DIVERSITY AND TAXONOMY OF DOTHIDEOMYCETES ON WOODY LITTER IN NORTHERN THAILAND

SARANYAPHAT BOONMEE

DOCTOR OF PHILOSOPHY
IN
BIOSCIENCES

SCHOOL OF SCIENCE
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ABSTRACT

Dothideomycetes is one of the largest fungal groups mainly characterised by the distinct "bitunicate" asci feature. Most Dothideomycetes are saprobes, endophytes and pathogens; besides, they can also be present as fungicolous, coprophilous or lichenicolous. In this study, we aimed to investigate the diversity of saprobic Dothideomycetes from dead wood in northern Thailand. In Thailand, the study of this fungal group is limited and thus our data are expected to provide an insight on Dothideomycetes's taxonomy.

Initially, the fungal strains isolated from dead wood specimens were morphologically characterised and those showing the "bitunicate" characteristics were further studied using multigene analysis (i.e., ITS, LSU, RPB2, SSU and TEF1-α). As a result, classification and phylogenetic relationships of Thai Dothideomycetes were presented.

Based on this study, we introduced the new order *Tubeufiales* on the basis of the family *Tubeufiaceae*. This order was to accommodate the fungal group distinct

from the *Pleosporales*. Besides, we also revised the taxonomic classification within the family *Tubeufiaceae*. For this, 15 genera were included in this family. Twelve new species were discovered from this study; these included *Acanthostigma chiangmaiensis, Acanthostigma fusiforme, Acanthostigma lignicola, Acanthostigmina piniraiensis, Chlamydotubeufia huaikangplaensis, Chlamydotubeufia khunkornensis, Helicoma chiangraiense, Helicoma fagacearum, Helicoma siamense, Thaxteriella inthanonensis, Tubeufia chiangmaiensis* and *Tubeufia khunkornensis*.

The new family *Kirschsteiniotheliaceae* was also proposed based on morphological and molecular phylogeny. This family was founded to accommodate the fungal taxa grouping with *Kirschsteiniothelia aethiops*. Two new species *Kirschsteiniothelia emarceis* and *Kirschsteiniothelia lignicola*, including their asexual states were described and illustrated. The new genus *Halokirschsteiniothelia* was also introduced to accommodate *Kirschsteiniothelia maritima* and placed in the *Mytilinidiaceae* (*Mytilinidiales*).

Additionally, the type species of nine Dothideomycetes families were studied using the herbarium type specimen. Their detailed descriptions were provided as well as a key to genera of accepted species.

Keywords: Bitunicate/Phylogeny/Saprobic/Sexual and Asexual States/Type Species

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ABBREVIATIONS AND SYMBOLS

ca.	about, approximately
cf.	compare
cm	centimeter
com.	combination
diam.	diameter
e.g.	for example
et al.	and others
etc.	and so forth
g	gram
gen.	genus (singular) and genera (plural)
i.e.	that is
m.	meter
ml	milliliter
mm	millimeter
nov.	novum (Latin for new thing)
sp.	species
spp.	species (plural)
$\bar{x}$	average
°C	degree centigrade
=	heterotypic (taxonomic, facultative) a synonym
≡	homotypic (nomenclatural, obligate) a synonym
µm	micrometer
µl	microliter
%	percent

CHAPTER 1

INTRODUCTION

1.1 Introduction

The class Dothideomycetes was introduced by Eriksson and Winka (1997) based on the ordinal type *Dothideales* Fries (1818). Schoch et al. (2006) later, divided it into two subclasses, Pleosporomycetidae and Dothideomycetidae. Both subclasses are distinguished by the presence or absence of pseudoparaphyses, with the subclass Pleosporomycetidae having well-developed pseudoparaphyses, as in *Pleosporales*, while members of the subclass Dothideomycetidae lack pseudoparaphyses, as in the orders *Capnodiales, Dothideales* and *Myriangiales* (Kirk, Cannon, Minter & Stalpers, 2008; Lumbsch & Huhndorf, 2010; Schoch et al., 2006). Currently, the class Dothideomycetes has more than 11 orders; they are divided on the basis of morphological structures and molecular phylogeny (Lumbsch & Huhndorf, 2010; Wu et al., 2011; Zhang et al., 2011). Dothideomycete members are commonly named as the "bitunicate fungi", meaning that the ascus (pl. asci) is distinctly two-layered wall. They are characterised by sexually produced ascospores within a sac or ascus. In some cases, at maturity the asci have a fissitunicate function, called "jack-in-the-box" mechanism, where two separable wall layers of ascus dehisce and ascospores are discharged (Eriksson, 1981; Luttrell, 1951; Reynolds, 1989; Sivanesan, 1984). Previously, the Dothideomycetes were called "Ascoloculares" by Nannfeldt (1932), a name for sexual fungi characterized by multilocular ascoma. Luttrell (1951, 1955, 1981) later used "Bitunicate", "Loculoascomycetes", and "Loculoascomycetidae"; these names imply that the asci have a two-layered wall and developed in locules inside of the ascostroma

Members of bitunicate fungi are widely distributed in nature, mainly in terrestrial habitats, although some species live in aquatic habitats (freshwater or marine) (Barr, 1987; Jones, Wong, Sivichai, Au & Hywel-Jones, 1999; Pang, Abdel-Wahab, Sivichai, El-Sharouney & Jones, 2002; Shearer et al., 2009; Suetrong et al., 2009; Zhang et al., 2011; Zhang, Crous, Schoch & Hyde, 2012). They occupy several life modes as saprobes, endophytes, epiphytes, pathogens (plants, animals and humans) and have associations with lichens (Boehm, Schoch & Spatafora, 2009; Boonmee et al., 2011, 2012; Chomnunti et al., 2011; Crous et al., 2009; Hofmann, Kirschner & Piepenbring, 2010; Liu et al., 2012; Nelsen et al., 2009; Ruibal et al., 2009; Schoch et al., 2009; Shearer et al., 2009; Suetrong et al., 2009; Tanaka et al., 2009; Wu et al., 2011; Zhang et al., 2012). Additionally, a few species occur on dung, or rocks and other inorganic substrates (Cannon & Kirk, 2007; Ruibal et al., 2009; Tanaka et al., 2009; Zhang et al., 2012).

Saprobic Dothideomycetes comprise numerous species widely distributed in tropical to subtropical areas (Boehm, Mugambi et al., 2009; Boonmee et al., 2011, 2012; Pang et al., 2002; Promputtha & Miller, 2010; Suetrong, Hyde, Ahang, Bahkali & Jones, 2011; Wu et al., 2011, Zhang et al., 2011). Most members are commonly found in all parts of plant debris, especially woody litter, where they degrade cellulose and lignin for their nutrients (Hyde, Jones, Leaño, Ponting & Vrijmoed, 1998; Meier, Rapp, Bowers, Silman & Fierer, 2010; Vrijmoed, 2000). Members are the families *Hysteriaceae, Kirschsteiniotheliaceae, Pleosporaceae, Tubeufiaceae* and many other (Boehm, Mugambi et al., 2009; Boonmee et al., 2011, 2012; Zhang et al., 2011).

Dothideomycetes are poorly studied and some novel species have been discovered in Thailand. Chomnunti et al. (2011) reported many epiphytic species, *Capnodiaceae* (*Capnodiales*) on living leaves of various hosts. Liu et al. (2011) reported several species of *Astrosphaeriella* found on monocotyledon plants (bamboo and palms). Species of *Noemicrothyrium* (*Microthyriales*) have been published by Wu et al. (2011). Boonmee et al. (2011, 2012) reported on several species of woody fungi belonging to *Botryosphaeriaceae*, *Kirschsteiniotheliaceae* and *Tubeufiaceae*. Liu et al. (2012) reported on several species of *Botryosphaeria* (*Botryosphaeriales*). New species were also descibed in *Botryosphaeriales*, *Jahnulales*, *Microthyriales* and *Pleosporales*, most being saprobes (Boonmee et al., 2011, 2012; Liu et al., 2012;

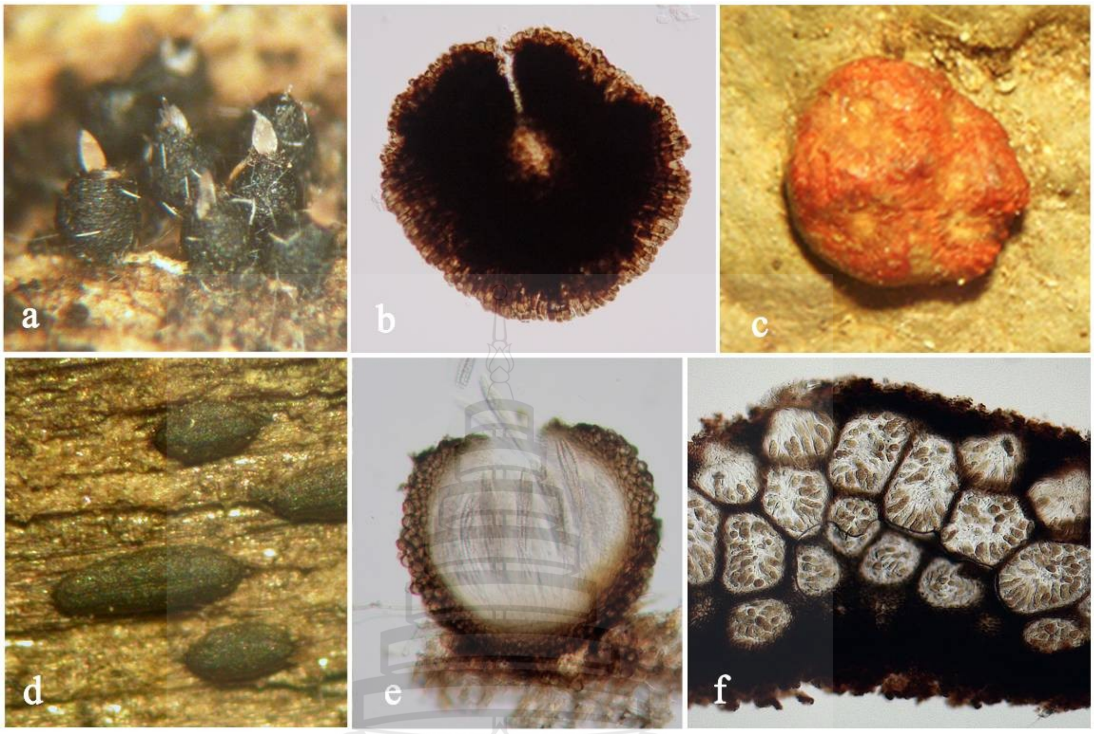
Wu et al., 2011). However, there are still numerous undecribed species yet to be found in Thailand.

1.2 Morphology of Dothideomycetes

Most fungal structures developed from filamentous hyphae. Sexual ascomata develop from ascogenous hyphae or pseudoparenchymatous cells (Eriksson, 1981). Morphologically, main structures comprise ascomata, hamathecial pseudoparaphyses, asci and ascospores.

1.2.1 Ascomata

Ascomata are stromatic, they can be multiloculate or uniloculate. Multilocular ascostromata can be found in members of *Dothideales* and *Myriangiales*, while uniloculate structures are common in *Pleosporales* (Barr, 1987; Miller, 1938; Zhang et al., 2012). Ascomata may be pseudothecium, hysterothecium, thyriothecium, crustose or cushion-like (Fig. 1.1). Their shapes vary being globose, subglobose, conical to flask-like, cup-like, circular, elongate, or even irregular-shaped. Most have dark pigmentation, while bright coloured froms can be found in members of *Tubeufiaceae* (Boonmee et al., 2011).

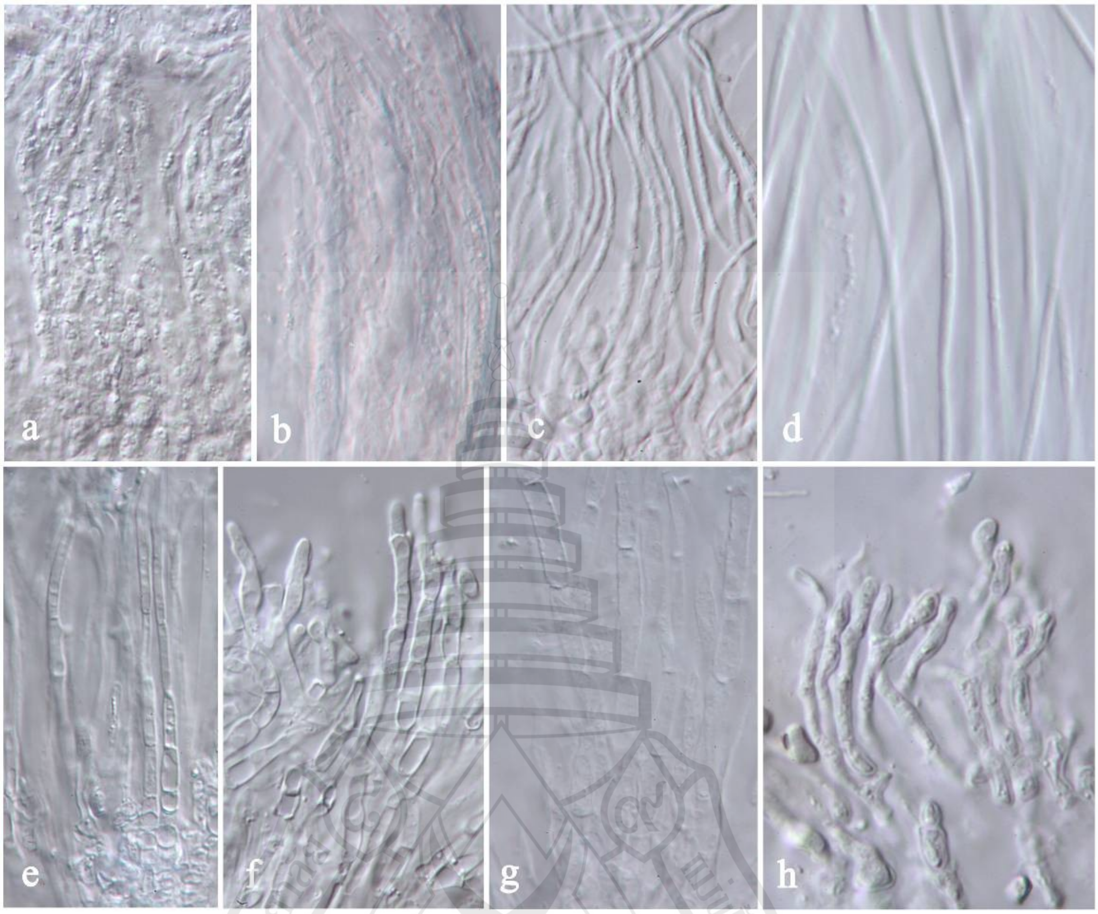


Note. (a) Pseudothecium. (b) Thyriothecium. (c) Crustose or cushsion-like ascostromata. (d) Hysterothecium (e) Uniloculate ascoma. (f) Multilocular ascostromatic.

Figure 1.1 Ascomatal Types

1.2.2 Hamathecial Pseudoparaphyses

Hamathecial pseudoparaphyses are sterile tissues within the ascomata (Luttrell, 1965). These cells are a widely presenced in many orders, and especially persistent in most members of *Pleosporales* (Zhang et al., 2012), while in the orders *Capnodiales*, *Dothideales* and *Myriangiales* these structures are lacking (Chomnunti et al., 2011, Liew, Aptroot & Hyde, 2000; Liu et al., 2012; Miller, 1938). Most cells are hyaline, septate or aseptate, branched or unbranched, and can be thin or thick (Fig. 1.2).

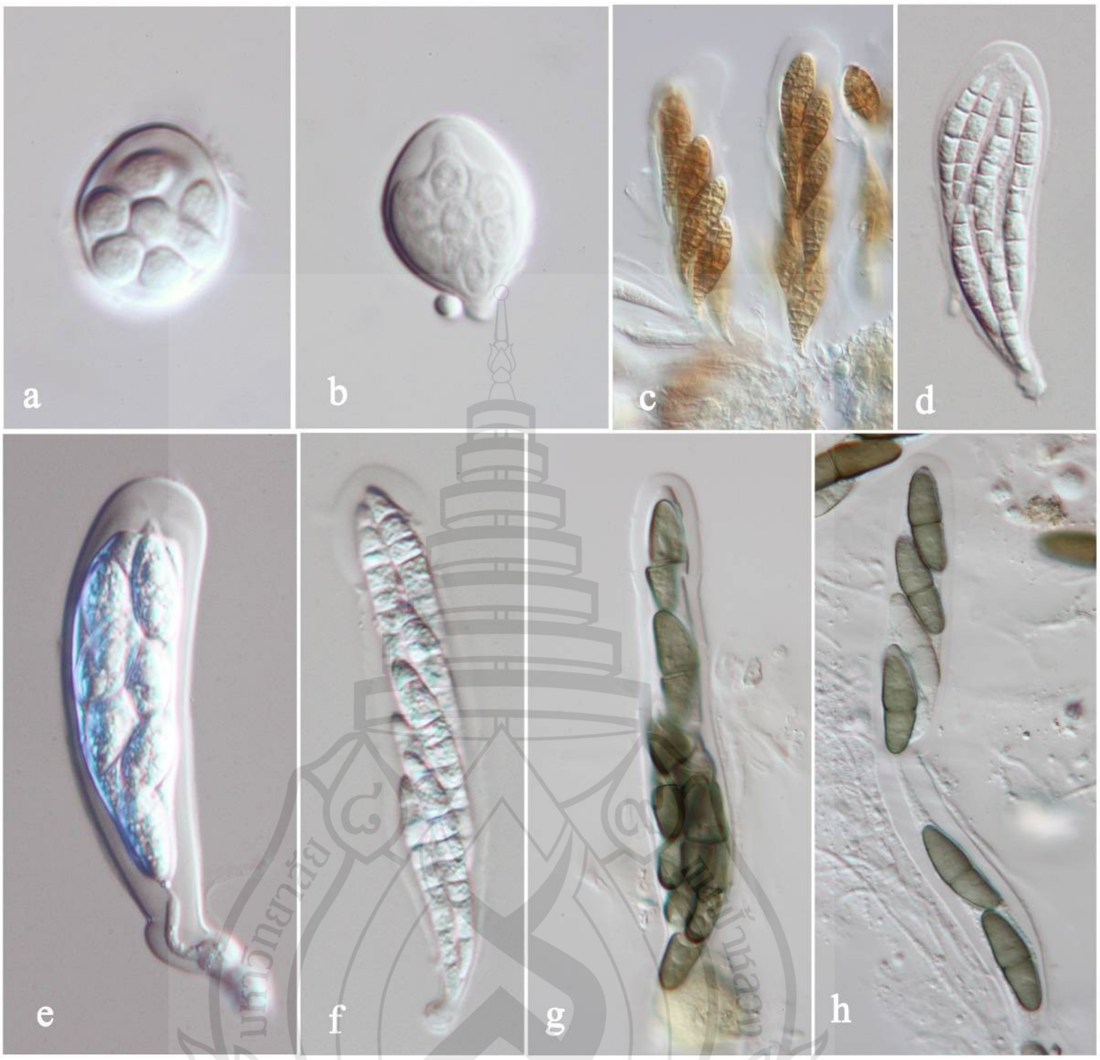


Note. (a)-(d) Thin-walled, cellular or trabeculate pseudoparaphyses. (e)-(g) Thick-walled, clearly septate (h) branched cells of pseudoparaphyses.

Figure 1.2 Pseudoparaphysis Types

1.2.3 Bitunicate Asci

The asci of Dothideomycetes are definitely bitunicate (two-layered wall), some species realese spores through a fissitunicate mechanism (Fig. 1.3). There vary in shape, which can be globose, subglobose, cylindrical, cylindro-clavate, or saccate. Some species are characterized by apical ocular chambers (Fig. 1.3b, d-e). The pedicels may be long, distinct or lacking.

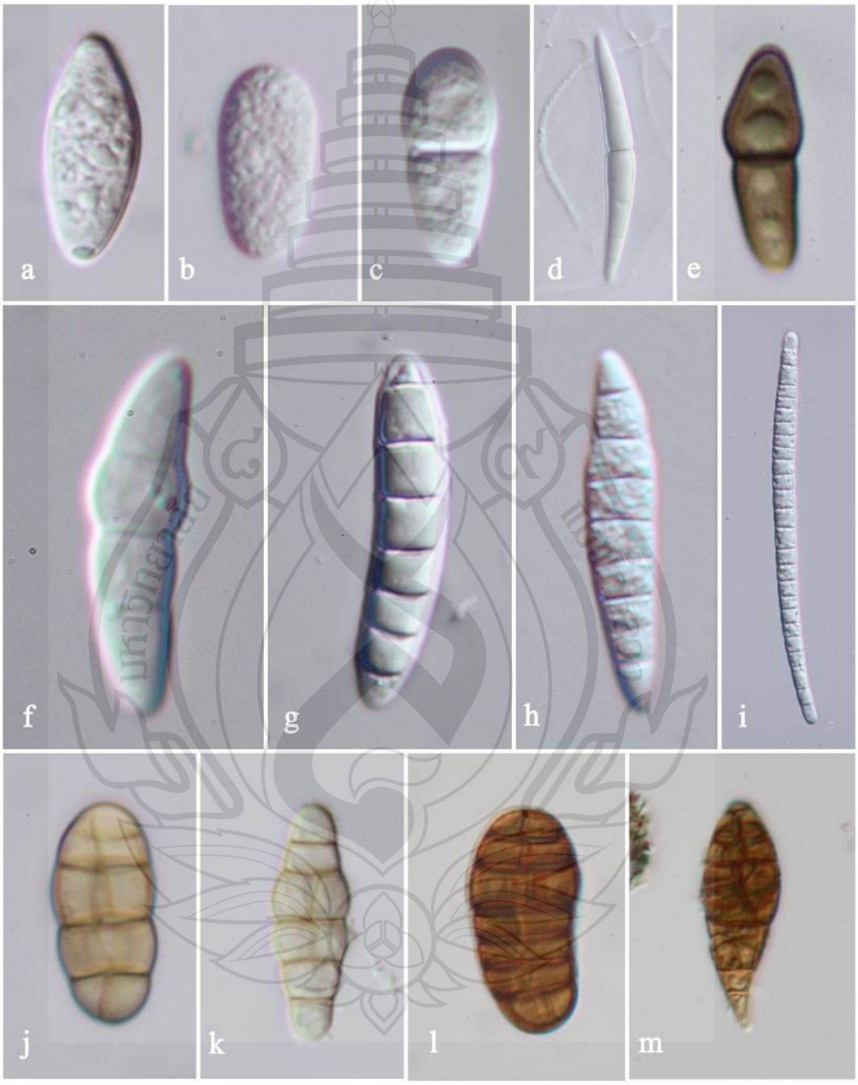


Note. (a)-(e) Two-layered wall of asci: globose, subglobose, cylindrical or saccate.
(f)-(h) Fissitunicate mechanism (jack-in-the-box: Asci splitting at discharge)

Figure 1.3 Morphological Characteristics of Bitunicate Asci in Dothideomycetes

1.2.4 Ascospores

Ascospores are of various types; they can be unicellular (amerosporous), bicellular (didymosporous), or multicellular (phragmosporous or dictyosporous) (Fig. 1.4). They are usually either hyaline (colourless), while some species have brownish, brown, dark brown or purplish pigment.



Note. Types of ascospores. (a)-(b) Amerospores. (c)-(e) Didymerospores. (f)-(i) Phragmospores. (j)-(m) Dictyospores

Figure 1.4 Ascospore Characters

1.2.5 Asexual States of Dothideomyces

Many speices of coelomycetous and hyphomycetous asexual fungi are connected to dothidomycetous sexual morphs (Hyde, McKenzie & KoKo, 2011). For example, the family *Tubeufiaceae* is connected with helicosporous several genera of hyphomycetes (Boonmee et al., 2011; Tsui & Berbee, 2006; Tsui, Sivichai & Berbee, 2006).

1.3 Orders of Dothideomycetes

The Dothideomycetes comprise 15 orders, mostly accepted based on molecular phylogenetic studies (Lumbsch & Huhndorf, 2010), and are listed in the Table 1.1. Each order is briefly discussed below. Six families *Ascoporiaceae, Coccoideaceae, Cookellaceae, Perisporiopsidaceae, Protoscyphaceae* and *Pseudoperisporiaceae* are included under family's *incertae sedis* because they lack molecular data and thus their position in Dothideomycetes cannot be resolved.

1.3.1 *Acrospermales*

Acrospermales Minter, Peredo & A. T. Watson, Boln Soc. argent. Bot. 42(1-2): 112 (2007)

Acrospermaceae has been placed in various orders at various times (Barr, 1990; Brandriff, 1936; Eriksson, 1982; Eriksson & Hawksworth, 1991; Hawksworth, Kirk, Sutton & Pegler, 1995; Kirk, Cannon, David & Stalpers, 2001; Sherwood, 1977). Minter, Peredo and Watson (2007) introduced the order *Acrospermales* to accommodate the family *Acrospermaceae* Fuckel., which is typified by *Acrospermum compressum* Tode. Lumbsch and Huhndorf (2010) placed the *Acrospermales* in Dothideomycetes, orders *incertae sedis*. Stenroos et al. (2010) recently, indicated that the *Acrospermaceae*, *Acrospermales* should be placed in Dothideomycetes, and also closely clustered with strains of Arthoniomycetes.

1.3.2 *Asterinales*

Asterinales M. E. Barr ex D. Hawksw. & O. E. Erikss., Syst. Ascom. 5(1): 177 (1986)

The order *Asterinales* was invalidly introduced by Barr (1976) because of lack of Latin diagnosis, and validated by Hawksworth and Eriksson (1986). The *Asterinales* is based on the type family *Asterinaceae* and is characterized by superficially, thyriothecial, dimidiate, appressed hyphae, with radial mycelium and often hyphopodiate, black ascomata, globose-subglobose asci and 1-septate, brown ascospores (Barr, 1987; Hosagoudar, 2012). Recently, molecular phylogenetic analyses confirmed that the *Asterinaceae*, *Asterinales* can be included in the Dothideomycetes with good support (Hofmann et al., 2010; Wu et al., 2011). Wu et al. (2011) also suggested the families *Aulographaceae* and *Parmulariaceae* should be included in this order. Therefore, the *Asterinales* presently includes three families *Asterinaceae*, *Aulographaceae* and *Parmulariaceae*.

1.3.3 *Botryosphaeriales*

Botryosphaeriales C. L. Schoch, Crous & Shoemaker, in Schoch et al., Mycologia 98(6): 1050 (2007) [2006]

= *Phyllostictales* Seaver, North American Flora 6(1): 3 (1922)

The order *Botryosphaeriales* was introduced by Schoch et al. (2006) based on phylogenetic classification and this has been verified in several recent papers. The order comprises three families; *Botryosphaeriaceae*, *Planistromellaceae* and *Phyllostictaceae* (Liu et al., 2012; Monkai et al., 2013). The order is defined by relatively thick-walled, dark-celled ascostromata, with one to several locules, bitunicate and fissitunicate asci and 1-2-celled ascospores, which are hyaline when immature and become brown in some genera at maturity. Cut ascostromata of dried specimens usually reveal distinct white contents. The asexual states are coelomycetous and produce hyaline to brown conidia from phialidic conidiogenous cells in conidiomata that usually form initially in the ascostromata.

1.3.4 *Capnodiales*

Capnodiales Woron., Annls mycol. 23(1/2): 177 (1925)

The order *Capnodiales* was introduced by Woronichin (1925), and accepted in Dothideomycetes based on phylogenetic studies (Chomnunti et al., 2011; Crous et al., 2009; Schoch et al., 2006, 2009). Lumbsch and Huhndorf (2010) list the order as comprising eight families: *Antennulariellaceae* Woron., *Capnodiaceae* (Sacc.) Höhn. ex Theiss., *Coccodiniceae* Hohn. ex O. E. Erikss., *Davidiellaceae* C. L. Schoch, Spatafora, Crous & Shoemaker, *Dissoconiaceae* Crous & de Hoog, *Metacapnodiaceae* Hughes & Corlett, *Mycosphaerellaceae* Lindau and *Piedraiaceae* Viegas ex Cif., Bat. & Campos. The *Capnodiales*, are mainly defined by their shared ecological niche as leaf epiphytes associated with the honey dew produced by insects, and saprobes or leaf pathogens, and are an ascostromatal order without pseudoparaphyses (Chomnunti et al., 2011; Crous et al., 2009; Hughes, 1976).

1.3.5 *Dothideales*

Dothideales Lindau, in Engler & Prantl, Nat. Pflanzenfam., Teil. I (Leipzig) 1: 373 (1897)

The order *Dothideales* was introduced as early as 1897 by Lindau (1897) and typified by *Dothidea sambuci* (Pers.) Fr. Molecular phylogenetic studies indicate that the order *Dothideales* belongs to Dothideomycetes with bootstrap value high support (Boehm, Mugambi et al., 2009; Schoch et al., 2009; Shearer et al., 2009; Suetrong et al., 2009). Lumbsch and Huhndorf (2010) list the order as comprising three families; *Dothideaceae*, *Dothioraceae* and *Teratosphaeriaceae*. However, *Dothioraceae* is shown to be a synonym of *Dothideaceae* in this study, while Crous et al. (2009) placed *Teratosphaeriaceae* in *Capnodiales*. The order is characterised by immersed, erumpent or superficial, uniloculate or multiloculate ascostromata, with or without a papillate apex, bitunicate asci, and usually septate, symmetric or longitudinally asymmetrical, sometimes muriform, hyaline or pigmented ascospores. The asexual states are coelomycetous.

1.3.6 *Hysteriales*

Hysteriales Lindau, in Engler & Prantl, Nat. Pflanzenfam., Teil. I (Leipzig) 1: 265 (1896)

The order *Hysteriales* was introduced as early as 1896 by Lindau (1896) and has been placed among the pyrenomycetes and the discomycetes at different times (Rehm, 1896). Molecular data now places *Hysteriales* into Dothideomycetes (Boehm, Mugambi et al., 2009; Boehm, Schoch et al., 2009; Shearer et al., 2009; Suetrong et al., 2009). This order consists of a single family, *Hysteriaceae*, with characteristics morphological features including elongate hysterothecium, opening by a longitudinal slit, and completely dark ascospores at maturity; species in the *Hysteriales* are also commonly found on wood (Boehm, Mugambi et al., 2009; Boehm, Schoch et al., 2009).

1.3.7 *Jahnulales*

Jahnulales K. L. Pang et al., in Pang et al., Mycol. Res. 106(9): 1033 (2002)

Pang et al. (2002) introduced the order *Jahnulales* for species with ascomata with well-developed long hyphal stalks or pedicels. The placement of *Jahnulales* in Dothideomycetes is confirmed by molecular analysis (Campbell, Ferrer, Raja, Sivichai & Shearer, 2007; Pang et al., 2002; Schoch et al., 2009; Shearer et al., 2009; Suetrong et al., 2009; 2011). *Jahnulales* comprises three families: *Aliquandostipitaceae*, *Kirschsteiniotheliaceae* and *Manglicolaceae* (Boonmee et al., 2012; Suetrong et al., 2011). Members of *Jahnulales* are aquatic species which are lignicolous, saprobic, and occur on rotting or submerged woody debris and palms (Campbell et al., 2007; Inderbitzin, Landvik, Abdel-Wahab & Berbee, 2001; Pang et al., 2002; Raja & Shearer, 2006, 2008; Suetrong et al., 2011). The order is characterized by mycelium that is wide, *ca*. 40 µm; ascomata sessile or borne on a septate, long wide stalks (up to 450 × 25–80 µm); hypha-like or trabeculate, septate, unbranched pseudoparaphyses up to 4 µm wide; bitunicate asci with an ocular chamber; and ascospores that are 1-septate, pale to dark brown, with bipolar apical appendages or surrounded by a thin or elaborate wide gelatinous sheath (Suetrong et al., 2011).

1.3.8 *Microthyriales*

Microthyriales G. Arnaud, Les Astérinées: 85 (1918)

The order *Microthyriales* was introduced by Arnaud (1918) with the family type *Microthyriaceae*. Schoch et al. (2009) provided molecular evidence based on a strain of *Microthyrium microscopicum* (the type species of *Microthyriaceae*) and *Stomiopeltis betulae* J. P. Ellis (*Micropeltidaceae*) to representative the *Microthyriales*, to show the order can be placed in Dothideomycetes. However, the molecular data based on these limited sequences did not provide enough clarity to support the order. Lumbsch and Huhndorf (2010) placed *Microthyriaceae* and *Micropeltidaceae* in Dothideomycetes family *incertae cedis*, and *Microthyriales* was not listed. In this study we accept the *Microthyriales* based on morphological data and include the families *Microthyriaceae* and *Micropeltidaceae*. The *Microthyriales* are characterised by dark, circular, thyriothecial ascomata with poorly developed bases and central rounded ostioles (Wu et al., 2011). The bitunicate asci are inclined from the base and outer rim towards the central ostiole and pseudoparaphyses may be present or lacking. Ascospores and hyaline or brown, mostly 2-celled or with a few to several transverse septa. Species are foliar epiphytes, biotrophs, saprobes or epiphytes on dead or living leaves and stems of plants. These group of fungi are relatively poorly studied (Wu et al., 2011), and thus molecular data is needed to confirm the relationships of these families and their genera, which are generally poorly know. Asexual states coelomycetous, (*Asterostomula*, *Cyclopeltella, Leptothyrium, Sirothyriella*); and hyphomycetous (*Hansfordiella*, *Holubovaniella, Isthmospora, Xenogliocladiopsis, Zalerion*) (Kirk et al., 2008; Seifert, Morgan-Jones, Gams & Kendrick, 2011; Wijayawardene, Mckenzie & Hyde, 2012; Wu et al., 2011).

1.3.9 *Myriangiales*

Myriangiales Starbäck, Bih. K. svenska VetenskAkad. Handl., Afd. 3 25(no. 1): 37 (1899)

Starbäck (1899) introduced the order *Myriangiales* for the species characterised by having crustose ascostromata and muriform ascospores, similar to the type species, *Myriangium duriaei* Mont. & Berk. (Berkeley, 1845; Miller, 1938). Phylogenetic studies have indicated that *Myriangiales* always cluster with Dothideomycetes, based on the multi-gene analysis (Boehm, Mugambi et al., 2009; Phillips et al., 2008; Schoch et al., 2009; Zhang et al., 2011). Kirk et al. (2008) included three families, *Cookellaceae, Elsinoaceae* and *Myriangiaceae* in *Myriangiales*, while Lumbsch and Huhndorf (2010) accepted only *Elsinoaceae* and *Myriangiaceae* in the order based on phylogenetic results, while *Cookellaceae* was treated in Dothideomycetes family *incertae cedis*. The order is characterized by pulvinate, irregular ascostromata in which the asci are irregularly arranged in one or more layers in locules. Locules are with single or multiple asci within each locule. Asci have minute pedicels and indistinct ocular chambers. Ascospores which are irregularly arranged are liberated only by the breakup of the stromatal layers above them. Asexual states are coelomycetous.

1.3.10 *Mytilinidiales*

Mytilinidiales Boehm, C. L. Schoch & Spatafora, Mycol. Res. 113(4): 468 (2009)

The *Mytilinidiaceae* was separated from the order *Pleosporales* and introduced to the higher taxonomic rank, *Mytilinidiales* by Boehm, Schoch et al. (2009) with molecular phylogenetic classification. The order comprises *Gloniaceae* and *Mytilinidiaceae* (Boehm, Schoch et al., 2009; Lumbsch & Huhndorf, 2010). Morphologically, the order is characterized by rigid, brittle, hysterothecial, carbonaceous ascomata with the appearance of bivalve shells or wedge-shaped, and ascospores of various septate types; didymosporous, phragmosporous and/ or dictyosporous (Alexopoulos, Mims & Blackwell, 1996; Boehm, Mugambi et al., 2009; Boehm, Schoch et al., 2009; Sivanesan, 1984). Most are pantropical species, saprobic on wood, bark, stem, twigs, gymnosperms and some species are parasitic on leaves (Barr, 1975, 1987, 1990; Boehm, Mugambi et al., 2009; Pereira & Barreto, 2003).

1.3.11 *Patellariales*

Patellariales D. Hawksw. & O. E. Erikss., Syst. Ascom. 5(1): 181 (1986)

The order *Patellariales* was introduced by Hawksworth and Eriksson (1986) based on morphological data to accommodate the families *Patellariaceae* Corda, *Arthrorhaphidaceae* Poelt & Hafellner and *Phillipsiellaceae* von Hohnel., while Barr (1987) only accepted *Patellariaceae* in *Patellariales*. Luttrell (1951) had considered

Hysteriaceae and *Patellariaceae* to be Discomycetes with bitunicate asci and placed them in the Bitunicate group. Kutorga and Hawksworth (1997) later monographed several genera of *Patellariaceae* and 17 genera were accepted. Morphologically features of the *Patellariales* comprises apothecial or hysterothecial, cup-shaped ascomata, bitunicate asci and hyaline, septate ascospores (Kutorga & Hawksworth, 1997). Boehm, Mugambi et al. (2009), Schoch et al. (2009) subsequently showed that the *Patellariales* belonged to Dothideomycetes using molecular data.

1.3.12 *Pleosporales*

Pleosporales Luttr. ex M. E. Barr, Prodr. Cl. Loculoasc. (Amherst): 67 (1987)

The order *Pleosporales* was invalidly introduced by Luttrell (1955), and later validated by Barr (1987), and is based on the family *Pleosporaceae* with the type species *Pleospora herbarum* (Barr, 1987). It is the largest order of Dothideomycetes (Kirk et al., 2008). Molecular studies indicated that *Pleosporales* should comprise 20 accepted families (Boehm, Mugambi et al., 2009; Mugambi & Huhndorf, 2009; Schoch et al., 2009; Shearer et al., 2009; Suetrong et al., 2009; Tanaka et al., 2009; Zhang, Fournier, Crous, Pointing, & Hyde, 2009, Zhang, Schoch et al., 2009), however, recently Zhang et al. (2012) included 26 families. *Pleosporales* presently comprises 26 families (Zhang et al., 2012), while *Venturiaceae* was excluded from the *Pleosporales* by Zhang et al. (2011) and placed in *Venturiales*. Members of *Pleosporales* are saprobic on dead plant material in fresh water, marine, or terrestrial environments, and a large number, especially asexual states are pathogens on living plants (Boonmee et al., 2012; Zhang, Fournier et al., 2009). *Pleosporales* comprises the suborders *Pleosporineae* and *Massarineae*. Zhang, Schoch et al. (2009) introduced *Pleosporineae*, which is a phylogenetically well supported suborder of *Pleosporales* and includes seven families i.e. *Cucurbitariaceae*, *Didymellaceae*, *Didymosphaeriaceae*, *Dothidotthiaceae*, *Leptosphaeriaceae*, *Phaeosphaeriaceae* and *Pleosporaceae*, while Ariyawansa et al. (2013) and Liu et al. (2013) included two new families, *Halojulellaceae* and *Shiraiaceae*. *Pleosporineae* contains economically important plant pathogens (de Gruyter et al., 2010; Zhang, Fournier et al., 2009) and is characterized by broadly to narrowly oblong ascomata, downward growing pseudoparaphyses with 1- to multi-septate ascospores. Recent studies using multigene phylogeny of *Phoma* and its closely related genera, indicated that their representative species clustered in different subclades of *Pleosporineae* (de Gruyter et al., 2009, 2010). *Massarineae* is characterized by immersed or superficial ascomata, cylindrical asci with a short pedicel and 1- to multi-septate ascospores. Zhang et al. (2012) included five families in *Massarineae* viz. *Lentitheciaceae*, *Massarinaceae*, *Montagnulaceae*, *Morosphaeriaceae* and *Trematosphaeriaceae*, among them most of the species are saprobic in terrestrial or aquatic environments.

1.3.13 *Trypetheliales*

Trypetheliales Lücking, Aptroot & Sipman, in Aptroot et al., Biblthca Lichenol. 97: 13 (2008)

The order *Trypetheliales* was introduced by Aptroot, Lücking, Sipman, Umaña and Chaves (2008) to accommodate a single family *Trypetheliaceae* of lichenised fungi. This order was accepted in the Dothideomycetes based on molecular phylogenetic results (del Prado et al., 2006; Nelsen et al., 2009, 2011; Schoch et al., 2009). The order includes mostly lichenized species characterized by a branched and anastomosing hamathecium embedded in a distinct gelatinous matrix, and by usually hyaline ascospores that feature a combination of eusepta and angular distosepta, giving the lumina a diamond-shaped appearance. These characters are not constant, however, as some lineages produce ascospores with eusepta only and in some lineages they are dark brown. Also the degree of anastomosing of the hamathecium can vary, especially in basal lineages.

1.3.14 *Tubeufiales* (Chapter 5)

This order has recently been proposed based on our current study (Chapter 5). The order *Tubeufiales* based mainly on a distinct habit, most being saprobes on decaying lignocellulosic material, sexual characters and helicosporous asexual morphs. The distinction from other orders in Dothideomycetes is also strongly supported by molecular data. Tubeufialean members usually occur on decaying wood or other plant debris and are saprobes. The ascomata are characterized by superficial, light brown, dark brown to black, uniloculate pseudothecia, and asexual states are helicosporous hyphomycetous (Barr, 1979, 1980; Boonmee et al., 2011; Kodsueb, Jeewon et al., 2006; Sivanesan, 1984; Tsui et al., 2006; Tsui & Berbee, 2006).

1.3.15 *Venturiales*

Venturiales Yin. Zhang, C. L. Schoch & K. D. Hyde, Fungal Diversity 51(1): 252 (2011)

The *Venturiales* was introduced by Zhang et al. (2011), based on morphological and ecological characteristics, as well as strong support from multi-gene phylogenetic analyses. This order presently includes *Sympoventuriaceae* and *Venturiaceae* (Zhang et al., 2011). Some species are plant pathogens and others are saprobic, mostly on leaves or stems of dicotyledons. Important characters are small to medium-sized ascomata, with or without seta, a mostly evanescent hamathecium, 8-spored, broadly or usually obclavate asci, usually lacking a pedicel, hyaline, light greenish olivaceous to brown, 1-septate, symmetrical, asymmetrical or apiosporous ascospores and coelomycetous asexual states.

Table 1.1 Orders and Families in The Dothideomycetes

Order	Family
Acrospermales Minter	Acrospermaceae Fuckel
Asterinales M. E. Barr ex D. Hawksw. & O. E. Erikss.	Asterinaceae Hansf.
	Aulographaceae Luttr. ex P. M. Kirk, P. F. Cannon & J. C. David
	Parmulariaceae
Botryosphaeriales C. L. Schoch, Crous & Shoemaker	Botryosphaeriaceae Theiss. & H. Syd.
	Planistromellaceae M. E. Barr
Capnodiales Woron.	Antennulariellaceae Woron.
	Capnodiaceae (Sacc.) Hohn. ex Theiss.
	Coccodiniaceae Ho hn. ex O. E. Erikss.
	Davidiellaceae C. L. Schoch, Spatafora, Crous & Shoemaker
	Dissoconiaceae Crous & de Hoog
	Metacapnodiaceae Hughes & Corlett
	Mycosphaerellaceae Lindau
	Piedraiaceae Viegas ex Cif., Bat. & Campos
Dothideales Lindau	Dothideaceae Chevall.
	Dothioraceae Theiss. & H. Syd.
	Teratosphaeriaceae Crous & U. Braun
Hysteriales Lindau	Hysteriaceae Chevall.
Jahnulales Pang, Abdel-Wahab, El-Sharouney, E. B. G. Jones & Sivichai	Aliquandostipitaceae Inderbitzin
	Kirschsteiniotheliaceae Boonmee & K. D Hyde
	Manglicolaceae Suetrong & E. B. G. Jones
Microthyriales G. Arnaud	Micropeltidaceae Clem. & Shear
	Microthyriaceae Sacc.
Myriangiales Starbäck	Elsinoaceae Höhn. ex Sacc. & Trotter
	Myriangiaceae Nyl.
Mytilinidiales Boehm, C. L. Schoch & Spatafora	Gloniaceae (Corda) Boehm, C. L. Schoch & Spatafora
	Mytilinidiaceae Kirschst.
Patellariales D. Hawksw. & O. E. Erikss.	Patellariaceae Corda

Table 1.1 (continued)

Order	Family
Pleosporales Luttrell ex M. E. Barr	Aigialaceae Suetrong, Sakayaroj, E. B. G. Jones, Kohlm., Volkm.-Kohlm. & C. L. Schoch
	Amniculicolaceae Yin. Zhang, C. L. Schoch, J. Fourn., Crous & K. D. Hyde
	Cucurbitariaceae G. Winter
	Delitschiaceae M. E. Barr
	Diademaceae Shoemaker & C. E. Babc.
	Didymellaceae Gruyter, Aveskamp& Verkley
	Didymosphaeriaceae Munk
	Dothidotthiaceae Crous & A. J. L. Phillips
	Halojulellaceae Ariyawansa et al.
	Hypsostromataceae Huhndorf
	Lentitheciaceae Yin. Zhang, C. L. Schoch, J. Fourn., Crous & K. D. Hyde
	Leptosphaeriaceae M. E. Barr
	Lindgomycetaceae K. Hiray., Kaz. Tanaka & Shearer
	Lophiostomataceae Sacc.
	Massariaceae Nitschke
	Massarinaceae Munk
	Melanommataceae G. Winter
	Montagnulaceae M. E. Barr
	Morosphaeriaceae Suetrong, Sakayaroj, E. B. G. Jones & C. L. Schoch
	Phaeosphaeriaceae M. E. Barr
	Pleomassariaceae M. E. Barr
	Pleosporaceae Nitschke
	Shiraiaceae Liu et al.
	Sporormiaceae Munk
	?Teichosporaceae M. E. Barr
	Tetraplosphaeriaceae Kaz. Tanaka & K. Hiray

Table 1.1 (continued)

Order	Family
	Trematosphaeriaceae Suetrong, C. L. Schoch, Spatafora, Kohlm., Volkm.-Kohlm., Sakay., Phongp., K. Tanaka, K. Hiray. & E. B. G. Jones
	?Zopfiaceae G. Arnaud ex D. Hawksw.
Tubeufiales S. Boonmee, A. Y. Rossman & K. D. Hyde	Tubeufiaceae M. E. Barr
Trypetheliales Lucking, Aptroot & Sipman	Trypetheliaceae Zenker
Venturiales Yin. Zhang, C. L. Schoch & K. D. Hyde	Venturiaceae E. Mull. & Arx ex M. E. Barr
	Sympoventuriaceae Yin. Zhang, C. L. Schoch & K. D. Hyde
Dothideomycetes, families *incertae sedis*	Ascoporiaceae Kutorga & D. Hawksw.
	Coccoideaceae P. Henn. ex Sacc. & D. Sacc.
	Cookellaceae Höhn. ex Sacc. & Trotter
	Perisporiopsidaceae E. Müll. & Arx ex R. Kirschner & T. A. Hofm.
	Protoscyphaceae Kutorga & D. Hawksw.
	Pseudoperisporiaceae Toro

1.4 Research Objectives

1) To study the diversity of species in families *Kirschsteiniotheliaceae* and *Tubeufiaceae* on woody litter in northern Thailand.

