occasionally four different conidial states in addition to the perithecia. Furthermore, species in *Metacapnodiaceae* do not grow on agar media. Although sequence data can now help solve these problems, unfortunately only a few sequences for sooty moulds were available in GenBank. We identified some of the sooty moulds based on Hughes various publications. Reynolds also carried out interesting studies on sooty moulds especially in family *Capnodiaceae,* but his publications are unclear and resulted in a confused taxonomy of this family (Reynolds 1986; 1999). In the present study, morphological characters and phylogenetic analysis of type specimens was carried out to identify sooty moulds and the relationships between sexual and asexual states. *Aithaloderma, Ceramoclasteropsis, Hyalocolecostroma* and *Trichomerium* are excluded from *Capnodiaceae* based on type specimens characters. Five genera; *Capnodium, Leptoxyphium, Phragmocapnias* and *Scorias* are well-defined in *Capnodiaceae*, while *Leptoxyphium* is an asexual state of *Capnodiaceae*. Using phylogenetic analysis we resolved the connections between sexual and asexual morphs, such as *Phragmocapnias, Capnodium* having asexual morph as *Conidiocarpus* and *Polychaeton* respectively.

Brief monographic descriptions, rough illustrations, and a simple key to *Trichomerium* species were provided by Batista and Ciferri (1963a) and the genus was placed in *Capnodiaceae*. Hughes (1976) later transferred *Trichomerium* to *Triposporiopsidaceae,* based on the characteristic asexual phialides, its apparent asexual state *Triposporiopsis* and the presence of *Tripospermum* conidia. This is not followed by other mycologists. In point of view of our studies on sooty moulds, multiple species of sooty moulds were found on living leaves, with some collections having more than five species on a single leaf, such as *Capnodium* sp., *Phragmocapnias* sp., *Cheatothyrium* sp., *Scorias* sp. *Trichomerium* sp. (Hughes, 1976; Hughes & Seifert, 2012; Reynold & Gilbert, 2005). In this study we have often found *Trichomerium* sp. occurring on leaves with other sooty moulds fungi. We introduce the new family *Trichomeriaceae* based on phylogenetic analyses which is clearly distinguished from *Capnodiaceae* and *Chaetothyriaceae*. The new family is characterized by having a brown, sessile, globose to subglobose ascostromata with abundant setae and central ostioles, bitunicate, cylindrical to clavate asci with an

apical ring, and hyaline, septate, fusiform, ascospores which are narrowly rounded at both ends and widest in the centre, and with or without a mucilaginous sheath. Unfortunately, the fungi are slow growing and we did not observe the asexual state in culture. We also found *Tripospermum* conidia surrounding the ascostromata but could not isolate the former in culture. Nevertheless, we predict that this is the asexual state of *Trichomerium* sp. and therefore future work is needed to isolate ascospores and conidia and to prove by phylogenetic result that they have connection with each other.

6.3 Significance and Publications Resulting from this Thesis

During the course of this study I went on more than 20 field trips and collected more than 70 specimens of sooty moulds. In this thesis I deal with 58 specimens which I identified to 11 species. I isolated 44 strains from my collections and sequenced five genes of 44 isolates with 46 new sequences deposited in GenBank. I also worked on other collections, which are not included in the thesis, but will be written up and published in future work. As a result of morphological and molecular studies I described nine new species and resolved the classification of species of sooty moulds and their placement at higher taxonomy levels. I have also increased the understanding of the relationships between sexual and asexual morphs of sooty moulds. This is the first study seriously incorporating morphology and molecular data to understand sooty moulds at the higher taxonomic levels and resulted in resolving the genera of *Capnodiaceae* (Capnodiales) and *Chaetothyriaceae* (Chaetothyriales) and showed the need for the introduction of the new family *Trichomeriaceae* (Chaetothyriales). I also studied herbarium material of 27 genera in 11 families of Dothideomycetes and this data is presently in preparation in a monograph of the families of Dothideomycetes. The results of my study have been published in four peer reviewed papers in international journals. I am first author of three of the publications and each of these publications introduce ground breaking findings which have improved the understanding of sooty moulds. I also co-authored six publications improving the understanding of Dothideomycetes and several other publications, including a major monograph, have yet to be completed. As a result of this study and

the publications herein, it will be easier for future researchers to study and understand the sooty moulds and Dothideomycetes in general. Therefore I believe the contribution that I have made to the field of science, particularly in relation to mycology and specifically sooty moulds is highly significant and thus worthy of a PhD.