2) To validate the taxonomic status in selected families of Dothideomycetes based on the type of herbarium materials.

3) To define the systematic families of Dothideomycetes on the basis of morphology and phylogenetic analysis, including sexual and asexual connections.

1.5 Research Contents

This thesis is divided into six chapters.

Chapter 1 is the review chapter which comprises of introduction, history of phylogenetic study, orders in the Dothideomycetes and objectives of this research.

Chapter 2 presents the diversity of *Tubeufiaceae* saprobic taxa in Thailand. Fresh materials were investigated from dead wood in terrestrial forests throughout northern Thailand. The morphological taxonomy is given with descriptions and illustrations. Herbarium type specimens were loaned from several herbaria to establish characters of genera. Molecular phylogenetic data were used to identify the new taxa.

Chapter 3 studies saprobic fungi of the *Kirschsteiniotheliaceae*, Dothideomycetes. Fresh material was surveyed on dead wood in northern Thailand. New collections were determined and isolated by single spore. The morphological taxonomy is provided by description and illustration. Molecular phylogenetic data were used to identify the new taxa.

Chapter 4 Morphological taxonomy of fungal herbarium type specimens were loaned from several herbaria. Nine representative families of the Dothideomycetes are redescribed, illustrated, discussed and keyed on the basis of re-examination of type material.

Chapter 5 introduces the new order *Tubeufiales*. The objective of this study is to reexamine the taxonomic status of *Tubeufiaceae* based on morphological, molecular phylogeny and anamorphic states. This is to evaluate its ordinal placement and relationships with Dothideomycetes.

Chapter 6 provides the overall conclusion of the finding of the thesis and suggestion for future work.

CHAPTER 2

REVISION OF LIGNICOLOUS *Tubeufiaceae* BASED ON MORPHOLOGICAL REEXAMINATION AND PHYLOGENETIC ANALYSIS

2.1 Introduction

The family *Tubeufiaceae* sensu Barr (1979) included the type genus *Tubeufia* and four representative genera (*Letendraea, Melioliphila, Podonectria,* and *Thaxteriella*) and was considered to be pleosporaceous. The representatives were saprobic, hyperparasitic or hypersaprobic on ascomycetes and scale insects, and characterized by brightly pigmented, fleshy, superficial ascomata, bitunicate asci, and mostly hyaline to pale brownish, narrowly-elongate, obovoid or oblong septate ascospores. Barr (1980) included ten genera while Rossman (1987) studied the family in detail and included 12 genera (Table 2.1). Other genera have since been added and the family now encompasses 23 genera (Lumbsch & Huhndorf, 2010) or 22 genera (Kirk et al., 2001). Most taxa of *Tubeufiaceae* are commonly found on woody litter, although some species can also be found on leaf litter or even decaying cloth and some are associated with other fungi or scale insects (Barr, 1980; Kodsueb, Jeewon et al., 2006; Promputtha & Miller, 2010; Rossman, 1987; Sánchez & Bianchinotti, 2010). The anamorphs of *Tubeufiaceae* have been well-studied and are mostly related to helicosporous taxa such as *Helicoma, Helicomyces* and *Helicosporium* (Hyde et al., 2011; Tsui et al., 2006, Tsui, Sivichai, Rossman & Berbee, 2007).

In this study, the generic types or authentic specimens of several tubuefiaceous genera were examined, described and illustrated. The woody litter in forest throughout northern Thailand was surveyed and obtained several new collections of these fungi, which were also described, isolated and sequenced. We compared the sequence data of these new collections to those from the generic types and to provide insights on the inter-generic and species relationships in *Tubeufiaceae*. One new genus and five new species encountered during the course of the field work were described.

2.2 Materials and Methods

2.2.1 Sample Collection and Specimen Examination

Decaying wood was randomly collected from forests at various sampling sites in northern Thailand (i.e. Chiang Mai, Chiang Rai, Lam Pang and Pha Yao). Material was examined under a Motic SMZ 168 Series microscope. Micromorphological structures were photographed using a Nikon ECLIPSE 80i compound microscope with a Canon 450D digital camera, and measurements were made using the Tarosoft (R) Image Frame Work program. Images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems Inc., The United States). The type herbarium material is deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand, and cultures are deposited in the Mae Fah Luang University Culture Collection (MFLUCC), BIOTEC Culture Collection (BCC) and IFRD culture collections, International Fungal Research & Development Centre, Kunming, China, the latter under material transfer agreement No. 4/2010 (MTA). Isolation of fungi Single spore isolates were cultured on water agar (WA) or malt extract agar (MEA, Difco Laboratories, Detroit, Michigan, USA).

Herbarium type specimens of *Acanthophiobolus, Allonecte, Byssocallis, Kamalomyces, Podonectria, Taphophila, Thaxteriella, Thaxteriellopsis* and *Thaterina* were obtained from the repositories of U.S. National Fungus Collections (BPI), Farlow Reference Library and Herbarium of Cryptogamic Botany (FH), Karl-Franzens-Universität Graz (GZU), CABI Bioscience UK Centre (IMI), New York Botanical Garden (NY), Swedish Museum of Natural History (S), Tropical Forest Research Institue (TFRI) and Naturhistorisches Museum Wien (W). The herbarium specimens were rehydrated by 5% KOH and/or distilled water prior to examination. For genera *Acanthostigma, Chaetosphaerulina, Letendraeopsis* and *Tubeufia* were not able to locate the type specimens, these are provided by drawing based on protologues. The classification of *Tubeufiaceae* members is presently shown in Table 2.1.

2.2.2 Isolation of Fungi

Germinating spores were transferred to MEA media and incubated at room temperature for 1 week. Isolates were grown for 1-2 months when morphological characters in culture, such as the extent of the mycelium, colour, shape and texture were recorded. These cultures were also used for DNA extraction and sequencing.

2.2.3 DNA Extraction, PCR Amplification and Sequencing

Fungal mycelium was scraped from surface colonies grown on MEA 1 month. DNA was extracted with a Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, China) following the manufacturer's instructions (Hangzhou, P.R. China). The DNA amplification was performed by polymerase chain reaction (PCR). Primer pairs used were ITS5/ ITS4, NS1/ NS4 and LROR/ LR5 (White, Bruns, Lee & Taylor. 1990; Vilgalys & Hester 1990, see also Table 2.2). PCR amplification was performed in 50 µl reaction volume, consisting of 1 µl Taq DNA polymerase, 5 µl 10x PCR buffer, 1 µl dNTP, 5 µl $MgCl_2$, 4 µl of each forward and reverse primer, 5 µl template DNA, and dH_2O. The PCR conditions were initial denaturation at 95^{o}C for 2 min, followed by 35 cycles of 94^{o}C, 1 min; 50^{o}C, 1 min; and 72^{o}C, 1 min; and a final extension step of 72^{o}C for 10 min. PCR products were visualized by staining with red gel on 1% agarose gel electrophoresis for purity, and sent for sequencing by Shanghai Sangon Biological Engineering Technology & Services Co Ltd. (Shanghai, P.R. China). Phylogenetic analyses using a BLAST search was performed to verify identities of the cultures and to look for closely related sequences in the GenBank database.

Table 2.1 Various Classifications of *Tubeufiaceae*

Barr (1979)	Barr (1980)	Rossman (1987)	Kirk et al. (2001)	Lumbsch & Huhndorf (2010)	This study
Letendraea	*Allonectria*	*Allonectria*	*Acanthophiobolus*	*Acanthostigma*	*Acanthostigma*
Melioliphila	*Boerlagiomyces*	*Boerlagiomyces*	*Acanthostigmella*	*Acanthophiobolus*	*Acanthophiobolus*
Podonectria	*Byssocallis*	*Byssocallis*	*Allonecte*	*Acanthostigmella*	*Acanthostigmella*
Rebentischia	*Letendraea*	*Letendraea*	*Amphinectria*	*Allonecte*	*Amphinectria*
Thaxteriella	*Melioliphila*	*Malacaria*	*Boerlagiomyces*	*Amphinectria*	*Boerlagiomyces*
Tubeufia	*Paranectriella*	*Melioliphila*	*Borinquenia*	*Boerlagiomyces*	*Chaetocrea*
	Podonectria	*Paranectriella*	*Byssocallis*	*Byssocallis*	*Chaetosphaerulina*
	Puttemansia	*Podonectria*	*Chaetocrea*	*Chaetocrea*	*Glaxoa*
	Rebentischia	*Puttemansia*	*Glaxoa*	*Chaetosphaerulina*	*Kamalomyces*
	Tubeufia	*Rebentischia*	*Letendraea*	*Glaxoa*	*Malacaria*
		Tubeufia	*Letendraeopsis*	*Letendraeopsis*	*Melioliphila*
		Uredinophila	*Malacaria*	*Malacaria*	*Paranectriella*
			Melioliphila	*Melioliphila*	*Podonectria*
			Paranectriella	*Paranectriella*	*Puttemansia*
			Podonectria	*Podonectria*	*Rebentischia*
			Puttemansia	*Puttemansia*	*Thaxteriella*
			Rebentischia	*Rebentischia*	*Thaxteriellopsis*
			Taphrophila	*Taphrophila*	*Tubeufia*
			Thaxterina	*Thaxteriella*	*Uredinophila*
			Tubeufia	*Thaxteriellopsis*	
			Uredinophila	*Thaxterina*	
				Tubeufia	
				Uredinophila	

Table 2.2 Primers Used for PCR Amplification and Sequencing in This Study

Primers	Sequences (5'--->3')	References
ITS1	TCC GTA GGT GAA CCT GCG G (ITS, forward)	White, et al. (1990)
ITS4	TCC TCC GCT TAT TGA TAT GC (ITS, reverse)	White et al. (1990)
LROR	ACC CGC TGA ACT TAA GC (LSU, forward)	Vilgalys & Hester (1990)
LR5	TCC TGA GGG AAA CTT CG (LSU, reverse)	Vilgalys & Hester (1990)
NS1	GTA GTC ATA TGC TTG TCT C (SSU, forward)	White et al. (1990)
NS4	CTT CCG TCA ATT CCT TTA AG (SSU, reverse)	White et al. (1990)

2.2.4 Phylogenetic Analysis

Analysis of sequence data (Table 2.3) were aligned using BioEdit (Hall, 1999) and Clustal X (Larkin et al., 2007). The aligned datasets were analyzed using PAUP* v. 4.0b10 (Swofford, 2002). Ambiguous regions in the alignments were excluded from the phylogenetic analyses. Maximum Parsimony (MP) was performed with stepwise additions of sequences using 1,000 random replicates and tree-bisection-reconnection (TBR) branch-swapping algorithm, with MAXTREES setting at 1000. The parsimony tree scores including tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were also calculated. Bootstrap support for the branches was estimated based on 500 MP replicates with a single sequence addition replicates in each bootstrap replicate. Models of nucleotide substitution for each gene were determined using MrModeltest 2.3 (Nylander, 2004). GTR + I + G evolutional model were selected for likelihood analysis using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003). The Markov Chain Monte Carlo (MCMC; Rannala & Yang, 1996) algorithm was used to estimate posterior probabilities (PP). Six MCMC chains were run from a random starting tree for 1,000,000 generations and trees sampled every 100 generations. The first 2,000 trees were discarded as burn-in prior to convergence of the chains. The remaining 10,001 trees based on 1,000 replicates were used to construct 50% majority rule consensus tree and to calculate posterior probabilities were determined more than 95% PP given each on branches.

Table 2.3 Fungal Taxa Used for Phylogenetic Analysis with GenBank Accession Numbers and New sequences of *Tubeufiaceae* from Thailand are marked by an asterisk (*)

Fungal isolates	Sources of culture	GenBank accession numbers		
		ITS	LSU	SSU
*Acanthostigma chiangmaiensis**	MFLUCC10-0125	JN865209	JN865197	JN865185
Acanthostigma filiforme	ANM 514	GQ856146	GQ850494	–
Acanthostigma filiforme	ANM 101	–	GQ850495	–
Acanthostigma minutum	ANM 880	–	GQ850486	–
Acanthostigma minutum	ANM 238	–	GQ850487	–
Acanthostigma minutum	ANM 818	–	GQ850488	–
Acanthostigma patagonica	BBB MVB573	JN127358	JN127359	–
Acanthostigma perpusillum	UAMH 7237	AY916492	AY856892	AY856937
Acanthostigma scopulum	ANM 386	–	GQ850489	–
Acanthostigma scopulum	ANM 95	–	GQ850490	–
Acanthostigma septoconstrictum	ANM 536.1	GQ856143	GQ850491	–
Botryosphaeria stevensii	CBS 431.82	–	DQ678064	DQ678012
Botryosphaeria viticola	CBS 117009	–	DQ678087	DQ678036
Byssothecium circinans	CBS 675.92	–	AY016357	AY016339
Byssolophis sphaerioides	IFRDCC2053	–	GU301805	GU296140
*Chlamydotubeufia huaikangplaensis**	MFLUCC10-0926	JN865210	JN865198	JN865186
*Chlamydotubeufia khunkornensis**	MFLUCC10-0117	JN865201	JN865189	JN865177
*Chlamydotubeufia khunkornensis**	MFLUCC10-0118	JN865202	JN865190	JN865178
Cochliobolus heterostrophus	CBS 134.39	–	AY544645	AY544727
Didymella bryoniae	CBS 133.96	–	GU301863	–
Didymella bryoniae	CBS 133.96	–	GU301863	–
Helicoma ambiens	UAMH 10533	–	AY856916	AY856955
Helicoma chlamydosporum	CBS 160.69	–	AY856875	AY856923
Helicoma conicodentatum	UAMH 10534	–	AY856869	AY856918
Helicoma dennisii	NBRC 30667	AY916455	AY856897	–
Helicoma intermedium	ATCC 22621	–	AY856912	–
Helicoma vaccinii	CBS 216.90	AY916486	AY856879	AY856926
Helicomyces lilliputeus	NBRC 32664	AY916483	AY856899	AY856942
Helicosporium guianense	UAMH 1699	AY916479	AY856891	AY856936
Helicosporium guianense	CBS 269.52	AY916487	AY856893	AY856938
Helicosporium linderi	NBRC 9207	AY916454	AY856895	–
Helicosporium pallidum	CBS 962.69	AY916460	AY856886	AY856932
Leptosphaerulina australis	CBS 317.83	–	GU301830	GU296160
Leptosphaeria doliolum	CBS 505.75	–	GU301827	GU296159
Lindgomyces ingoldianus	ATCC200398	–	AB521736	AB521719
Lophiotrema brunneosporum	CBS 123095	–	GU296165	GU301835
Lophiotrema nucula	CBS 627.86	–	GU301837	GU296167

Table 2.3 (continued)

Fungal isolates	Sources of culture	GenBank accession numbers		
		ITS	LSU	SSU
Lophiostoma arundinis	CBS 621.86	–	DQ782384	DQ782383
Lophiostoma compressum	IFRD 2014	–	GU301834	GU296164
Massariosphaeria phaeospora	CBS 611.86	–	GU301843	GU296173
Massariosphaeria typhicola 2	KT 797	–	AB521747	AB521730
Pleomassaria siparia	CBS 279.74	–	DQ678078	DQ678027
Pleospora herbarum	CBS 191.86	–	DQ247804	DQ247812
*Thaxteriella inthanonensis**	MFLUCC11-0003	JN865211	JN865199	JN865187
*Thaxteriellopsis lignicola**	MFLUCC10-0122	JN865206	JN865194	JN865182
*Thaxteriellopsis lignicola**	MFLUCC10-0123	JN865207	JN865195	JN865183
*Thaxteriellopsis lignicola**	MFLUCC10-0124	JN865208	JN865196	JN865184
*Thaxteriellopsis lignicola**	MFLUCC10-0121	JN865205	JN865193	JN865181
*Thaxteriella helicoma**	JCM 2739	–	AY787939	–
*Tubeufia amazonensis**	ATCC 42524	AY916458	AY856911	AY856951
Tubeufia cerea	CBS 941.72	AY916488	AY856883	AY856930
Tubeufia cerea	NBRC 9014	AY916489	AY856903	AY856947
Tubeufia cylindrothecia	BCC 3559	–	AY849965	–
Tubeufia helicomyces	CBS 271.52	AY916461	AY856887	AY856933
*Tubeufia khunkornensis**	MFLUCC10-0119	JN865203	JN865191	JN865179
Tubeufia paludosa	ANM 953	GQ856139	GQ850483	–
Tubeufia paludosa	ANM 1169	–	GQ850484	–
Tubeufia paludosa	HKUCC 9118	–	AY849966	–
Westerdykella cylindrica	CBS 454.72	–	AY004343	AY016355
Westerdykella ornata	CBS 379.55	–	GU301880	GU296208
Schismatomma decolorans	DUKE 0047570	–	AY548815	AY548809

Note. Abbreviations of isolates and culture collections. A.N. Miller: ANM; American Type Culture Collection, Virginia, U.S.A: ATCC; Bahía Blanca Biology Herbarium: BBB; BIOTEC Culture Collection, Bangkok, Thailand: BCC; Centralbureau voor Schimmel cultures, Utrecht, Netherlands: CBS; Duke University Herbarium, Durham, North Carolina, U.S.A: DUKE; The University of Hong Kong Culture Collection, Hong Kong, China: HKUCC; Culture Collection, International Fungal Research & Development Centre, Chinese Academy of Forestry, Kunming, China: IFRD; Japan Collection of Microorganisms: JCM; K. Tanaka: KT; Mae Fah Luang University Culture Collection, Thailand: MFLUCC; NITE Biological Resource Centre, Japan:

NBRC; University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, Canada: UAMH.

2.3 Results and Discussion

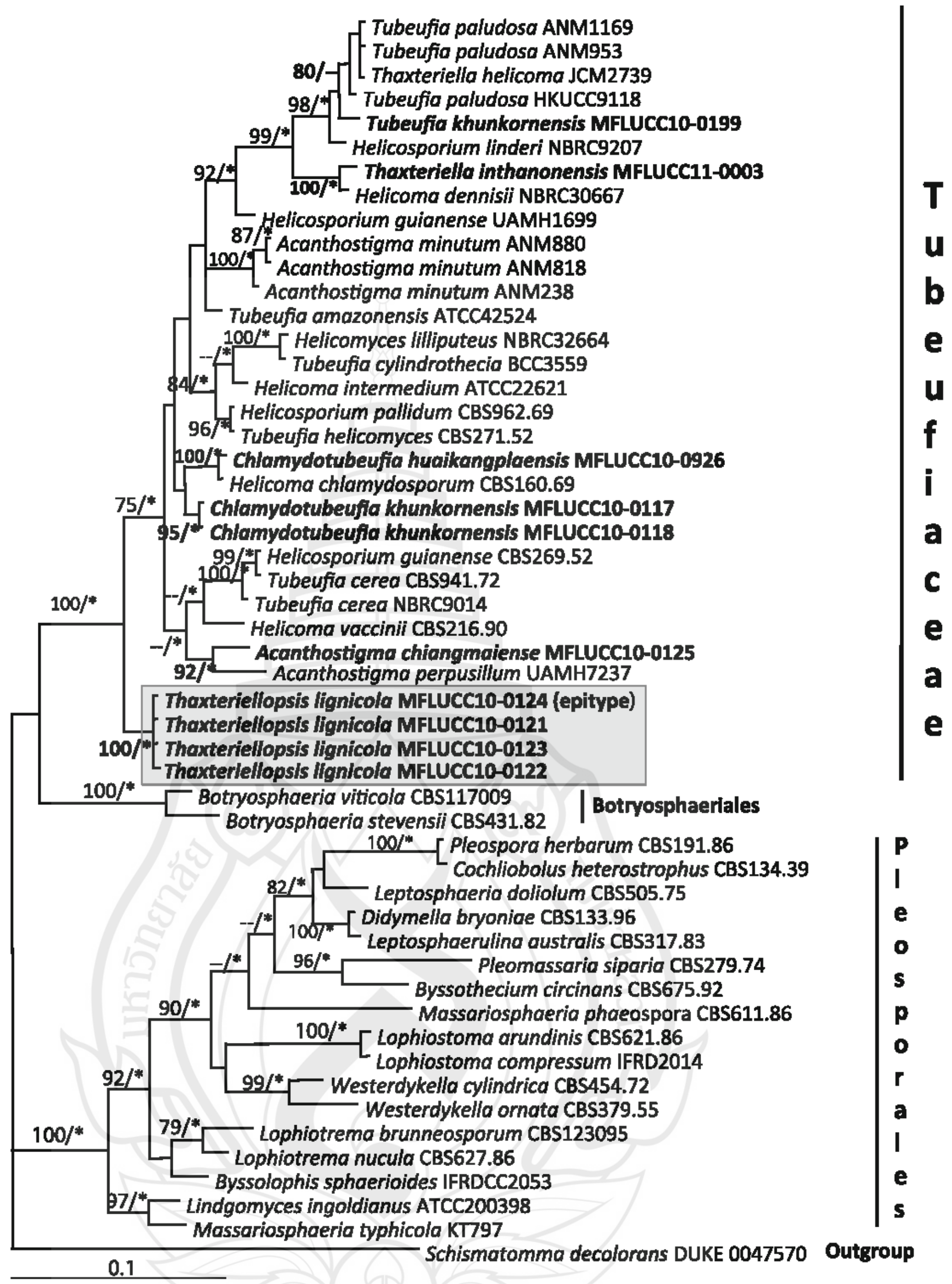
2.3.1 Phylogenetic Analysis

Ten new sequences were generated in this study. The new sequences of SSU and LSU were aligned with sequences from 42 taxa retrieved from GenBank (Table 2.2), using *Schismatomma decolorans* as an outgroup. The alignment of combined SSU and LSU gene sequences contained 52 taxa and 1046 characters. Parsimony analysis of the dataset resulted in 90 trees (759 steps). Out of 1046 characters, 765 characters were constant and 222 were parsimony informative. A likelihood tree was generated by Bayesian analysis with both parsimony bootstrap values and posterior probabilities shown on each branch (Fig. 2.1). The overall tree topology did not differ from previous analyses of Dothideomycetes (Schoch et al., 2009), with the fact that the placement of *Tubeufiaceae* was not clearly resolved. *Tubeufiaceae* appeared to form a distinct sister relationship to *Botryosphaeriales* and *Tubeufiaceae* might represent a new order (Fig. 2.1). Most of the newly collected fungi nested in the *Tubeufiaceae*, forming a strong monophyletic cluster (Fig. 2.1). Surprisingly, *Thaxteriellopsis* lignicola did not cluster with other representatives in *Tubeufiaceae* but became a sister taxon in the family *Tubeufiaceae* with 100% bootstrap support and 100% PP.

Our material of *Thaxteriellopsis lignicola* highly resembled the generic type of *Thaxteriellopsis*, which also belonged to *Tubeufiaceae*, in terms of morphological features (Figs. 2.19-2.20). Therefore we designated one of our strains as the epitype of *Thaxteriellopsis lignicola* (see below).

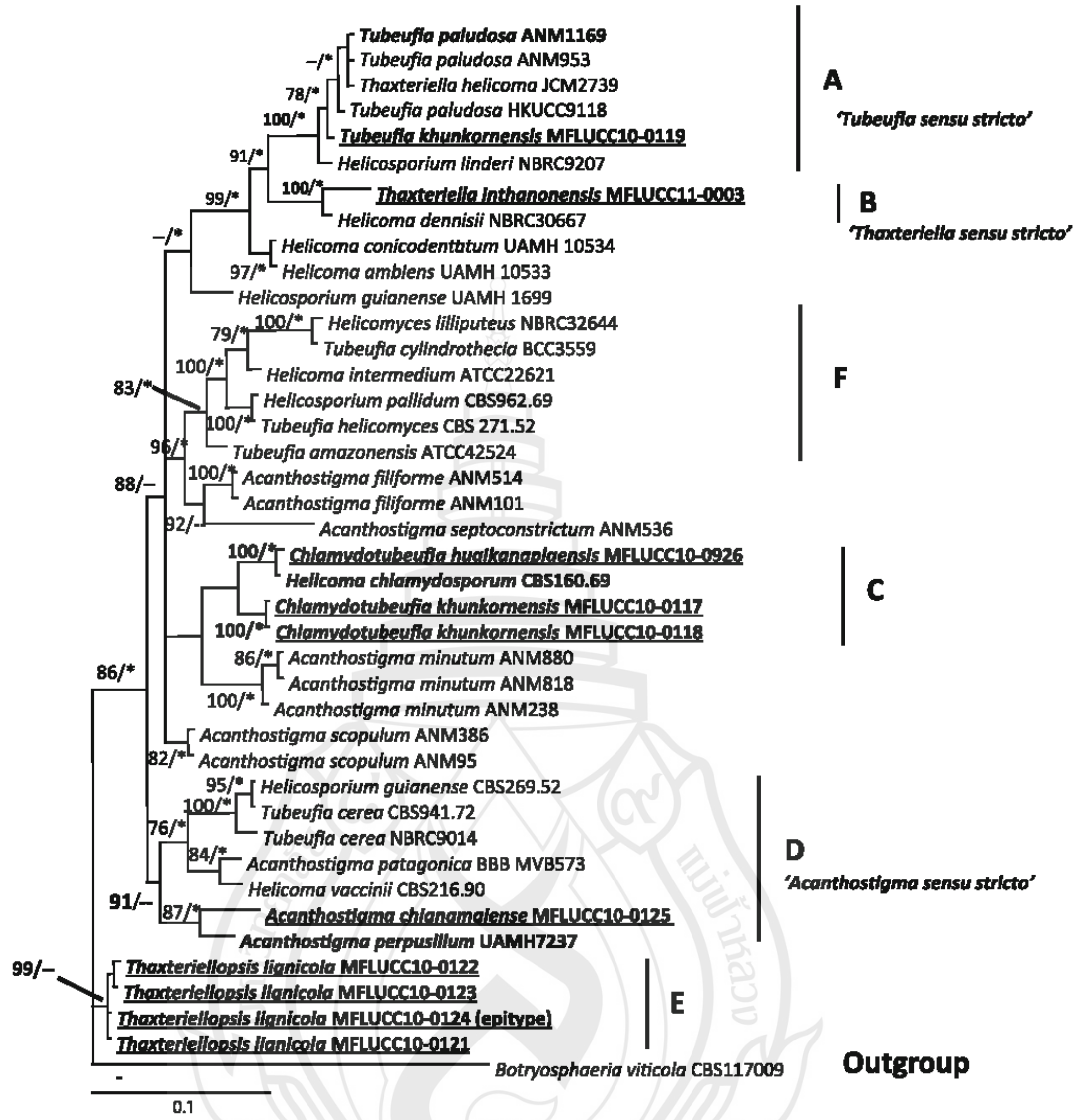
The combined ITS and LSU sequence dataset consisted of 41 taxa and 1141 characters. MP analysis of the dataset resulted in 5 trees (1325 steps), and out of 1141 characters, 703 characters were constant and 334 were parsimony informative. Based on the results of parsimony and Bayesian analysis (Fig. 2.2), representatives of *Tubeufiaceae* can be divided into multiple well-supported clades (>95% PP). Herein, we concentrated on clades A-F since we have performed thorough morphological examination of specimens within these clades, including five new taxa described in the current investigation (Fig. 2.2). Clade A comprised of six taxa and received strong bootstrap support (100%). This clade includes three strains of *Tubeufia paludosa*, the generic type of *Tubeufia*, the new species *T. khunkornensis* and *Helicosporium linderi*, plus a putative strain of *Thaxteriella helicoma*. This clade may represent *Tubeufia* sensu stricto. *Thaxteriella inthanonensis* clustered with *Helicoma dennisii* with high confidence support in clade B (100% PP). We consider this clade as *Thaxteriella* sensu stricto because *T. inthanonensis* is morphologically very similar to the *T. corticola*, the generic type of *Thaxteriella*. *Helicosporous* anamorphs such as *Helicoma ambiens, Helicoma conicodentatum*, and *Helicosporium guianense* are closely related to members in clades A and B (Fig. 2.2).

Members in clade C produced dictyochlamydosporous asexual stages, albeit the fact the clade received moderate bootstrap support (66%, data not shown on Fig. 2.2). Two species are described under a new genus *Chlamydotubeufia* as *Chlamydotubeufia huaikangplaensis* and *C. khunkornensis* (Figs. 2.10, 2.12). Clade D was considered as *Acanthostigma* sensu stricto because it contained *Acanthostigma perpusillum*, the type species. This clade also included a new species of *Acanthostigma* described in this paper as well as five other taxa. Clade E contained multiple strains of *Thaxteriellopsis lignicola*. *Tubeufia amazonensis, T. helicomyces*, *T. cylindrothecia, Helicosporium pallidum, Helicomyces lilliputeus* and *Helicoma intermedium* clustered in a clade F with strong support (85% bootstrap support and 99% PP). Since the clade contained three *Tubeufia* species, this casts doubt on the monophyly of the genus *Tubeufia* because the type species nested in clade A.



Note. Parsimony bootstrap percentages ≥75% and Bayesian posterior probabilities of ≥95% (marked by an asterisk "*") are shown above the branches. The designated outgroup is *Schismatomma decolorans*. New collections of *Tubeufiaceae* from Thailand are indicated in bold

Figure 2.1 Phylogenetic Tree of *Tubeufiaceae*, Based on a Maximum Likelihood Analysis of SSU and LSU Gene Sequences



Note. Bootstrap percentage values (≥75%) generated from 500 replicates from maximum parsimony and posterior probabilities (≥95%) from Bayesian analysis (marked by an asterisk "*") are shown above the branches. The designated outgroup is *Botryosphaeria viticola*. *Tubeufiaceae* new collections are indicated in under line and italic

Figure 2.2 Phylogenetic Relationships of Taxa in *Tubeufiaceae*, Based on a Maximum Likelihood Analysis of ITS and LSU Gene Sequences

2.3.2 Taxonomy

Tubeufiaceae M.E. Barr

Generic type: *Tubeufia* Penz. & Sacc.

Saprobic on dead plant material, especially wood, fungi or scale insects. *Ascomata* mostly forming on a subiculum, superficial, solitary, clustered to gregarious, globose to subglobose or turbinate, light yellow, orange, brown, dark brown to black, shiny, occasionally collapsing when dry, central ostiolate, papillate, with or without setae, or with mycelial covering. *Peridium* relatively wide, comprised of relatively thick-walked angular to globose cells. *Pseudoparaphyses* cellular, filiform, branched, anastomosing, attached to the hymenium and to the ascomata wall above. *Asci* 8-spored, bitunicate, fissitunicate, usually cylindrical to broadly clavate, rounded at the apex, with or without an ocular chamber, with or without a pedicel. *Ascospores* filiform, cylindrical to narrowly fusiform, tapering towards the rounded to sub-acute ends, trans-septate, hyaline, pale yellow or brown, smooth-walled.

Anamorphs hyphomycetes, helicosporous, staurosporous or dictyosporous, belonging to *Annellospermosporella, Aquaphila, Araneomyces, Guelichia, Helicoma, Helicoon, Helicomyces, Helicosporium, Kamalomyces, Monodictys*-like*, Pendulispora, Peziotrichum, Tetracrium, Titaea* and *Xenosporium* (Hyde et al., 2011; Kirk et al., 2008).

Members of the *Tubeufiaceae* are easily recognized and usually occur on decaying woody plant material, including cloth, while there is a group that is fungicolous or occur in association with scale insects. The ascomata mostly form in a gregarious mass on a dark subiculum or are often covered in mycelium. Ascomata are usually dark and globose, but may also be light coloured. Asci are bitunicate, cylindrical and ascospores are fusiform to filamentous, trans-septate and hyaline or pale yellow or brown. The distinction between genera, is however, not clear and previous molecular studies have not resolved genera well.

Genera accepted in *Tubeufiaceae*

Tubeufia Penz. & Sacc., Malpighia 11: 517 (1898) [1897]

= *Linobolus* Syd. & P. Syd., Annls Mycol. 15(3/4): 204 (1917)

Saprobic on dead wood. *Ascomata* without a subiculum, superficial, solitary to gregarious, globose to subglobose, or clavate to obclavate, yellow to orange, becoming dark, occasionally hairy. *Peridium* composed of pseudoparenchymatous cells. *Asci* 8-spored, clavate to broadly cylindrical, apex rounded, short pedicellate. *Ascospores* cylindrical to fusiform, elongate, ends rounded, trans-septate, not constricted at septa, hyaline to pale yellow or brown, smooth-walled.

Anamorphs reported for genus: *Aquaphila*, *Helicoma*, *Helicosporium*, *Monodictys*-like, *Pendulispora* (Hyde et al., 2011).

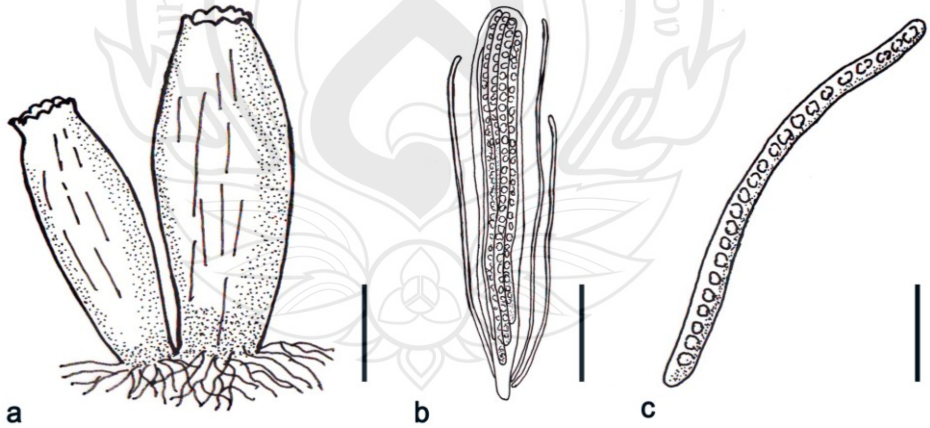
Type species: *Tubeufia javanica* Penz. & Sacc.

Tubeufia javanica Penz. & Sacc. Malpighia 11: 517 (1898) [1897] (Fig. 2.3)

Saprobic on culms of bamboo. *Ascomata* 350 × 150 µm, without a subiculum, superficial, solitary, gregarious, globose to subglobose or clavate to obclavate, white, orange, black, ostiole central occasionally hairy. *Peridium* pseudoparenchymatous. *Asci* 150–200 × 18–25 µm, (4–)8-spored, clavate to broadly cylindrical, apically rounded, short pedicellate. *Ascospores* 5–6 µm wide (spores length not reported), cylindrical to fusiform, elongated, ends rounded, trans-septate, hyaline to pale yellow or brown, smooth walled.

The type material was not able to locate, therefore the description and drawing are based on those provided by Penzig and Saccardo (1904). The generic type *Tubeufia javanica* was considered as a synonym of *T. paludosa* (Crouan & H. Crouan) Rossman, which is an earlier name (Rossman, 1977). However, Barr (1980) considered Rossman's (1977) concept of *T. paludosa* to be rather broad and suggested that the collections grouped under this species eventually might be separated. Certainly, the illustrations of Penzig and Saccardo (1904) of *T. javanica* and those by Rossman (1977) of *T. paludosa* and Sivanesan (1984) of *T. paludosa* are quite different (perhaps stylized) and may not be the same species. Sequence data for a few strains of *Tubeufia*, including *T. paludosa*, are available in GenBank; however, it is difficult to establish if these are correctly named. The three strains of *T. paludosa* included in this study cluster together with *Thaxteriella helicoma*, with 65% bootstrap support. Fresh collections of *T. javanica* from Java are needed to establish if this is the same species as *T. paludosa* from a dead stem of Rubus in a stream bed in France, and both species need epitypifying with living cultures.

In this study, *Tubeufia* is accepted in a narrow sense, based on *T. javanica* and *T. paludosa*. Ascomata of *Tubeufia* are often globose to subglobose or clavate to obclavate, lack setae and may form on a subiculum, while asci have a wide thickened region at the apex and the ascospores are cylindrical to fusiform and trans-septate, and there is a helicosporous asexual state. Our new collections of *Tubeufia* also resemble *T. paludosa*; however, the ascomatal shape is slightly different. It does, however, cluster with putative strains of *Tubeufia paludosa* and *Thaxteriella helicoma* (Fig. 2.2). Therefore, *Tubeufia khunkornensis* sp. nov. is considered belong to *Tubeufia*. The anamorphs of *Tubeufia* are recorded as mostly helicosporous. *Aquaphila* is tubeufiaceous but did not cluster with the *Tubeufia paludosa* clade in the phylogenetic analyses (Promputtha & Miller, 2010; Tsui et al., 2006, 2007) and appears to be a distinct genus. *Helicoma* and *Helicosporium* are shown to be polyphyletic in this and other studies (Promputtha & Miller, 2010; Tsui et al., 2006, 2007). The anamorphs of the genera *Tubeufia* will need to be reconsidered following this study.



Note. Redrawn from Penzig and Saccardo (1904). (a) Ascomata. (b) Ascus with pseudoparaphyses. (c) Ascospore. Scale bars: (a) = 100 µm, (b)-(c) = 50 µm

Figure 2.3 *Tubeufia javanica*

Tubeufia khunkornensis Boonmee & K. D. Hyde, Fungal Diversity, 51: 63-102, (2011), MycoBank 563498 (Figs. 2.4-2.5)

= *Tubeufia paludosa* (Cr. & Cr.) Rossman. similis, sed ascospores (99–)111–118(–123) × (4–)5–7(–8) µm et 20–23-septate.

Etymology: from the Latin *–ensis* referring to an association with the *Khun Korn* Waterfall, the place of collection.

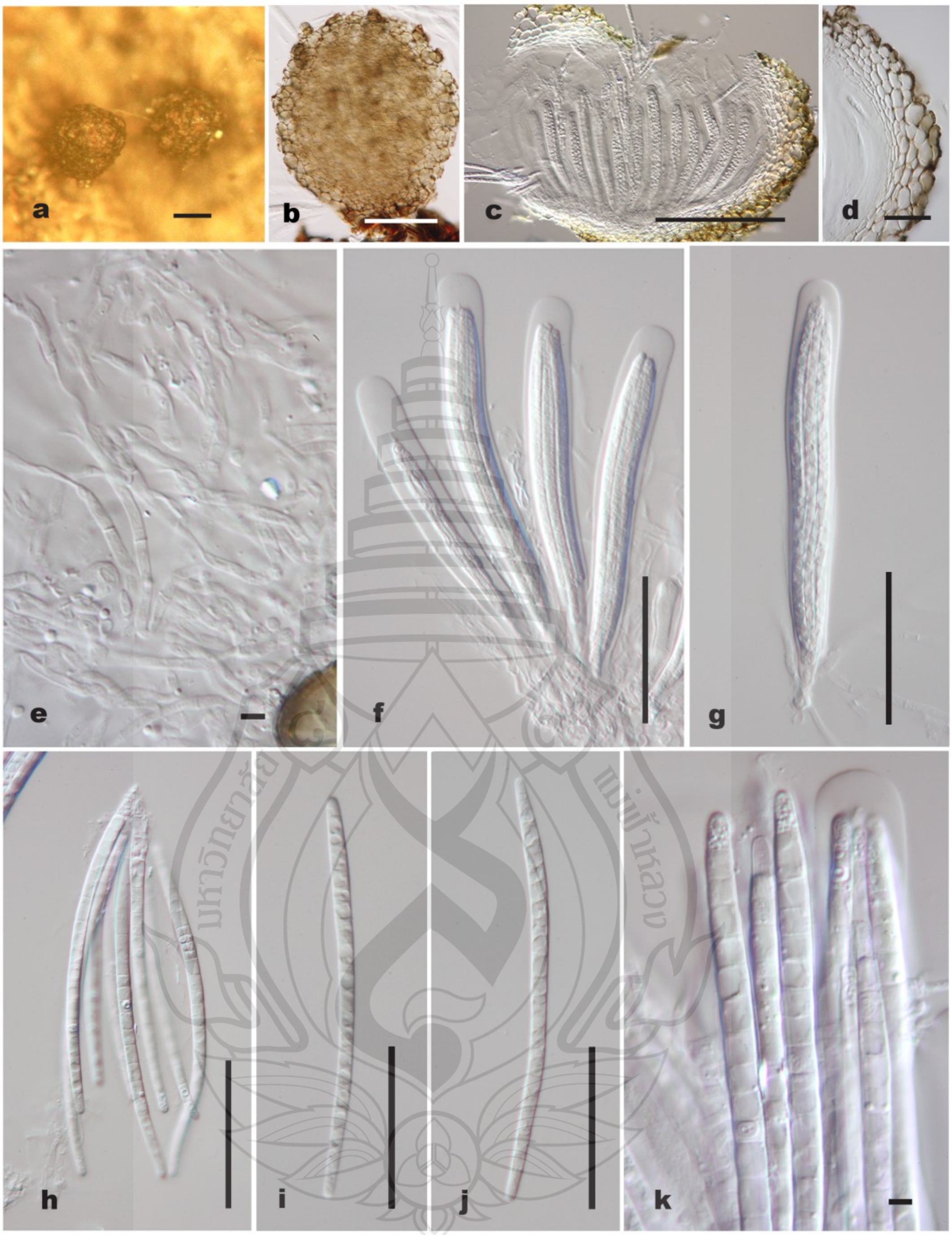
Saprobic on dead wood. *Ascomata* (133–)203–240 µm high × (193–)207–230 µm diam. ($\bar{x}$ = 192 × 210 µm, n = 5), borne on a dark brown subiculum, superficial, solitary or scattered, globose to subglobose, oval, to widely obclavate, brown to dark brown, ostiole unknown. *Peridium* 39–42 µm thick, comprised of several-layers of cells of textura angularis; inner cells hyaline to pale brown and angular, outer layer cells brown to dark brown and subglobose. *Hamathecium* cellular, filiform, *ca*. 2 µm wide, hyaline pseudoparaphyses, embedded in a gelatinous matrix. *Asci* (128.5–)130–153(–161) × (13–)16–19 µm ($\bar{x}$ = 141 × 17 µm, n = 20), 8-spored, bitunicate, cylindrical, apex thickened and rounded, large apical area lacking contents, ocular chamber not observed, with a 20–25 µm long pedicel. *Ascospores* (99–)111–118(–123) × (4–)5–7 (–8) µm ($\bar{x}$ = 114 × 6 µm, n = 20), 3–4-seriate, cylindrical to fusiform, narrowly elongate, straight or slightly curved, 20–23-septate, tapering towards the sub acute to rounded ends, not constricted at the septa, hyaline to pale grayish, smooth-walled.

Cultural characteristics: *Ascospores* germinating on MEA within 24-36 hours and germ tubes produced from both ends. Colonies growing slowly on MEA, reaching 9 mm in 1 week at 28°C, effuse, velvety to hairy, edge fimbriate, olive to olive brown, pigmented in media. *Mycelium* superficial and immersed, branched, septate, smooth, pale yellowish brown to reddish brown. Club-shaped, brown, muriform conidia-like structures formed on hyphae.

Material examined: THAILAND, Chiang Rai, Muang, Khun Korn Waterfall, N19°51–54' E 99° 35.39', 671 m., on dead wood of unidentified trees, 13 November 2009, S. Boonmee KK-08 (MFLU10–0052, holotype), extype culture MFLUCC10–0119 = IFRD2180 = BCC.

Habitat: terrestrial, saprobic, on dead wood of unidentified trees.

Known distribution: Chiang Rai, Thailand.



Note. (a) Ascomata. (b) Squash mount of ascoma. (c) Section through ascoma. (d) Peridium. (e) Pseudoparaphyses. (f)-(g) Asci. (h)-(k) Ascospores. Scale bars: (a)-(c) = 100 µm, (d) = 40 µm, (e), (k) = 5 µm, (f)-(i) = 50 µm

Figure 2.4 *Tubeufia khunkornensis* (MFLU10–0052, holotype)

Note. (a) Germinating ascospore. (b)-(c) Colonies on MEA from above and below. (d)-(i) Mycelium on culture. Note the brown, club-shaped, muriform conidia-like structures formed at the ends of hyphae. Scale bars: (a) = 50 μm, (b)-(c) = 10 mm, (d)-(i) = 10 μm

Figure 2.5 Cultural Characteristics of *Tubeufia khunkornensis* (MFLUCC10–0119, holotype)

Tubeufia khunkornensis is different from *T. javanica* and *T. paludosa* in ascomatal shape and colour, being clavate to obclavate and pale yellow to orange for the latter and dark for the former (Fig. 2.2; Penzig & Saccardo, 1904; Samuels, Rossman & Müller, 1979). Based on DNA sequences comparisons, *T. khunkornensis* is related to *T. paludosa* and *Thaxteriella helicoma* and receives 80% Bayesian support (Fig. 2.1).

Acanthostigma De Not. , Sfer. Ital., 85 (1863)

Saprobic on dead plant debris. *Ascomata* superficial, scattered, globose to subglobose, reddish-brown to dark brown, collapsing when dry, sparsely setose on the upper part, setae dark brown to opaque. *Peridium* consisting of several-layers of polyhedral cells. *Hamathecium* numerous, cellular, branching and anastomosing pseudoparaphyses developing from the basal hymenium. *Asci* 8-spored, bitunicate, fissitunicate, clavate, broadly rounded and thickened at the apex, short pedicellate. *Ascospores* fusiform, narrowly rounded at both ends, one of middle cells often broader than the others, trans-septate, straight or slightly curved, not-constricted or slightly constricted at the septa, hyaline.

Anamorphs reported for genus: *Helicomyces, Helicosporium* (Hyde et al., 2011)

Type species: *Acanthostigma perpusillum* De Not.

Acanthostigma perpusillum De Not., Sfer. Ital.: 207 (1863) (Fig 2.6)

= *Acanthostigma clintonii* (Peck) Sacc., Syll. fung. (Abellini) 2: 210 (1883)

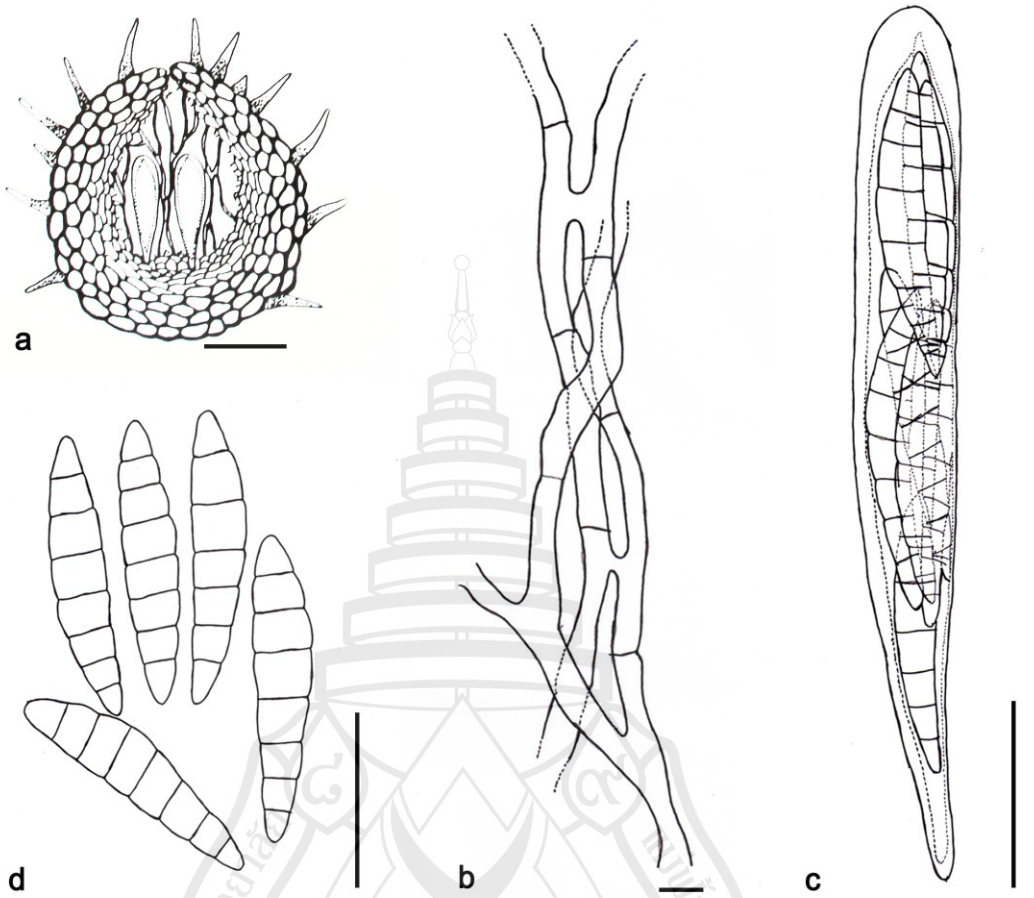
= *Sphaeria clintonii* Peck, Ann. Rep. N. Y. St. Mus. nat. Hist. 30: 65, tab. 2, Figs. 19 and 20 (1878) [1877]

= *Tubeufia clintonii* (Peck) M. E. Barr, Mycotaxon 12(1): 163 (1980)

Saprobic on dead bark. *Ascomata* 100–110 µm high × 150–155 µm diam., superficial, scattered, globose to subglobose, reddish-brown to dark brown, collabent towards when dry, sparsely setose on the upper part. *Setae* 1-celled, dark brown, opaque, acute, (10–)28–97 µm long × 5–6 µm wide at the base. *Peridium* 15–22 µm thick, comprising 3–4 layers of polyhedral, pale brown to brown, thick-walled cells. *Hamathecium* 2–3.5 µm wide, numerous, cellular, branched, anastomosing pseudoparaphyses developing from the basal hymenium. *Asci* 77–79 × 14–16 µm, 8-spored, bitunicate, fissitunicate, cylindro-clavate, broadly rounded and thickened at the apex, ocular chamber not observed, short-pedicellate. *Ascospores* 30.5–35.5(–42) × 5–6 µm, 2–4-seriate in the ascus, fusiform to clavate, narrowly rounded at both ends, one of middle cells often broader than the others (5–)6–7(–8)-septate, straight or slightly curved, not-constricted or slightly constricted at the septa, hyaline.

Description and drawing based on Réblová and Barr (2000).

Acanthostigma was monographed by Réblová and Barr (2000) and six species were accepted. Berlese (1894), and Réblová and Barr (2000) redescribed the type and provided illustrations and a detailed description. The type species was not able to locate during the time frame of this study and therefore refer to the excellent description of the type provided by Réblová and Barr (2000). *Acanthostigma* was confirmed as belonging to *Tubeufiaceae* based on LSU and SSU, ITS and LSU sequence analyses (Schoch et al., 2009; Promputtha & Miller, 2010; Tsui et al., 2007). This study, *Acanthostigma* is polyphyletic within *Tubeufiaceae* with representatives scattered all over the tree (Fig. 2.2). *Acanthostigma chiangmaiense* sp. nov. clustered with *A. perpusillum* (strain no. UAMH 7237; Tsui & Berbee, 2006) with 87% bootstrap support, and can be considered to belong in *Acanthostigma* sensu stricto. *Acanthostigma minutum, A. scopulum, A. filiforme* and *A. septoconstrictum* however, were distant from *A. perpusillum* and clustered in other clades (Fig. 2.2). Promputtha and Miller (2010) also assigned *A. perpusillum* in *Tubeufiaceae*, although Kodsueb, Jeewon et al. (2006) placed one collection of *A. perpusillum* (AY856892) in the *Herpotrichiellaceae* clade with high bootstrap support. Both *Helicomyces* and *Helicosporium* have been reported as anamorphs of *Acanthostigma* and are polyphyletic (Promputtha & Miller, 2010; Tsui et al., 2007) and *A. chiangmaiense* only formed chlamydospores in culture. Thus, further work is required to establish the anamorphs of *Acanthostigma sensu stricto*.



Note. Redrawn from Réblová and Barr (2000). (a) Ascomata cross section on substrate. (b) Pseudoparaphyses. (c) Ascus. (d) Ascospores. Scale bars: (a) = 50 µm, (b) = 5 µm, (c)-(d) = 20 µm

Figure 2.6 *Acanthostigma perpusillum*

Acanthostigma chiangmaiense Boonmee & K. D. Hyde, Fungal Diversity, 51: 63-102, (2011), MycoBank 563499 (Figs 2.7-2.8)

Acanthostigma perpusillum De Not. similis sed ascosporis (35–)37–44(–48) × 5–8 µm differt.

Etymology: From the Latin *–ensis*, meaning associated with and Chiang Mai in reference to the type locality of Chiang Mai Province.

Saprobic on dead wood. *Ascomata* (75–)108–118.5 µm high × (71–)113–123 (–146.5) µm diam. ($\bar{x}$ = 99 × 108.5 µm, n = 5), superficial, solitary, scattered, globose to subglobose, dark brown to black, ostiole central, covered with black 26.5–74 µm long, 1-celled, tapering setae. *Peridium* 11.5–18 µm wide, dark brown, a single stratum comprised of 3–4 cell layers of *textura angularis*. *Hamathecium ca*. 2 µm wide, filiform, cellular, anastomosing pseudoparaphyses surrounded by a gelatinous matrix, forming from the hymenium at the base/and sides of the ascoma. *Asci* (62.5–70–92 (–94) × (16–)17.5–20(–24.5) µm ($\bar{x}$ = 79 × 18 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindro-clavate, broadly rounded at the apex (some with broadly rounded upper part), with a small ocular chamber, short pedicellate. *Ascospores* (35–)37–44(–48) × 5–8 µm ($\bar{x}$ = 40 × 6 µm, n = 20), 3–4-seriate, fusiform-clavate, slightly tapering toward and sub acute ends, straight or slightly curved, 8–9-septate, widest at the third to fourth cell from the apex, not noticeably constricted or slightly constricted at the septa, hyaline, smooth-walled.

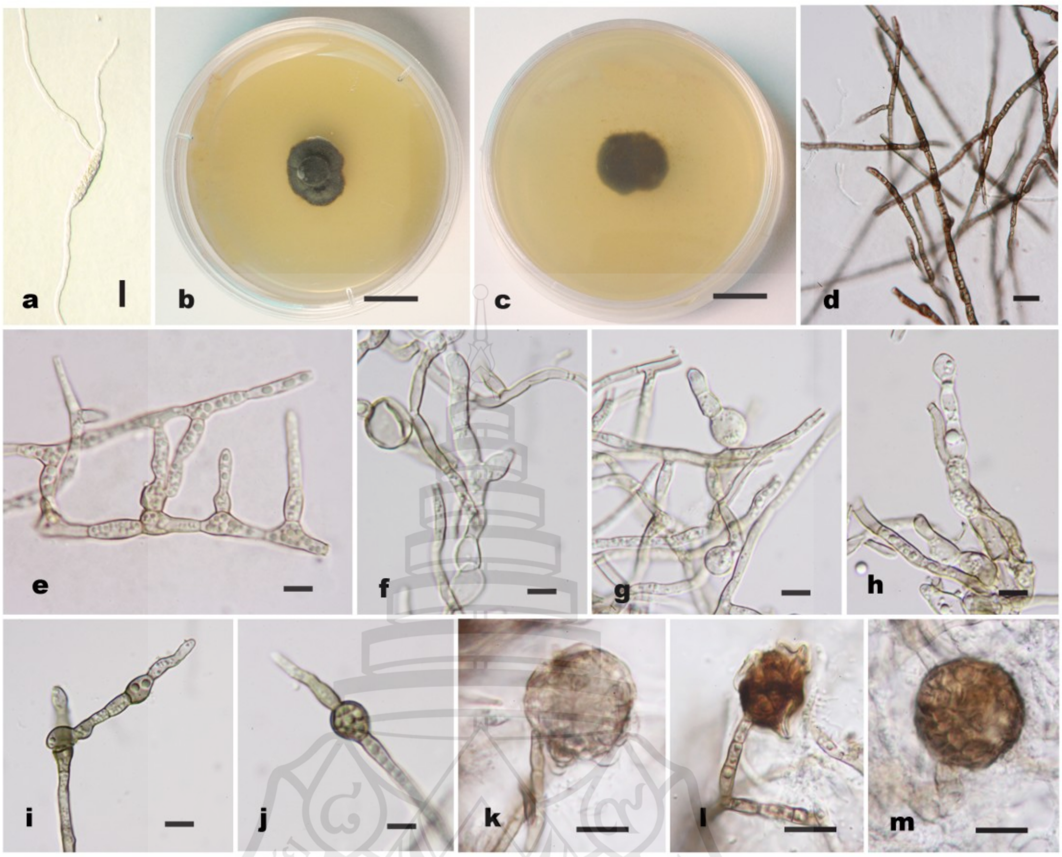
Cultural characteristics: *Ascospores* germinating on WA within 12 hours and germ tubes produced from both ends. Colonies growing slowly on MEA, reaching a diam of 2 mm after 1 week at 28°C, effuse, velvety, entire to slightly undulate edge, dark to blackish. *Mycelium* superficial, branched or anastomosing, septate, smooth, subhyaline to pale brownish and olivaceous brown. *Chlamydospores* forming between hyphae, olive brown, becoming dark brown with age, globose, at first single celled, becoming multicellular, muriform, (16–)23–37 µm diam.

Material examined: THAILAND, Chiang Mai, Mae Rim, Mae Sa, Pong Yaeng, *ca*. 1000 m., on dead wood of unidentified trees, 7 September 2009, S. Boonmee, PYW-02 (MFLU10–0058, holotype), ex-type culture MFLUCC10–0125 = IFRD2198 = BCC.

This new species belongs to *Acanthostigma* because it has similar morphological features such as a darkened ascomata surrounded by setae, cylindric-clavate asci and clavate-fusiform, trans-septate hyaline ascospores; it is also very similar to *Acanthostigma perpusillum* (Réblová & Barr, 2000). The taxon grouped with *A. perpusillum*, the generic type in the phylogenetic analysis with high support (100% PP). *A. chiangmaiense* can be distinguished from *A. perpusillum* by dimensions and shape of the ascospores, being (35–)37–44(–48) × 5–8 µm and 8–9-septate in the former and 30.5–35.5(–42) × 5–6 µm and (5–)6–7(–8) septate in *A. perpusillum*. The species are however closely related. It is not similar to any other species of *Acanthostigma* (Promputtha & Miller, 2010; Réblová & Barr, 2000).

Note. (a) Ascomata. (b) Section through of ascoma. (c) Seta. (d). Peridium. (e) Pseudoparaphyses. (f)-(h) Asci. (i)-(l). Ascospores. Scale bars: (a)-(c) = 50 µm, (d), (i)-(l) = 10 µm, (e) = 5 µm, (f)-(h) = 30 µm

Figure 2.7 *Acanthostigma chiangmaiense* (MFLU10–0058, holotype)



Note. (a) Germinating ascospore. (b)-(c) Colonies on MEA from surface and reverse. (d)-(m) Mycelium culture. Note the formation of chlamydospores. Scale bars: (a), (k)-(m) = 10 μm, (b)-(c) = 5 mm, (d)-(j) = 5 μm

Figure 2.8 Cultural Characteristics of *Acanthostigma chiangmaiense* (MFLUCC10–0125, holotype)

Acanthophiobolus Berl., Atti Congl. Bot. Intern. di Genova, 1892: 571 (1893) [1892]

Saprobic on cloth. *Ascomata* superficial, globose to subglobose, reddish brown to dark brown, ostiole central, with red brown to dark brown setae, which are septate, and taper towards the apex. *Peridium* comprising several-layers; inner layer composed of *textura prismatica-porrecta* and hyaline to pale, outer layer composed of 2–3 layers of dark brown cells of textura angularis. *Hamathecium* comprising filiform pseudoparaphyses. *Asci* 8-spored, bitunicate, elongate, cylindro-clavate, short pedicellate. *Ascospores* filiform, septate, spiral in the ascus, hyaline.

Anamorphs reported for genus: none.

Type species: *Acanthophiobolus helminthosporus* (Rehm) Berl.

Acanthophiobolus helminthosporus (Rehm) Berl., Die Pilze des Weinstockes, Vienna: 571 (1893) [1892] (Fig. 2.9)

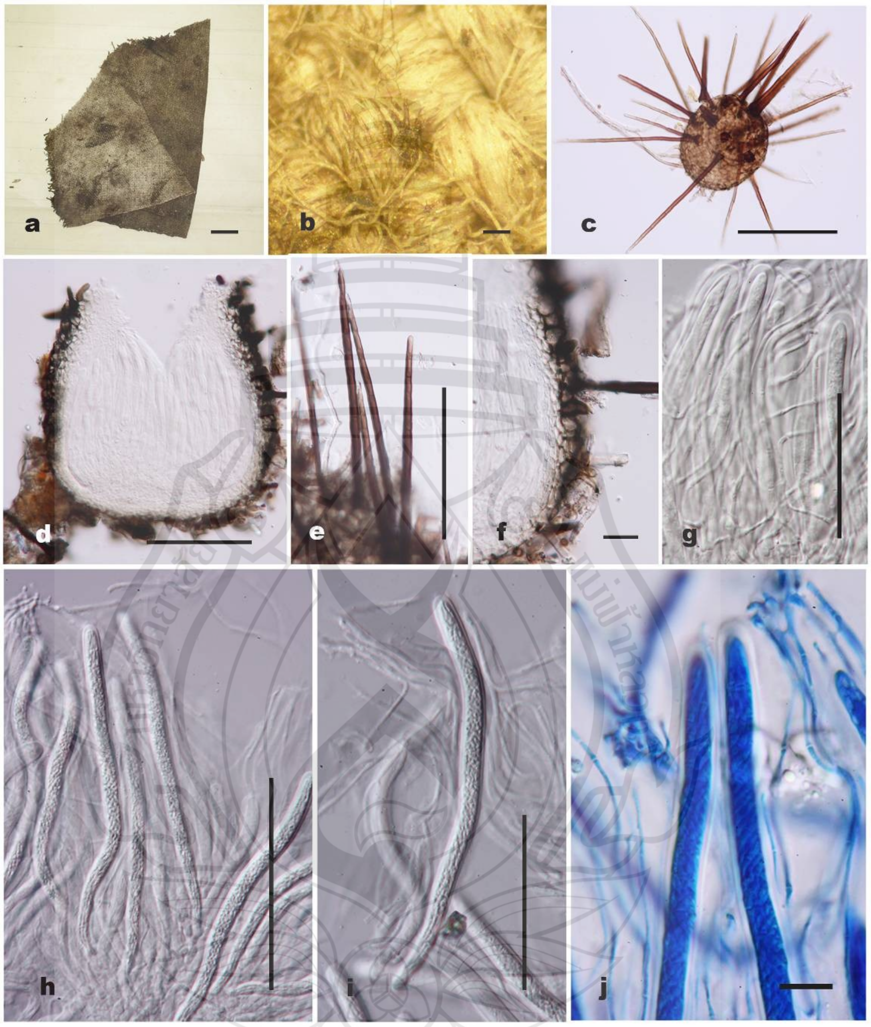
≡ *Leptospora helminthospora* Rehm

Saprobic on rotten cloth. *Ascomata* 82–122 × 68–111 µm ($\bar{x}$ = 101 × 96 µm, n = 5), superficial, globose to subglobose, brown to dark brown or red brown, ostiole central, covered in setae which are 82–163.5 µm long, brown to red brown, darkened at the base, septate and taper towards the apex. *Peridium* comprising two substrata, an inner layer composed of hyaline to pale cells of *textura prismatica-porrecta* and an outer layer composed of 2–3 layers of dark brown cells of textura angularis. *Hamathecium* filiform, 1–1.5 µm wide, pseudoparaphyses developing from the basal hymenium. *Asci* 126–168 × 8–10 µm ($\bar{x}$ = 148 × 9 µm, n = 20), 8-spored, bitunicate, elongate cylindric-clavate, ocular chamber not observed, short pedicellate. *Ascospores* filiform, spiral in the ascus, hyaline (ascospores length could not be recorded but they were longer than asci).

Material examined: GERMANY, Augsburg, on rotten cloth, 14 February 1880, M. Britzelmayr No. F12985 (S, holotype).

Acanthophiobolus was introduced by Berlese (1893) and six species are listed in Index Fungorum. An earlier name was found in *Acanthophiobolus helicosporus* (Berk. & Broome) J. Walker based on *Sphaeria helicospora* Berk. & Broome and *Acanthophiobolus helminthosporus* was considered to be a synonym (Walker, 1980). Ascomata are superficial on the substrate and globose to subglobose, covered by red brown to dark brown and long setae. Asci are cylindrical with filiform and spiral ascospores. It was not possible to obtain single ascospores from the asci for measurement as they were spiraled in the asci (Fig. 2.8h-j; Walker, 1980).

No molecular phylogenetic study has been conducted to *Acanthophiobolus*. Although the ascoma characters are somewhat similar to *Acanthostigma*, *Acanthophiobolus* clearly differs in having bright colour ascomata, septate setae which are much longer, and cylindrical, elongate asci with long filiform, trans-septate ascospores (Figs. 2.9h-j). We therefore maintain *Acanthophiobolus* is a distinct genus in *Tubeufiaceae*. The type needs recollecting and epitypifying.



Note. (a) Habit of fungus on rotten cloth. (b) Ascoma. (c) Squash mount of ascoma. (d) Section through of ascoma. (e) Setae. (f) Peridium. (g) Pseudoparaphyses. (h)-(i). Asci. (j). Ascospores spiraled in asci. Scale bars: (a)-(e), (g)-(i) = 100 µm, (f), (j) = 10 µm

Figure 2.9 *Acanthophiobolus helminthosporus* (S, holotype)

Chaetosphaerulina I. Hino, Bulletin Miyazaki Coll. Agric. Forest. 10: 62 (1938)

Chaetosphaerulina (generic type = *C. yasudai* I. Hino 1938). *Saprobic* on dead wood. *Subiculum* superficial, attenuate apex, velutinous, dark to black, irregular, effuse brown hyphae, septate, attenuate apex. *Ascomata* on subiculum, ovoid to ellipsoid, ostiolate, hairy surrounded. *Hamathecium* filiform, cellular pseudoparaphyses. *Asci* 8-spored, bitunicate, cylindrical, slightly truncate apex. *Ascospores* hyaline, multiseptate, constricted at the median. *Chlamydospores* at the apex of hyphae, with verticillate production, 2–8-septate, oblong, fusoid to vermiform, frequently curved.

Anamorph reported for genus: *Xenosporium* (Hyde et al., 2011)

Type species: *Chaetosphaerulina yasudai* I. Hino

Chaetosphaerulina yasudai I. Hino, Canad. J. Plant Sci. 10: 62 (1938)

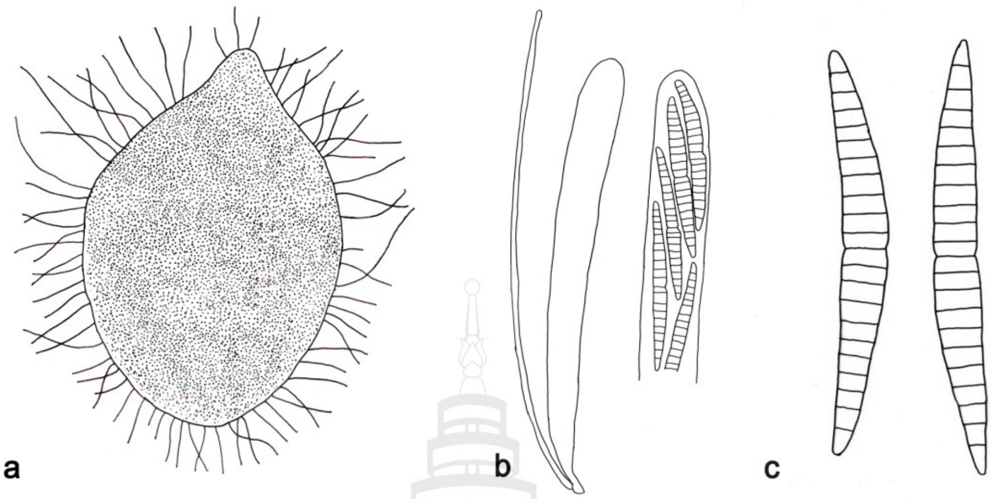
(Fig. 2.10)

≡ *Herpotrichia yasudai* (I. Hino) Piroz. [as 'yasudae'], Mycol. Pap. 129: 16 (1972)

≡ *Tubeufia yasudai* (I. Hino) Sivan. [as 'yasudae'], Bitunicate Ascomycetes and their Anamorphs (Vaduz): 589 (1984)

Saprobic on culms of bamboo. *Subiculum* 311–555 × 3–6 µm, superficial, dark to black, comprising effuse brown septate hyphae, attenuate at the apex. *Ascomata* 289–366 × 244–333 µm, forming on a subiculum, ovoid to ellipsoid, ostiolate, hairy. *Hamathecium* filiform, cellular pseudoparaphyses. *Asci* 208–221 × 10–16 µm, 8-spored, bitunicate, cylindrical, with slightly truncate apex. *Ascospores* 61–67 × 6 µm, hyaline, 21-septate, constricted at the median. *Chlamydospores* 11–61 × 5–16 µm, forming at the ends of hyphae, oblong, fusoid to vermiform, frequently curved, verticillate, 2–8-septate.

Thaxteriellopsis bambusicola Sivan. & N. D. Sharma is very similar to *Chaetosphaerulina yasudai* and was transferred to *Chaetosphaerulina bambusicola* (Sivan. & N. D. Sharma) by Crane et al. (1998). The ascospores overlap in size but *C. bambusicola* differs in have a few longitudinal septa. These may well turn out to be the same species but needs recollecting.



Note. Redrawn from Hino (1938). (a) Ascoma. (b) Asci with paraphyses. (c) Ascospore

Figure 2.10 *Chaetosphaerulina yasudai*

Chlamydotubeufia Boonmee & K. D. Hyde, Fungal Diversity, 51: 63-102, (2011), MycoBank 563500

Acanthostigma De Not. similis sed status anamorphosis dictyochlamydosporae differt.

Etymology: *A combination* of *Chlamydo-* and *–tubeufia* in reference to the similarity to the genus *Tubeufia* but dictyochlamydosporous anamorphs.

Saprobic on dead wood. *Ascomata* superficially, solitary, scattered or in small groups, globose-subglobose, with or without an ostiolate, surrounded by darkened setae. *Peridium* dark brown to black. *Hamathecium* numerous cellular pseudoparaphyses, embedded in a mucilaginous matrix. *Asci* 8-spored, bitunicate, fissitunicate, cylindro-clavate, broadly clavate, rounded at the apex short-pedicellate. *Ascospores* 2–3-seriate, hyaline, narrowly fusiform, broad at supra-median, slightly curved, multiseptate, slightly constricted septum, ends asymmetrical. *Anamorph* helicosporous and also producing a dictyochlamydosporous state in culture and often on wood. *Dictyochlamydospores* broadly-oblong, elongate, multiseptate, at first red brown becoming darkening to black.

The new genus is introduced to accommodate *Tubeufia*-like species with globose ascomata, a helicosporous anamorph form as well as producing dictyochlamydospores. *Helicoma chlamydosporum* Shearer (1987) also have dictyochlamydospores and clustered with the three *Tubeufia*-like species producing dictyochlamydospores in culture. This cluster clearly separates from *Tubeufia* species in Clade A which include the generic type of *Tubeufia paludosa* and therefore the new genus *Chlamydotubeufia* is introduced based on morphological and molecular data. The helicospores produced in *H. chlamydosporum* are produced on blunt-tipped denticles on swellings on the hyphae, directly on hyphae, or on short lateral 0–3-septate conidiophores whereas in most typical *Helicoma* species conidiophores are rather long (Shearer, 1987; Zhao, Liu & Wu, 2007).

This study, two new *Chlamydotubeufia* species are introduced, while *H. chlamydosporum* and *H. depressispora* are transferred to *Chlamydotubeufia*, the latter also producing dictyochlamydospores. Another genus with similar chlamydospores and an aquatic habitat is *Intercalispora* and this may be an earlier name for *Chlamydotubeufia*. The type *I. nigra* is illustrated in Seifert et al. (2011) and although quite similar to *Chlamydotubeufia*, the septation in *Intercalarispora nigra* J. L. Crane & Schokn. is irregular, while in *Chlamydotubeufia* species the septa is distinctly transverse with up to two vertical septa. It is not apparent if this different has any taxonomic value in separating genera but until *Intercalarispora* is recollected, isolated and subjected to sequence analysis we prefer to use the new name *Chlamydotubeufia*. Another species with *Intercalarispora*-like chlamydospores is *Tubeufia amazonensis* Samuels, Rossman & E. Müll.; the ascomata differ from *Chlamydotubeufia* as they lack setae in this species (Samuels & Müller, 1978). Seifert et al. (2011) also illustrate a *Monodictys* species as being anamorphic *Tubeufiaceae*, this however, may also be a species of *Intercalarispora*, but with the apical hyphae missing giving it the appearance of *Monodictys*.

Type species: *Chlamydotubeufia huaikangplaensis* Boonmee & K. D. Hyde.

Chlamydotubeufia huaikangplaensis Boonmee & K. D. Hyde, Fungal Diversity, 51: 63-102, (2011), MycoBank 563501 (Figs. 2.11-2.12)

Acanthostigma perpusillum De Not. similis sed ascospores 32–41.5 × 5–8 µm et 5–6-septate et status anamorphosis dictyochlamydosporae (45–)50–77 × (25–)39–42 µm differt.

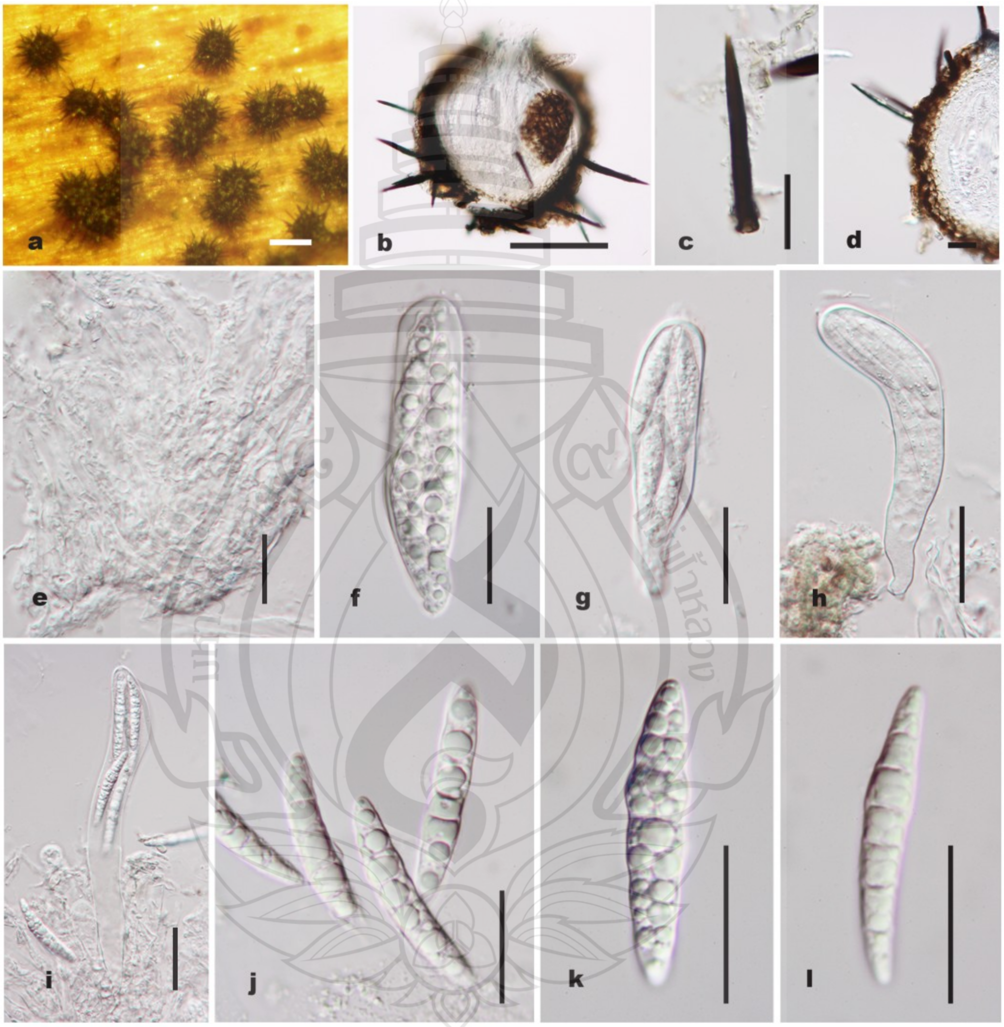
Etymology: from *–ensis* meaning pertaining to and *Huai Kang Pla*, the place of collection in reference to the collecting site.

Saprobic on dead wood of Pinus. *Ascomata* 161–192 µm high × 142–184 µm diam. ($\bar{x}$ = 173 × 160 µm, n = 5), superficial, solitary or scattered, globose to subglobose, black, with a central ostiolate, covered with darkened and one-celled setae. *Peridium* 17–23 µm thick, a single substratum, composed of 3–4 layers of cells of *textura angularis*. *Hamathecium* 2–2.5 µm wide, branched, hyaline pseudoparaphyses embedded in a gelatinous matrix. *Asci* 50–83 × 16–21 µm ($\bar{x}$ = 64 × 18 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical to clavate or saccate, apically rounded, ocular chamber not apparent, short pedicellate. *Ascospores* 32–41.5 × 5–8 µm ($\bar{x}$ = 37 × 7 µm, n = 20), fusiform, slightly curved, guttulate, 5–6-septate, slightly constricted at the septa, hyaline to pale gray.

Cultural characteristics: *Ascospores* germinating on MEA within 24-36 hours and germ tubes produced from both ends. Colonies growing on MEA slowly, reaching 9 mm in 1 month at 28°C, flat to slightly effuse, edge entire-erose or dentate, darkened to blackish. *Mycelium* mostly superficial, branched, septate, smooth, subhyaline to pale brown, hyphae becoming dark brown due to the development of dictyochlamydospores. *Conidia* (45–)50–77 µm long, (25–)39–42 µm wide ($\bar{x}$ = 56 × 35 µm, n = 20), blastic, broadly oval to ellipsoid, dictyoseptate, light coloured when immature, becoming darkened to black when mature.

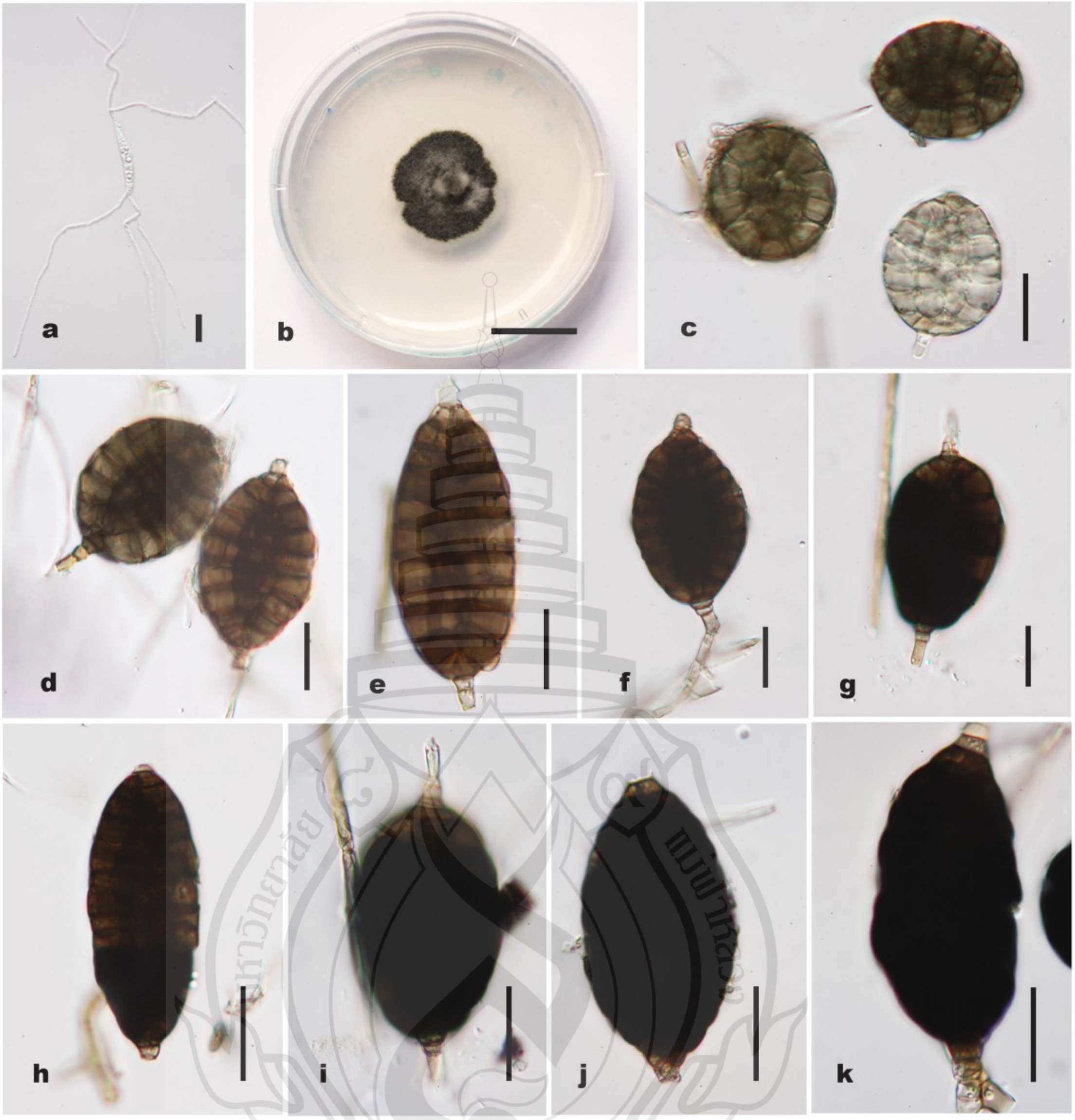
Material examined: THAILAND, Chiang Rai, Mae Chan, Huai Kang Pla Waterfall, N19°51–54' E 99° 35.39', 512 m., on dead wood of Pinus, 25 October 2010, S. Boonmee, HKP-01 (MFLU10–0972, holotype), ex-type culture MFLUCC10–0926 = BCC.

Chlamydotubeufia huaikangplaensis has superficial, globose to subglobose, black ascomata covered with 1-celled darkened setae, clavate asci, and fusiform-oblong, hyaline, trans-septate ascospores. In addition, dictyochlamydospores form in culture (Fig. 2.12c-k). In molecular analysis (Fig. 2.2) *C. huaikangplaensis* clusters with *Helicoma chlamydosporum* with strong support (100% PP).



Note. (a) Ascomata. (b) Section through of ascoma. (c) Seta. (d) Peridium comprising of textura angularis. (e) Pseudoparaphyses. (f)-(i). Asci. (j)-(l) Ascospores. Scale bars: (a)-(b) = 100 µm, (c)-(l) = 20 µm

Figure 2.11 *Chlamydotubeufia huaikangplaensis* (MFLU10–0972, holotype)



Note. (a) Germination of ascospore. (b) Colonies on MEA in surface view. Note colonies are dark brown. (c)-(k) Dictyochlamydospores. Scale bars: (a)-(b) = 10 mm, (c)-(k) = 20 µm

Figure 2.12 Asexual State of *Chlamydotubeufia huaikangplaensis* in Culture (MFLUCC10–0926, holotype)

Chlamydotubeufia khunkornensis Boonmee & K. D. Hyde, Fungal Diversity, 51: 63-102, (2011), MycoBank 563502 (Figs. 2.13-2.14)

Acanthostigma perpusillum De Not. similis sed ascospores 34–42 × 5–7 et 6–7-septate et status anamorphosis dictyochlamydosporae (60–)73.5–130.5(−150) × (39–)48–65.5 (−70) µm differt.

Etymology: from *–ensis* meaning pertaining to and *Khun Korn*, the place of collection in reference to the collecting site.

Ascomata 180–250 µm high × 199–249 µm diam. ($\bar{x}$ = 210.5 × 220 µm, n = 5), superficial, solitary or scattered, globose to subglobose, with a central ostiolate 20–30 µm wide, covered with dark brown setae, which taper to an acute apex. *Peridium* 18–28 µm thick, composed of 3–5 layers of light brown cells of compressed *textura angularis*. *Hamathecium* numerous cellular, filiform pseudoparaphyses, hyaline, embedded in a gelatinous matrix. *Asci* 79.5–106 × 13–21 µm ($\bar{x}$ = 96.5 × 15.5 µm, n = 20), 8-spored, bitunicate, cylindrical to clavate, apically rounded, ocular chamber not observed, short pedicellate. *Ascospores* 34–42 × 5–7 µm ($\bar{x}$ = 38 × 6 µm, n = 20), 2–3-seriate, fusiform, broad at the supra-median cell, 6–7-septate, ends asymmetrical, not constricted at any septum, hyaline, smooth-walled.

Cultural characteristics: Ascospores germinating on MEA within 24–36 hours with germ tubes produced from both ends. Colonies growing on MEA slowly, reaching 5 mm in 1 month at 28°C, flat to slightly effuse, sparsely hairy, with entire to fimbriate edge, darkened to blackish. Mycelium partly superficial, partly immersed, composed of branched, septate, smooth, subhyaline to olivaceous brown, dark brown hyphae, due to the development of dictyochlamydospores. *Conidia* (60–)73.5–130.5(−150) µm length, (39–)48–65.5(−70) µm wide ($\bar{x}$ = 97.5 × 56.5 µm, n = 20), dictyochlamydospores, multicellular, darkening to black.

Material examined: THAILAND, Chiang Rai, Muang, Khun Korn Waterfall, N19°51–54' E 99° 35.39', 671 m., on dead wood of *Aleurites moluccana* (Candlenut tree), 13 November 2009, S. Boonmee KK-06 (MFLU10–0051, holotype), ex-type culture MFLUCC10–0118 = IFRDCC2186 = BCC.

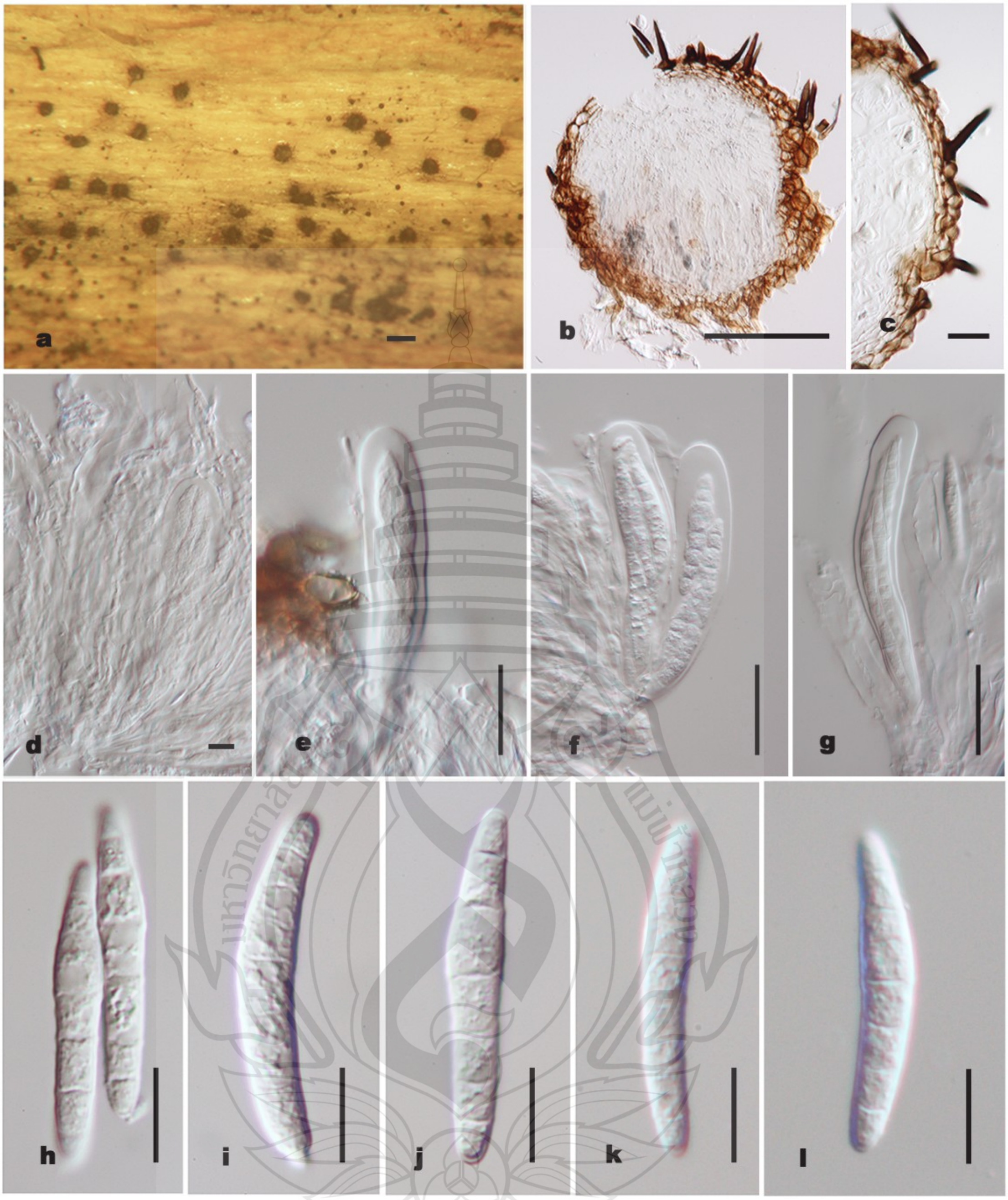
The teleomorph state of *Chlamydotubeufia khunkornensis* is morphologically similar to *C. huaikangplaensis*, but differs by bright coloured ascomata, short-setae, and ascospores without guttulates (Figs. 2.11, 2.13). The dictyochlamydospores are also larger being 97.5 × 56.5 µm in *C. khunkornensis* and 56 × 35 µm in *C. huaikangplaensis*. The two species cluster together with low support (Fig. 2.1).

Chlamydotubeufia chlamydospora (Shearer) Boonmee & K. D. Hyde, Fungal Diversity, 51: 63-102, (2011), MycoBank 563503

≡ *Helicoma chlamydosporum* Shearer, Mycologia 79: 468, 1987

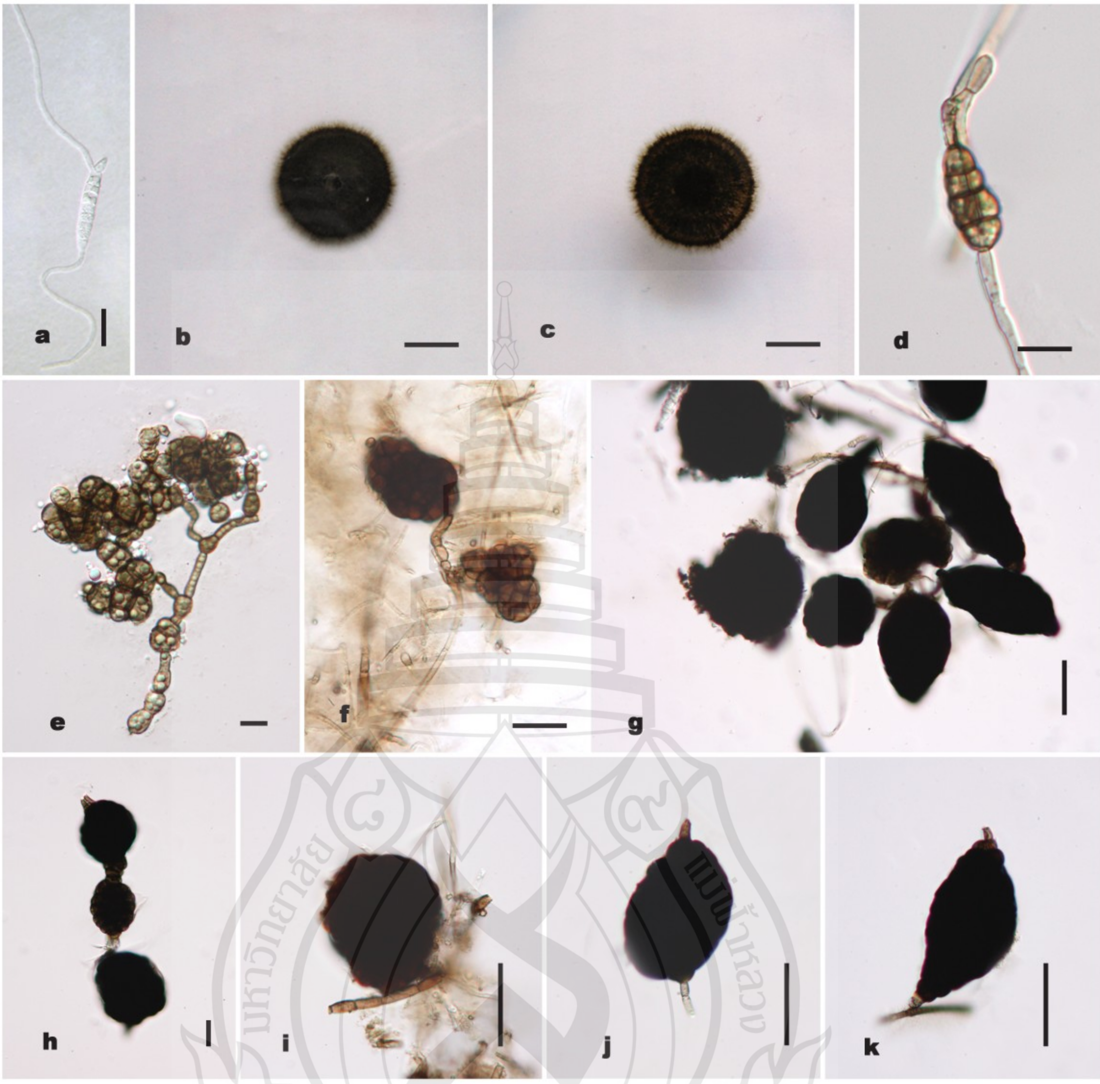
Chlamydotubeufia depressispora (Matsush.) Boonmee & K. D. Hyde, Fungal Diversity, 51: 63-102, (2011), MycoBank 563504

≡ *Helicoma depressispora* Matsush., Matsush. Mycol. Mem. 7: 52 1993



Note. (a) Ascomata. (b)-(c) Section through of ascoma and peridium. Note ascomata covered with dark brown setae. (d) Pseudoparaphyses. (e)-(g) Asci at young and mature stages. (h)-(l) Ascospores. Scale bars: (a)-(b) = 100 μm, (c), (e)-(g) = 20 μm, (d) = 5 μm, (h)-(l) = 10 μm

Figure 2.13 *Chlamydotubeufia khunkornensis* (MFLU10–0051, holotype)



Note. (a) Germinating ascospore. (b)-(c) Colonies on MEA from surface and in reverse. Note darkened colonies. (d)-(f). Mycelium development on culture. (g)-(k) Dictyochlamydospores. Scale bars: (a)-(c) = 10 mm, (d)-(f) = 10 µm, (g)-(k) = 20 µm

Figure 2.14 Asexual State of *Chlamydotubeufia khunkornensis* in Culture (MFLUCC10–0118, holotype)

Kamalomyces R. K. Verma, N. Sharma & Soni, Forest Fungi of Central India: 196 (2008)

Saprobic on dead bamboo. *Ascomata* forming on a subiculum of crowded black mycelium, superficial, clustered to solitary, globose to subglobose, stalked. *Peridium* comprising 3–4 layers of darkened cells of *textura angularis*, dull at margin. *Hamathecium* cellular, filiform, branched pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, thick-walled, saccate-clavate, with an ocular chamber, pedicellate. *Ascospores* fusiform to clavate, slightly tapering toward the rounded ends, trans-septate with crowded septa, straight or slightly curved, upper part broad, hyaline.