List of publications

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6.4 Future Work

Thailand is a tropical region and the environment and conditions are suitable for growth of epiphytic sooty moulds. Abundant sooty moulds occur on economically important plant crops and a large number of sooty moulds are waiting to be identified and classified. Hitherto, only a very few reports on sooty moulds were known from Thailand as they are difficult to isolate. With newer efforts and methods, we are now able to grow them in culture and, with this; we will continue to collect sooty moulds in Thailand and to provide more data and discovery of new taxa of these fungi. The relationship between asexual and sexual states of sooty moulds are poorly established and with the new techniques now available to induce asexual or sexual morphs in culture, the taxonomy and classification of these fungi can be resolved. Sooty moulds have been reported as fungi associated with sap feeding insects, but not as a state specific between sooty mould, insect and host. Our study did not establish the relationship further. Therefore, studies on their relationships are required for future understanding and management of the distribution of fungi and their control. Of the fungi associated with insects, ingestion of sooty mould fungi by freshwater stream insects has been reported for the first time from New Zealand, further experiments on

insects with sooty moulds are needed to prove that sooty moulds fungi are really source of food or harmful to insects. Whether sooty moulds are host-specificity is still an unanswered question; we found sooty moulds on every host plant where the honey dew occurs. Further experiments are needed to investigate the host-specificity of sooty moulds, by spraying sugary solutions on leaves to test which sooty moulds appear. This may be very useful to control fungal disease caused by sooty moulds.

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APPENDICES

APPENDIX A

STAINS AND REAGENTS FOR MICROSCOPIC EXAMINATION OF FUNGI

Chemical Reagent.

Examination of fungi morphology from fresh specimens and herbarium specimens, various chemical reagents were used in the present study and preparation as following.

Indian ink was used for observed gelatinous appendages and sheaths of spores that appeared in some species

Lactogleceral was used routinely for observing fungi and preparing semipermanent slide. It was prepared as following formula;

Lactic acid	10 ml
Gleceral	10 ml
Distilled water	10 ml

Melzer's reagent was used in examination of ascomycetes. Frequently coour-change reactions occur in Melzer's reagent. The amyloid reacition (blue) of apical pore is often of taxonomic importance. It was prepared as following formula;

Chloral hydrate	100 g
Potassium Iodide	5 g
Iodine	1.5 g
Distilled water	100 ml

Potassium Hydroxide 5% was routinedly used in the rehydration of the herbarium specimens. It was prepared as following formula;

Potassium hydroxide	0.5 g
Distilled water	10 ml

APPENDIX B

REAGENTS FOR MOLECULAR STUDY

Reagent for DNA extraction: Biospin Fungus Genomic DNA

LE buffer	20 ml
DA Buffer	7.5 ml
E Binding Buffer	14 ml
G Binding Buffer	25 ml
Wash Buffer	25.2 ml
Elution Buffer	10 ml
Spin column	50

*Kit Components (50 T)

PCR Amplification

Concentrations for a PCR mixture (volume for single reaction)

Template DNA	1 µL
ddH2O	8.825 µL
MgCl2	0.625 µL
dNTPs	0.250 µL
Forward Primer	0.250 µL
Reverse Primer	0.250 µL
Taq DNA polymerase	0.050 µL

Agarose Gel Electrophoresis

Preparation of a 1% agarose gel 50 ml volume.

Agarose	0.5 g.
0.5xTBE buffer	50 ml
Ethidium bromide	1 µL

APPENDIX C

ABBREVIATIONS OF FUNGAL COLLECTION

Table C1 Name of fungal collection.

Abbreviations	Full name of herbaria
B	Botanischer Gerten und Botanisches Museum Berlin-Dahlem, Zentraleinrichtung der Freien Universität Berlin
BPI	U.S. National Fungus Collections
FH	Harvard University
G	Conservatoire et Jardin botaniques de la Ville de Genève
IMI	CABI Bioscience UK Centre
K.	Royal Botanic Garden, Kew
LEP	All-Russian Research Institute of Plant Protection
NY	New York Botanical Garden
S	Swedish Museum of Natural History
URM	Universidade Federal de Pernambuco

APPENDIX D

PUBLICATIONS

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Phylogeny of Chaetothyriaceae in northern Thailand including three new species

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Abstract: In a recent study unusual taxa of epiphyllous ascomycota belonging to Chaetothyriaceae (Eurotiomycetes) were collected in northern Thailand. This family is poorly understood due to morphological confusion and lack of phylogenetic studies. This paper deals with three new species, *Ceramothyrium thailandicum*, *Chaetothyrium brischofiacola* and *Phaeosaccardinula ficus*, which are fully described and illustrated. A DNA sequence analyses of LSU and ITS rDNA genes shows that the new species cluster in the Chaetothyriaceae. This paper adds six sequences for Chaetothyriaceae to GenBank, providing much needed data for the family.