Anamorphs reported for genus: none.

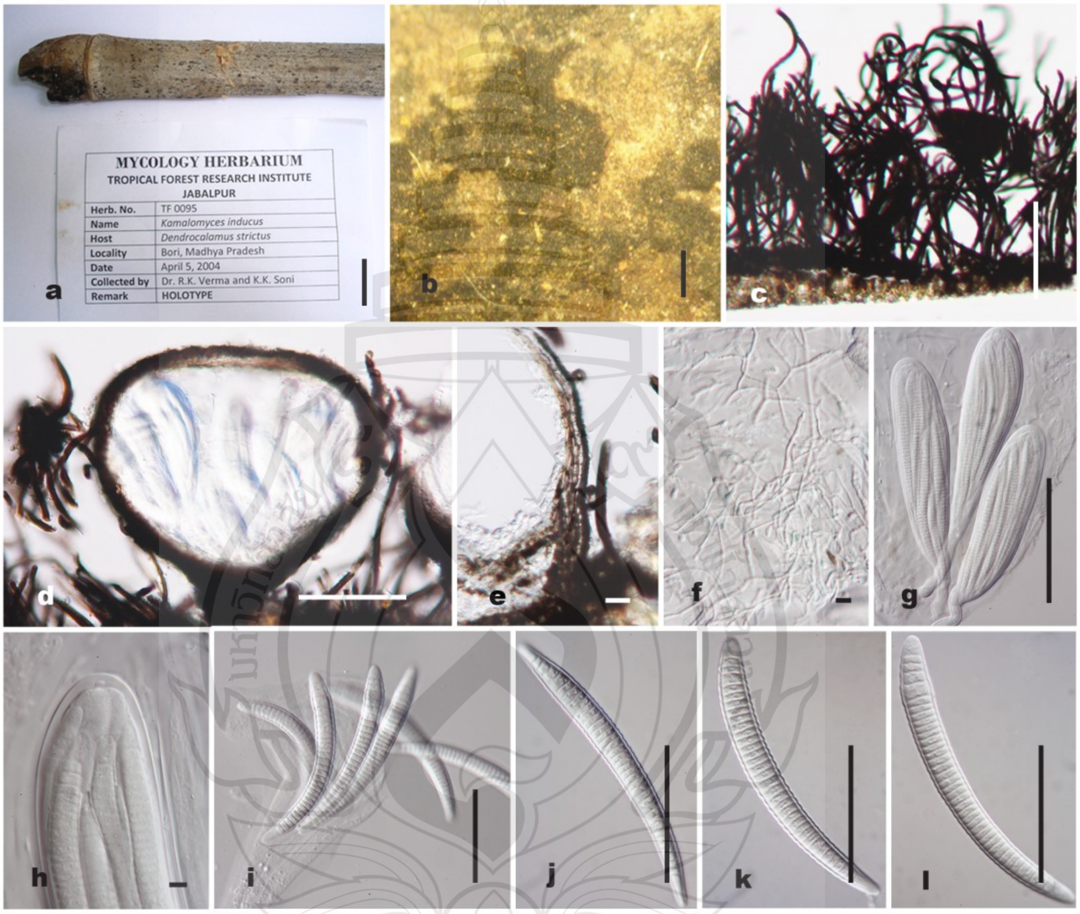
Type species: *Kamalomyces indicus* R. K. Verma, N. Sharma & Soni

Kamalomyces indicus R. K. Verma, N. Sharma & Soni, Forest Fungi of Central India: 196 (2008) (Fig. 2.15)

Saprobic on dead culms of bamboo (*Poaceae*). *Ascomata* (169–)216.5–253 µm high, (184–)225.5–295.5(−331) µm diam. ($\bar{x}$ = 218 × 263 µm, n = 5), forming on a subiculum of crowded black mycelium, superficial, clustered to solitary, subglobose-globose, stalked. *Peridium* (14.5–)19–28 µm, comprising of 3–4 layers of darkened cells of *textura angularis*, dull at margin. *Hamathecium* 1–1.5 µm wide, cellular, filiform, hyaline, branched pseudoparaphyses, embedded in a gelatinous matrix. *Asci* (140–)157–214(−223) × (27.5–)29–36(−38) µm ($\bar{x}$ = 185 × 34 µm, n = 20), 8-spored, bitunicate, clavate, cylindric-clavate, subclavate or broadly obovoid, with an ocular chamber, pedicel (21–)25–49.5(−68.5) µm long. *Ascospores* (92–)95.5–104.5(−107) × 8–10.5 µm ($\bar{x}$ = 99 × 9 µm, n = 20), 3–4-seriate in the ascus, fusiform-clavate, slightly tapering towards the rounded ends, straight or slightly curved, upper part broad, (34–)38–42-trans-septate with crowded septa, constricted at the septum, hyaline.

Material examined: INDIA, Madhya Pradesh, Bori, on culms of *Dendrocalamus strictus* Nees (*Poaceae*), 5 April, 2004, R.K. Verma and K.K. Soni, No. TF0095 (TFRI, holotype).

This taxon appears to be typical of *Tubeufiaceae* as it has superficial, globose to subglobose, dark brown to black ascomata which form on a subiculum of crowded black mycelium, clavate asci and cylindrical-fusiform to elongate, trans-septate hyaline ascospores. No other genus in *Tubeufiaceae* have such a suite of characters and the crowded septa are particular distinct.



Note. (a) Appearance of fungus on bamboo. (b)-(c) Ascomata in subiculum on substrate. (d) Section of ascoma with stalk. (e) Peridium. (f) Pseudoparaphyses. (g)-(h) Asci showing pedicel (in Fig. 2.14g) and ocular chamber (in Fig. 2.14h). (i)-(l) Ascospores with crowded septa. Scale bars: (a)-(c) = 150 µm, (d), (g) = 100 µm, (e) = 10 µm, (f), (h) = 5 µm, (i)-(l) = 50 µm

Figure 2.15 *Kamalomyces indicus* (TFRI, holotype)

Podonectria Petch, Trans. Br. Mycol. Soc. 7: 146 (1921)

Habit occurring in associations with scale insects. *Ascomata* superficial, scattered, solitary, subglobose-globose, light yellow-reddish, covered with light brown mycelium. *Peridium* composed of several-layers of pale yellowish brown cells of *textura angularis*. *Hamathecium* filiform, branched, septate, hyaline pseudoparaphyses embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, cylindro-clavate, short pedicellate. *Ascospores* 2–3-seriate, clavate to fusiform, hyaline to pale brown, trans-septate, wall minutely verruculose.

Anamorphs reported for genus: *Peziotrichum*, *Tetracrium* (Hyde et al. 2011)

Lectotype species: *Podonectria coccicola* (Ellis & Everh.) Petch

Podonectria coccicola (Ellis & Everh.) Petch, Trans. Br. Mycol. Soc. 7: 146 (1921) (Fig. 2.16)

≡ *Nectria coccicola* Ellis & Everh., J. Mycol. 2(4): 39 (1886)

≡ *Ophionectria coccicola* (Ellis & Everh.) Berl. & Voglino, in Saccardo, Syll. fung., Addit. I-IV (Abellini): 218 (1886)

≡ *Puttemansia coccicola* (Ellis & Everh.) Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 127: 625 (1918)

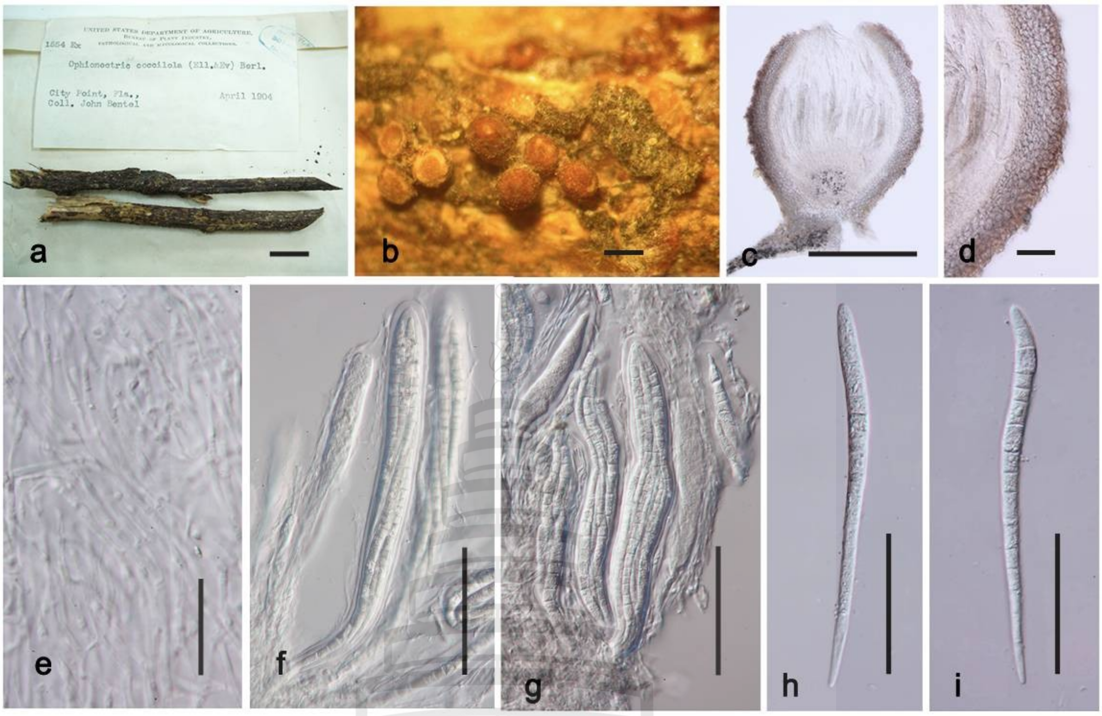
≡ *Scoleconectria coccicola* (Ellis & Everhart) Seaver, Mycologia 1(5): 198 (1909)

≡ *Tubeufia coccicola* (Ellis & Everh.) Lar.N. Vassiljeva, Nizshie Rasteniya, Griby i Mokhoobraznye Dalnego Vostoka Rossii, Griby. Tom 4. Pirenomitsety i Lokuloaskomitsety (Sankt-Peterburg): 317 (1998)

Habit occurring in association with scale insects. *Ascomata* (440.5–)476–496.5(−524.5) µm high × 357.5–458(−599) µm diam. ($\bar{x}$ = 487 × 433 µm, n = 5), superficial, scattered, solitary, subglobose-globose, light yellow to reddish brown, brighter or reddish at the centre, covered with light brown mycelium. *Peridium* 51–69(−74) µm thick, composed several layers of pale yellowish brown cells of *textura angularis*. *Hamathecium ca*. 1 µm wide, cellular, filiform, branched, septate pseudoparaphyses embedded in a gelatinous matrix. *Asci* (133.5–)142–183(−202) × 14–16(−21.5) µm ($\bar{x}$ = 165 × 16.5 µm, n = 10), 8-spored, bitunicate, cylindric-clavate, ocular chamber not obvious, short pedicellate. *Ascospores* (101–)114–134.5(−139) × 5.5–8.5(−10) µm ($\bar{x}$ = 124 × 8 µm, n = 10), 2–3-seriate, narrowly clavate to fusiform, (12–)14–18-trans-septate, ends rounded to sub acute, hyaline to pale grayish, wall minutely verruculose.

Material examined: USA, Florida, City Point, on scale insects on bark of orange trees, April 1904, John Bentel, ex BPU 1554 (NY); Gainesville, Florida, on scale insects on orange leaves, J. Matz, Herb. U. Mass. 632 (NY); Florida, Melbourne, on scale insects, 28 June 1937 J. Young (NY); Florida, Southworth, on dead scale insects on the bark of living orange trees, 24 January to 5 April 1923, Fred J. Seaver and Carlos E. Chardon No. 1064 (NY).

Podonectria is a genus that occurs on scale insects with long trans-septate ascospores and has been monographed by Rossman (1978) with eight accepted species. A collection determined as *P. coccicola* by Rossman (1978) was examined and is illustrated here. *Podonectria* was introduced by Petch (1921) for a taxon with light-reddish brown, hairy ascomata with clavate-fusiform, pale hyaline to grayish ascospores There are eleven species recorded in Index fungorum (www.indexfungorum.org/). *Podonectria coccicola*, the type species of the genus, was described from scale insects on orange trees from Florida (Rossman, 1978). The habitat of the fungus and colour of the ascomata are unusual for *Tubeufiaceae* and this species needs recollecting to confirm its placement in the family.



Note. (a) Material label. (b) Orange ascomata on substrate. (c) Section of ascoma. (d) Peridium. (e) Pseudoparaphyses. (f)-(g) Asci. (h)-(i) Ascospores with minutely verruculose walls. Scale bars: (a)-(c) = 200 µm, (d) = 40 µm, (e) = 20 µm, (f)-(i) = 50 µm

Figure 2.16 *Podonectria coccicola* (NY, ex BPU 1554)

Thaxteriella Petr., Annls Mycol. 22(1/2): 63 (1924)

Saprobic on dead bark. Mycelium forming a dense velvety subiculum over the surface of the bark, comprising black hyphae. *Ascomata* superficially develop on the basal subiculum or slightly embedded, solitary or densely gregarious, globose to turbinate, black, shiny, often with small apical papillae at the central ostiolate apex. *Peridium* comprising two strata, outer layer of thick-walled, dark brown cells of *textura angularis* and inner layer of brown cells of *textura globosa*. The apical region is composed of less thick-walled cells which disintegrate to form an ostiole. *Hamathecium* cellular, filamentous, branched, anastomosing pseudoparaphyses attached to the hymenium and to the ascomata wall above. *Asci* bitunicate, 8-spored, cylindrical to broadly clavate or subclavate, thickened at the apex with an ocular chamber, usually with a short pedicel. *Ascospores* cylindrical to long fusiform, tapering towards the rounded to sub-acute ends, trans-septate, hyaline.

Anamorphs reported for genus: *Helicoma* (Hyde et al., 2011)

Type species: *Thaxteriella corticola* Petr.

Thaxteriella corticola Petr., Annls Mycol. 22(1/2): 63 (1924) (Fig. 2.17)

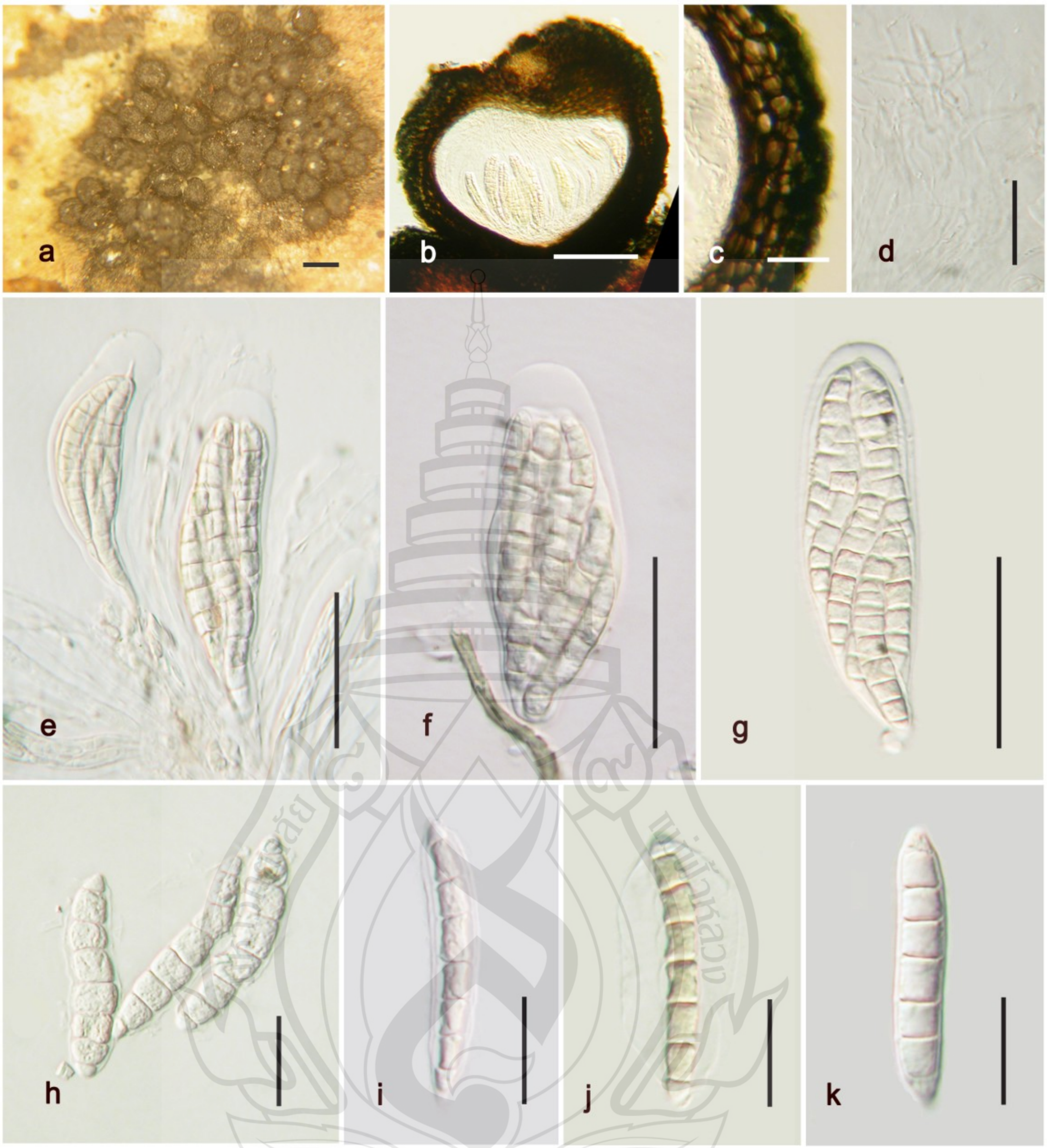
Saprobic on dead bark. Mycelium forming a dense velvety subiculum over the surface of the bark, comprising black hyphae. *Ascomata* 230–320 × 230–310 µm diam. ($\bar{x}$ = 284 × 296 µm, n = 10), superficially developing on a basal subiculum or slightly embedded, solitary or densely aggregated, globose to turbinate, black, shiny, often with small apical papillae at the apex. *Ostiole* (10–)12.5–15 µm diam. ($\bar{x}$ = 13.5 µm, n = 5), central. *Peridium* 30–60 µm diam. ($\bar{x}$ = 39 µm, n = 10), comprising two layers, outer layer of thick-walled, dark brown cells of *textura angularis* and inner layer of brown cells of *textura globosa*. The apical region is composed of less thick-walled cells which disintegrate to form an ostiole. *Hamathecium* 1–2 µm wide, cellular, filamentous, filiform, branched, anastomosing pseudoparaphyses attached to the hymenium and to the ascomata wall above. *Asci* (67.5–)77.5–101.5(−112.5) × 25–35 µm ($\bar{x}$ = 92 × 28 µm, n = 10), 8-spored, bitunicate, cylindrical to broadly clavate or subclavate, thickened at the apex with an ocular chamber, usually with a short 5–10 × 5 µm ($\bar{x}$ = 7.5 × 5 µm, n = 5) pedicel. *Ascospores* 45–55 × (7.5–)10–12.5 µm ($\bar{x}$ = 49.5 × 11 µm, n = 10), 3–6-seriate or fasciculate, cylindrical to long fusiform, tapering towards the rounded to sub acute ends, 7-septate, straight or slightly curved, sometimes constricted in some of the septa, hyaline to pale-yellowish.

Material examined: PUERTO RICO, Vega Baja., on dead bark, 15 January 1916, B. Fink No. 2145, F. Petrak, Pilzherbarium, Acqu. 1974 No. 24118 (W, holotype).

Thaxteriella was introduced by Petrak (1924) characterized by globose, black ascomata forming on a subiculum comprising smooth, thick-walled hyphae and bitunicate asci resembling *Herpotrichia* Fuckel (Bose, 1961). Petrak (1953) proposed a new combination with *Thaxteriella pezizula* (Berk. & M. A. Curtis) Petr. and considered

Thaxteriella corticola a synonym. Morphologically, *Thaxteriella* is similar to *Tubeufia* and has a similar anamorph (Samuels et al., 1979). *Thaxteriella* is typified by *T. corticola* Petr. (Petrak, 1924) which Crane et al. (1998) considered to be a lost species, but is identical to *T. pezizula* (Berk. & M. A. Curtis) Petr. We have been able to locate type material and therefore redescribe *T. corticola*. Crane et al. (1998) mentioned that *Thaxteriella* differs from *Tubeufia* in pigmentation and structure of the peridium and he transferred *T. helicoma* and *T. amazonensis* to *Thaxteriella* based on morphological characters.

Thaxteriella is treated as a distinct genus in *Tubeufiaceae* because ascomata are subglobose, shiny and become cupulate when dry, in having a distinct subiculum, and cylindrical to long fusiform ascospores which tapering towards the rounded to sub-acute ends, and a *Helicoma* anamorph. In the phylogenetic tree (Fig. 2.2) *T. inthanonensis* forms a distinct cluster with *Helicoma dennisii*. *T. inthanonensis* is characteristic of *Thaxteriella* and anamorphs are typical of *Helicoma*. *Thaxteriella helicoma* however clusters with *Tubeufia paludosa*, but we cannot confirm the correct identity of this taxon from GenBank. The anamorph of *Thaxteriella* is reported as *Helicoma* (Hyde et al., 2011; Sivanesan, 1984), which is also polyphyletic.



Note. (a) Superficial gregarious ascomata on bark. (b) Section through of ascomata. (c) Peridium. (d) Pseudoparaphyses. (e)-(g) Asci. Note the ocular chamber and short pedicels. (h)-(k) Ascospores. Notes the sheath in Fig. j. Scale bars: (a)-(b), (d) = 100 µm, (c), (e)-(g) = 40 µm, (d) = 100 µm, (h)-(k) = 20 µm

Figure 2.17 *Thaxteriella corticola* (W, holotype)

Thaxteriella inthanonensis Boonmee & K. D. Hyde, Fungal Diversity, 51: 63-102, (2011), MycoBank 563505 (Figs. 2.18-2.19)

Thaxteriella corticola Petr. similis sed ascospores (29.5–)31.5–36.5(−37.5) × (5.5–)7–8 µm, 7-septate, anamorphosis helicosporae (10–)13–20 × 4–7 µm.

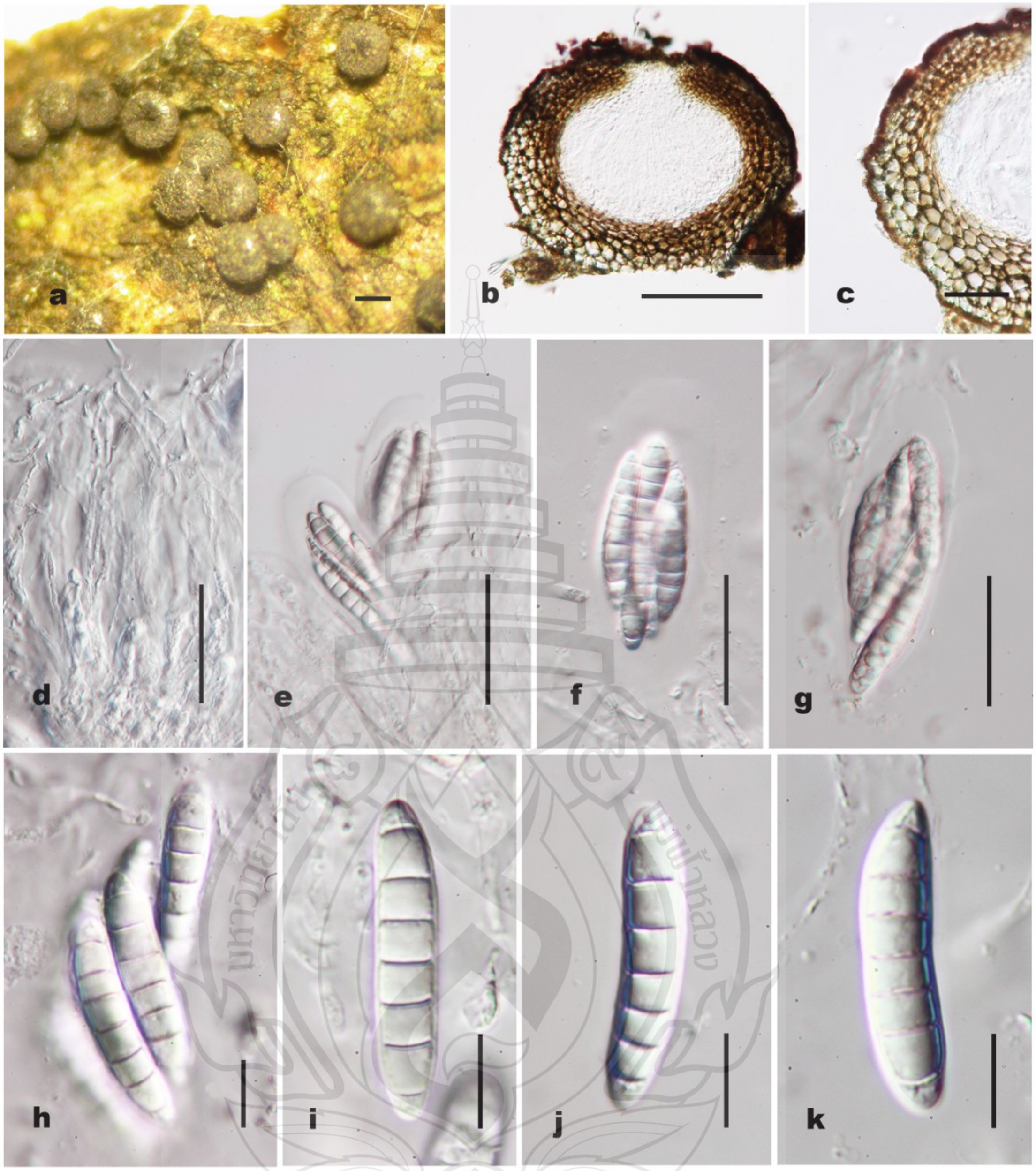
Etymology: from the Latin –ensis referring to an association and *Inthanon* the place of collection.

Saprobic on dead bark. *Ascomata* (216–)227–247 µm high × 258.5–307(−322) µm diam. ($\bar{x}$ = 232 × 293.5 µm, n = 5), superficial, solitary or scattered, globose to subglobose, subiculum or turbinate, collapsing when mature or dry, dark brown, shiny, without setae, central ostiolate. *Peridium* (42–)61–70 µm thick, comprising several layers of thickened cells of *textura angularis* to *globularis;* inner wall having subhyaline cell layers, become brown to dark brown inwardly. *Hamathecium ca*. 2 µm wide, hyaline, filiform, septate, anastomosing pseudoparaphyses embedded in a gelatinous matrix. *Asci* (73–)80–91 × (21–)24–30(−32.5) µm ($\bar{x}$ = 83.5 × 28 µm, n = 10), 8-spored, bitunicate, clavate-broadly, rounded and thickened at the apex, without a pedicel, 2–3-seriate in the ascus. *Ascospores* (29.5–)31.5–36.5(−37.5) × (5.5–)7–8 µm ($\bar{x}$ = 33 × 7 µm, n = 20) allantoid, cylindrical to long fusiform, tapering towards the ends, slightly curved, 7-septate, slightly constricted at septa, symmetrical, granular when immature, hyaline to pale, smooth-walled at maturity.

Cultural characteristics: Ascospores germinating on MEA within 12–24 hours and germ tubes produced from both ends. Colonies growing on MEA slowly, reaching 8 mm in 1 week at 28°C, slightly effuse, edge entire to erose or dentate, darkened to blackish. Mycelium mostly superficial, branched, septate, smooth, subhyaline to pale brown. *Conidiophores* (14.5–)26.5–34(−42) µm in diam., 3 µm wide, septate, smooth, pale brown. *Conidia* (10–)13–20 µm in diam., 4–7 µm wide ($\bar{x}$ = 14 × 6 µm), acropleurogenous, helicosporous, at first hyaline to pale yellowish brown, becoming brown, 7-septate, smooth or rough.

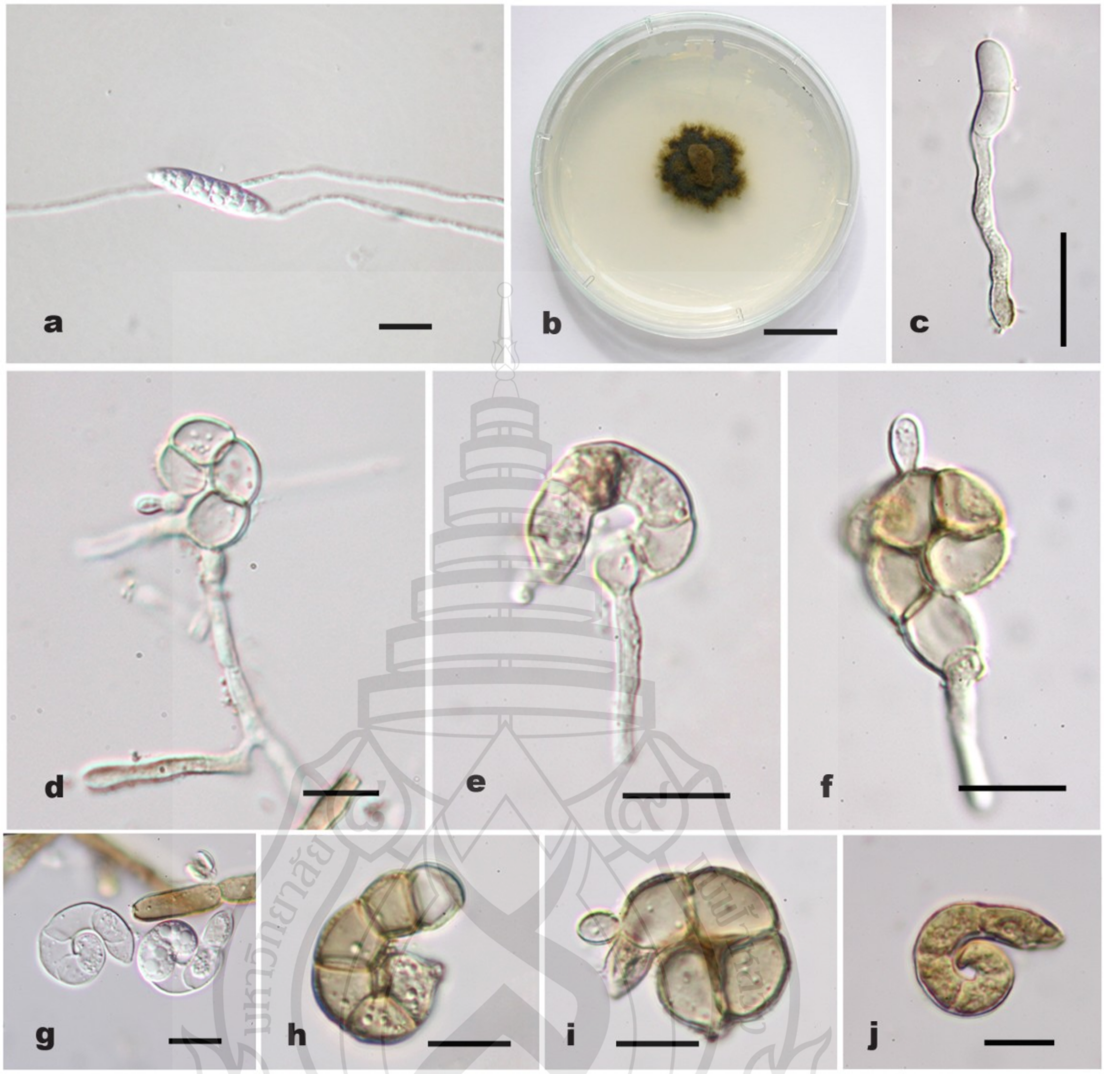
Material examined: THAILAND, Chinag Mai, Doi Inthanon, Jom Thong, N18°31.576' E 98°29.790', *ca*. 800–1000 m., on dead bark of unidentified trees, 16 November 2010, Rungtiwa Phookamsak (MFLU11–0003, holotype), ex-type culture MFLUCC11–0003 = BCC.

Thaxteriella inthanonensis is very similar to *T. corticola* and obviously represents the same genus. *Thaxteriella inthanonensis* however, differs in the colour of the ascomata (Figs. 17a, 18a; Sivanesan, 1984) being dark-brown in the new species and black in *T. corticola*. In *T. inthanonensis* ascospores are also smaller than *T. corticola* (33 × 7 µm vs 49.5 × 11 µm). In addition *T. inthanonensis* produces pale brownish, septate *Helicoma*-like conidia (Fig. 2.19c-j), while in *T. corticola* no anamorphs are reported. *T. inthanonensis* clusters in an individual clade with 100% PP (Fig. 2.2) and is representative of *Thaxteriella* which is a distinct genus from *Tubeufia*.



Note. (a) Ascomata. (b) Ascoma cross section. (c) Peridium. (d) Pseudoparaphyses. (e)-(g) Asci. (h)-(k) Ascospores. Scale bars: µm, (a)-(b) = 100 µm, (c)-(g) = 40 µm, (h)-(k) = 10 µm

Figure 2.18 *Thaxteriella inthanonensis* (MFLU11–0003, holotype)



Note. (a) Germination of ascospore. (b) Colonies on MEA in surface view. Notes colonies are dark brown. (c)-(f) Conidiophores with conidia. (g)-(j). Conidia. Scale bars: (a), (d)-(j) = 10 µm, (b) = 10 mm, (c) = 20 µm

Figure 2.19 Asexual State of *Thaxteriella inthanonensis* in Culture (MFLUCC11–0003, holotype)

Thaxteriellopsis Sivan., Panwar & S.J. Kaur, Kavaka 4: 39 (1977) [1976]

Saprobic on dead wood. *Ascomata* born on a thin dark brown subiculum, superficial, solitary or scattered, globose to subglobose, reddish brown to dark brown, with brown to dark brown, septate, setae mostly on the top. *Peridium* comprising 3–4 layers of red brown to dark brown cells of *textura angularis*. *Hamathecium ca*. 2 µm wide, cellular, filiform pseudoparaphyses embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, cylindrical to clavate, apically rounded, short pedicellate, with long apical region with amorphous contents. *Ascospores* 2–3-seriate in an ascus, fusiform to clavate, broader above, straight to slightly curved, 5-septate, constricted at the septum, hyaline, smooth-walled.

Anamorphs reported for the genus: *Helicosporium, Moorella, Xenosporium* (Hyde et al., 2011)

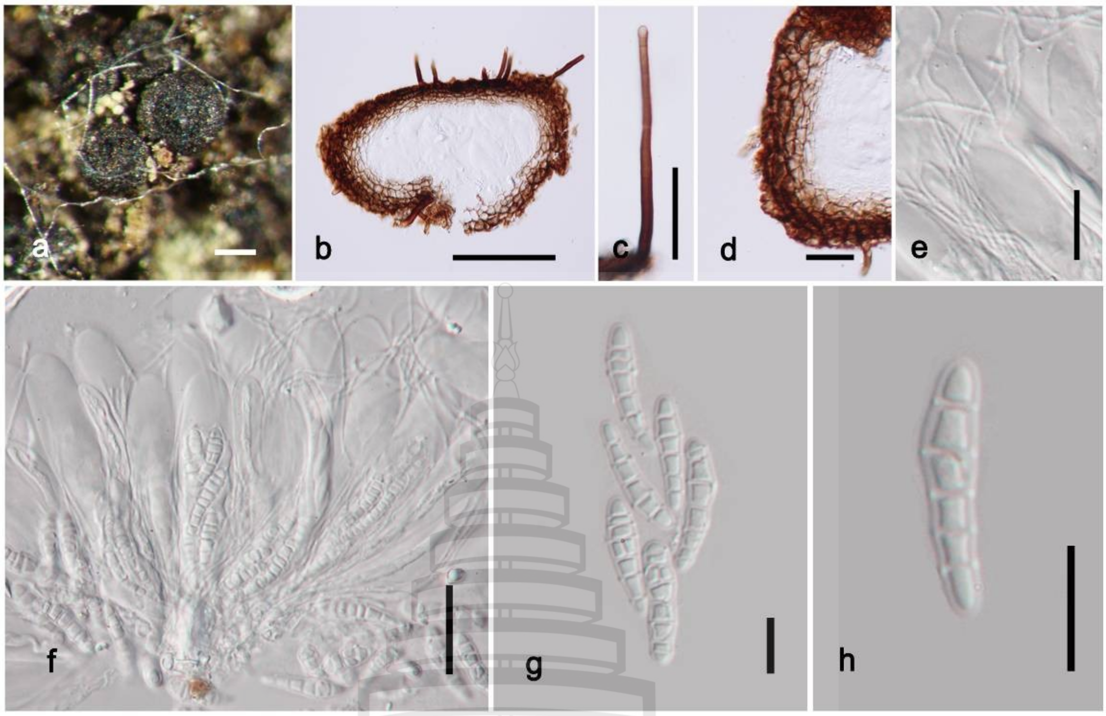
Type species: *Thaxteriellopsis lignicola* Sivan.

Thaxteriellopsis lignicola Sivan., Panwar & S. J. Kaur, Kavaka 4: 39 (1977) [1976] (Figs. 2.20)

≡ *Chaetosphaerulina lignicola* (K. S. Panwar & S. J. Kaur), J. L. Crane, Shearer & M.E. Barr, Can. J. Bot. 76(4): 608 (1998)

Description from holotype (Fig. 2.20)

Saprobic on dead wood. *Ascomata* (142.5–)150–165(−179) µm high × (182–) 204.5–248.5(−258.5) µm diam. ($\bar{x}$ = 157 × 217.5 µm, n = 5), borne on a thin dark brown subiculum, superficial, solitary or scattered, globose to subglobose, reddish brown to dark brown, with brown to dark brown, septate setae, mostly on the top. *Peridium* (35–)38–45 µm thick, comprising 3–4 layers of red brown to dark brown cells of *textura angularis*. *Hamathecium ca*. 2 µm wide, cellular, filiform pseudoparaphyses embedded in a gelatinous matrix. *Asci* (73.6–)76–92(−99) × (13–) 15–18(−19) µm ($\bar{x}$ = 84 × 16 µm, n = 20), 8-spored, bitunicate, cylindrical to clavate, apically rounded with long apical region with amorphous contents, short pedicellate. *Ascospores* (23–) 24.5–27(−29) × 6–8(−9) µm ($\bar{x}$ = 25.5 × 7 µm, n = 20), 2–3-seriate, fusiform to clavate, broader above, straight to slightly curved, 5-septate, constricted at then septum, hyaline, smooth-walled.



Note. (a) Ascomata on substrate. (b) Section of ascoma. (c) Single seta. (d) Section of peridium. (e) Pseudoparaphyses. (f) Squash mount showing asci. Note the long apical region with amorphous contents. (g)-(h) Ascospores. Scale bars: (a)-(b) = 100 µm, (c) = 50 µm, (d)-(e) = 20 µm, (f)-(g) =10 µm

Figure 2.20 *Thaxteriellopsis lignicola* (IMI: holotype)

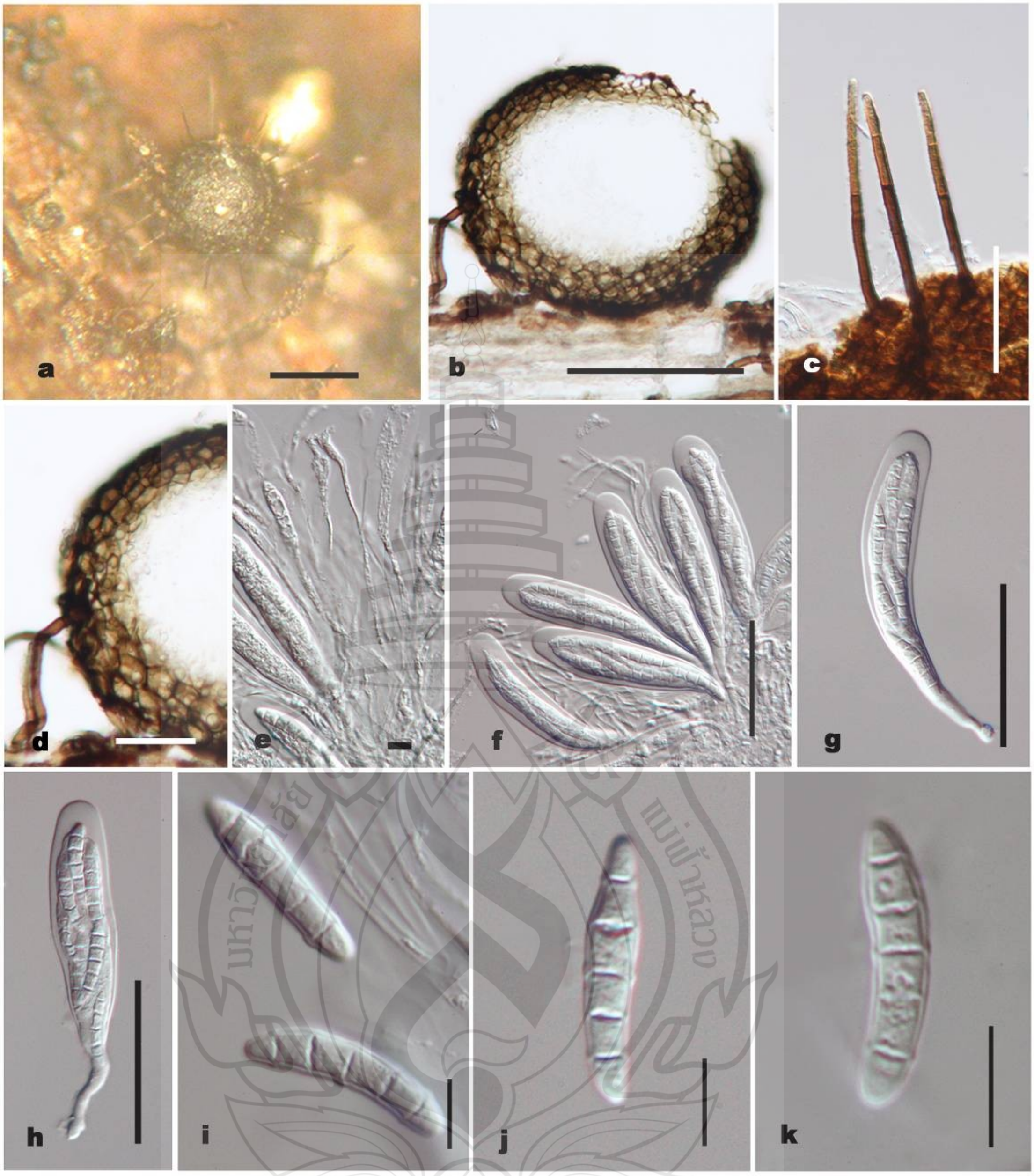
Description from epitype (Figs. 2.21-2.22)

Ascomata 116–118(−148) µm high × 130–132(−179.5) µm diam. ($\bar{x}$ = 127.5 × 147 µm, n = 3), superficial, solitary and scattered, globose to subglobose, shiny, reddish brown to dark brown, collapsed when dry, with central ostiole 17–19 µm wide, with dark brown setae up to 83.5 µm long with an acute apex. *Peridium* 17.5–28 µm thick, comprising up to 4-layers of dark brown cells of *textura angularis*. *Hamathecium ca*. 1.5 µm wide, cellular, filiform, hyaline pseudoparaphyses. *Asci* (78.5–)82–98(−106) × (14.5–)15–17(−18.5) µm ($\bar{x}$ = 90 × 16 µm, n = 20), 8-spored, bitunicate, cylindrical to clavate, apically rounded, and amorphous thickened apical region, ocular chamber low, with a 13–24 µm long pedicel. *Ascospores* (21.5–)23.4–28.5(−31) × 5–6(−7) µm ($\bar{x}$ = 26 × 5.5 µm, n = 20), 2–3-seriate in the ascus, clavate-fusiform, upper part broad, straight or slightly curved, ends rounded, 5-septate, slightly constricted at the septa, hyaline, smooth-walled.

Cultural characteristics: Ascospores germinating on WA within 12 hours and germ tubes produced from both ends. Colonies growing slowly on MEA, reaching 6 mm in 1 week at 28°C, effuse, edge entire to fimbriate, dark to blackish, aerial mycelium raised. Mycelium superficial, composed of branched, septate, smooth, subhyaline to pale yellow brown hyphae. Micronematous conidia-like structures (29–)40–80(−96) µm long, 5–7 µm wide, develop directly from hyphae.

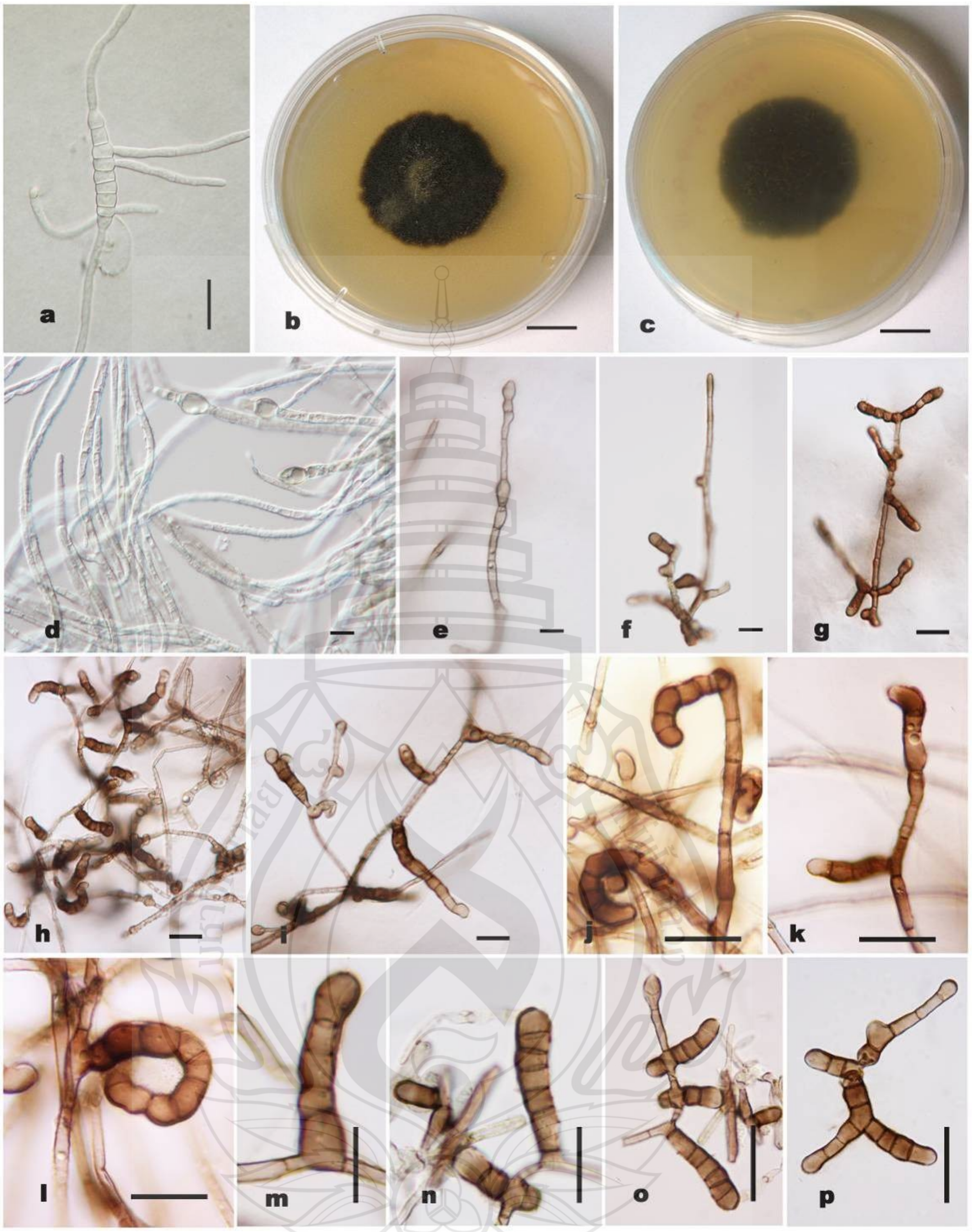
Material examined: INDIA, Rajasthan, Mount Abu, on dead wood of *Lingo emortuo*, 10 September 1975, leg. K. S. Panwar 555 (IMI 197065, holotype); THAILAND, Chiang Mai Province, Mae Rim, Pong Yaeng, elev. *ca*. 1000 msl., on dead wood of Zizyphus mauritiana Lamk (Jujube), 7 September 2009, S. Boonmee, PYJJ-01 (MFLU10–0057, epitype designated here), ex-epitype culture MFLUCC10–0124 = IFRD2197 = BCC; Lam Pang Province, Wang Neua, elev. *ca*. 800–900 msl., on dead wood of unidentified trees, 7 January 2011, S. Boonmee, LP-01 (MFLU10–0055), living culture MFLUCC10–0122 = IFRD2175 = BCC; Chiang Rai Province, Muang, Khun Korn Waterfall, N19°51–54' E 99°35.39', 671 m., on dried bark of unidentified trees, 18 December 2009, S. Boonmee, KK-10 (MFLU10–0054), living culture MFLUCC10–0121 = IFRD2167 = BCC.

Thaxteriellopsis was introduced by Sivanesan, Panwar and Kaur (1976) with *Thaxteriellopsis lignicola* as the type species and *T. eucalypti* A. Pande & V. G. Rao and *T. bambusicola* Sivan. & N. D. Sharma have since been added (Crane et al., 1998). General characters as well as the ability to produce helicosporous anamorphs is are similarity to *Thaxteriella* (Fig. 2.19; Sivanesan, 1984; Subramanian & Sekar, 1982), but *Thaxteriellopsis* differs in having ascomata with long setae up to 180 µm and ascospores occasionally with longitudinal septate and becoming pale brown at maturity (Sivanesan et al., 1976). *Thaxteriellopsis bambusicola* has been transferred to *Chaetosphaerulina* by Crane et al. (1998) and this is probably correct, however we doubt that *Thaxteriellopsis lignicola* is a species of *Chaetosphaerulina*. The short ascospores, globose reddish-brown ascomata which collapse when dry and large thickened amorphous region at the ascus apex lead us to believe *Thaxteriellopsis* and *Chaetosphaerulina* are distinct genera. We did not find ascospores of *Thaxteriellopsis lignicola* with as many septa as reported by Sivanesan et al. (1976), but have clearly examined the same taxon. We did however find three new collections of *T. lignicola* which we consider to be the same taxon as the type and therefore epitypify the species here. *Thaxteriellopsis lignicola* forms a distinct clade in the phylogenetic tree with 99% PP (Fig. 2.1) and thus is justified as a genus distinct from *Thaxteriella*. *Thaxteriellopsis lignicola* was associated with *Moorellalike* anamorphs (Subramanian & Sekar, 1982).



Note. (a) Ascomata. (b) Section through of ascoma. (c) Peridium. (d) Setae. (e) Pseudoparaphyses. (f)-(h) Asci. Note the amorphous thickened apical region, low ocular chamber and long pedicel. (i)-(k) Ascospores. Scale bars: (a)-(b) = 100 μm, (c), (f)-(h) = 50 μm, (d) = 20 μm, (e) = 5 μm, (i)-(k) = 10 μm

Figure 2.21 *Thaxteriellopsis lignicola* (MFLU10–0057, epitype)



Note. (a) Germinating ascospore. (b)-(c) Colonies on MEA from surface and reverse. (d)-(g) Mycelium development in culture. (h)-(p) Conidia-like structures. Scale bars: (a) = 20 µm, (b)-(c) = 10 mm, (d)-(g) = 5 µm, (h)-(p) = 20 µm

Figure 2.22 Cultural Characteristics of *Thaxteriellopsis lignicola* (MFLUCC10–0124, epitype)

Genera excluded from *Tubeufiaceae*

Allonecte Syd., Annls Mycol. 37(4/5): 378 (1939)

Parasitic on living leaves. *Ascomata* superficial, subglobose, arranged in small clusters forming on a black, basal subiculum, ostiole central. *Peridium* composed of cells of *textura angularis*, outer layers dark brown to black, inner layers brown. *Hamathecium* filiform, branched, septate, hyaline pseudoparaphyses, mostly longer than asci. *Asci* 8-spored, bitunicate, cylindrical to fusiform, with a knob-like pedicel. *Ascospores* ellipsoid to broadly-fusiform, slightly obovoid, rounded ends, 1-septate, constricted at the septum, hyaline, thick-walled.

Anamorphs reported for genus: none.

Type species: *Allonecte lagerheimii* (Pat.) Syd., Annls Mycol. 37(4/5): 379 (1939)

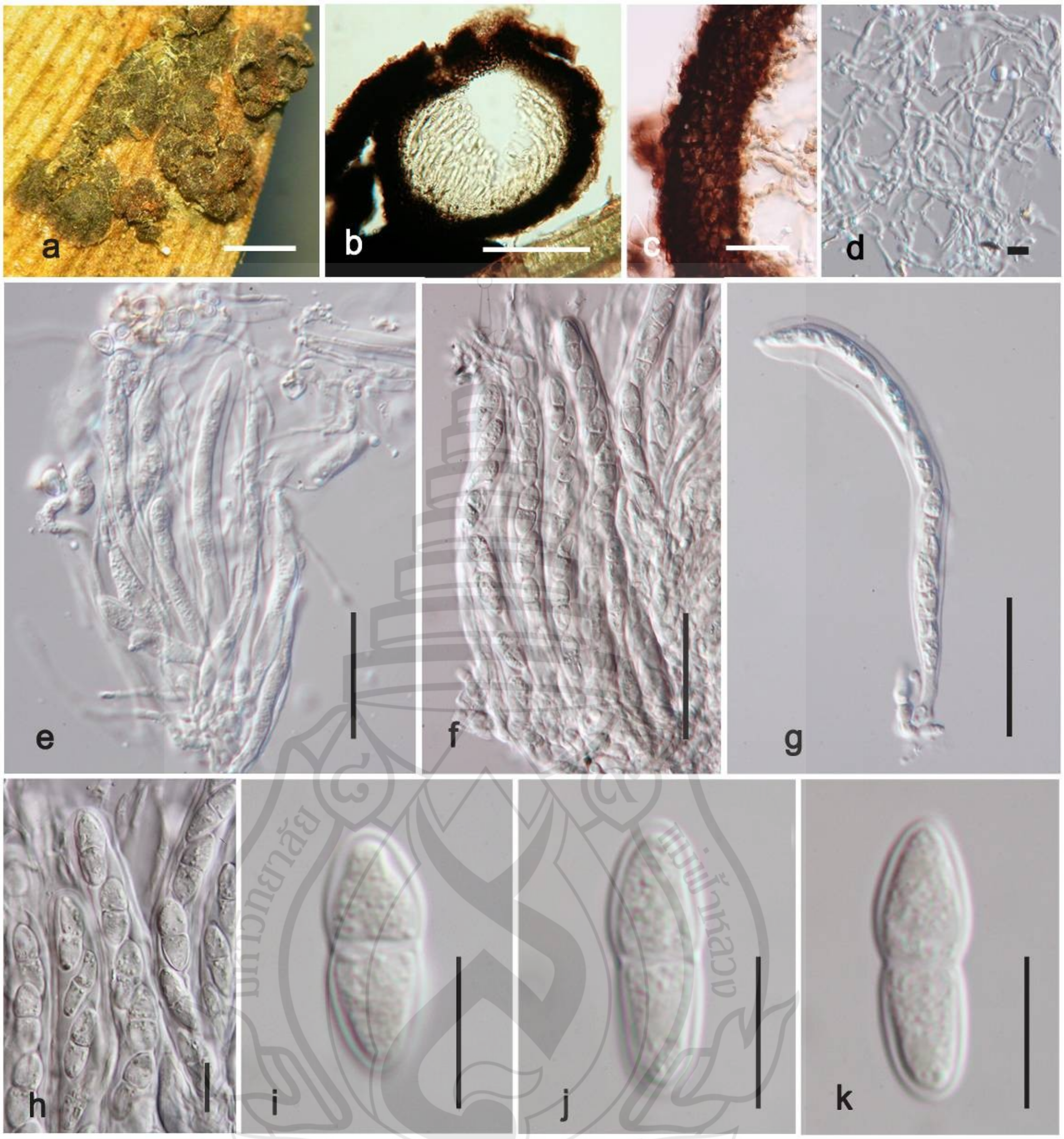
Allonecte lagerheimii (Pat.) Syd., Annls Mycol. 37(4/5): 379 (1939) (Fig. 2.23)

≡ *Broomella lagerheimii* Pat., Bull. Soc. Mycol. Fr. 11(4): 229 (1895)

Parasitic on living leaves of *Poaceae* (bamboo). *Ascomata* 240–300 μm high × 210–310 μm diam. ($\bar{x}$ = 266 × 254 μm, n = 5), superficial, subglobose, arranged in small clusters on a black, basal subiculum ostiole central. *Peridium* 40–60 μm. wide, comprising cells of *textura angularis*, outer cells dark brown to black and inner cells brown. *Hamathecium* 1 μm wide, hyaline pseudoparaphyses, mostly longer than asci. Asci 125–177.5 × 7.5–12.5 μm. ($\bar{x}$ = 150 × 12 μm, n = 10), 8-spored, bitunicate, fissitunicate, cylindrical, pedicellate, with an ocular chamber. *Ascospores* 17.5–20 × 7.5 μm ($\bar{x}$ = 19.4 × 7.5 μm, n = 25), uniseriate, ellipsoid to broad fusiform, slightly obovoid, rounded ends, two-celled, constricted at the septum, hyaline, thick-walled.

Material examined: ECUADOR, on living leaves of *Poaceae* (bamboo), July 1892, Lagerheim San Jorge (FH, holotype).

This genus was erected by Sydow (1939) to accommodate *Allonecte lagerheimii*. It differs from *Tubeufiaceae* because it has ellipsoid, 2-celled ascospores with thick walls and asci are cylindrical and more typical of *Pleosporaceae* (Fig. 2.23; Crane et al., 1998; Müller & von Arx, 1962; Rossman, 1987).



Note. (a) Ascomata. (b) Section of ascoma. (c) Peridium. (d) Pseudoparaphyses. (e)-(h) Asci with ocular chamber. (i)-(k) Ascospores. Scale bars: (a)-(b) = 100 µm, (c), (e)-(g) = 40 µm, (d) = 5 µm, (h)-(k) = 10 µm

Figure 2.23 *Allonecte lagerheimii* (FH, holotype of *Broomella lagerheimii*)

Byssocallis Syd., Annls Mycol. 25(1/2): 14 (1927)

Parasitic on leaves. *Ascomata* superficial, scattered, solitary, subglobose-globose, light yellow-reddish, with a opening at the centre, covered with light brown mycelia. *Peridium* composed of pale yellow cells of *textura angularis*. *Hamathecium* filiform, branched, septate, hyaline pseudoparaphyses embedded in gelatinous matrix. *Asci* 8-spored, bitunicate, cylindrical to clavate, thick-walled, slightly curved, shorts-talked. *Ascospores* biseriate in the ascus, obovoid, broadly-narrowly, usually 3-trans-septate, occasionally with vertical septate, symmetrical ends, hyaline to grayish, smooth walled.

Anamorphs reported for genus: none.

Type species: *Byssocallis phoebes* Syd.

Byssocallis phoebes Syd., Annls Mycol. 25(1/2): 14 (1927) (Fig. 2.24)

≡ *Puttemansia phoebes* (Syd.) Petr., Annls Mycol. 29(5/6): 343 (1931)

Parasitic on mycelium of Meliolaceae on leaves of *Phoebes jonduzii*. Ascomata (110–)166–200(−208.5) µm high × 163–281 (−306.5) µm diam. ($\bar{x}$ = 180 × 249 µm, n = 5), superficial, scattered, solitary, subglobose-globose, light yellow-reddish, with a opening at the centre, covered with light brown mycelia. *Peridium* 27.5–36 µm thick, composed 3–4 layers of pale yellow cells of *textura angularis*. *Hamathecium* 1–1.5 µm wide, hyaline, filiform, branched, septate pseudoparaphyses embedded in a gelatinous matrix. *Asci* (83–)96–121(−126) µm × 18–22(−24.5) µm ($\bar{x}$ = 106 × 20 µm, n = 20), 8-spored, bitunicate, cylindrical to clavate, slightly curved, short pedicellate. *Ascospores* (35–)36.5–45(−48) µm × 9–12.5 µm ($\bar{x}$ = 42 × 10.5 µm, n = 20), fusiform-clavate, upper part widest, 3-trans-septate, occasionally vertically septate, slightly constricted, hyaline to grayish, smooth-walled.

Material examined: COSTA RICA, Grecia, on leaves of *Phoebes jonduzii* on *Meliolaceae*, 19 January 1925, H. Sydow 160a, ILL 8149 (S, BPI, isolectotype).

Byssocallis has yellow to orange ascomata and clavate ascospores, which is atypical of *Tubeufiaceae* (Barr, 1980). Thus, its taxonomic status needs to be reevaluated. It was treated as a member of Dothideomycetes *genera incertae cedis*.

Note. (a) Type material. (b) Ascomata. (c) Section through of ascoma. (d) Peridium. (e) Pseudoparaphyses. (f)-(g) Asci. (h)-(j) Ascospores. Scale bars: (a)-(c) = 100 µm, (d), (h)-(j) = 20 µm, (e)-(g) = 50 µm

Figure 2.24 *Byssocallis phoebes* (S, isolectotype)

Letendraeopsis K. F. Rodrigues & Samuels, Mycologia 86(2): 255 (1994)

Endophytic in living leaves. *Ascomata* superficial, scattered to gregarious, globose to subglobose, without papilla or ostiole, colorless, becoming yellowish, smooth, glabrous. *Peridium* comprising a few layers of small, compressed cells. *Hamathecium* cellular, branched and anastomosing pseudoparaphyses, present at earliest stages of ascal development, attached apically and basally, persisting among mature asci arranged in a basal layer. *Asci* bitunicate, subglobose to obclavate at early stages, becoming clavate to broadly cylindrical, few in each ascoma, apex with an angular ocular chamber. *Ascospores* biseriate in ascus, fusiform, 1-septate, septum submedian, each cell with one to three globules, hyaline becoming yellow brown at maturity, with a broad hyaline sheath, smooth-walled.

Anamorphs reported for genus: none.

Type species: *Letendraeopsis palmarum* K. F. Rodrigues & Samuels

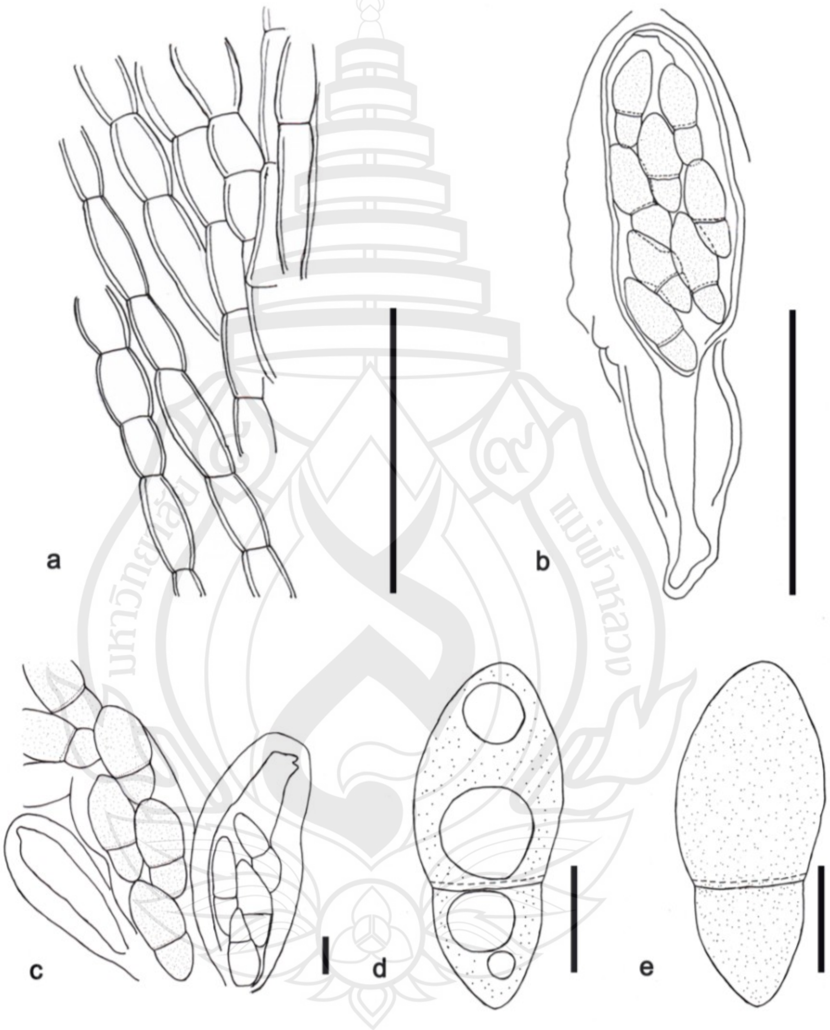
Letendraeopsis palmarum K. F. Rodrigues & Samuels, Mycologia 86(2): 255 (1994) (Fig. 2.25)

Endophytic in living leaves. *Ascomata* (88–)113–227(−293) μm high × (100–) 138–227(−293) μm diam., superficial, scattered to gregarious, globose to subglobose, without papilla or ostiole, colourless, becoming yellowish, smooth, glabrous. *Peridium* less than 20 μm wide, comprised a few layers of small, compressed cells. *Hamathecium* of 2.5–3.5 μm wide, cellular, branched, anastomosing pseudoparaphyses, present at earliest stages of ascal development, persisting among mature asci, arranged in a basal layer. *Asci* 60–80 × (19–)22–38(−40) μm, subglobose to obclavate at the early stages, becoming clavate to broadly cylindrical, few in each ascoma, with an angular ocular chamber. *Ascospores* (14–)19.2–26.4(−27.5) × (7.2–)8.2–13.3(−14.7) μm, biseriate in ascus fusiform, 1-septate, septum submedian, each cell with one to three globules; at maturity, hyaline, becoming yellow brown, with a broad, hyaline sheath, smooth-walled.

Description revised from Rodrigues and Samuels (1994)

Material examined: BRAZIL, Para, Belém, Ilha Combu, on leaflets of Euterpe oleracea, September 1990, K. F. Rodrigues 427i3d, dry culture BPI (holotype).

The description follows Rodrigues and Samuels (1994). *Letendraeopsis* comprises globose to subglobose, colourless to yellowish ascomata, containing clavate to broadly cylindrical asci, with fusiform, hyaline to yellow brown, 1-septate ascospores (Fig. 2.25). These characters are atypical of *Tubeufiaceae* and *Letendraeopsis* are more typical of *Pleosporales* (sensu Zhang et al., 2012) where it is transferred.



Note. Redrawn from Rodrigues and Samuels (1994). (a) Pseudoparaphyses. (b) Ascus with 8-spores. (c) Ascospores released. (d)-(e) Ascospores. Scale bars: (a)-(b) = 40 µm, (c)-(e) = 10 µm

Figure 2.25 *Letendraeopsis palmarum*

Taphrophila Scheuer, Biblthca Mycol. 123: 171 (1988)

Saprobic on rotten plants. *Ascomata* superficial, solitary, globose to subglobose, with apically branched setae on the top, ostiole central. *Peridium* comprising 1–2 layers of brown cells of *textura angularis*. *Hamathecium* lacking. *Asci* 8-spored, bitunicate, broadly-clavate, slightly curved, short pedicellate. *Ascospores* multiseriate in ascus, elongate-fusiform, trans-septate, ends rounded, hyaline to grayish, wall roughened.

Anamorph reported for genus: *Mirandina* G. Arnaud ex Matsush. (1975)

Type species: *Taphrophila cornu-capreoli* Scheuer

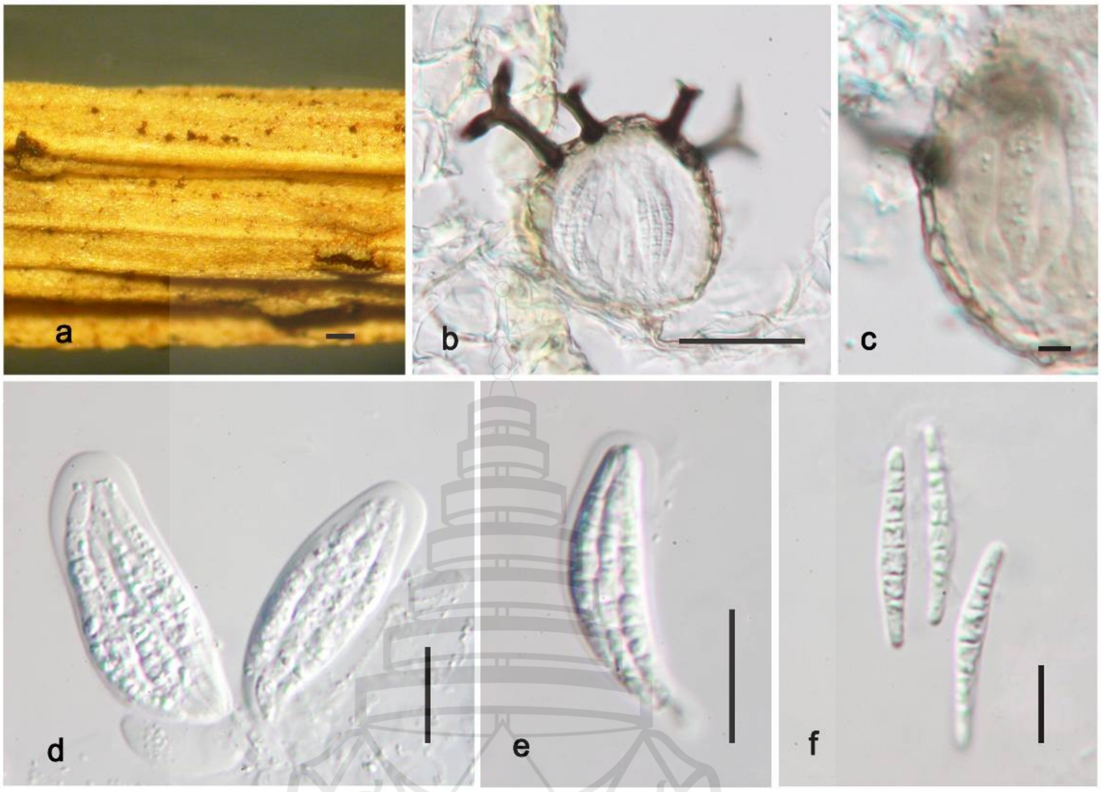
Taphrophila cornu-capreoli Scheuer, Biblthca Mycol. 123: 172 (1988)

(Fig. 2.26)

Saprobic on rotten leaves or stems of *Carex paniculata*. *Ascomata* 27–33(−44) µm high × 27–34(−47) µm diam. ($\bar{x}$ = 33 × 34 µm, n = 5), globose to subglobose, superficial, solitary, branched setae on the top, ostiole central. *Hamathecium* lacking. *Peridium* 2–3 µm thick, composed of 1–2 layers of brown cells of *textura angularis*. *Asci* 19.5–24 × 7–9 µm ($\bar{x}$ = 21.5 × 8 µm, n = 20), 8-spored, bitunicate, broadly-clavate, slightly curved, short pedicellate. *Ascospores* 13–18 × 1–2 ($\bar{x}$ = 15 × 2 µm, n = 10), multi-seriate in ascus, narrowly fusiform, 5–6-septate, ends rounded, hyaline to grayish, wall-roughened.

Material examined: AUSTRIA, Styria, Rottenmanner Tauern, Pöls valley, along the brook Pöls S of St. Johann am Tauern, 47°20'32" N, 14°28'17, 1020 m., grid ref. 8652/4; soggy brookside with *Alnus incana*, Filipendula ulmaria, Cirsium oleraceum, Scirpus sylvaticus, on rotten leaves and stems of *Carex paniculata*, 12 August 1984, Ch. Scheuer Nr. 1151 (GZU, holotype).

Taphrophila was introduced by Scheuer (1988) as a genus of *Tubeufiaceae* because of its ascomata having dark brown setae (Fig. 2.24b; Scheuer, 1991). The very small ascomata, thin peridium, branching setae around the ascomata apex, clavate to saccate asci and lack of pseudoparaphyses are atypical of *Tubeufiaceae* and *Taphrophila* should be placed in Dothideomycetes *incertae cedis* until it can be sequenced (Scheuer, 1991). The anamorph was reported as *Mirandina* by Matsushima (1975).



Note. (a) Ascomata. (b) Section through of ascoma and illustrating the branched setae. (c) Thin-walled peridium. (d)-(e) Asci. (f) Ascospores. Scale bars: (a)-(b), (d)-(e) = 20 μm, (c), (f) = 5 μm

Figure 2.26 *Taphrophila cornu-capreoli* (GZU, holotype)

Moristroma A. I. Romero & Samuels, Sydowia 43: 246 (1991)

Thaxterina Sivan., R. C. Rajak & R. C. Gupta, Trans. Br. mycol. Soc. 90(4): 662 (1988)

Saprobic on dead wood. *Ascomata* forming on a subiculum, superficial, clustered, gregarious, globose to subglobose, red brown to dark brown, ostiole not observed, with setae, collapsed when dry. *Peridium* comprising several-layers of red brown to dark brown cells of *textura angularis*. *Hamathecium* hyaline, filiform, cellular pseudoparaphyses embedded in a gelatinous matrix. *Asci* multisporous, bitunicate, wide fusiform to saccate, rounded and thickened at the apex, with an ocular chamber.

Ascospores numerous, oblong to elliptic-fusiform, ends rounded, 1–3-septate, constricted at septum, thick-walled, hyaline, smooth-walled.

Anamorph reported for genus: none.

Type species: *Thaxterina multispora* Sivan.

Thaxterina multispora Sivan., R. C. Rajak & R. C. Gupta

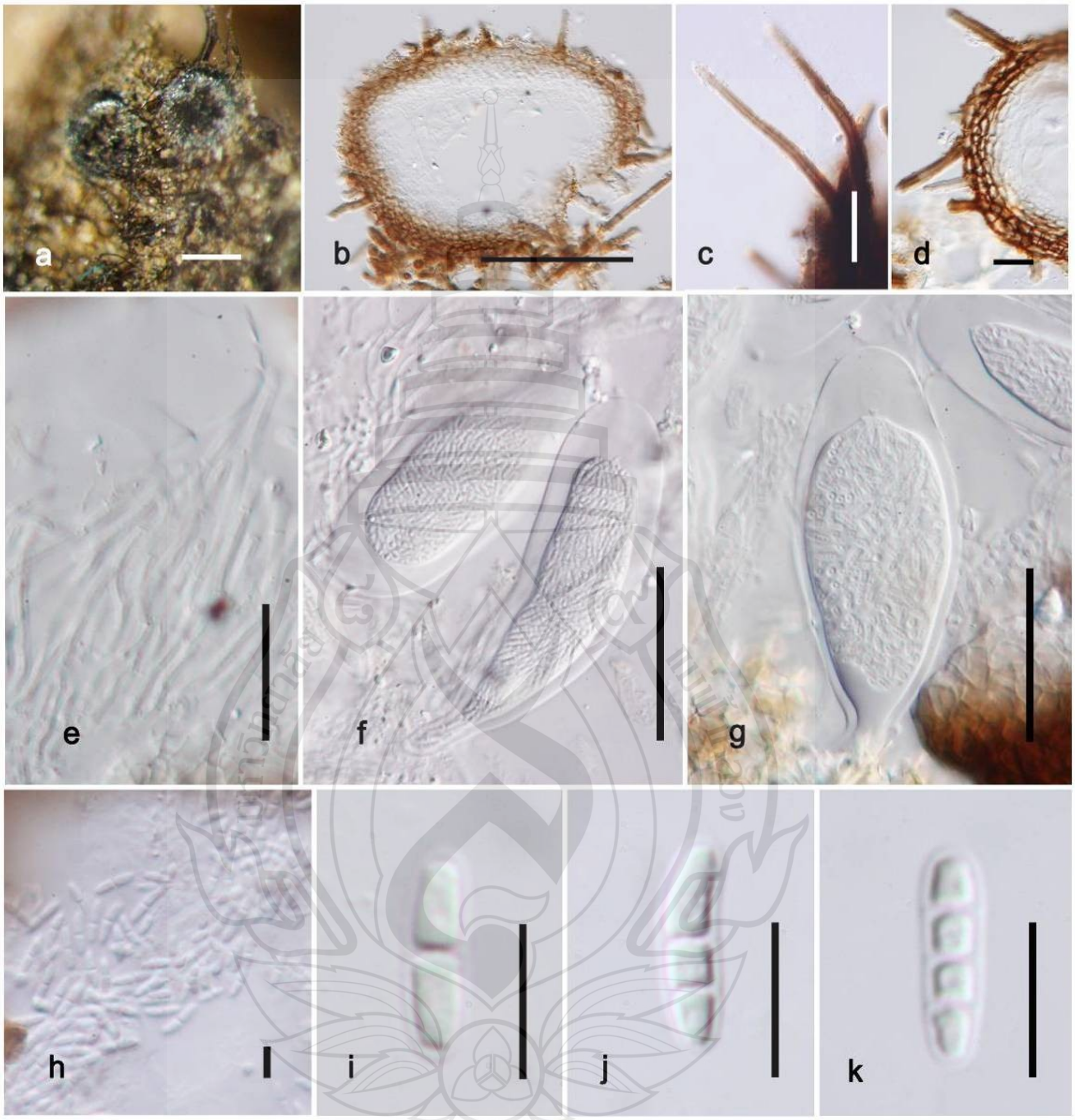
Moristroma multisporum (Sivan., R. C. Rajak & R. C. Gupta), Boonmee & K.D. Hyde, comb. nov. (Fig. 2.27)

≡ *Thaxterina multispora* Sivan., R. C. Rajak & R. C. Gupta, Trans. Br. mycol. Soc. 90(4): 662 (1988), MycoBank 563506

Saprobic on dead wood of *Terminalia arjuna*. *Ascomata* (96–)128–196(−236) µm high × 129–147(−292) µm diam. ($\bar{x}$ = 160 × 194 µm, n = 5), superficial, clustered, gregarious, globose to subglobose, forming on a subiculum, red brown to dark brown, collapsed when dry, ostiole not observed, with 39–145 µm long setae. *Peridium* (19.5–)27–38.5 µm thick, comprising 3–4 layers of red brown to dark brown cells of *textura angularis*. *Hamathecium ca*. 2 µm wide, cellular, filiform, hyaline pseudoparaphyses, embedded in a gelatinous matrix. *Asci* (102–)104–138.5(−144) × (28–)37–47(−48) µm ($\bar{x}$ = 121 × 42 µm, n = 10), multisporous, bitunicate, wide fusiform to saccate, rounded and thickened at the apex, with an ocular chamber and short pedicel. *Ascospores* numerous, 7–10(−12) × 2–3.5 µm ($\bar{x}$ = 9 × 3 µm, n = 20), oblong to elliptic-fusiform, 1–3-septate, constricted at septum, thick-walled, hyaline, smooth-walled.

Material examined: INDIA, Madhya Pradesh, on dead wood of *Terminalia arjuna*, March 1987, RC Gupta 5 (IMI, holotype).

Sivanesan, Rajak and Gupta (1988) introduced *Thaxterina* as the genus in *Tubeufiaceae* having ascomata characters similar to *Thaxteriella* and *Thaxteriellopsis*, but differing in being multisporous (Sivanesan et al., 1988). This taxon is very similar to *Moristroma polysporum* (Zhang et al., 2012) and thus we transfer *T. multispora* to this genus. Phylogenetic analysis of *Moristroma* by Nordén, Sunhede and Larsson, (2005) showed it to be closely related to Chaetothyriomycetes instead of Dothideomycetes. The ordinal status of *Moristroma* has not been resolved. *Thaxterina* was also considered to be similar to *Moristroma polysporum* because it produced multispores in the ascus (Romero & Samuels, 1991).



Note. (a) Ascomata. (b) Section through of ascoma. (c) Setae. (d) Peridium. (e) Pseudoparaphyses. (f)-(g) Asci. (h)-(k) Ascospores. Scale bars: (a)-(b) = 100 µm, (c), (e)-(g) = 50 µm, (d) = 20 µm, (h)-(k) = 5 µm

Figure 2.27 *Thaxterina multispora* (IMI, holotype)

There have been several molecular studies of *Tubeufiaceae* and its anamorphs (Kodsueb et al., 2004; Promputtha & Miller, 2010; Sánchez, Miller & Bianchinotti, 2012; Tsui et al., 2006, 2007) but these studies have only partially resolved the generic concepts used within the family. This is mainly because these studies were unable to use strains of generic types or other species because they did not exist. They also used sequences downloaded from GenBank and it has not always been established if these taxa were correctly named. In other genera such as *Colletotrichum* the names in GenBank have been shown to be highly erroneous (Cai et al., 2009; KoKo, Stephenson, Bahkali & Hyde, 2011). Our study represents an effort to resolve this problem, at least in part, by epitypifying genera and species and providing sequences of species very similar to the generic types.

The study makes some progress towards establishing the generic concepts in the *Tubeufiaceae* and define five distinct genera based on morphology and molecular data and accept a further 14 genera based on morphology only. We also suspect that *Aquaphila* should be retained as a distinctly aquatic genus and consider *Tubeufia asiana* as a synonym of *Aquaphila* (*Aquaphila albicans* Goh, K. D. Hyde & W. H. Ho, Mycol. Res. 102(5): 588 (1998) ≡ *Tubeufia asiana* Sivichai & K. M. Tsui, Mycologia 99(6): 885 (2008)[2007]). We have also linked anamorphs to their teleomorph genera, although the anamorphic genera are still mostly unresolved. It is important that generic types are recollected and sequenced and designated as epitypes so that the generic boundaries can be further defined based on molecular data analysis as has been suggested by Hyde et al. (2010) and Vasilyeva and Stephenson (2010).

An alternative approach would be to clump all *Tubeufia*-like species illustrated above and anamorphs in a single large speciose genus (see Promputtha & Miller, 2010; Sánchez et al., 2012). We however feel that there is enough morphological differences and molecular evidence to support the differentiation of genera and follow this option.

The anamorph genera of *Tubeufiaceae* are mostly helicosporous and most of these genera have been shown to be polyphyletic (Figs. 2.1-2.2; Tsui et al., 2006, 2007). In the present study we have shown that species with dictyochlamydospores form a distinct clade and, as a consequence, are placed in the new genus *Chlamydotubeufia*. This genus however, also produces helicosporous anamorphs (e.g. *Helicoma chlamydosporum*).

Tsui et al. (2007) showed *Aquaphila albicans* to be an anamorph of a species of *Tubeufia* that they described as *T. asiana*, however our studies would suggest this might represent a unique genus. On the other hand, *Helicoma, Helicosporium* and *Helicomyces* were shown to be highly polyphyletic by Tsui et al. (2006) and Tsui et al., (2007), clustering throughout seven and nine clades, respectively, based on an analysis of ITS and partial LSU ribosomal sequences.

Several genera included in *Tubeufiaceae* by Lumbsch and Huhndorf (2010) were not studied because we have been unable to obtain a loan of type material during the time frame of this study and have not collected fresh material. Several of these genera were discussed by Barr (1980), Rossman (1987), Rossman, Samuels, Rogerson and Lowen (1999) and Kodsueb, Jeewon et al. (2006), and only brief mention is made here. These taxa can be split into two groups, the first made up of those growing on dead plant material and the second consisting of those growing on other fungi.

Genera of *Tubeufiaceae* growing on dead plant material

Acanthostigmella (generic type = *Acanthostigmella genuflexa* Höhn.) was monographed by Barr (1977), and the ascomata are small, with setae around the ostioles, and develop on a delicate hyphal subiculum. Pseudoparaphyses are lacking and ascospores are 1-several septate. The genus has seven species (www.indexfungorum.org/). The lack of pseudoparaphyses would be unusual for *Tubeufiaceae* and Crane et al. (1998) suggested that this genus should be retained in *Herpotrichiellaceae* which is the concept followed herein. *Acanthostigmella brevispina* M.E. Barr & Rogerson clustered with members of *Pleosporales* in Untereiner, Straus and Malloch, (1995); however, this species was transferred to *Tubeufia* by Crane et al. (1998), based on the fact it has pseudoparaphyses.

Amphinectria (generic type = *Amphinectria portoricensis* Speg.) consists of two species. The genus has been studied by various authors (Petrak, 1951; Rossman, 1987; Rossman et al., 1999) and is considered an ambiguous member of *Tubeufiaceae* as no good type material had been located. *Boerlagiomyces* is a well defined genus typified by *B. velutinus* (Penz. & Sacc.) Butzin and has been reviewed by Crane et al.

(1998). There are several freshwater species (Cai, Hyde & Tsui, 2006). The genus is characterized by superficial, setose, membranous ascomata with a peridium of large pseudoparenchymatous cells and hyaline to pale brown ascospores with numerous transverse septa and with vertical septa in most cells (Crane et al., 1998). One sequence in GenBank of *Boerlagiomyces websteri* Shearer & J. L. Crane does not cluster with *Tubeufiaceae* (Kodsueb, Jeewon et al., 2006) and this genus needs recollecting and sequencing to confirm if this result is correct.

Glaxoa (generic *G. pellucida* P. F. Cannon [Cannon 1997]) is also a monotypic genus, having been described from scales of dead cones of *Cupressus sempervirens* in the United Kingdom. We have examined the type material (IMI 362099) and it is in poor condition. The taxon has hyaline, rounded ascomata, and two-celled hyaline ascospores. Cannon (1997) stated that this genus is most similar to *Letendraea* which Kodsueb, Jeewon et al. (2006) showed it to cluster in *Pleosporales* where *Glaxoa* should also be placed. However, fresh collections are needed so that the genus can be sequenced and analyzed. *Rebentischia* (generic type = *R. pomiformis* P. Karst.) has two species reviewed by Barr (1980) that appear to belong in *Tubeufiaceae* but are unusual in having a setiform basal appendage and a septate, pale brown upper part (Barr, 1980).

Fungicolous genera

We have examined only the generic type of *Podonectria* associated with scale insects in this study, and there is a question as to whether or not this and other fungicolous genera belong to *Tubeufiaceae*. *Chaetocrea* (generic type *C. parasitica* Syd.) is a monotypic genus and is discussed and illustrated by Rossman et al. (1999) who placed it in *Tubeufiaceae*. The taxon grows on the stroma of other fungi. *Malacaria* (generic type = *M. meliolicola* Syd. was described by Sydow (1930) and is parasitic on *Meliolaceae*. There are eight species recorded in Index Fungorum (www.indexfungorum.org/). *Malacaria* is unusual in *Tubeufiaceae* in having pale smoke-grey ascospores and unbranched septate pseudoparaphyses (Rossman, 1987). *Melioliphila* (generic type = *M. graminicola* (F. Stevens) Speg. was introduced by Spegazzini (1924) and is also parasitic on *Meliolaceae*. It differs from *Malacaria* in having hyaline, fusiform to clavate ascospores (Rossman, 1987). *Paranectriella* (generic type = *P .juruana* [Henn.] Höhn.) is also parasitic on other fungi and differs from other members of *Tubeufiaceae* by having a distinct papilla or cellular appendages at each end of the ascospore (Rossman, 1987). *Puttemansia* (generic type = *P. lanosa* Henn.) also occurs on fungi and its ascospores are similar to those of *Paranectriella*. *Uredinophila* (generic type = *U. tropicalis* (Speg.) Rossman was introduced by Rossman (1987) to accommodate two species parasitic on rusts and has narrowly cylindrical to fusiform ascospores. Representative species from each of these fungicolous genera need recollecting, epitypifying and sequencing to establish if the genera are distinct and whether or not they belong to the *Tubeufiaceae*.

2.4 Conclusion

In this study, we revisit the family *Tubeufiaceae* with notes on genera that we have re-examined where possible. Generic type specimens of *Acanthophiobolus, Kamalomyces, Podonectria, Thaxteriella* and *Thaxteriellopsis* were re-examined, described and illustrated and shown to belong to *Tubeufiaceae*. Notes are provided on *Acanthostigma, Chaetosphaerulina, Thaxterina* and *Tubeufia*, which are retained in *Tubeufiaceae*; however, we were unable to locate the types of these genera during the time frame of this study. *Allonecte* is excluded from the *Tubeufiaceae*, as the ascospores are fusiform-ellipsoidal, grey-brown and 1-septate and the asci are cylindrical, all of which are features more typical of *Pleosporaceae*, where it is transferred. *Byssocallis* has yellow to orange ascomata and clavate ascospores which is atypical of *Tubeufiaceae*. Thus its taxonomic status needs to be reevaluated. *Letendraeopsis* has an endophytic habit, cylindro-clavate asci and two-celled ascospores more typical of *Pleosporales*, where it is transferred. *Taphrophila* has small ascomata, a thin peridium, branching setae around the apex of the ascomata, clavate to saccate asci and lacks pseudoparaphyses. These are features atypical of the *Tubeufiaceae*, and *Taphrophila* should be placed in the Dothideomycetes *incertae cedis*. Twelve new collections of *Tubeufiaceae* from Thailand were isolated, and their DNA was extracted. The sequence data of LSU, SSU and ITS rDNA were amplified and analyzed using parsimony and likelihood methods. The results of phylogenetic analysis was used to establish the inter-generic relationships in *Tubeufiaceae*. *Thaxteriellopsis lignicola*, epitypified in this investigation, is a sister taxon in the family *Tubeufiaceae* based on phylogenetic analysis of rRNA sequence data. *Chlamydotubeufia* is introduced as a new genus based on the production of dictyochlamydosporous anamorphs, including two new species. Three new species, one each in *Acanthostigma, Tubeufia* and *Thaxteriella* are also described and illustrated. The phylogenetic placement of these genera is also discussed.

CHAPTER 3

TWO NEW *Kirschsteiniothelia* SPECIES WITH *Dendryphiopsis* ANAMORPHS CLUSTER IN *Kirschsteiniotheliaceae* fam. nov.

3.1 Introduction

Kirschsteiniothelia was introduced by Hawksworth (1985a) and is represented by the type *Kirschsteiniothelia aethiops* based on *Sphaeria aethiops* Berk. & M.A. Curtis. The genus is characterized by superficial to semi-immersed, hemispherical or subglobose, dark brown to black ascomata, cylindrical-clavate asci that develop among numerous pseudoparaphyses, and mostly one septate (in some species two-septate), ellipsoidal, dark-brown ascospores; there are presently 18 species recorded in Index Fungorum and seven estimated species in Kirk et al. (2008). *Kirschsteiniothelia* D. Hawksw. is a genus of the Dothideomycetes (Hawksworth, 1985a), although its ordinal and familial placements are uncertain and it is currently classified as *Dothideomycetes incertae sedis* in Index Fungorum and in Lumbsch and Huhndorf (2010). In MycoBank (Crous, Gams, Stalpers, Robert & Steghuis, 2004; Robert, Stegehuis & Stalpers, 2005) this genus is placed in the *Pleosporaceae*, while its known hyphomycete anamorphs are referred to the *Pleomassariaceae* (Hyde et al., 2011). Thus placement of the genus is uncertain. Schoch et al. (2006) analyzed molecular data for *Kirschsteiniothelia aethiops*, which did not cluster close to *Pleosporaceae*, and it was suggested that this genus should be transferred to a separate family. In a recent molecular phylogenetic analysis of the Dothideomycetes, *Kirschsteiniothelia elaterascus* Shearer clustered in the same clade as *Morosphaeria* (*Morosphaeriaceae*) while *Kirschsteiniothelia maritima* (Linder) D. Hawksw. clustered in the *Mytilinidiaceae* clade, as a sister group of *Mytilinidion* (Schoch et al., 2009; Suetrong et al., 2009).