Key words: *Ceramothyrium*, Chaetothyriaceae, *Chaetothyrium*, LSU, *Phaeosaccardinula*, phylogeny

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Introduction

The Chaetothyriaceae are typical of capnodiaceous Dothideomycetes because they form on the surface of leaves and resemble typical sooty molds (Batista and Ciferri 1962). Species of Chaetothyriaceae are mostly epiphytes, colonizing the surface of living leaves with mycelium appressed to the host cuticle without penetrating host tissues (Batista and Ciferri 1962, von Arx and Müller 1975). Ascomata are surrounded by a thin pellicle of superficial mycelium forming black sooty mold-like areas on leaves that are easily detached from the cuticle (Batista and Ciferri 1962). However the ecology of many species of Chaetothyriaceae is poorly studied and it is unclear whether they are saprotrophic or biotrophic (Barr 1987). Members of Chaetothyriaceae often are confused with capnodiaceous sooty molds due to their similar morphology and habitat preferences, however these fungi are never associated with insects such as several Capnodiaceae (Hansford 1946). Sooty molds are a general taxonomic term for capnodiaceous and/or chaetothyriaceous fungi; common genera from both these groups often are found growing together in sooty mold complexes in plant exudates or the sugary honeydew secreted by insects, for example *Aithaloderma* (*Leptoxyphium*), *Aureobasidium*, *Capnodium*, *Cladosporium*, *Microxyphium*, *Podoxyphium*, *Scorias* and *Trichomerium* (*Tripospermum*) (Thaung 2006).

Studies on Chaetothyriaceae were conducted mainly by Hansford (1946), Batista and Ciferri (1962), von Arx and Müller (1975) and Hughes (1976), and few studies have been undertaken since. Members of Chaetothyriaceae are primarily tropical species characterized by dark mycelium forming as a loose net of hyphae over the substrate, and they produce ascomata beneath a mycelial pellicle with or without setae (Batista and Ciferri 1962, Hughes 1976, Pereira et al. 2009). The family is poorly circumscribed and most work comprised brief descriptions with line drawings (e.g. Hansford 1946, Batista and Ciferri 1962). The arrangement of genera often seem subjective because individual authors emphasize certain characters, such as spore septation, presence of ascomata setae and mycelium color (Batista and Ciferri 1962, Hughes 1976). Batista and Ciferri (1962) considered the family Chaetothyriaceae to be the type family in order Chaetothyriales. This group share a number of centrum characters with members of the Dothideomycetes, such as the presence of bitunicate

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Capnodiaceae

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Abstract In this paper we revisit the *Capnodiaceae* with notes on selected genera. Type specimens of the ascomycetous genera *Aithaloderma*, *Anopeltis*, *Callebaea*, *Capnodaria*, *Echinothecium*, *Phragmocapnias* and *Scorias* were re-examined, described and illustrated. *Leptoxyphium* is anamorphic *Capnodiaceae* and *Polychaeton* is a legitimate and earlier name for *Capnodium*, but in order to maintain nomenclatural stability we propose that the teleomorphic name should be considered for the approved lists of names currently in preparation for fungi. Notes are provided on the ascomycetous genus *Scoriadopsis*. However, we were unable to locate the type of this genus during the time frame of this study. The ascomycetous genera *Aithaloderma*, *Ceramoclasteropsis*, *Hyaloscolecostroma* and *Trichomerium* are excluded from *Capnodiaceae* on the basis of having ascostromata and trans-septate hyaline ascospores and should be accommodated in *Chaetothyriaceae*. *Callebaea* is excluded as the ascomata are thyriothecia and the genus is placed in *Micropeltidaceae*. *Echinothecium* is excluded as synonym of *Sphaerellothecium* and is transferred to *Mycosphaerellaceae*. The type specimen of *Capnophaeum* is lost and this should be considered as a doubtful genus. The coelomycetous *Microxiphium* is polyphyletic, while the status of *Fumiglobus*, *Polychaetella* and *Trispermium* is unclear. Fourteen new collections of sooty moulds made in Thailand were isolated and

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Trichomeriaceae, a new sooty mould family of Chaetothyriales