The anamorph of *Kirschsteiniothelia* has been listed as *Dendryphiopsis atra* (Corda) S. Hughes (Kirk et al., 2008), a connection proven by morphology and molecular data (Hernandez, Heredia, Arias, Portales & Ruiz, 2008; Schoch et al., 2006). The aim of the present study is to establish the taxonomic placement of *Kirschsteiniothelia* with morphological and phylogenetic characters and to describe two new species collected in Thailand.

3.2 Materials and Methods

3.2.1 Sample Collection, Specimen Examination and Photography

Collections of Dothideomycetes were made in northern Thailand and two species of *Kirschsteiniothelia* were collected from Chiang Mai and Chiang Rai provinces during June-October 2009. The specimens were characterised morphologically as described and illustrated in Chapter 2 (see Section 2.1.1). Single spore isolates were done as previously explained in Section 2.2.2. DNA extraction, PCR amplification and DNA sequencing were performed as described in Section 2.2.3

3.2.2 Phylogenetic Analyses

A BLAST query was performed to find the possible similarity of the generated *Kirschsteiniothelia* sequences in the GenBank database. Analysis of combined ITS, SSU and LSU gene sequences data were aligned with BioEdit (Hall, 1999) and Clustal X 2 (Larkin et al., 2007) with available sequences of the orders *Capnodiales, Jahnulales, Mytilinidiales* and *Pleosporales* (Dothideomycetes) obtained from the GenBank (Table 3.1) and *Schismatomma decolorans* (Arthoniomycetes) as an outgroup. The aligned dataset was analyzed with PAUP* 4.0b10 (Swofford, 2002). Maximum parsimony (MP) analyses on different settings (unweighted, weighted [step matrix] and reweighted) produced the same topology for all analyses. Stepwise additions of sequence used 1000 replicates of random and tree-bisection-reconnection (TBR) branch-swapping algorithm, with MAXTREES set at 1000. The parsimony tree scores including tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) also were calculated.

Table 3.1 GenBank Accession Numbers of Taxa in Combined Genes Used in This Study and New sequences of *Kirschsteiniothelia* from Thailand are marked by an asterisk (*)

Species	Strain no.	GenBank Accession mumber		
		LSU	SSU	ITS
Capnodium coffeae	CBS147.52	GU214400	DQ247808	AJ244239
Dendryphiopsis atra	DAOM 231155	DQ678046	–	–
Jahnula aquatica	R68–1	EF175655	EF175633	–
Jahnula bipileata	F49–1	EF175657	EF175635	–
Kirschsteiniothelia aethiops	CBS109.53	AY016361	AY016344	–
Kirschsteiniothelia elaterascus	HKUCC7769	AY787934	–	–
*Kirschsteiniothelia lignicola**	MFLUCC10–0105	HQ441568	HQ441569	HQ441567
Kirschsteiniothelia maritima	CBS221.60	AY849943	–	–
Kirschsteiniothelia maritima	CBS221.60	GU323203	–	–
*Kirschsteiniothelia emarceis**	MFLUCC10–0106	HQ441571	HQ441572	HQ441570
Lophiostoma arundinis	CBS621.86	DQ782384	DQ782383	AJ496633
Lophiostoma macrostomum	HHUF27290	AB433273	–	–
Lophium mytilinum	CBS114111	–	EF596818	EF596819
Massaria anomia	M47 (WU30509)	HQ599378	HQ599453	HQ599378
Massaria conspurcata	M14 (WU30519)	HQ599393	HQ599441	HQ599393
Massaria inquinans	M15 (WU30526)	HQ599400	HQ599442	HQ599400
Massaria inquinans	M19 (WU30527)	HQ599402	HQ599444	HQ599402
Massaria ulmi	M25 (WU30566)	HQ599428	HQ599446	HQ599428
Massarina eburnea	CBS473.64	GU301840	GU296170	–
Massarina igniaria	CBS845.96	GU301841	GU296171	–
Morosphaeria ramunculicola	BCC18404	GQ925853	GQ925838	–
Morosphaeria velataspora	BCC17059	GQ925852	GQ925841	–
Mycosphaerella africana	CPC794	GU214433	GU214536	–
Mycosphaerella punctiformis	CBS113265	NG027571	–	EU167569
Mytilinidion acicola	EB0379	GU397346	GU397362	–

Table 3.1 (continued)

Species	Strain no.	GenBank Accession mumber		
		LSU	SSU	ITS
Mytilinidion californicum	EB0385	GU323208	GU323186	–
Mytilinidion mytilinellum	CBS303.34	FJ161184	FJ161144	HM163570
Preussia funiculata	CBS659.74	GU301864	GU296187	–
Preussia terricola	CBS317.65	GQ203725	–	GQ203765
Schismatomma decolorans	DUKE 0047570	AY548815	AY548809	AY548808

Note. Abbreviations of isolates and culture collections: American Type Culture Collection, Virginia, U.S.A: ATCC; BIOTEC Culture Collection, Bangkok, Thailand: BCC; Centralbureau voor Schimmelcultures, Utrecht, Netherlands: CBS; P.W. Crous: CPC; National Mycological Herbarium, Department of Agriculture, Ottawa, Ontario, Canada: DAOM; Duke University Herbarium, Durham, North Carolina, U.S.A: DUKE; Original designation for culture and dried specimens from Eric W.A. Boehm, presently deposited with CBS and BPI as designated: EB; Herbarium of Hirosaki University, Japan: HHUF; The University of Hong Kong Culture Collection, Hong Kong, China: HKUCC; K. Tanaka: KT; Mae Fah Luang University Culture Collection, Thailand: MFLUCC; T. Grafenhan: M47; H. Voglmayr: M14, M15, M19 and M25

Bootstrap support for branches were based on 1000 MP replicates with a single sequence addition replicates for each bootstrap replicate. The phylogenetic tree was visualized with Treeview (Page, 1996). Models of nucleotide substitution for each genes were determined with MrModeltest 2.3 (Nylander, 2004). GTR+I+G evolutional model was selected (Ronquist & Huelsenbeck, 2003). The Metropolis-coupled Markov chain Monte Carlo (MCMCMC, Rannala & Yang, 1996) algorithm was used to estimate posterior probabilities (PP). Six Markov chains were run from a random starting tree for 1 000 000 generations and trees sampled every 100 generations. The first 2000 trees were discarded as burn-in before convergence of the four chains. The remaining trees were used to construct 50% majority rule consensus tree and to calculate posterior probabilities (PP) with those equal or more than 0.95 given below each branch (Fig. 3.2). The consensus tree obtained for the Bayesian analyses confirmed the tree topology obtained from parsimony analysis.

3.3 Results and Discussion

3.3.1 Phylogenetic Analysis

The phylogenetic tree (Fig. 3.1) was constructed with 260 Dothideomycete taxa, mostly from Schoch et al. (2009), plus *Kirschsteiniothelia* species sequenced in this study and outgroup taxa and is based on combined genes (ITS, LSU and SSU). This yielded a maximum parsimony (MP) tree length of 8982 steps (CI = 0.242, RI = 0.697, RC = 0.169, HI = 0.758). Clades with greater than or equal to 50% bootstrap support are shown on the upper nodes. In the MP analysis (Fig. 3.1) *Kirschsteiniothelia aethiops*, *K. lignicola*, *K. emarceis* and *Dendryphiopsis atra* formed a monophyletic group that we designated as a new family, *Kirschsteiniotheliaceae*, within the *Jahnulales*. The ITS, LSU and SSU gene sequences of 30 taxa (Table 3.1) were combined and aligned to establish the phylogeny of *Kirschsteiniothelia* species, especially their ordinal and familial positions. The combined dataset after alignment comprised 1877 characters including gaps. This yielded a maximum parsimony (MP) tree length of 1909 steps (CI = 0.620, RI = 0.672, RC = 0.417, HI = 0.380). Bayesian analysis generated similar tree topology and the Bayesian values are marked below branches (Fig. 3.2). Twenty-nine fungal strains were grouped in Dothideomycetes within four well defined orders, *Mytilinidiales, Pleosporales, Capnodiales* and *Jahnulales*, with the exception of the outgroup *Schismatomma decolorans* (Arthoniomycetes). The phylogenetic analysis revealed an affiliation between the two new species of *Kirschsteiniothelia* from Thailand, described as *K. lignicola* and *K. emarceis*, the generic type *K. aethiops* and the anamorph *D. atra*. *Kirschsteiniothelia maritima* and *K. elaterascus* however were placed in different families, with *K. elaterascus* closely related to *Morosphaeria* with strong statistical support (99% BT, 1.00 PP) within *Morosphaeriaceae* (*Pleosporales*). *K. elaterascus* is thus transferred to *Morosphaeria*. *K. maritima* clustered with *Mytilinidion* spp.

as a sister group in the *Mytilinidiaceae* clade. Thus, it is introduced as a new genus *Halokirschsteiniothelia* in *Mytilinidiales*.

Clade A: *Mytilinidiaceae*. *Kirschsteiniothelia* maritima groups in the *Mytilinidiaceae* clade (*Mytilinidiales*) and as a sister group to *Mytilinidion* species (Fig. 3.2). *K. maritima* differs from *Mytilinidion* in appearance and in its marine habitat (Figueira & Barata, 2007; Hawksworth, 1985b; Kohlmeyer, J. & Kohlmeyer, E., 1979). Therefore a new genus, *Halokirschsteiniothelia* (*Mytilinidiales*), is introduced to accommodate *K. maritima*. The characters of *Halokirschsteiniothelia* are unusual for *Mytilinidiaceae* and therefore its inclusion in the family is tentative.

Halokirschsteiniothelia S. Boonmee & K. D. Hyde. Mycologia, 104(3): 705, (2012), MycoBank MB561017

Etymology: from Greek *hals* = salts, in reference to the marine origin of the fungus.

Ascomata erumpentia ad superficialia, hemisphaerica ad subglobosa, dispersa, leviter papillata. *Pseudoparaphyses* sparsis, septata, ramosis. *Asci* bitunicate, octaspori, cylindri-clavati, cubiculum ocular, pedicellati. *Ascosporae* ellipsoideae, pallide brunnae, uniseptatae, constrictae ad septae.

Ascomata erumpent to superficial, hemispherical to subglobose, scattered, with a small papillate ostiole. *Pseudoparaphyses* cellular, persistent, sparse, septate, branched, 2–3 µm thick. *Asci* 64–85 × 8–12 µm, eight-spored, bitunicate, clavate, pedicellate, and with a small ocular chamber. *Ascospores* 16–20 × 4.5–6 µm, ellipsoid, pale brown, one-septate, the apices tending to be attenuated, constricted at the septum.

Type species: *Halokirschsteiniothelia maritima* (Linder), S. Boonmee & K. D. Hyde.

Halokirschsteiniothelia maritima (Linder) S. Boonmee & K. D. Hyde. Mycologia, 104(3): 705, (2012), MycoBank MB561018

≡ *Amphisphaeria maritima* Linder, Farlowia 1:4 11, 1944.

= *Kirschsteiniothelia maritima* (Linder) D. Hawksw., J. Linn. Soc., Bot. 91: 193 (1985).

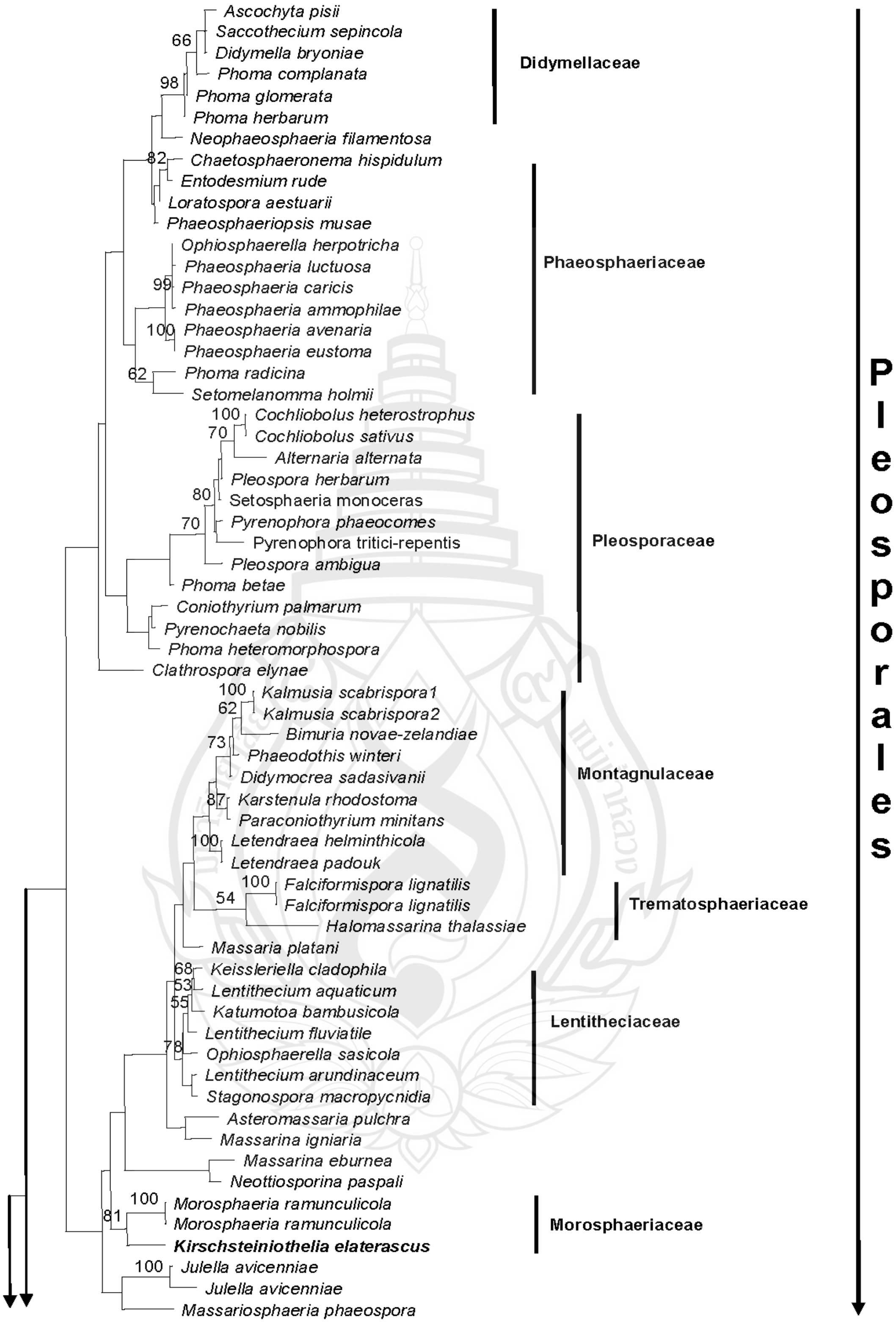


Figure 3.1 Phylogenetic Tree of *Kirschsteiniotheliaceae*, Based on Maximum Parsimony Analysis of ITS, LSU and SSU Gene Sequences

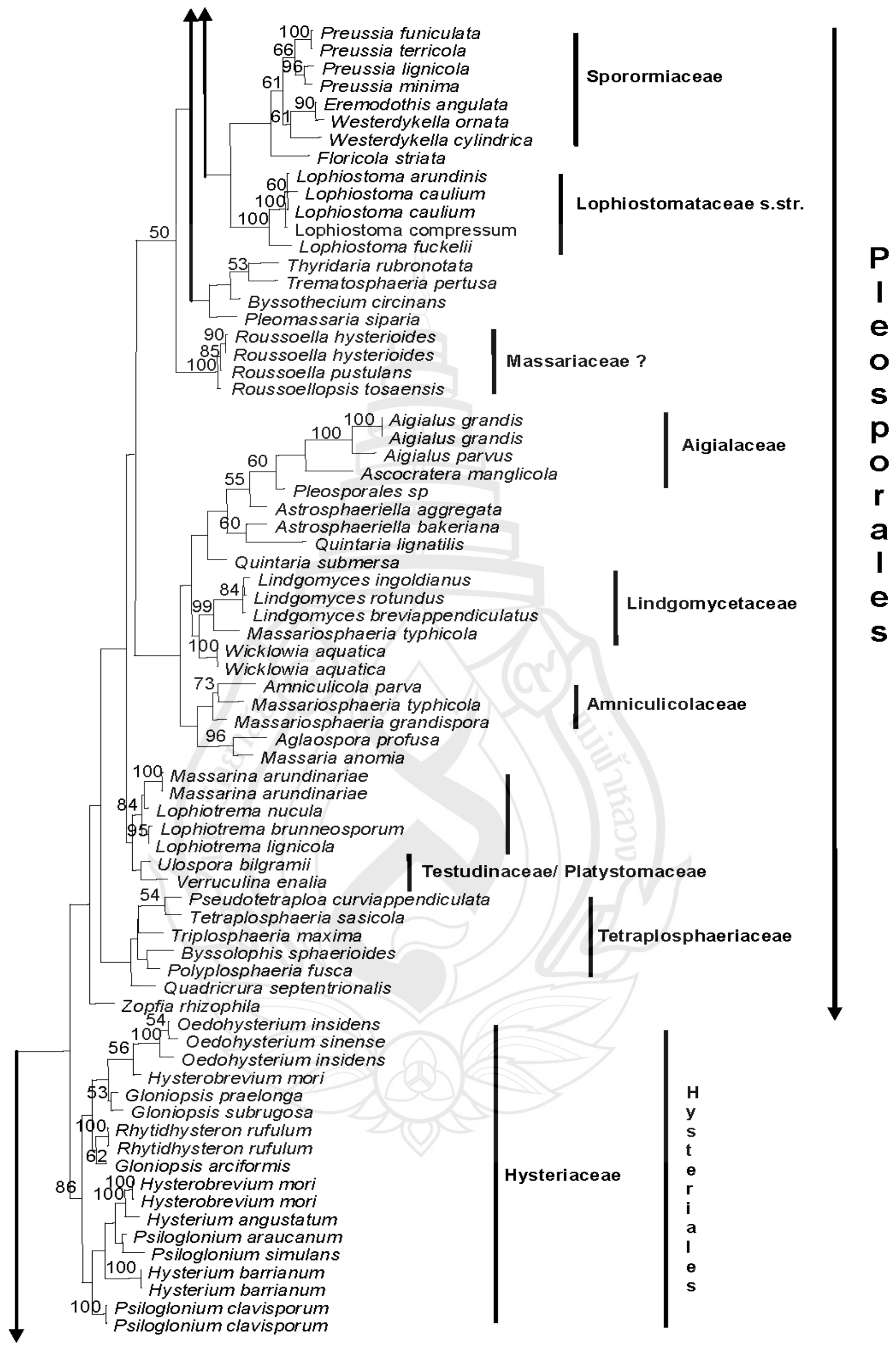


Figure 3.1 (continued)

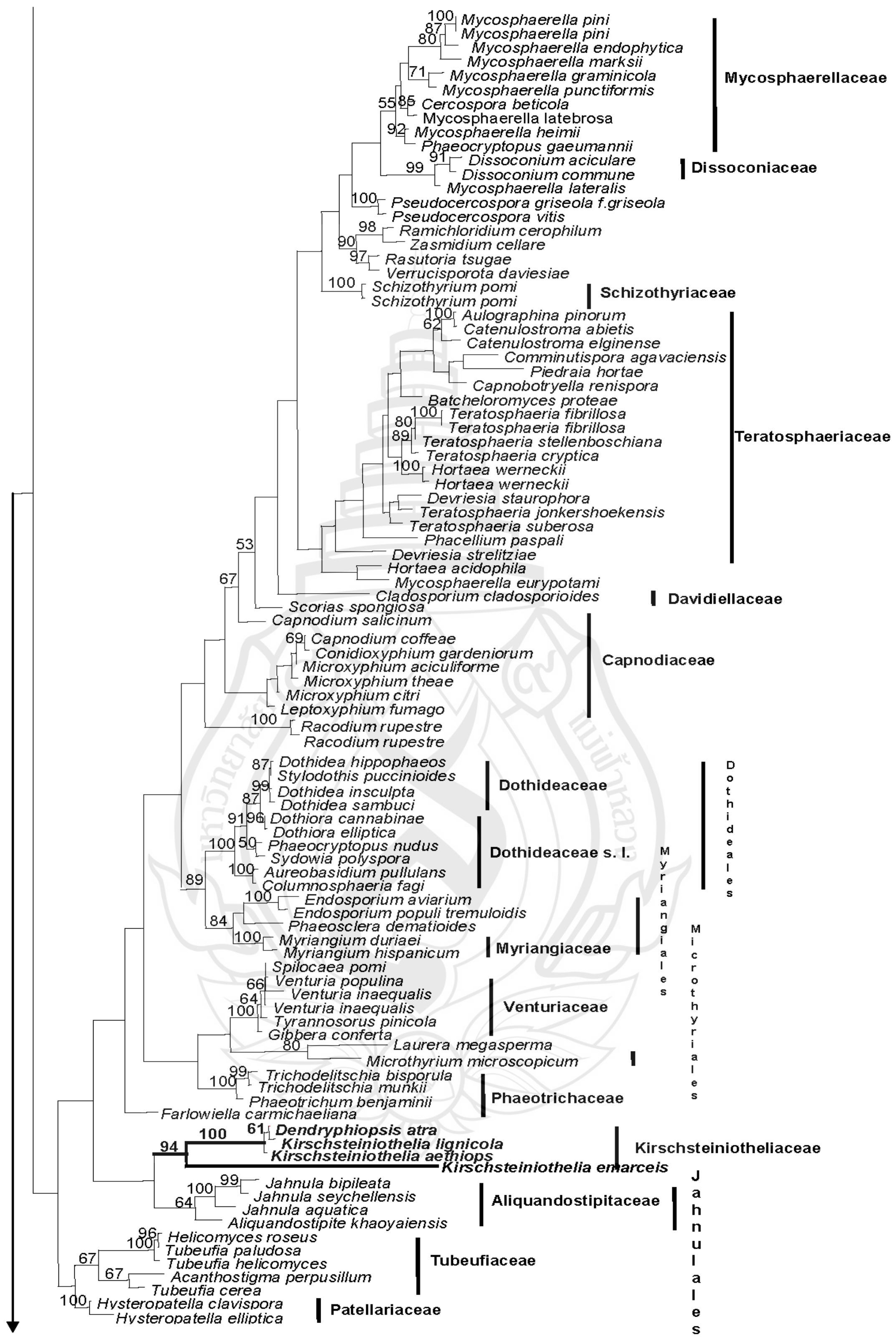
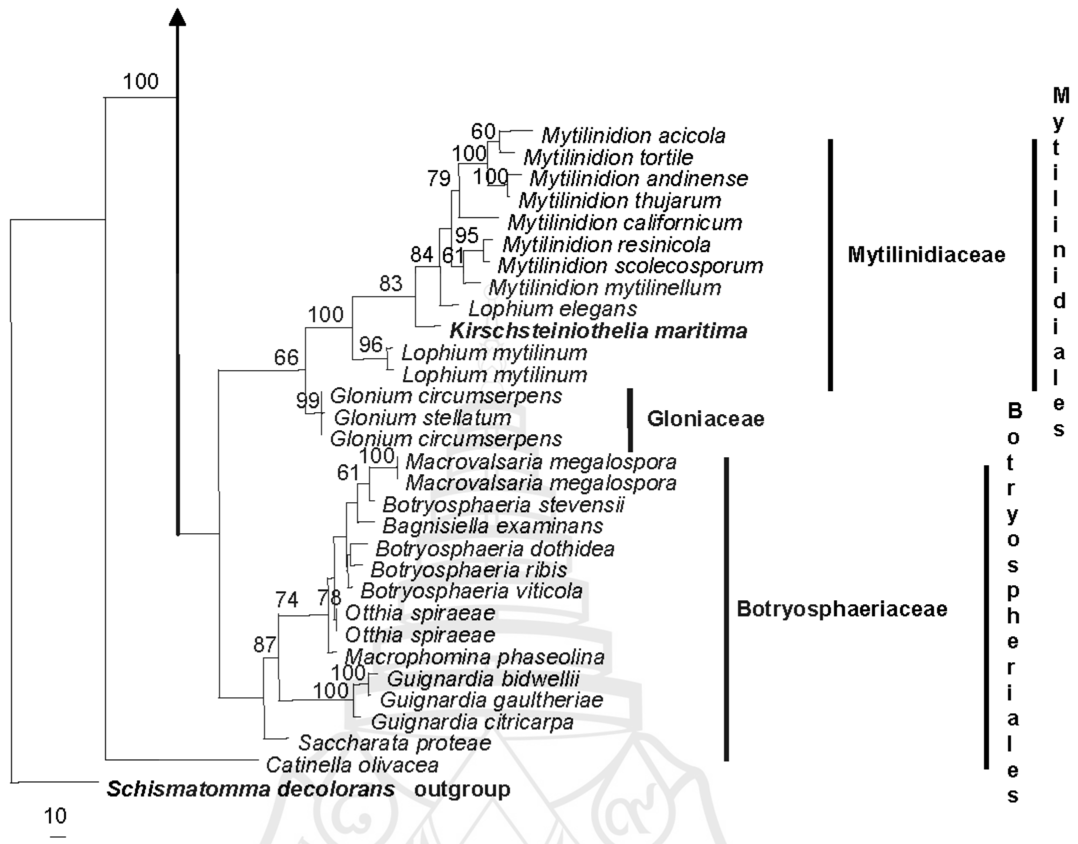
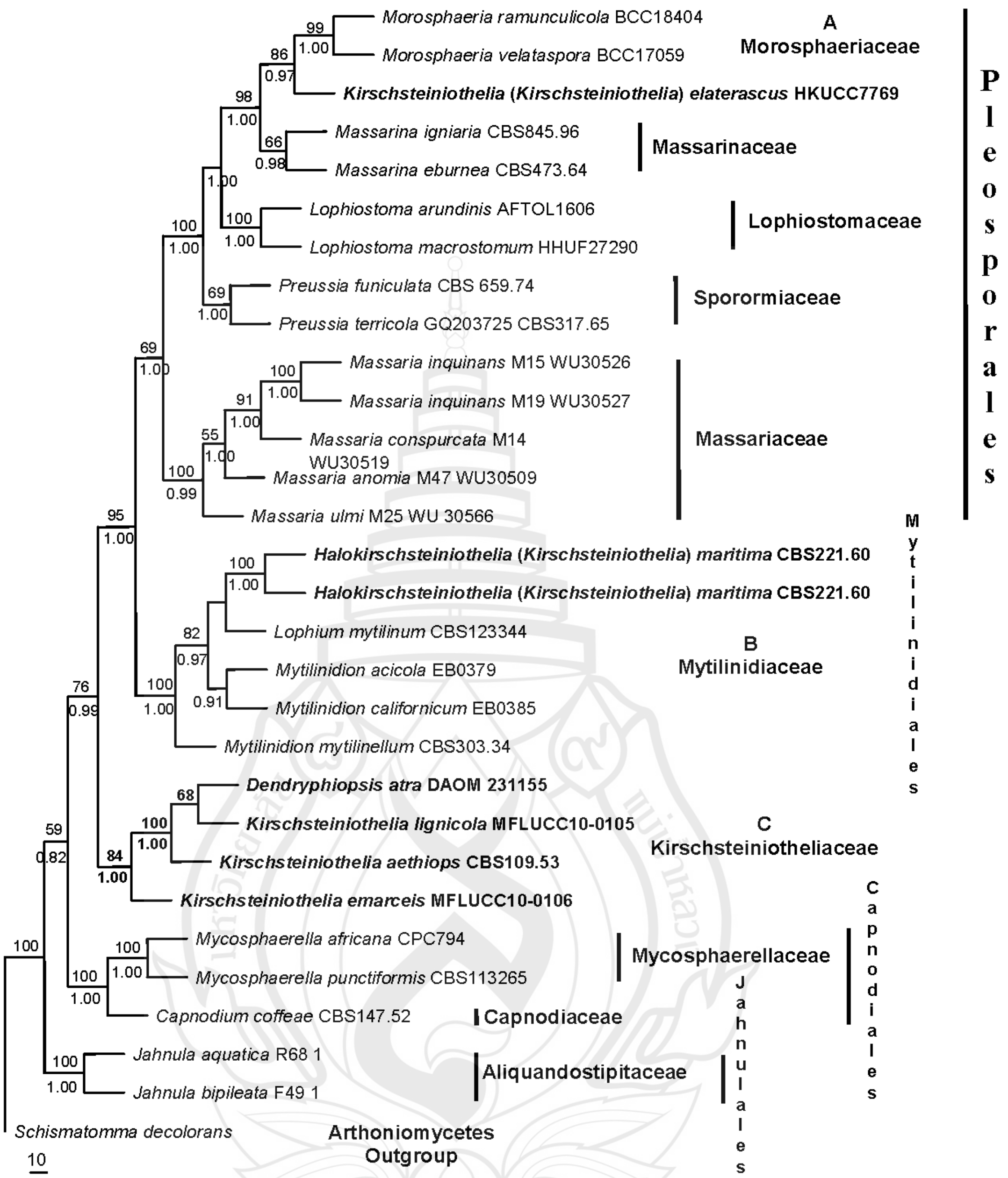


Figure 3.1 (continued)



Note. Maximum parsimony tree constructed with 259 Dothideomycete taxa, mostly from Schoch et al. (2009), plus *Kirschsteiniothelia* species sequenced in this study. Bootstrap percentage values greater ≥ 50% are shown on the upper nodes, based on 500 replicates

Figure 3.1 (continued)



Note. The *Kirschsteiniothelia* lineage is indicated in bold. Bootstrap support values higher than 75% are shown at the relevant nodes, based on 1000 replicates. Bayesian posterior probabilities greater than 0.95 are indicated under thickened branches

Figure 3.2 Phylogenetic Relationships of *Kirschsteiniothelia* species, Based on Maximum Parsimony Analysis of LSU, SSU and ITS Gene Sequences

= *Microthelia maritima* (Linder) Kohlm., Nova Hedwigia 2: 32, 1960.

= *Microthelia linderi* Kohlm., Trans. Br. Mycol. Soc. 57: 483, 1971.

Illustrations: Wang, Aptroot & Hyde, 2004 (Plate 61, Figs. 1-7). Illustrated as *M. maritima* Linder Kohlm. in Icones Fungorum Maris, plate 39, 1964. This species has been described by Kohlmeyer & Kohlmeyer in Marine Mycology, the Higher Fungi p 430-431, 1979, as *Microthelia linderi* Kohlm. with *K. maritima* (Linder) Kohlm. Trans. Br. Mycol. Soc. 57: 483, 1971. as a synonym. Hawksworth (1985a) however excluded it from *Microthelia* and placed it in *Kirschsteiniothelia*.

Material examined: UNITED STATES. MASSACHUSETTS, Woods Hole, on test block immersed *ca*. 7 ft. below low water, 13 Oct 1941, E.S. Barghoorn (FH, HOLOTYPE of *Amphisphaeria maritima*). This species is widespread in the oceans (Kohlmeyer, J. & Kohlmeyer, E. 1979) and differs from *Kirschsteiniothelia* in having ostiolate ascomata and small ascospores with acute ends (Wang et al., 2004).

Clade B: *Morosphaeriaceae*. The *Morosphaeriaceae* clade comprises four marine species (*M. ramunculicola*, *M. velatispora*, *Helicascus kanaloanus*, *H. nypae*) and the freshwater species *K. elaterascus*, all commonly found on dead wood (Suetrong et al., 2009). Shearer (1993) distinguished *K. elaterascus* from *K. aethiops* by its ascospores, which are surrounded by a sheath and asci whose pedicel stretch in water. Phylogenetic analyses indicate that *K. elaterascus* groups with *Morosphaeria* (*Morosphaeriaceae*) with high support (Kodsueb, Dhanasekaran et al., 2006; Schoch et al., 2009; Shearer et al., 2009; Suetrong et al., 2009). In this study *K. elaterascus* grouped with *Morosphaeria* as a sister group with high support (99% BT, 1.00 PP). Based on these results, *K. elaterascus* is transferred to *Morosphaeria*, *Morosphaeriaceae*.

Morosphaeria elaterascus (Shearer) S. Boonmee & K. D. Hyde. Mycologia, 104(3):705, (2012), MycoBank MB561019

≡ *Kirschsteiniothelia elaterascus* Shearer, Mycologia 85: 963, 1993.

The placement of *K. elaterascus* in *Morosphaeria* is supported by both molecular data (Fig. 3.2) and morphology. *Morosphaeria elaterascus* is similar to other species in the genus in having immersed to superficial ascomata, clavate to cylindrical asci and sheathed ascospores, which are not hyaline as in other *Morosphaeria* species

(Lumbsch & Huhndorf, 2010; Suetrong et al., 2009). *K. elaterascus* differs from *Morosphaeria* species in the structure and mode of dehiscence of the ascus, its brown ascospores and freshwater habitat (Shearer, 1993).

Clade C: *Kirschsteiniotheliaceae*. The clustering of *Kirschsteiniothelia aethiops*, *K. lignicola*, *K. emarceis* and *Dendryphiopsis atra* in a basal position to *Pleosporales* is shown in the phylogenetic analysis (Fig. 3.2), and a new family *Kirschsteiniotheliaceae* is introduced to accommodate this group.

3.3.2 Taxonomy

Kirschsteiniotheliaceae S. Boonmee & K. D. Hyde Mycologia, 104(3): 705, (2012), MycoBank: MB 561021

Familia Pleosporalium, Ascomycetum. *Ascomata* superficialia, subglobosa ad globosa, atrobrunnea ad nigra. Peridium texturam angularem. *Hamathecium* numerosae, pseudoparaphysum. *Asci* bitunicati, cylindrici vel clavati, apicali rotundati, cum camera ocular. *Ascosporae* ellipsoideae, 1–2-septatae, viridi, brunnea ad atrobrunnea, constrictae. *Anamorpha*: *Conidiophora* longa stipula, recta vel leniter curvata, atrobrunnea. *Conidia* producta apice, obovata ad late, fusiformis ad obclavata, ferruginea ad atrobrunnea, griseobrunnea, septatae, constrictae.

Type genus: *Kirschsteiniothelia* D. Hawksw.

Genus of *Pleosporales*: *Ascomata* superficial, subglobose to globose, dark brown to black, solitary. *Peridium* comprising textura angularis. *Hamathecium* of numerous filiform pseudoparaphyses. *Asci* cylindrical-clavate, apically rounded with a small ocular chamber. *Ascospores* biseriate, ellipsoidal, 1–2-septate, dull green, olive brown to dark brown, smooth-walled. *Anamorph*: *Conidiophores* long-stalked, straight and slightly curved, thickened at the base, transversely septate, apex branched and dark brown. *Conidia* produced apically, broadly obovoid, fusiform to obclavate, reddish brown to dark brown, grayish brown, septate, constricted at septa, rounded at each end.

Notes: A new family, *Kirschsteiniotheliaceae*, is introduced to accommodate the genus *Kirschsteiniothelia* and its anamorph *Dendryphiopsis*. Also the high support indices in the phylogram (Fig. 3.2) indicate its placement separate from other families of *Pleosporales*. It differs from other families in the *Pleosporales* having mostly ellipsoid, septate, colored ascospores, septate and colored and a *Dendryphiopsis* anamorph. Two new species from Thailand are introduced.

Type species: *Kirschsteiniothelia aethiops* (Berk. & M. A. Curtis) D. Hawksw.

Kirschsteiniothelia aethiops (Berk. & M. A. Curtis) D. Hawksw., J. Linn. Soc. Bot., 91: 185 (1985)

≡ *Sphaeria aethiops* Berk. & M. A. Curtis, Grevilla 4: 143, 1876

Anamorph: *Dendryphiopsis atra* (Corda) S. Hughes, Can. J. Bot. 31: 655, 1953

Kirschsteiniothelia lignicola S. Boonmee & K. D. Hyde. Mycologia, 104(3): 706, (2012), MycoBank: MB 561030 (Figs. 3.3-3.5)

Etymology: The species is named in reference to the habitat on wood.

Ascomata superficialia, 152–178 μm alta × 156–214 μm diam., subglobosa ad globosa, atrobrunnea ad nigra, fasciculatae. *Pseudoparaphyses* numerosae, filiformia, hyalinae. *Asci* 107–163 × 19–28.5 μm, bitunicati, octaspori, fissitunicati, cylindrici-clavati, apice rotundatae. *Ascosporae* 27–30(–33) × 10–12(–13) μm, ellipsoideae, viridi, brunnea ad atrobrunnea, constrictae. *Conidiophora* erecta, septatae, brunnea ad atrobrunnea. *Conidia* obovata, oblonga ad obclavata, septatae, constrictae, pallide brunneis ad obscure fuscum.

Ascomata 152–178 μm high × 156–214 μm diam., superficial, subglobose to globose, dark brown to black, membranaceous, clustered, solitary, with central papilla 24 μm wide with 24 μm high. *Peridium* 18–22 μm thick, composed of one strata, comprising 3–4 layers of cells of textura angularis; inner layer cells pale brown, but outer layer cells dark. *Pseudoparaphyses* numerous, filiform, 1–2 μm wide, hyaline, embedded in a gelatinous matrix. *Asci* 107–163 × 19–28.5 μm, eight-spored, bitunicate, fissitunicate, cylindrical-clavate, with a long pedicel 14.5–24 μm, apically rounded, with an ocular chamber 2 µm wide. *Ascospores* 27–30(–33) × 10–12(–13) µm ($\bar{x}$ = 30 × 11 µm, n = 20), biseriate, ellipsoidal, slightly curved, 1(–2) septate, with median septum or in lower part, some ascospores with secondary septum, dull green, brown to dark brown at maturity, thick-walled, lacking a mucilaginous sheath.

Cultural characteristics: *Ascospores* germinating on MEA within 12 hours. Colonies on MEA, reaching up to 2 cm diam in 1 week at 25-28 °C, within a month covering the Petri dish, effuse, hairy, mycelium radiating outwards, edged fimbriate, colony dense, dark green to dark brown. *Mycelium* immersed and superficial; hyphae dark brown, septate, *ca*. 5 µm wide. *Conidiophores* 39–148 µm long, 4–7 µm thick, septate, branched at apex, brown to dark brown, smooth. *Conidia* 24.5–35(–41) × 14–16 (–19) µm, broadly obovoid, reddish brown to dark brown, 1–2-transverse septa, slightly constricted at septa, smooth-walled, rounded at ends.

Anamorph growing on type material. *Conidiophores* erect, 287–406 × 11–13 µm, branched apically, dark brown, smooth, conidium forming at apex. *Conidia* obovoid to broadly, 39–48(–52) × 21–25(–28) µm, 1–2-transverse septa, constricted at septa, smooth-walled, dark brown, rounded at ends.

Material examined: THAILAND, Chiang Rai, Muang, Khun Korn Waterfall, (N19°51–54′ E99°35.39′), 671 m., on decaying wood of unidentified tree, 15 August 2009, Saranyaphat Boonmee (HOLOTYPE MFLU10–0036), ex-type culture: MFLUCC10–0105 and IFRDC2179.

Habitat: On decaying wood of unidentified tree.

Mode of life: Saprobic.

Distribution: Chiang Rai, Thailand.

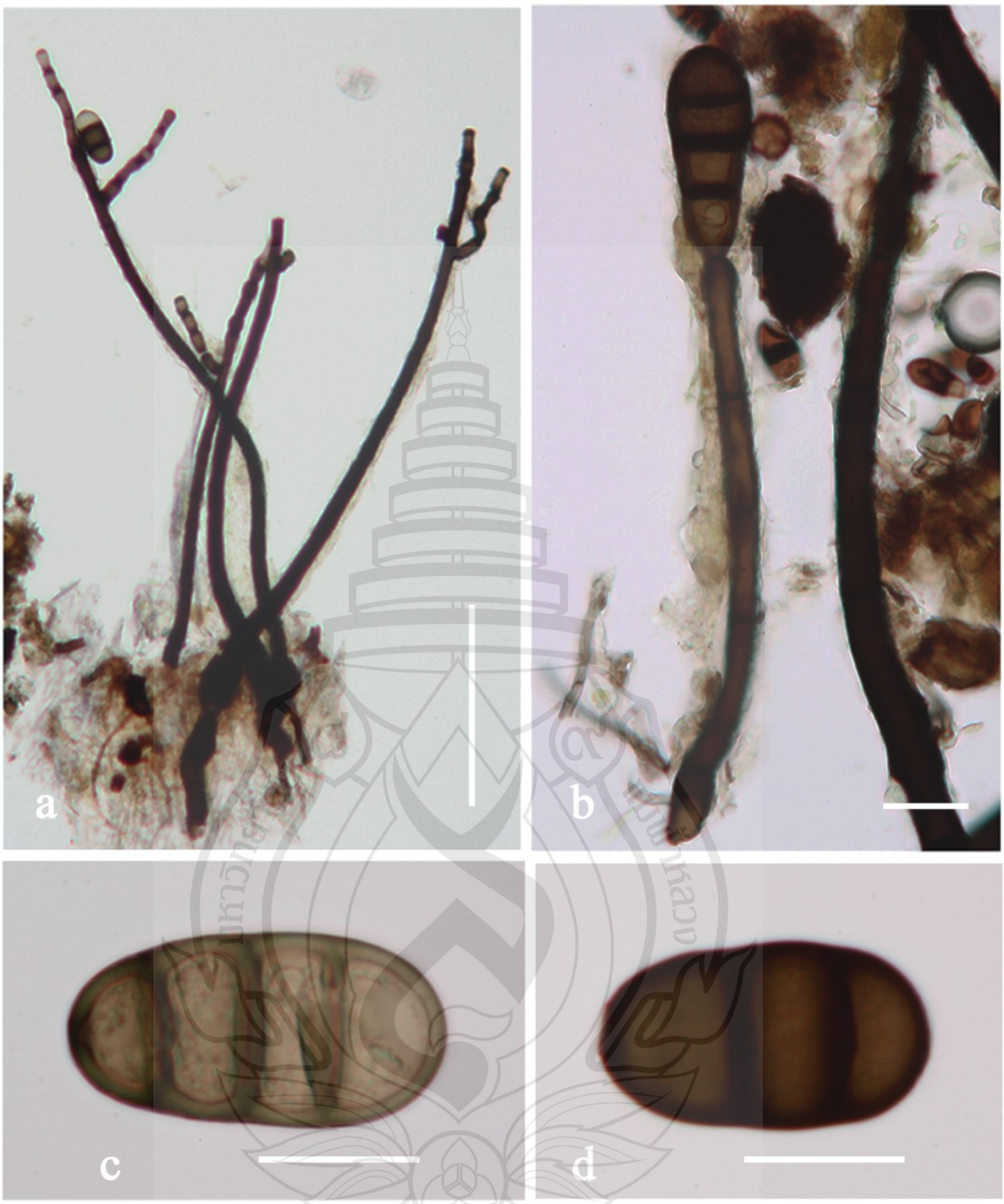
Notes: This species is characterized by superficial ascomata, with mycelia present at the base, a coriaceous wall, short-pedicellate asci, pigmented ascospores, which form secondary septa when mature (Fig. 3.3a-b, j-m). *K. lignicola* is similar to *K. populi* (Tracy & Earle) You Z. Wang, Aptroot & K. D. Hyde, *K. recessa* (Cooke & Peck) D. Hawksw., *K. umbrinoidea* (Pass.) You Z. Wang, Aptroot & K. D. Hyde, and *K. xera* (Fairm.) You Z. Wang, Aptroot & K. D. Hyde because ascospores are smooth-walled and lack a sheath (Hawksworth, 1985a; Wang et al., 2004) but differs in having thick-walled and larger ascospores with secondary septa. The anamorph was derived from single ascospores, and also found on the substrate (Figs. 3.4-3.5). The conidia of the anamorph in culture were smaller than those produced on the natural substrate. It is not unusual that characters in culture differ from the natural substrate, but because we were unable to isolate the anamorph we cannot unequivocally state these are the same. The characters of conidiophores and conidia are similar to the genus *Dendryphiopsis*, in agreement with the inferred phylogenetic relationships (Hughes, 1953, Fig. 3.2).

Note. (a) Habitat. (b) Appearance of ascomata on host surface. (c) Section through ascoma. (d) Peridium. (e) Pseudoparaphyses. (f)-(i) Asci. (j)-(m) Ascospores. Scale bars: (b)-(c) = 100 μm, (d) = 20 μm, (f)-(i) = 40 μm, (e) = 5 μm, (j)-(m) = 10 μm

Figures 3.3 *Kirschsteiniothelia lignicola* (MFLU10–0036, holotype)

Note. (a) Germinating ascospore. (b) Colony on MEA. (c) Mycelium on MEA. (d)-(f). Conidiogenous cells and conidia. (g)-(l). Conidia. Scale bars: (a) = 10 μm, (b) = 1 cm, (c)-(e) = 5 μm, (f)-(l) = 20 μm

Figures 3.4 Asexual State of *Kirschsteiniothelia lignicola* in Culture (MFLU10–0036, holotype)



Note. (a) Conidiophores. (b)-(c) Conidia. Scale bars: (a) = 100 μm, (b)-(d) = 20 μm

Figures 3.5 Asexual State of *Kirschsteiniothelia lignicola* on Natural Substrate (MFLU10–0036, holotype)

Kirschsteiniothelia emarceis S. Boonmee & K. D Hyde. Mycologia, 104(3): 708, (2012), MycoBank: MB 561033 (Figs. 3.6-3.8)

Etymology: The species name refers to MRC (Mushroom Research Centre) where the specimen was collected.

Ascomata superficialia, 109–280 µm alta × 101–318 µm diam., subglobosa ad globosa, atrobrunnea ad nigra, fasciculatae, solitarii vel dispersa, paucis sparsis capillis-like. Hamathecium numerosae, filiformibus pseudoparaphysum, hyalinae. *Asci* 88–140 × 18–24 µm, bitunicati, octaspori, cylindrici-clavati, fissitunicati, pedicellis parvis, cubiculum ocular. *Ascosporae* (23.5–)25–28 × 8–9(–10) µm, ellipsoideae, leniter curvatae, viridi, brunnea ad atrobrunnea, uniseptatae, constrictae. *Conidiophora* apice ramosis, brunneae ad atrobrunnea, laeves. *Conidia* fusiformia-obclavati, ferruginea ad atrobrunnea, septatae, constrictae.

Ascomata 109–280 µm high × 101–318 µm diam., dark brown to black, clustered, solitary or scattered, superficial, subglobose to globose, slightly immersed, base flattened, with central ostiole, but unclear, with a few sparse hair-like setae. *Peridium* 12–40 µm thick, composed of 3–4 layers, comprising cells of textura angularis; inner layer cells pale brown, but cells of outer layer black. *Hamathecium* comprising numerous narrow filiform pseudoparaphyses, 1–2 µm wide, hyaline, embedded in a gelatinous matrix. *Asci* 88–140 × 18–24 µm, eight-spored, bitunicate, fissitunicate, cylindrical to clavate, apically rounded, pedicel *ca*. 3–13 µm long and with small ocular chamber *ca*. 2 µm wide. Ascospores (23.5–)25–28 × 8–9(–10) µm ($\bar{x}$ = 27 × 9 µm, n = 20), biseriate, ellipsoidal, slightly curved, septum median to supra-median, dull green, becoming brown to dark brown at maturity, one-septate, two-celled, smooth-walled, lacking a mucilaginous sheath.

Cultural characteristics: *Ascospores* germinating on MEA within 12 hours. Colonies on MEA, reaching up to 2 cm diam in 1 week at 25-28 C and within a month covering Petri dish. Aerial mycelium, radiating outward, effuse, hairy, edge fimbriate, colony dense, dull green to dark brown. *Mycelium* immersed and superficial, dark brown; hyphae septate, *ca*. 5 µm wide. *Conidiophores* produced on MEA 32–92 µm long, 5–7 µm thick, branched at apex, brown to dark brown, smooth. *Conidia* (21–)27–28(–36) × 9–13(–15) µm, reddish brown to dark brown, 1–2(–3)-transverse septate, constricted at septa, smooth-walled, rounded at the ends, fusiform to obclavate.

Anamorph also growing on type material. *Conidiophores* erect, 162–271 × 7–14 µm, branched apically, brown to dark brown, smooth, conidia forming at apex. *Conidia* (40–)45–56(–67) × (10–)14–15(–17) µm, oblong to obclavate, grayish brown to dark brown, 3–4(–5) septate, constricted at septa, smooth-walled.

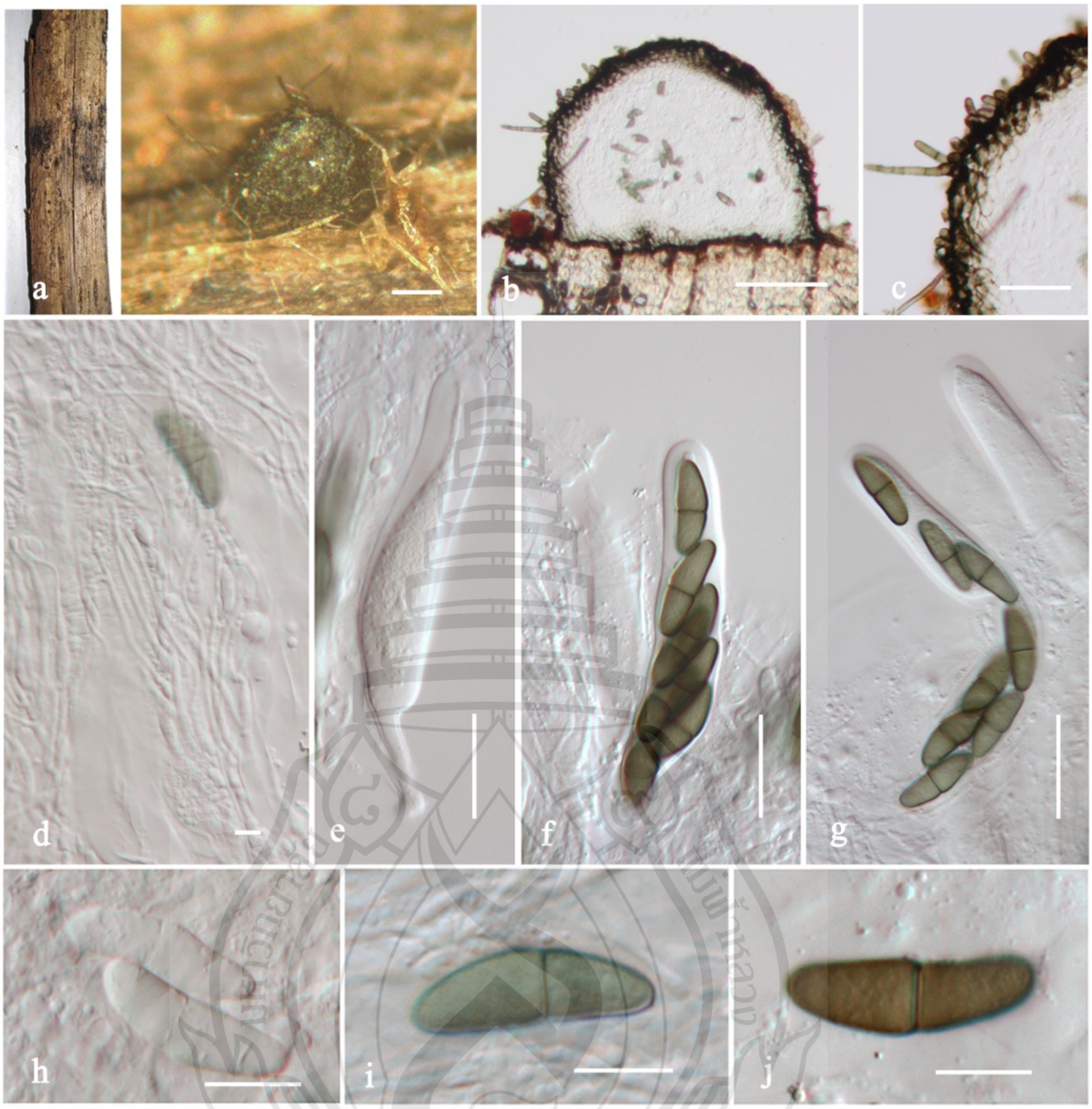
Material examined: THAILAND, Chiang Mai: Mae Taeng, Ban Pha Pae, Mushroom Research Center, N19°17.123′ E 98°44. 009′, 900 m., on dead wood of unidentified tree, 6 September 2009, Saranyaphat Boonmee (HOLOTYPE MFLU10–0037), ex-type culture: MFLUCC10–0106 and IFRDC2181.

Habitat: On dead wood of unidentified tree.

Mode of life: Saprobic.

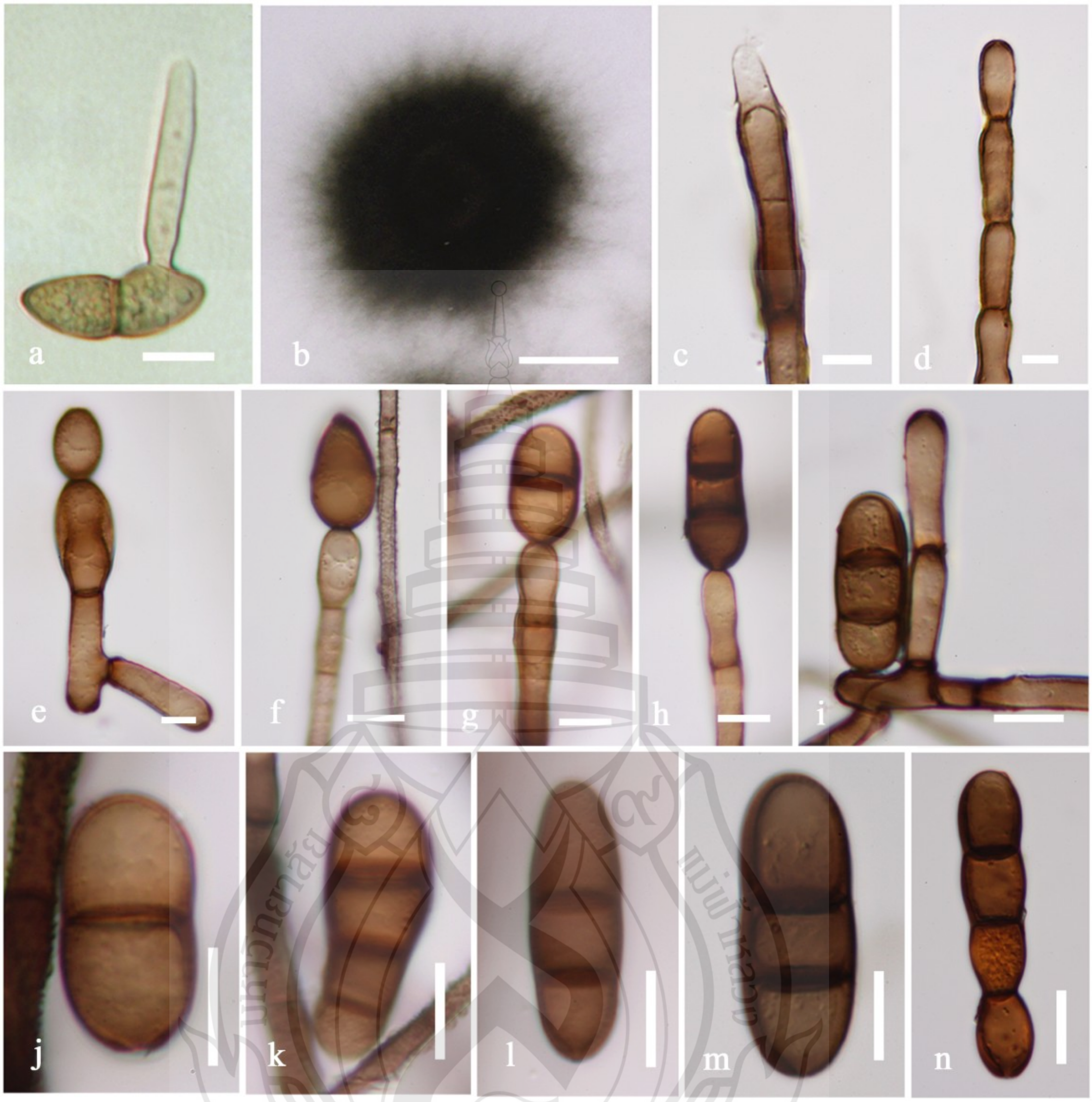
Distribution: Chiang Mai, Thailand.

Notes: *Kirschsteiniothelia emarceis* ascospores are similar to that of *K. populi* (Tracy & Earle) You Z. Wang, Aptroot & K. D. Hyde, *K. recessa* (Cooke & Peck) D. Hawksw., *K. umbrinoidea* (Pass.) You Z. Wang, Aptroot & K. D. Hyde, and *K. xera* (Fairm.) You Z. Wang, Aptroot & K. D. Hyde, being brown to dark brown, one-septate and have asymmetrical ends (Hawksworth, 1985a; Wang et al., 2004). It differs from all other *Kirschsteiniothelia* species by having sparse ascomatal hairs (Fig. 3.6a-c). Its anamorph in culture and on the natural substrate produce oblong to obclavate, dark brown conidia with several septa, typical of *Dendryphiopsis* (Figs. 3.7-3.8; Hughes, 1953). The conidia of the anamorph in culture were smaller and lighter than on the substrate as with *K. lignicola*. It is not unusual that characters in culture differ from those of the substrate, but because we were unable to obtain an isolate from the anamorph we cannot unequivocally state these are the same species. The phylogenetic analysis shows *K. emarceis* to form a sister relationship with *K. aethiops* and *K. lignicola*, with a long branch length (Fig. 3.2).



Note. (a) Ascomata on host surface. Note the dome-shaped ascomata with a flattened base. (b) Section through ascoma covered in sparse hairs. (c) Peridium. (d) Pseudoparaphyses. (e)-(g) Asci. (h)-(j) Ascospores. Scale bars: (a)-(b) = 100 µm, (c) = 40 µm, (d) = 5 µm, (e)-(g) = 20 µm, (h)-(j) = 10 µm

Figures 3.6 *Kirschsteiniothelia emarceis* (MFLU10–0037, holotype)



Note. (a) Germinating ascospore. (b) Colony on MEA. (c)-(e) Conidiophores and conidiogenous cells. (f)-(n) Conidia. Scale bars: (a), (f)-(n) = 10 μm, (b) = 1 cm, (c)-(e) = 5 μm

Figures 3.7 Asexual State of *Kirschsteiniothelia emarceis* in Culture (MFLU10–0037, holotype)

Note. (a)-(e) Conidiophores. (f)-(h) Conidia. Scale bars: (a)-(e) = 100 µm, (f)-(h) = 20 µm

Figures 3.8 Asexual State of *Kirschsteiniothelia emarceis* on Natural Substrate (MFLU10–0037, holotype)

Morphological characters of two new species of *Kirschsteiniotheliaceae*. An additional two new species (*K. lignicola* and *K. emarceis*) are described herein from Thailand and included in this family. Molecular phylogenetic analyses have demonstrated that the genus, as currently understood, is polyphyletic (Fig. 3.2) with *M. elaterascus* and *K. maritima* grouping in the *Morosphaeriaceae* and *Mytilinidiaceae* respectively. The two new species from Thailand (*K. lignicola* and *K. emarceis*) grouped with the type species *K. aethiops* in a clade that is distinct from other families in the Dothideomycetes with good bootstrap support. Therefore a new family is introduced to accommodate these species. Morphologically they share common features as well as having anamorphs similar to the genus *Dendryphiopsis* (Hughes, 1953). The anamorphs share straight to slightly curved, darkened conidiophores and oval-oblong, 2–3-septate, dark brown conidia (Figs. 3.4-3.8).

Species currently classified in *Kirschsteiniothelia* are scattered in *Capnodiales, Jahnulales, Mytilinidiales* and *Pleosporales* (Fig. 3.2). Studies on morphology and phylogeny of *Kirschsteiniothelia* highlighted its uncertain status (Barr, 1987; Hawksworth, 1985a, 1985b; Lumbsch & Huhndorf, 2010; Lumbsch & Lindemuth, 2001; Nelsen et al., 2009; Ruibal et al., 2009; Schoch et al., 2006; Shearer et al., 2009; Wang et al., 2004). Phylogenetic studies have indicated its polyphyly (Suetrong et al., 2009). *K. elaterascus* thus is transferred to the genus *Morosphaeria* (*Morosphaeriaceae*) based on morphological and molecular evidence. This is in agreement with studies by Shearer et al. (2009), Schoch et al. (2009) and Suetrong et al. (2009). *K. maritima* has been shown to be affiliated with the *Mytilinidiaceae* in a well supported monophyletic clade (Schoch et al., 2009, Suetrong et al., 2009) and a new genus *Halokirschsteiniothelia* is erected to accommodate it. However *Halokirschsteiniothelia maritima* has few morphological characters in common with the *Mytilinidiaceae*, except they are all saprobic fungi found on non-living plants, especially on deadwood (Boehm, Mugambi et al., 2009; Boehm, Schoch et al., 2009; Hawksworth, 1985a).

Finally, *Kirschsteiniothelia aethiops* is the type of the new family *Kirschsteiniotheliaceae* in a well supported clade along with the two new species described in this paper with a *Dendryphiopsis* anamorph. The taxonomic position of four species has been confirmed, while the affiliation of other taxa in the genus remains to be resolved. This study has advanced our understanding of the higher order taxonomy of a genus previously regarded as polyphyletic.

3.4 Conclusion

In this study, two new *Kirschsteiniothelia* species are proposed based on morphological and molecular phylogenetic analysis; both were collected on decaying wood from Chiang Mai and Chiang Rai provinces in northern Thailand. The taxa were isolated and the morphological characters are described and illustrated. ITS, LSU and SSU combined sequence analysis showed taxa of *Kirschsteiniothelia* separating into three lineages: (i) *K. elaterascus* grouped within *Morosphaeriaceae* (*Pleosporales*); (ii) *K. maritima* clustered with *Mytilinidion* spp. as a sister group in the *Mytilinidiaceae* clade; and (iii) the two new *Kirschsteiniothelia* species, which produce *Dendryphiopsis* anamorphs in culture, clustered with *K. aethiops* (the generic type) and the anamorph *D. atra*. The new family *Kirschsteiniotheliaceae* is introduced to accommodate taxa grouping with *K. aethiops*. *K. elaterascus* is transferred to *Morosphaeria* (*Morosphaeriaceae*) and a new genus *Halokirschsteiniothelia* is introduced to accommodate *K. maritima* (*Mytilinidiaceae*).

CHAPTER 4

TAXONOMY OF THE INTERESTING FAMILIES OF DOTHIDEOMYCETES BASED ON THE HERBARIUM TYPE MATERIAL

4.1 Introduction

Many genera in Dothideomycetes are placed in the Dothideomycetes *genera incertae sedis*, because molecular data is not available to confirm their placement (Cannon & Kirk, 2007; Kirk et al. 2008; Lumbsch & Huhndorf, 2010). Dothideomycetes are characterised on the basis of having bitunicate ascus with two separable wall layers (Barr, 1987, 1990; Eriksson, 1981; Luttrell, 1955; von Arx & Müller, 1975), while, ascomata, pseudoparaphyses and ascospores differ and can be found in other groups of the fungi. The types of ascomata are important is separating some families. For example, multilocular ascostromata are found in *Ascoporiaceae, Coccoideaceae, Cookellaceae* and *Protoscyphaceae*, while unilocular ascomata are found in *Aulographaceae, Kirschsteiniotheliaceae, Parodiopsidaceae, Pseudoperisporiaceae*, *Tubeufiaceae* (Barr, 1979; Boonmee et al., 2012; Hennings, 1904a; von Höhnel, 1909b; Kirschner et al., 2010; Kutorga & Hawksworth, 1997; Luttrell, 1973; Samuels & Romero, 1991; Seaver & Chardén, 1926). The Dothideomycetes are well known as saprobes or pathogens, developing structures on wood or leaves of most plants and are distributed throughout temperate to tropical areas. There are only a few species and genera of Dothideomycetes that have previously been reported from Thailand (Boonmee et al., 2011, 2012; Seephueak, Phongpaichit, Hyde & Petcharat, 2011; Tsui et al., 2007; Wang, Hyde, Soytong & Lin, 2008). The present work, studies the Dothideomyces, particularly on woody litter in northern Thailand.

4.2 Materials and Methods

Herbarium type specimens of *Ascoporiaceae, Aulographaceae, Coccoideaceae, Cookellaceae, Kirschsteiniotheliaceae, Parodiopsidaceae, Protoscyphaceae, Pseudoperisporiaceae, Tubeufiaceae* were obtained from the repositories of U.S. National Fungus Collections (BPI), Kew Royal Botanic Gardens (K), Mae Fah Luang University (MFLU), Università degli Studi di Padova (PAD) and Muséum National d'Histoire Naturelle (PC). The herbarium specimens were rehydrated by 5% KOH and/ or distilled water prior to examination. Morphologically, the type specimens were provided descriptions and illustrations as previously processes in Chapter 2 (see in Section 2.1.1).

4.3 Results and Discussion

4.3.1 *Ascoporiaceae*

Saprobic on decorticated wood in terrestrial habitats. *Ascostromata* relatively large, superficial, circular or cupulate, slightly raised, stipitate, solitary, exterior surrounded by brown mycelium, orange to red-brown at the margin or edge, dark brown to black in the centre, cells of ascostromata comprising a maze-like arrangement of mixed, brown-walled cells of *textura angularis* to *subglobosa* or *irregulata*, multiloculate. *Locules* in a single layer at the periphery of the ascostromata, subcylindric-elongate, obpyriform to ovoid, crowded, opening by small, narrow, vertical ostiole. *Hamathecium* comprising numerous filiform, branched, septate pseudoparaphyses embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, narrowly cylindric-clavate, pedicellate, apically rounded with a small ocular chamber, staining pinkish to purplish in 5% KOH. *Ascospores* biseriate, fusiform, 1-septate, constricted at the septum, hyaline to yellowish when immature, olive-brown to dark brown when mature. *Asexual state*: see notes under genus.

Notes: *Ascoporia* was introduced by Samuels and Romero (1991) and placed in *Patellariaceae*. There is considerable variation in ascostroma structures, ascus shape and ascospore colour of *A. lateritia*. Typically the ascostromata are darkly pigmented in section, although bright coloured features may be seen on the substrate as well; asci are obviously bitunicate with a small apical ocular chamber; ascospores are typically fusiform, 1-septate and dark brown at maturity (Samuels & Romero, 1991). Kutorga and Hawksworth (1997) investigated the isotype of *A. lateritia* and found that overall mophologies distinguish it from the *type species* of *Patellariaceae* (*Patellaria* Fr. 1882). Therefore, they separated *Ascoporia* from *Patellariaceae* and introduced the family *Ascoporiaceae* (Kutorga & Hawksworth, 1997) comprising a single genus *Ascoporia*. An earlier name for *Ascoporia* was founded in *Pseudosolidum* by Rossman et al. (1999) when they examined a portion of the type specimen of *Pseudosolidum* at Kew herbarium (K).

Family type: *Pseudosolidum* Lloyd (as McGinty), Mycol. Writ. 7: 1206 (1923), MycoBank: MB 26458 (Fig. 4.1)

= *Ascoporia* Samuels & A. I. Romero, Bolm Mus Paraense 'Emilio Goeldi', Ser. Bot. 7(2):264 (1993) [1991]

Saprobic on decorticated wood in terrestrial habitats. *Ascostromata* relatively large up to 10 mm diam., superficial, circular or cupulate, slightly raised, with surface inwardly concaved, stipitate, solitary, exterior surrounded by brown mycelium, orange to red-brown at the margin, dark brown to black in the centre, cells of ascostromata comprising a maze-like arrangement of mixed, brown-walled cells of *textura angularis* to *subglobosa* or *irregulata*, multiloculate. *Locules* in a single layer at the periphery of the ascostromata, subcylindric-elongate, obpyriform to ovoid, crowded, opening by small narrow vertical ostiole, black, with orange to reddish brown inner tissues. *Hamathecium* comprising numerous filiform, branched, septate pseudoparaphyses embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, narrowly cylindric-clavate, with a foot-like pedicel, apically rounded with a small ocular chamber, staining pinkish to purplish in 5% KOH. *Ascospores* biseriate in the ascus, fusiform, tapering towards the narrowly rounded ends, 1-septate, constricted at the septum, hyaline to yellowish when immature, olive-brown to dark brown when mature. *Asexual state* see under notes.

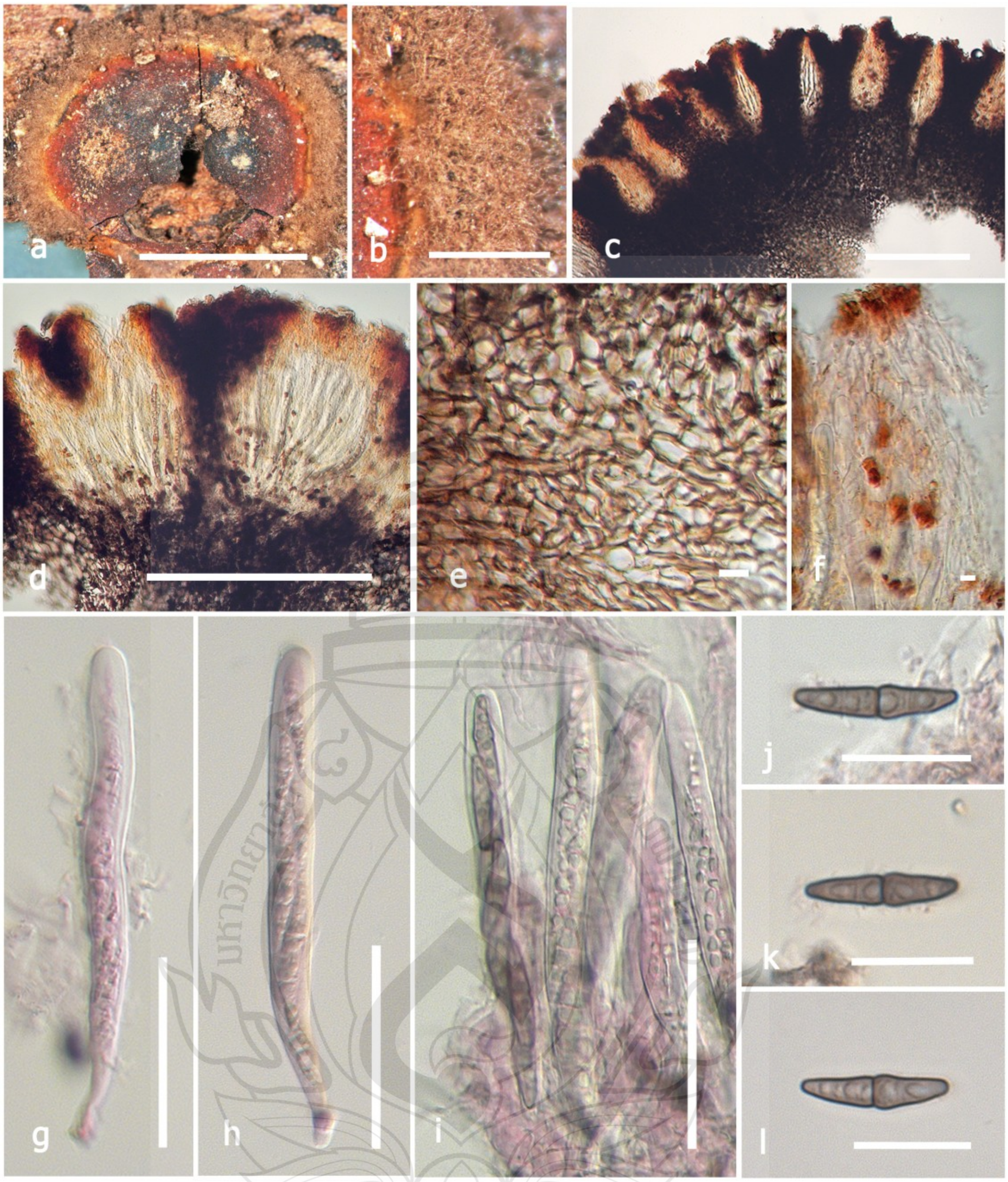
Material examined: BRAZIL: Belem, Iha do Combu, Estacao Experimental Combu, Para. 01d30's, 48d27'w, on decorticated wood, January 1989, G.J. Samuels; K.F. Rodrigues (6207), BPI1109903, isotype of *Ascoporia lateritia*.

Notes: *Pseudosolidum* (= *Ascoporia*) is a unique genus with locules lying in a peripheral layer around the cushion-like ascostromata, but is typical of *Dothideomycetes* in its hamathecium characters (Fig. 4.1). The most similar genus may be *Shiraia* which also has large orange ascostromata, however the habit (an endophyte on bamboo) and ascospores (muriform) are distinct (Hennings, 1900; Liu et al., 2012). *Pseudosolidum* contains a single species *P. solidum* (Fig. 4.1). Rossman et al. (1999) noted that *Ascoporia lateritia*, which is illustrated here, is a synonym of *Pseudosolidum solidum*. Kutorga and Hawksworth (1997) in their examination of the isotype specimen of *A. lateritia*, found a coelomycete similar to *Plectophomella* Moesz, which they considered as a possible asexual stage. This coelomycetous asexual morph has cylindrical, hyaline, smooth, phialidic conidiogenous cells and ellipsoid to ovate, 1-celled, hyaline conidia and was described by Kutorga and Hawksworth (1997). There is no molecular data to support this link.

Type species: *Pseudosolidum solidum* (Berk. & M. A. Curtis) Lloyd (as McGinty), Mycol. Writ. 7: 1206 (1923), MycoBank: MB 360813

≡ *Hypoxylon solidum* Berk. & M. A. Curtis, J. Acad. Nat. Sci. Philad., N. S. 2(6): 286 (1854) [1853]

= *Ascoporia lateritia* Samuels & A. I. Romero, Bolm Mus paraense 'Emílio Goeldi', Sér. Bot. 7(2): 264 (1993) [1991]



Note. (a) Ascostroma. (b) Crowded brown mycelium surrounding ascostroma. (c)-(d) Section through ascostroma showing arrangement of locules. (e) Cells of ascostromata. (f) Hamathecium. (g)-(i) Immature and mature asci in 3% KOH. Note colour changes. (j)-(l) Ascospores. Scale bars: (a) = 5 mm, (b) = 1 mm, (c)-(d) = 200 µm, (e) = 10 µm, (f) = 5 µm, (g)-(i) = 50 µm, (j)-(l) = 20 µm

Figure 4.1 *Pseudosolidum solidum* (BPI, isotype of *Ascoporia lateritia*)

4.3.2 *Aulographaceae*

Aulographaceae Luttrell ex P. M. Kirk, P. F. Cannon & J. C. David, in Kirk, Cannon, David & Stalpers, Ainsworth & Bisby's Dictionary of the Fungi, Edn 9 (Wallingford): ix (2001), MycoBank: MB 82122

Saprobic on dead leaves. *Ascomata* superficial, a thyriothecium, solitary, some fused, scattered, usually elongate, slightly convex or shield-like, mostly linear, some furcate or branched, anastomosed, triangular in shape, dehiscing by a slit, dark brown to black, red-brown at the margin, mycelium appressed towards the base. *Peridium* composed of very thin, tightly packed *textura epidermoidea*, somewhat brittle, easily broken. *Hamathecium* of asci and lacking pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate, broadly ellipsoid or subclavate-oblong, apex thickened, pedicel stumpy or lacking, ocular chamber not distinct. *Ascospores* 2-3-seriate, narrowly obovoid to clavate, 1-septate, not constricted at the septum, upper cell wider, hyaline, guttulate, smooth-walled. *Asexual state* unknown.

Notes: *Aulographaceae* was invalidly introduced by Luttrell (1973), and later validated by Kirk et al. (2001) with a Latin diagnosis. Subsequently, it was accommodated in *Microthyriales* based on the flattened thyriothecial ascomata (Kirk et al., 2008), but treated as a distinct family due to its opening by a longitudinal slit at maturity to expose the asci (George, 1895; Luttrell, 1973; Sierra, 2006). Presently, the family comprises *Aulographum* and *Polyclypeolina* (Lumbsch & Huhndorf, 2010). These genera occur on the surface of leaves (Fig. 4.2b-c). In *Polyclypeolina* ascomata form irregularly, are clustered and fused, without a longitudinal slit, and asci and ascospores differ from *Aulographum* (Batista, 1959; Hansford, 1945). Based on the morphological characters this genus can be excluded and placed in Ascomycetes genera *incertae sedis*.

Molecular data is needed to confirm its correct placement. Wu et al. (2011) added *Lembosiella* to *Aulographaceae* based on ascomatal structures resembling those of *Aulographum*. The ascomata are typically superficial thyriothecia and similar to *Asterinaceae* (Batista, 1960; Ellis, 1980). Ascomatal structures of *Asterinaceae* are generally circular with outwardly radiating mycelium which dehisce by irregular fissures (Batista, Maia & Farr, 1958; Hansford, 1946). In addition, they produce appressoria (Hofmann et al., 2010; Housagoudar, 2012), a character which may distinguish these families. *Aulographum* is similar to *Aulographina* and *Lembosia* in *Asterinaceae* and these genera may be synonyms. *Aulographum* may also be placed in *Asterinaceae*. For the proper placement of these taxa, molecular data is needed.

Family type: *Aulographum* Lib., Pl. Crypt. Arduenna, fasc. (Liège) 3: no. 272 (1834), MycoBank: MB 461 (Fig. 4.2)

Saprobic on dead leaves. *Ascomata* superficial, a thyriothecium, solitary, some fused, scattered, usually elongate, slightly convex or shield-like, mostly linear, some furcate or branched, or anastomosed and triangular in shape, dark brown to black, red brown at the margin, dehiscing by a longitudinal slit, mycelium appressed outwards from the base. *Peridium* composed of very tightly packed *textura epidermoidea*, somewhat brittle, easily broken. *Hamathecium* of asci, pseudoparaphyses lacking. *Asci* 8-spored, bitunicate, fissitunicate, broadly ellipsoid, subclavate-oblong, apex thickened, pedicel stumpy or lacking, ocular chamber not distinct. *Ascospores* 2-3-seriate, narrowly obovoid, clavate, 1-septate, not constricted at the septum, upper cell wider, hyaline, guttulate, smooth-walled. *Asexual state* unknown.

Material examined: on leaves of *Hedera* sp., BPI 1108478.

Notes: *Aulographum* was introduced by Libert (1834), and is typified by *Aulographum hederae*. The type specimen of *Aulographum hederae* was not available for study, therefore the illustration is derived from BPI 1108478which appears to be an authentic specimen (Fig. 4.2). In the protologue described by Libert (1834), thyriothecia are slightly linear, longitudinally splitting and produce oval-oblong, 1-septate, hyaline ascospores (Massee, 1895). This genus comprises 75 epithets (Index Fungorum, www.indexfungorum.org/). Several of the species however, were transferred to other families, such as *Asterinaceae*, *Elsinoaceae*, *Leptopeltidaceae*, or *Lophiostomataceae*. *Aulographum hederae* is charaterized by longitudinally splitting ascomata, and a peridial wall appressed by mycelium with bright-colours at the margin (Fig. 4.2a-d). These are different from *Polyclypeolina*, presently included in the same family (Batista, 1959; Hansford, 1945).

Type species: *Aulographum hederae* Lib., Pl. Crypt. Arduenna, fasc. (Liège) 3: no. 272 (1834), MycoBank: MB 161393

= *Aulographum vagum* Desm. Annls Sci. Nat., Bot., Sér. 2 19:362 (1843)

Other genera included

Lembosiella Sacc., Syll. Fung.9:1101 (1891)

Type species: *Lembosiella polyspora* (Pat.) Sacc., Syll. Fung. 9:1101 (1891)

Polyclypeolina Bat. & I.H. Lima, in Batista, Publções Inst. Micol. Recife 56: 457 (1959)

Type species: *Polyclypeolina brideliae* (Hansf.) Bat., Publções Inst. Micol. Recife 56:457 (1959)

Key to putative genera of *Aulographaceae*

1. Thyriothecia irregular, clustered or fused, with ostiole ....*Polyclypeolina*
1. Thyriothecia elongate, mostly linear, with longitudinal slit 2
2. Ascospores hyaline ....*Aulographum*
2. Ascospores brown to reddish brown...*Lembosiella*

Note. (a) Thyriothecia. (b)-(d) Squash mount of thyriothecium. (e)-(h) Immature and mature asci. (i)-(n). Ascospores. Scale bars: (a) = 500 μm, (d) = 50 μm, (e)-(n) = 10 μm

Figure 4.2 *Aulographum hederae* (BPI, authentic specimen)

4.3.3 *Coccoideaceae*

Coccoideaceae Henn. ex Sacc. & D. Sacc., Syll. Fung. (Abellini) 17: 860 (1905), MycoBank: MB 80616

Parasitic on living leaves. *Ascostromata* large up to 2 mm diam., single to solitary, or scattered, mostly superficial, slightly immersed in host tissue, circular to subcircular, discoid, cushion-like, semicircular in section, edge entire, slightly convex at the top, raised from the base, black, thickened at the base, soft, with numerous locules, in a layer in the upper part the ascostromata. *Cells of ascostromata* comprising heavily pigmented cells of *textura angularis*. *Locules* of ascomata completely immersed in ascostromata, globose-subglobose or obpyriform, with apical ostiole. *Hamathecium* of filiform, anastomosing, branched, septate, hyaline, relatively wide pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to subclavate, pedicellate, apically rounded with a distinct wide ocular chamber. *Ascospores* 2-seriate, apiosporous, ellipsoidal-obovoid, slightly oval, subglobose, yellowish to light brown, tapering toward narrow lower end, 1-septate at the lower end, not constricted at the septum, darkened at the septa, smooth. *Asexual state* unknown.

Notes: *Coccoideaceae* was invalidly introduced by Hennings (1904b) and was subsequently validly published in Saccardo, P. A. and Saccardo, D. (1905). These are mainly parasites on the lower surface of living leaves and have superficial, circular or discoid and darkly pigmented, ascostromata (Saccardo, P. A. & Saccardo, D., 1905). The family *Coccoideaceae* now includes three genera and is placed under *Dothideales* in Kirk et al. (2008). Lumbsch and Huhndorf (2010) however, list two genera, i.e. *Coccoidea* and *Coccoidella* placed in *Dothideomycetes incertae sedis*. There are no molecular data to confirm these placements. The family is based on the type species *C. quercicola* Henn. & Shirai from Japan, which we have not been able to locate. von Höhnel (1909a) added a second genus *Coccoidella*, having circular and flattened ascostromata and bitunicate, oblong asci (Berkeley, 1876). We have examined this which mainly differs in having 2-celled ascospores. Both genera are placed in the same family based on only morphological classification and the genera need to be re-studied.

Family type: *Coccoidea* Henn., Bot. Jb. 28: 275 (1900), MycoBank: MB 1144 (Fig. 4.3)

Parasitic on living leaves of *Quercus lamellosa*. *Ascostromata* large, up to 2 mm diam, single to solitary, or scattered, mostly superficial, slightly immersed in host tissue, circular to subcircular, discoid, cushion-like, semicircular in section, edge entire, slightly convex at the top, raised from the base, black, thickened at the base, soft, with numerous locules, in a layer in the upper part the ascostromata. *Cells of ascostromata* comprising heavily pigmented cells of *textura angularis*. *Locules* of ascomata completely immersed in ascostromata, globose-subglobose or obpyriform, with upper ostiole. *Hamathecium* of filiform, anastomosing, branched, septate, hyaline, relatively wide pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical-clavate, with short to long pedicels, with a wide ocular chamber. *Ascospores* 1-2-seriate, ellipsoidal-obovoid, slightly oval, subglobose, tapering towards narrow nipple-like base, hyaline and 1-celled when immature, at maturity, 1-septate near the lower end, not constricted and dark brown at the septum, yellowish to light brown, smooth-walled. *Asexual state*: unknown.

Material examined: INDIA, Sukliapokhari, Alt. 7000 ft., on living leaves of *Quercus lamellosa* Sm., 11 May 1967, M.K. Maity PCC 1252; BPI 643971.

Notes: *Coccoidea* was introduced by Hennings (1900), for species with superficial, circular-shaped and black ascostromata. The locules are reported to be in a layer at the top of the ascostromata. Besides the type species it contains three other epithets (Kishi, 1998; Kobayashi, Katsumoto, Abiko, Abe & Kakishima, 1992; Müller & Sanwal, 1954; Müller & von Arx, 1962; Sawada, 1959), one being the family type of *Coccoidella*. Both genera have been placed in *Venturiaceae*, *Pseudosphaeriales* (Müller & von Arx 1962). The specimen illustrated here is from India (Kar & Maity, 1971) and is different to the Japanese species in having many locules scattered throughout the larger ascostromata. The genus and family need to be restudied.

Type species: *Coccoidea quercicola* Henn. & Shirai, in Hennings, Bot. Jb. 28: 275 (1900), MycoBank: MB 167300

Other genera included

Coccoidella Höhn., Sber. Akad. Wiss. Wien, Math. Nat. Kl., Abt. 1 118: 847 [35 repr.] (1909)

Type species: *Coccoidella scutula* (Berk. & M. A. Curtis) Höhn., Sber. Akad. Wiss. Wien, Math. Nat. Kl., Abt. 1 118: 847 [35 repr.] (1909)

Key to genera of *Cookellaceae*

1. Asci slightly oval, septate at the lower end, yellowish-brown to light brown .... *Coccoidea*

1. Asci ellipsoidal-oblong to clavate, septate at the centre, hyaline-pale brown .... *Coccoidella*

Note. (a)-(b) Ascostromata. (c) Section through of ascostroma showing arrangement of locules (Note ostioles arrowheads). (d) Pseudoparaphyses. (e)-(f) Asci. (g) Fissitunicate ascus. (h)-(j) Ascospores. Scale bars: (a) = 5 mm, (b) = 1 mm, (c) = 200 μm, (d), (f)-(g) = 50 μm, (h)-(k) = 10 μm.

Figure 4.3 *Coccoidea quercicola* (BPI, authentic specimen)

4.3.4 *Cookellaceae*

Cookellaceae Höhn. ex Saccardo& Trotter, in Saccardo, Syll. Fung. 22: 585 (1913), MycoBank: MB 80637

Parasitic on leaves in terrestrial habitats, some possibly fungicolous. *Ascostromata* superficial, subglobose or irregular, pulvinate to effuse, cushion-shaped, surface rough, flat or applanate at the base, scattered, pigmented, containing locules with individual asci, non-papillate, opening by breaks in the upper surface; soft textured, cells of ascostromata comprising *textura angularis-subglobulosa*, with a basal hypostroma developing in the host tissue, appearing as stromal blocks and swollen cells, of compressed packages of *textura angularis-prismatica*. *Locules* scattered throughout ascostromata, small, globose-subglobose, containing single asci, wall not obvious. *Hamathecium* lacking pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate, globose-subglobose or saccate, apedicellate, lacking an ocular chamber. *Ascospores*-scattered in the ascus in overlapping rows of 3-5-seriate, muriform, ellipsoid to oblong, hyaline when immature, becoming brown at maturity, straight to slightly curved, ends rounded, with 3-transverse septa and 1-longitudinal septum in each cell, constricted at the septa, smooth-walled. *Asexual state* see notes under genus.

Notes: *Cookellaceae* was introduced by von Höhnel (1909a) as *Cookellaceen*, and formally published by Saccardo and Trotter (1913). The family is characterized by superficial pulvinate to effuse, cushion-shaped, pigmented, ascostromata, without pseudoparaphyses, forming hypostroma in the host tissues. Asci are arranged in individual locules, irregular, mostly globose-subglobose and bitunicate and fissitunicate. Ascospores are hyaline to brown, septate and muriform, and forcible discharge of ascospores occurs at maturity (Barr, 1979, 1987; von Höhnel, 1909a; von Arx, 1963; von Arx & Müller, 1975). von Höhnel (1909a) initially placed *Cookella* in *Myriangiaceae*, based on the superficial, disk-like ascostromata with flattened bases, and asci produced in locules in a hyphal tissue. von Höhnel (1909a, 1911) later considered that ascomata with a flattened base, and bright thin-walled peridium, were characteristics that differed from the *Myriangiaceae* and therefore introduced the family *Cookellaceae*. von Arx (1963) placed *Cookellaceae* in *Myriangiales* based on superficial stromatic ascomata and parasitic life form on leaves or other fungi. Later, the family was excluded from *Myriangiales*, and placed in *Dothideomycetes* of uncertain order (Eriksson, 2005). The *Cookellaceae* presently comprise three genera, *Cookella*, *Pycnoderma* and *Uleomyces* (Kirk et al., 2008; Lumbsch & Huhndorf, 2007). *Uleomyces* is a very poorly known genus that may be similar to *Cookella* and has pulvinate ascostromata, globose asci and hyaline to brown ascospores (Inácio & Cannon, 2008) and also occurs on oak leaves. *Uleomyces* is said to be fungicolous (Hennings, 1895). *Pycnoderma* is more like a member of *Brefeldiellaceae.* No molecular data are available for any of these taxa and their placement in the *Myriangiales* cannot be confirmed.

Family type: *Cookella* Sacc., Michelia 1(4):407 (1878), MycoBank: MB 1225 (Fig. 4.4)

Parasitic on living leaves in terrestrial habitats. *Ascostromata* superficially, subglobose, cushion-shaped, surface rough, flat or applanate at the base, scattered, pigmented, containing locules with individual asci, non-papillate, opening by breaks in the upper surface; ascostromata comprising *textura angularis-subglobulosa*, with a basal hypostroma developing in the host tissue, appearing as stromal blocks with swollen cells, compressed packages of *textura angularis-prismatica*. *Locules* scattered throughout ascostromata, small, globose-subglobose; peridium composed of *textura angularis-subglobulosa*, with developing hypostroma in the host tissue. *Hamathecium* lacking pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate, globose-subglobose or saccate, apedicellate, lacking an ocular chamber. *Ascospores* scattered in the ascus in overlapping rows of 3-5-seriate, muriform, ellipsoid to oblong, hyaline when immature, becoming brown at maturity, straight to slightly curved, ends rounded, with 3-transverse septa and 1-longitudinal septum in each cell, constricted at the septa, smooth-walled. *Asexual state*: see notes.

Material examined: ITALY, Erbario Patavinum, Centro Interdipartimentale Musei Scientifici, Università degli Studi di Padova, Via Orto Botanico 15, I-35123 Padova, PAD.

Notes: The type specimen obtained from PAD contains oak leaves lacking ascostromata. Therefore, we provide an account of *Cookella* based on the protologue and drawings in Arnaud (1925), and von Arx (1963) and from the drawing with the PAD type material. *Cookella* was established by Saccardo (1878) based on a description of *Cookella microscopica*. Five species of *Cookella* are listed in Index Fungorum (www.indexfungorum.org/) and are typically parasites on leaves (Barr, 1987; von Höhnel, 1909a). Saccardo (1878) found a coelomycetous taxon with muriform conidia, named *Stigmella* on leaves around ascostromata of *Cookella microscopica*, and considered that this might be the asexual state. The fruiting bodies of the two taxa are however dissimilar (ascostromata versus immersed individual conidiomata). The relationship of these two morphs, which are based on being found on the same material, must be treated with caution.