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Abstract *Trichomerium* is a genus of foliar epiphytes with the appearance of sooty moulds, mostly occurring on the surface of living leaves and apparently gaining their nutrients from insect exudates. Species have ascostromata with setae and develop on a loosely interwoven mycelial mass of dark brown hyphae, while asci have a bitunicate appearance with hyaline ascospores. In this study, we made 16 collections of *Trichomerium* from Thailand. All were isolated, and the LSU and ITS rDNA gene regions sequenced. Phylogenetic analysis indicated that the *Trichomerium* species form a monophyletic clade within *Chaetothyriales* and warrant the introduction of a new family *Trichomeriaceae*. Bootstrap support for the *Chaetothyriales* is 100 % and clearly separates *Trichomeriaceae* from *Capnodiales* which are morphologically very similar. A detailed account of *Trichomerium* is provided and we describe and illustrate three new species based on morphological and molecular data. We propose that *T. foliicola* is adopted as the generic type of *Trichomerium* because it has been impossible to obtain the holotype specimen of *T. coffeicola* and also no molecular data exists in worldwide databases for this species or genus.

Keyword Foliar epiphytes · Phylogeny · Sooty moulds · *Trichomerium*

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Introduction

The taxonomy of genera of foliar epiphytes is poorly known as they have not been well-studied. No molecular data is available for most genera and therefore an understanding of the higher level classification of these fungi is rather inadequate. We have, therefore, initiated a research program to collect and study these important taxa using morphology and phylogeny. Our initial study (Chomnunti et al. 2012) resulted in the transfer of the genus *Trichomerium*, previously placed in *Capnodiaceae* to *Chaetothyriaceae* in *Chaetothyriales*. We have also provided an account of *Microthyriaceae* (Wu et al. 2011) and are presently studying other genera of foliar epiphytes. Examples of foliar epiphyte genera with a sooty mold-like appearance are *Aithaloderma*, *Capnodaria*, *Phragmocapnias* and *Scorias*. Chomnunti et al. (2011) gave an account of the genera in *Capnodiaceae*, while Chomnunti et al. (2012) dealt with species of *Chaetothyriaceae*. The genus *Trichomerium* was placed in *Chaetothyriaceae* but no further data was provided (Chomnunti et al. 2011). Hughes and Seifert (2012) provided notes on the taxonomy and nomenclature of sooty mould names, but further work is required to resolve their interrelationship, especially at the molecular level.

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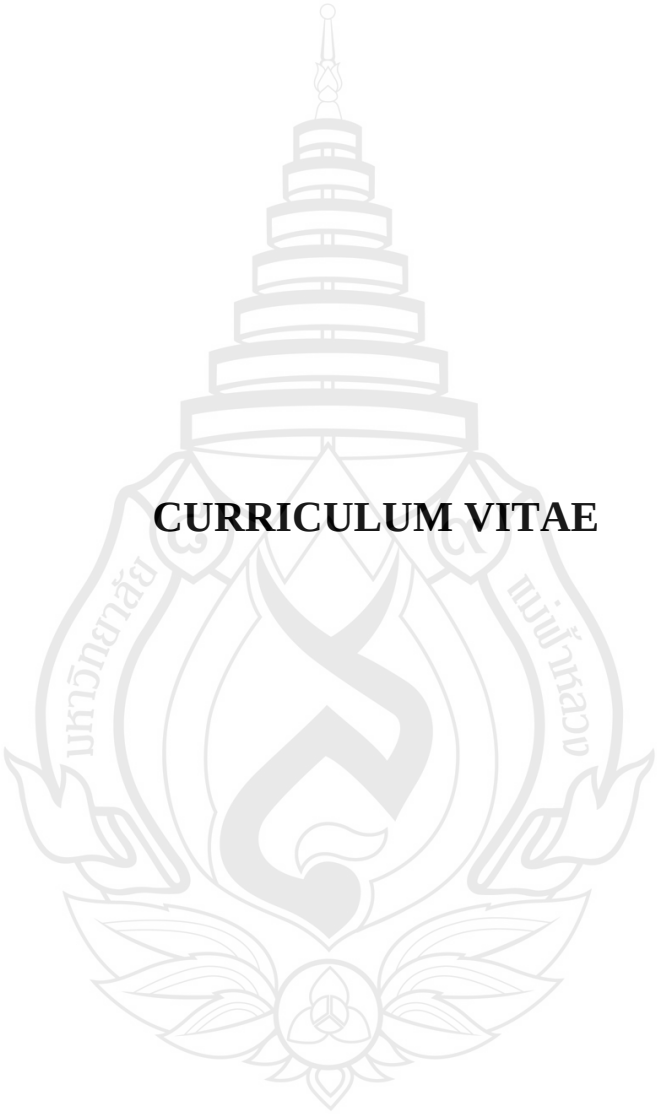

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