Type species: *Cookella microscopica* Sacc., Michelia 1(4): 407 (1878), MycoBank: MB 218808

Other genera included

Pycnoderma Syd. & P. Syd., Ann Mycol 12(6): 563 (1914)

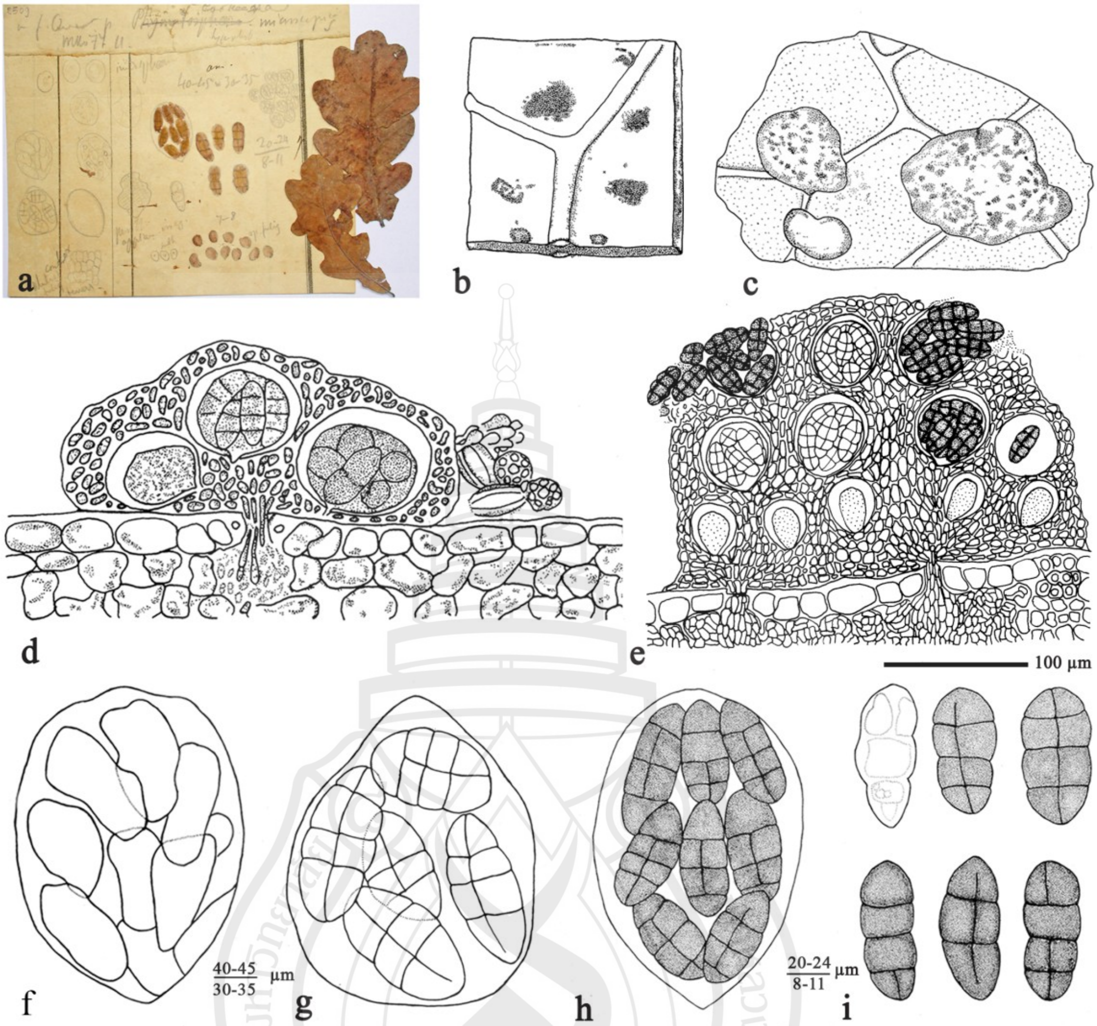
Type species: *Pycnoderma bambusinum* Syd. & P. Syd. Ann Mycol 12(6): 563 (1914)

Uleomyces Henn., Hedwigia 34:107 (1895)

Type species: *Uleomyces parasiticus*Henn., Hedwigia 34: 107 (1895)

Key to genera of *Cookellaceae*

1. Ascostromata superficial, cushion or disk-shaped...2
1. Thyriothecia superficial, disk-shaped, ... *Pycnoderma*
2. Ascospores brown.. *Cookella*
2. Ascospores hyaline .... *Uleomyces*



Note. (a) Reproduction of notes on herbarium envelope of *Cookella microscopica*. (b)-(c) Ascostromata on substrate. (d)-(e). Section through ascostroma to show distribution of locules (bar = 100 μm). (f)-(h) Immature and mature asci. (i) Immature and mature ascospores. Notes: Figs. (a)-(i) redrawn from PAD: no. 2509; Figs. (b)-(d) redrawn from Arnaud 1925; Fig. (e) redrawn from von Arx, 1963

Figure 4.4 *Cookella microscopica* (PAD, holotype)

4.3.5 *Kirschsteiniotheliaceae*

Kirschsteiniotheliaceae S. Boonmee & K. D. Hyde, Mycologia 104(3): 705 (2012), MycoBank: MB 561021

This family has recently been proposed by Boonmee et al. (2012). It is a part of this present study. The detail description of the fungal strains in this family was previously described in Section 3.3.2, Chapter 3.

Notes: The *Kirschsteiniotheliaceae* was recently proposed by Boonmee et al. (2012), for saprobic fungi occurring on dead wood, and this was supported by molecular data. This is a small family with a single genus that is represented by *Kirschsteiniothelia aethiops*, characteristically with superficial ascomata, globose, unilocular, heavily pigmented, bitunicate, fissitunicate asci, dull green, brown to dark brown, septate ascospores. Principally, the *Type species K. aethiops* was connected with asexual form *Dendryphiopsis* (Hughes, 1953), with mononematous, erect, branched, septate, coloured conidiophores, broadly ellipsoid-obovoid, septate, light to dark brown conidia (Fig. 4.5; Hawksworth, 1985a; Hughes, 1953). The introduced family *Kirschsteiniotheliaceae* is well supported by morphological characters and molecular phylogenetic analyses (Boonmee et al., 2012; Schoch et al., 2006; Shearer et al., 2009).

Family type: *Kirschsteiniothelia* D. Hawksw., J. Linn. Soc., Bot. 91: 182 (1985), MycoBank: MB 25723 (Fig. 4.5)

Synonyms

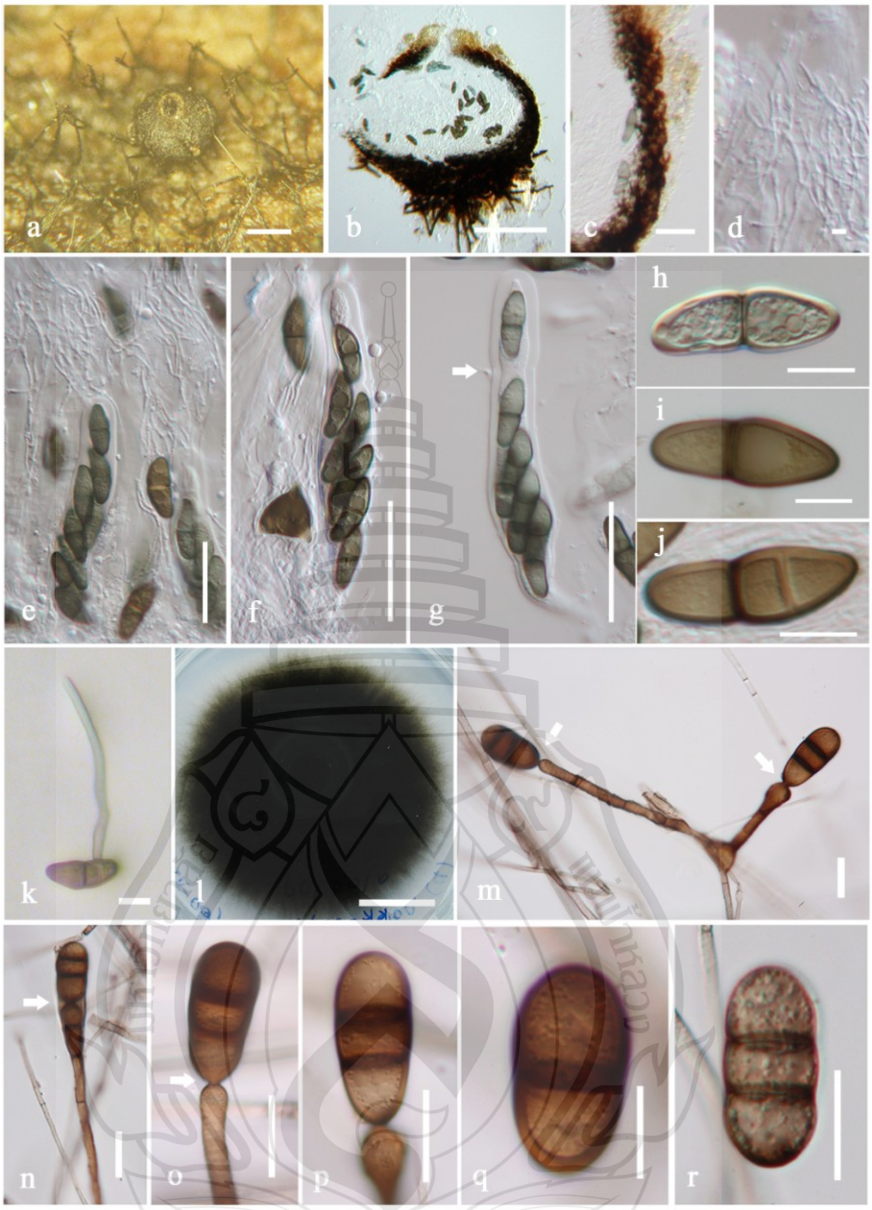
Dendryphiopsis S. Hughes, Can. J. Bot. 31: 655 (1953)

Notes: No genus description is provided as this is a monotypic family. Most species are widespread in tropical regions and commonly occur on dead wood. Additionally, both type species of sexual and asexual states are characteristic of the family (Boonmee et al., 2012). Currently three *Kirschsteiniothelia* species are assigned to the family: *K. aethiops*, *K. emarceis* and *K. lignicola* based on sequence data, while *K. maritima* (as *Halokirschsteiniothelia maritima*) groups in the *Mytilinidiaceae*, and *K. elaterascus* (as *Morosphaeria elaterascus*) in *Morosphaeriaceae* (Boonmee et al. 2012). The placement of other *Kirschsteiniothelia* species remains to be resolved.

Type species: *Kirschsteiniothelia aethiops* (Sacc.) D. Hawksw., J. Linn. Soc., Bot. 91(1-2): 185 (1985), MycoBank: MB 104401

Notes: Most species are widespread in tropical regions and commonly occur on dead wood. Additionally, both generic types of sexual and asexual states are characteristic of the family (Boonmee et al., 2012). Currently three *Kirschsteiniothelia* species are assigned to the family: *K. aethiops*, *K. emarceis* and *K. lignicola* based on sequence data, while *K. maritima* (as *Halokirschsteiniothelia maritima*) groups in the *Mytilindiaceae*, and *K. elaterascus* (as *Morosphaeria elaterascus*) in *Morosphariaceae* (Boonmee et al., 2012). The placement of other *Kirschsteiniothelia* species remains to be resolved.

Family type: *Kirschsteiniothelia aethiops* (Sacc.) D. Hawksw., J. Linn. Soc., Bot. 91(1-2): 185 (1985)



Note. (a) Ascoma appearance among its asexual morph. (b) Section through ascoma. (c) Peridium. (d) Pseudoparaphyses. (e)-(g) Asci and fissitunicate ascus arrowed in (g). (h)-(j) Ascospores. (k) Germinating ascospore. (l) Colony in culture. (m)-(p) Conidiophores and conidial attachment arrowed. (q)-(r) Conidia. Scale bars: (a)-(b) = 100 μm, (c), (m)-(r) = 20 μm, (d) = 5 μm, (e)-(g) = 40 μm, (h)-(k) = 10 μm, (l) = 1 cm

Figure 4.5 *Kirschsteiniothelia lignicola* (MFLU10–0036, holotype)

4.3.6 *Perisporiopsidaceae*

Perisporiopsidaceae E. Müll. & Arx ex R. Kirschner & T. A. Hofm., in Kirschner et al., Sydowia 62(2): 238 (2012), MycoBank: MB 81875

= *Parodiopsidaceae* Toro, J. Agric. Univ. P. Rico 36: 66 (1952)

Parasitic on living leaves or sooty moulds, pantropical. *Ascomata* superficial, solitary or gregarious, always unilocular, globose to subglobose, or ovate, brown to dark brown, with a dissolving pore, covered by hairy light brown hyphae, superficial mycelium, brown, septate, hyphopodia present or absent. *Peridium* dark brown, 3-4 cell layers of *textura angularis*. *Hamathecium* composed of hyaline, membranous tissues in a gelatinous matrix, pseudoparaphyses absent. *Asci* 8-spored, bitunicate, fissitunicate, ellipsoid-subclavate to broadly clavate, pedicellate, sometimes sessile. *Ascospores* 2–3-seriate, broadly ellipsoid-fusiform to oblong, 2–3-septate, not constricted at the septa, hyaline to pale brown. *Asexual state*: hyphomycetous represented by simple conidiophores, with monoblastic, annellidic or sympodial conidiogenous cells, producing pigmented, septate conidia.

Notes: The family *Parodiopsidaceae* was introduced (invalidly) by Arnaud (1920) to accommodate the genera *Parodiopsis*, *Perisporiopsis* and *Perisporina*, based mainly on their occurrence on leaf surfaces as mycelium with superficial ascomata. The family has since been compared with several families and orders (Arnaud, 1918, 1921; Barr, 1976, 1979, 1987; Hansford, 1946; Luttrell, 1951, 1955; von Arx & Müller, 1975). The type genus, *Parodiopsis* was found to be later homonym of *Perisporiopsis* by Arnaud (1915) and therefore, *Perisporiopsis* is the type representative of the family, which is based mainly on the original description of Hennings (1904a). The family includes species with globose-pigmented ascomata, usually present on superficial mycelium, and sometimes setose or hyphopodiate, a lack of pseudoparaphyses, broadly clavate asci and ellipsoidal fusiform, septate, hyaline ascospores which may be brownish when mature.

The family *Perisporiopsidaceae* was invalidly introduced by Müller and von Arx (1962) to combine the families of *Parodiellinaceae* and *Parodiopsidaceae*. Kirschner et al. (2010) validated *Perisporiopsidaceae* by providing a Latin diagnosis, while *Parodiopsidaceae* was treated as a synonym. Although the latter family name is younger it makes sense using *Perisporiopsidaceae* for a family represented by the genus *Perisporiopsis* (= *Parodiopsis*). Currently, there are 19 genera included in *Perisporiopsidaceae* which is grouped in *Dothideomycetes incertae sedis* (Lumbsch & Huhndorf, 2010). The family itself is certainly polyphyletic based on the morphology of its genera, the common denominator being small, often setose, ascomata on leaves. Molecular phylogenetic analysis is available for putative endophytic *Perisporiopsis* strains (Chaverri & Gazis, 2010), which shows the genus to be a possible member of *Pleosporales*. The placement of other genera in *Parodiopsidaceae* needs verification by molecular data.

The asexual states of most genera in *Perisporiopsidaceae* are reasonably well known and are hyphomycetous represented by simple conidiophores, with monoblastic, annellidic or sympodial conidiogenous cells, producing pigmented, septate conidia (Kirschner et al., 2010; Sivanesan, 1984). *Alina* and *Balladynopsis urtiaga* Sivan. have a *Tretospora* anamorphs (Kirschner et al., 2010; Sivanesan, 1981, 1984) and since the type of *Tretospora* is linked to *Balladynopsis* we synonymise these genera under *Balladynopsis* below.

Family type: *Perisporiopsis* Henn., Hedwigia 43: 83 (1904), MycoBank: MB 3827 (Fig. 4.6)

Parasitic causing leaf spots on living leaves of *Struthanthus* sp., pantropical. *Subiculum* comprising hairy, light brown, superficial mycelium covering ascomata, some hyphae entering the stomata and forming sausage-shaped haustoria in adjacent host cells. *Ascomata* immersed in subiculum, but superficial on host, solitary or gregarious, globose, subglobose to ovate, brown to dark brown, unilocular, opening by a pore in the upper lighter part of the ascomata, often stalked, hyphopodia not observed. *Peridium* comprising 3-4 layers of thick-walled, dark brown, cells of *textura angularis*. *Hamathecium* comprising hyaline, membranous tissue, in a gelatinous matrix, lacking pseudoparaphyses. *Asci* 8-spored, thick-walled, bitunicate, fissitunicate, ellipsoid-subclavate to broadly clavate, with a short indistinct pedicellate or sometime sessile, apically rounded and thickened, with an indistinct ocular chamber and apical ring. *Ascospores* 2-3-seriate, broadly ellipsoid-fusiform to oblong, 2-3-septate, not constricted at the septum, greenish hyaline to pale brown, minutely guttulate, smooth-walled. *Asexual state*: “Septoidium” hyphomycetous.

Material examined: BRAZIL, Rio de Janeiro, Serra dos Orgãos, on leaves of *Struthanthus* sp., August 1899, E. Ule no. 2631 (PC0084481, holotype).

Notes: The generic name *Perisporiopsis* was introduced by Hennings (1904a) with a single species *P. struthanthi* Henn. Maublanc (in Arnaud, 1915) also introduced *Parodiopsis* for species having aerial mycelium and producing haustoria, and included *P. melioloides* (Wint.) Maubl., *P. lateritia* (Speg.) Maubl., *P. struthanthi* (Henn.) G. Arnaud, *P. manaosensis* (Henn.) G. Arnaud and *P. viridescens* (Rehm.). Arnaud (1915) re-examined all type species of *Parodiopsis* and considered these taxa to be one genus (Eriksson, 1981). *Perisporiopsis* is the earlier name and so *Parodiella melioloides* was synonymized under *Perisporiopsis melioloides* (Berk. & M. A. Curtis) Arx (Müller & von Arx, 1962) and thus *Perisporiopsis struthanthi* is also the correct name. In this study, we examined and illustrate the fungal type specimen of *Perisporiopsis struthanthi* with ascospores that are 1-2-septate.

Perisporiopsis presently comprises 21 species epithets (Index Fungorum, www.indexfungorum.org/), although *P. wrightii* (Berk. & M. A. Curtis) F. Stevens was transferred to *Myriangiaceae* by Stevens (1917). Several species of *Perisporiopsis* including the generic type *P. struthanthi* are linked to hyphomycetous *Septoidium* asexual states (Arnaud, 1921; Chaverri & Gazis, 2010; Hansford, 1946; Kirk et al., 2008; Müller & von Arx, 1962; Sivanesan, 1984; Wijayawardene et al., 2012; von Arx & Muller, 1975).

Type species: *Perisporiopsis struthanthi* Henn., Hedwigia, Beibl. 43: 83 (1904), MycoBank: MB 216818

≡ *Parodiopsis struthanthi* (Henn.) G. Arnaud, Annls Épiphyt. 7: 54 (1921)

≡ *Perisporina struthanthi* (Henn.) Hansf., Proc. Linn. Soc. London 157: 144 (1946) [1944-45]

Other genera included

Alina Racib., Bull. int. Acad. Sci. Lett. Cracovie, Cl. Sci. Math. Nat. Sér. B, Sci. Nat. 3: 374 (1909) [link to *Septoidium*, *Tretospora* anamorphs]

Type species: *Alina jasmini* Racib., Bull. int. Acad. Sci. Lett. Cracovie, Cl. sci. Math. Nat. Sér. B, Sci. Nat. 3: 375 (1909)

Balladyna Racib., Parasit. Alg. Pilze Java's (Jakarta) 2: 6 (1900)

Type species: *Balladyna gardeniae* Racib., Parasit. Alg. Pilze Java's (Jakarta) 2: 6 (1900)

Balladynocallia Bat., in Batista, Silva & Bezerra, Atas Inst. Micol. Univ. Pernambuco 2: 216 (1965)

Type species: *Balladynocallia glabra* (Hansf.) Bat., in Batista, Silva & Bezerra, Atas Inst. Micol. Univ. Recife 2: 216 (1965)

Balladynopsis Theiss. & Syd., Annls Mycol. 15(6): 475 (1918) [1917] [*Tretospora* anamorph]

Type species: *Balladynopsis philippinensis* Syd. & P. Syd., Annls Mycol. 15(6): 476 (1918) [1917]

= *Alina* Racib., Bull. int. Acad. Sci. Lett. Cracovie, Cl. Sci. Math. Nat. Sér. B, Sci. Sat. 3: 374 (1909)

= *Tretospora* M.B. Ellis, More Dematiaceous Hyphomycetes (Kew): 374 (1976)

Chevalieropsis G. Arnaud, Annls Épiphyt. 9: 2 (1923). [*Septoidium* anamorph]

Type species: *Chevalieropsis ctenotricha* (Pat. & Har.) G. Arnaud, Annls Épiphyt. 9: 2 (1923)

Cleistosphaera Syd. & P. Syd., Annls Mycol. 14(1/2): 74 (1916)

Type species: *Cleistosphaera macrostegia* Syd. & P. Syd. [as 'macrostegiae'], Annls Mycol. 14(1/2): 75 (1916)

Dimeriella Speg., Revta Mus. La Plata 15(2): 12 (1908)

Type species: *Dimeriella hirtula* Speg., Revta Mus. La Plata 15(2): 12 (1908)

Dimerium (Sacc. & P. Syd.) McAlpine, Proc. Linn. Soc. N.S.W. 28: 98 (1903)

Type species: *Dimerium pulveraceum* (Speg.) Theiss., Beih. Bot. Zbl., Abt. 2 29: 66 (1912)

Dysrhynchis Clem., Gen. Fung. (Minneapolis): 32 (1909)

Type species: *Dysrhynchis pulchella* (Sacc.) Clem., in Clements & Shear, Gen. Fung., Edn 2 (Minneapolis): 253 (1931)

Hyalomeliolina F. Stevens, Illinois Biol. Monogr. (Urbana) 8:193 (1924)

Type species: *Hyalomeliolina guianensis* F. Stevens, Illinois Biol. Monogr. (Urbana) 8: 193 (1923)

Leptomeliola Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 128: 557 (1919)

Type species: *Leptomeliola hyalospora* (Lév.) Höhn., Sber. Akad. Wiss. Wien, Math. Nat. Kl., Abt. 1 128: 558 (1919)

Neoparodia Petr. & Cif., Annls Mycol. 30(3/4): 219 (1932)

Type species: *Neoparodia ekmanii* Petr. & Cif., Annls Mycol. 30(3/4): 219 (1932)

Ophiomeliola Starbäck, Bih. K. svenska VetenskAkad. Handl., Afd. 3 25(no. 1): 22 (1899)

Type species: *Ophiomeliola lindmanii* Starbäck, Bih. K. svenska VetenskAkad. Handl., Afd. 3 25(no. 1): 22 (1899)

Ophioparodia Petr. & Cif., Annls Mycol. 30(3/4): 223 (1932) [link to anamorphic *Septoidium*]

Type species: *Ophioparodia pulchra* Petr. & Cif., Annls Mycol. 30(3/4): 223 (1932)

Parodiellina Henn. ex G. Arnaud, Annals d'École National d'Agric. de Montpellier, Série 2 16(1-4): 21 (1918) [1917]

Type species: *Parodiellina manaosensis* (Henn.) G. Arnaud, Annals d'École National d'Agric. de Montpellier, Série 2 16(1-4): 21 (1918) [1917]

Pilgeriella Henn., Hedwigia 39(Beibl.): 137 (1900) [link to anamorphic *Septoidium*]

Type species: *Pilgeriella perisporioides* Henn., Hedwigia 39(Beibl.): 137 (1900)

Scolionema Theiss. & Syd., Annls Mycol. 15(6): 410 (1918) [1917]

Type species: *Scolionema palmarum* (Kunze) Theiss. & Syd., Annls Mycol. 15(6): 410 (1918) [1917]

Stomatogene Theiss., Annls Mycol. 14(6):406 (1917) [1916]

Type species: *Stomatogene agaves* (Ellis & Everh.) Theiss., Annls Mycol. 14(6): 406 (1918) [1917]

Key to genera of *Perisporiopsidaceae*

1. Ascospores non-septate..2
1. Ascospores septate 4
2. Ascomata globose-subglobose, cleistothecial........................ *Cleistosphaera*
2. Ascomata ovate to suboblong-ellipsoid, pseudothecial 3
3. Ascomata seated on hyphae, hypostroma from present............. *Parodiellina*
3. Ascomata not seated on hyphae, hypostroma absent.................... *Pilgeriella*
4. Ascospores 1-septate...5
4. Ascospores greater than 1-septate..13
5. Ascomata borne on a stroma...6
5. Ascomata with non stromatic development 7
6. Ascomata stroma, multi-loculate .... *Neoparodia*
6. Ascomata not stroma, uni-loculate ....*Chevalieropsis*
7. Ascomata without setae 8
7. Ascomata with setae 9
8. Ascomata associated with numerous hyphopodia ....*Balladynocallia*
8. Ascomata connected with long synnemata ....*Dysrhynchis*
9. Setae scattered around ascomata...10
9. Setae attached to ascomata...11
10. Hyphae with hyphopodia; ascospores olivaceous-brown to dark green.... *Balladyna*
10. Hyphae without hyphopodia; ascospores brown to dark brown ....*Stomatogene*
11. Ascospores smooth .... *Dimeriella*
11. Ascospores minutely verruculose 12
12. Mycelium with conidiophores ....*Alina*
12. Mycelium with hyphopodia ....*Balladnopsis*
13. Ascospores 7-8-septate 14
13. Ascospores 2-5-septate 17
14. Ascomata subglobose-ellipsoid; asci probably unitunicate .....*Ophiomeliola*
14. Ascomata globose-subglobose; asci clearly bitunicate.............................15
15. Ascospore with greater than 7-septate ....*Scolionema*
15. Ascospore with equally 7-septate 16
16. Ascospore narrowly cylindrical ....*Ophioparodia*

16. Ascospore broadly ellipsoid-fusiform................................... *Perisporiopsis*

17. Ascomata with crowded hyphae; ascospores dark brown ....*Hyalomeliolina*

17. Ascomata without appendage hyphae; ascospores hyaline 18

18. Ascospores slightly oblong-fusiform, 3-5-septate ....*Leptomelina*

18. Ascospores ellipsoid-subfusiform, (1-)-3-septate......................... *Dimerium*

Note. (a)-(b) Ascomata covered by brown hyphae. (c) Section of ascoma. (d) Peridium. (e)-(g) Asci. (h)-(k) Ascospores. Scale bars: (a) = 1 cm, (b) = 200 µm, (c) = 100 µm, (d) = 20 µm, (e)-(g) = 50 µm, (h)-(k) = 40 µm

Figure 4.6 *Perisporiopsis struthanthi* (PC, holotype)

4.3.7 *Protoscyphaceae*

Protoscyphaceae Kutorga & D. Hawksw., Syst. Ascom. 15(1-2):70 (1997), MycoBank: MB 81924

Parasitic on living leaves, pantropical. *Ascostromata* superficial, solitary, or scattered, discoid or cup-shaped, slightly convex or raised pulvinate, semi-immersed, flattened and thickened at the base, occurring in light brown lesions surrounded by external black mycelial areas, multilocular, opening by ruptured or cracking. *Peridium* thick, composed of dark cells arranged in a *textura angularis*. *Hamathecium* comprising relatively wide, anastomosing, septate pseudoparaphyses, often with swollen cells and intermixed with reddish brown colouration, surrounded by a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, subglobose-oblong, broadly cylindrical to subclavate, short pedicellate, with a wide, but short ocular chamber. *Ascospores* 2-3-seriate in the ascus, muriform, oblong to oval-subellipsoid, highly constricted at the septum, hyaline, pale brown or light brown. *Asexual state*: unknown.

Notes: The family *Protoscyphaceae* was introduced by Kutorga and Hawksworth (1997) for a fungal parasite on living leaves, with discoid ascomata similar to taxa in the *Cookellaceae*, *Elsinoaceae* and *Myriangiaceae* (Kutorga & Hawksworth, 1997; von Arx & Müller, 1975). The symptoms are most conspicuous on the leaf, with light brown and black areas, and roundish lesions up to 1 mm diam. The representative genus of this monotypic family is *Protoscypha* (Sydow, 1925). Ascostromata are typically darkened, containing a single row of monoascus locules, asci are bitunicate, broadly cylindrical to subclavate and thick-walled and ascospores are muriform, oblong to oval-subellipsoid, septate, hyaline to light brown, and covered by thin a gel. Kutorga and Hawksworth (1997) excluded *Protoscypha* from *Patellariaceae* and *Arthoniaceae* because of its distinctive morphology. *Protoscyphaceae* was placed as in *Dothideales incertae sedis* by Kutorga and Hawksworth (1997). There are no molecular data that confirms this relationship, however, Kirk et al. (2008), and Lumbsch and Huhndorf (2010) refer the genus to *Dothideomycetes incertae sedis*.

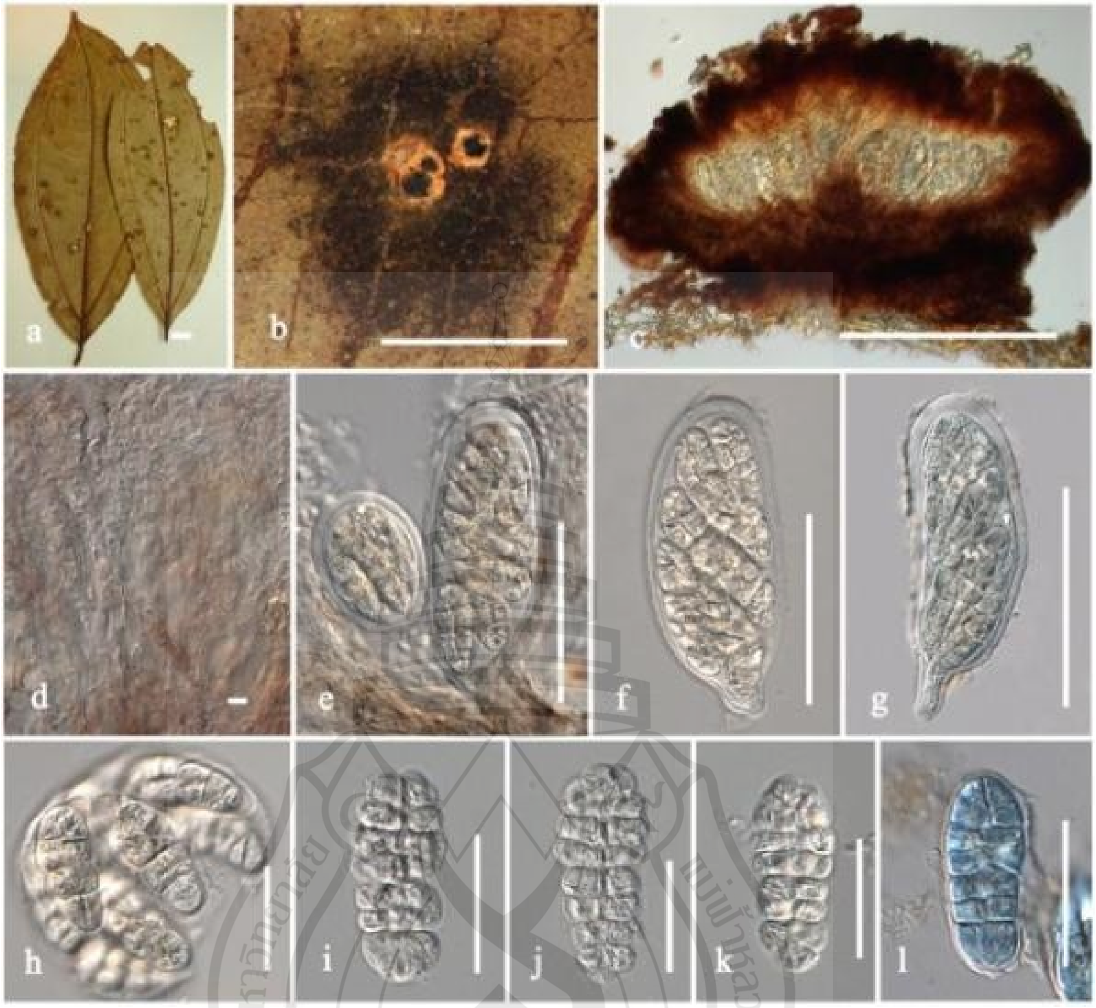
Family type: *Protoscypha* Syd., Annls Mycol. 23(3/6): 402 (1925), MycoBank: MB 4394 (Fig. 4.7)

Parasitic on living leaves of *Miconia thomasiana*. *Ascostromata* superficial, solitary, scattered, occurring on the lower leaf surface, discoid or cup-shaped, slightly convex or raised pulvinate, semi-immersed, flattened and thickened at the base, occurring in lesions of light brown regions and surrounded by external black mycelial areas up to 2-3 mm diam., multilocular, opening by rupturing or cracking of the apical ascostromata. *Peridium* thick, composed of dark cells arranged in a *textura angularis*. *Hamathecium* numerous, comprising hyaline, relatively wide, anastomosing, septate pseudoparaphyses, often with swollen cells and intermixed with reddish brown colouration, surrounded by a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, subglobose-oblong, broadly cylindrical to subclavate, somewhat thickened at the apex, short pedicellate, with a wide, but short ocular chamber. *Ascospores* 2–3-seriate in the ascus, muriform, oblong to oval-subellipsoid, 3–7-transeptate, with 1-longitudinal septa in each transverse row, highly constricted at the septum, hyaline, pale brown or light brown, surrounded by a thin gelatinous sheath. *Asexual state*: unknown.

Material examined: COSTA RICA, Los Angeles de San Ramon. on leaves of *Miconia thomasiana*, H. Sydow, 30 January 1925, BPI663043, holotype.

Notes: *Protoscypha* was introduced by Sydow (1925) based on the type species *Protoscypha pulla*. The genus comprises two tropical species (*P. pulla* Syd. and *P. subtropicum* (G. Winter) Petr.), and both are unusual as discussed above (Kutorga & Hawksworth, 1997; Sydow, 1925). Features of these two species are discoid or cup-like ascostromata, arranged as a single locule of oblong-subclavate asci, each locule separated by filamentous pseudoparaphyses.

Type species: *Protoscypha pulla* Syd., Annls Mycol. 23(3/6): 403 (1925), MycoBank: MB 275651



Note. (a)-(b) Ascostromata. (c) Section of ascostroma. (d) Pseudoparaphyses. (e)-(g) Asci. Note the rather thick walls and papillate pedicels. (h)-(l) Highly constricted muriform ascospores with 5-6 transverse septa and a single longitudinal septum in each transverse row. Note Figs. g and l were stained by lactophenol cotton blue . Scale bars: (a)-(b) = 2 mm, (c) = 200 µm, (d)-(g) = 50 µm, (h)-(l) = 20 µm

Figure 4.7 *Protoscypha pulla* (BPI, holotype)

4.3.8 ***Pseudoperisporiaceae***

Pseudoperisporiaceae Toro, in Seaver & Palacios Chardon, Scient. Surv. P. Rico 8(1): 40 (1926), MycoBank: MB 81227

Parasitic or *saprobic* on leaves, or other fungi, primarily in tropical regions. *Ascomata* superficial, solitary to gregarious, scattered, globose-subglobose, collapsed when dry, surrounded by brown mycelium at the base, central ostiole with 14-20 µm diam (on mounted slide) and surrounded by brown setae, septate, tapering to subacute at the apex. *Peridium* composed of *textura angularis*, brown to red brown, thin-walled, collapsed when dry. *Hamathecium* of cylindrical filiform, hyaline, branched, septate pseudoparaphyses. *Asci* bitunicate, fissitunicate, 8-spored, oblong-clavate, slightly curved, thickened at the apex, small ocular chamber, sessile, sometime knob-like, in a gelatinous matrix. *Ascospores* biseriate, fusoid-ellipsoid, rounded and subacute ends, 1-septate, slightly constricted at the septum, hyaline, becoming brownish at maturity, wall minutely verrucose. *Asexual state* coelomycetous *Chaetosticta*.

Notes: The family *Pseudoperisporiaceae* was introduced by Toro in Seaver and Chardén (1926) for mycoparasitic genera with superficial ascomata, mostly surrounded by external mycelium, with or without setae, darkly pigmented, asci and ascospores variously-shaped, with the genus *Pseudoperisporium* as family type and based on earlier taxon *Dimeriella erigeronicola* (Seaver & Chardón, 1926). *Pseudoperisporium erigeronicola* is synonym of *Lasiostemma melioloides*, the latter the type species of *Lasiostemma*, the earlier generic name. Finally, the genus *Pseudoperisporium* was replaced by *Lasiostemma* as correct name (Barr, 1987, 1997). Therefore, *Lasiostemma* is the generic type of the *Pseudoperisporiaceae*. The asexual state is known genus *Chaetosticta* (Coelomycetes), and related with two genera: *Lasiostemma* and *Nematostoma* (Hyde et al., 2011; Kirk et al., 2008). Lumbsch and Huhndorf (2010) list 22 genera in *Pseudoperisporiaceae* in Dothideomycetes *incertae sedis*. However additional molecular studies are required to elucidate the phylogenetic relationships of various genera in the family. Molecular data is available for the genera *Brychiton* and *Lizonia* (Stenroos et al., 2010).

Family type: *Lasiostemma* Theiss., Syd. & P. Syd., in Sydow & Sydow, Annls Mycol. 15(3/4): 218 (1917), MycoBank: MB 2660 (Fig. 4.8)

Saprobic on leaves of plants, or parastici on sooty moulds, primarily in tropical regions. *Ascomata* superficial, solitary to gregarious, scattered, globose-subglobose, surrounded by brown mycelium at the base, central ostiole and surrounded by brown setae, septate, tapering toward subacute at the apex, septate, peridial wall composed of angular-subglobosa cells, red brown, collapsing when dry. *Hamathecium* of wide, hyaline, branched, septate, pseudoparaphyses, often deliquescing. *Asci* 8-spored, bitunicate, fissitunicate, oblong-clavate, slightly curved, thickened at the apex, small ocular chamber, sessile or knob-like, embed in a gelatinous matrix. *Ascospores* biseriate, fusoid-ellipsoid, rounded and subacute ends, 1-septate, slightly constricted at the septum, hyaline, becoming brownish at maturity, minute verrucose. *Asexual state* coelomycetous e.g. *Chaetosticta*.

Material examined: VENEZUELA, El Limon, Puerto La Cruz Valley, Federal district, on leaves of *Erigeron bonariensis*, leg. Sydow H. (249), on 16 January 1928, BPI699548. Other herbaria: Fungi Venezuelani Sydow.

Notes: The genus *Lasiostemma* was finally accepted as generic type by Barr (1997) with type species *L. melioloides* (Berk. & M. A. Curtis) Theiss. & Syd. The typical characterized by ascomata superficial, spherical, numerous mycelium at the base, clearly central ostiole with surrounding setae; asci with fissitunicate, oblong-clavate, ocular chamber present, and ascospores mostly fusoid-ellipsoid, 1-septate, firstly hyaline and brownish when mature, with minutely verrucose-walled (Farr, 1979; Fig. 4.8k). Seventeen *Lasiostemma* species listed in Index Fungorum (www.indexfungorum.org/). While, two species *L. fimbriatus* and *L. irradians* were transferred to the genera *Wentiomyces* and *Epipolaeum,* respectively (Barr, 1968; Theissen & Sydow, 1918). The asexual state was reported as *Chaetosticta* (Crane, 1971; Hyde et al., 2011).

Type species: *Lasiostemma melioloides* (Berk. & Ravenel) Theiss., Syd. & P. Syd., in Sydow & Sydow, Annls Mycol. 15(3/4):218 (1917), MycoBank: MB 102249

≡ *Dimeriella melioloides* Berk. & Ravenel

Other genera included

Aphanostigme Syd., Annls Mycol. 24(5/6): 368 (1926)

Type species: *Aphanostigme solani* Syd., Annls Mycol. 24(5/6): 368 (1926)

Bryochiton Döbbeler & Poelt, in Döbbeler, Mitt. bot. StSamml., Münch. 14: 208 (1978)

Type species: *Bryochiton monascus* Döbbeler & Poelt, Mitt. Bot. St Samml., Münch. 14: 218 (1978)

Bryomyces Döbbeler, Mitt. Bot. St Samml., Münch. 14: 233 (1978)

Type species: *Bryomyces scapaniae* Döbbeler, Mitt. bot. StSamml., Münch. 14:254 (1978)

Epibryon Döbbeler, Mitt. bot. StSamml., Münch. 14: 260 (1978)

Type species: *Epibryon plagiochilae* (Gonz. Frag.) Döbbeler, Mitt. Bot. St Samml., Münch. 14: 293 (1978)

Episphaerella Petr., Annls Mycol. 22(1/2): 126 (1924)

Type species: *Episphaerella manihotis* (Henn.) Petr., Annls Mycol. 22(1/2): 126 (1924)

Eudimeriolum Speg., Anal. Mus. nac. Hist. Nat. B. Aires 23: 36 (1912)

Type species: *Eudimeriolum elegans* Speg., Anal. Mus. Nac. Hist. Nat. B. Aires 23: 36 (1912)

Eumela Syd., Annls Mycol. 23(3/6): 335 (1925)

Type species: *Eumela chiococcae* Syd., Annls Mycol. 23(3/6): 335 (1925)

Keratosphaera H.B.P. Upadhyay, Publções Inst. Micol. Recife 402: 5 (1964)

Type species: *Keratosphaera batistae* H.B.P. Upadhyay, Publções Inst. Micol. Recife 402: 6 (1964)

Lasiostemma Theiss., Syd. & P. Syd., in Sydow & Sydow, Annls Mycol. 15(3/4): 218 (1917)

Type species: *Lasiostemma melioloides* (Berk. & Ravenel) Theiss., Syd. & P. Syd., in Sydow & Sydow, Annls Mycol. 15(3/4): 218 (1917)

Lizonia (Ces. & De Not.) De Not., Sfer. Ital.: 72 (1863)

Type species: *Lizonia empirigonia* (Auersw.) De Not., Sfer. Ital.: 72 (1863)

Myxophora Döbbeler & Poelt, Mitt. bot. StSamml., Münch. 14: 315 (1978)

Type species: *Myxophora amerospora* Döbbeler & Poelt, Mitt. Bot. St Samml., Münch. 14: 315 (1978)

Nematostigma Syd. & P. Syd., Annls Mycol. 11(3): 262 (1913)

Type species: *Nematostigma obducens* Syd. & P. Syd., Annls Mycol. 11(3): 262 (1913)

Nematostoma Syd. & P. Syd., Annls Mycol. 12(2): 161 (1914)

Type species: *Nematostoma artemisiae* Syd. & P. Syd., Annls Mycol. 12(2): 161 (1914)

Nematothecium Syd. & P. Syd., Leafl. of Philipp. Bot. 5: 1534 (1912)

Type species: *Nematothecium vinosum* Syd. & P. Syd., Leafl. of Philipp. Bot. 5(76): 1534 (1912)

Neocoleroa Petr., Hedwigia 74: 38 (1934)

Type species: *Neocoleroa sibirica* Petr., Hedwigia 74: 38 (1934)

Ophiociliomyces Bat. & I.H. Lima, Anais Soc. Biol. Pernambuco 13(2): 29 (1955)

Type species: *Ophiociliomyces bauhiniae* Bat. & I.H. Lima, Anais Soc. Biol. Pernambuco 13(2): 30 (1955)

Phaeodimeriella Speg., Revta Mus. La Plata 15(2): 13 (1908)

Type species: *Phaeodimeriella occulta* (Racib.) Speg., Revta Mus. La Plata 15(2): 13 (1908)

Phaeostigme Syd. & P. Syd., Annls mycol. 15(3/4): 199 (1917)

Type species: *Phaeostigme picea* (Berk. & M.A. Curtis) Syd. & P. Syd., Annls Mycol. 15(3/4): 200 (1917)

Phragmeriella Hansf., Mycol. Pap. 15: 89 (1946)

Type species: *Phragmeriella ireninae* Hansf., Mycol. Pap. 15: 89 (1946)

Pododimeria E. Müll., Sydowia 12(1-6): 193 (1959) [1958]

Type species: *Pododimeria gallica* E. Müll., Sydowia 12(1-6): 195 (1959) [1958]

Raciborskiomyces Siemaszko, Acta Soc. Bot. Pol. 2: 270 (1925)

Type species: *Raciborskiomyces polonicus* Siemaszko, Acta Soc. Bot. Pol. 2(4): 270 (1925)

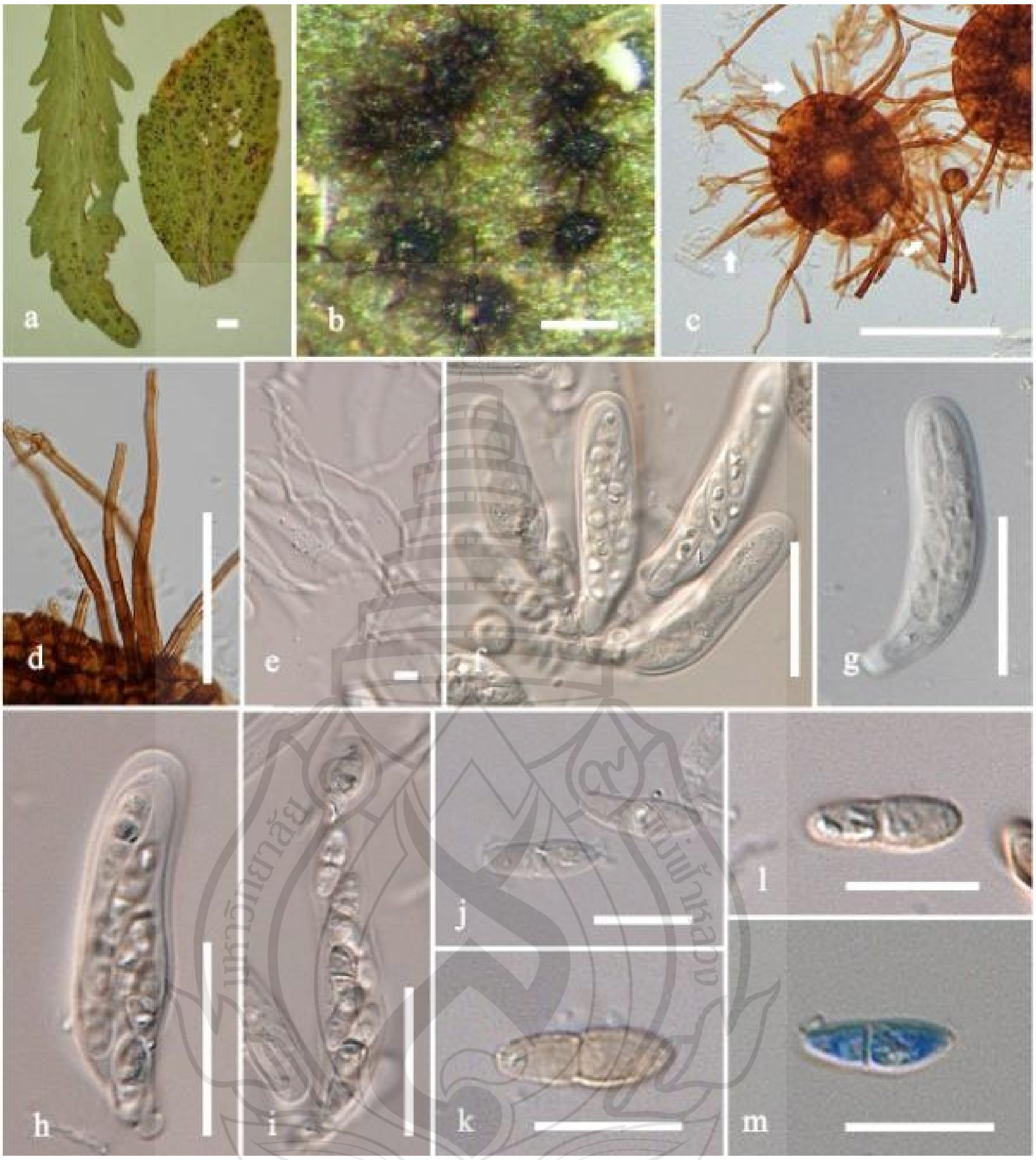
Toroa Syd., in Toro, J. Dept. Agric. Porto Rico 10(2): 19 (1926)

Type species: *Toroa dimerosporioides* (Speg.) Syd. [as 'dimerosporoides'], J. Dept. Agric. Porto Rico 2: 20 (1926)

Key to genera of *Pseudoperisporiaceae*

1. Ascomata superficial, with setae and/or hyphae..2
1. Ascomata superficial, without setae and/or hyphae...................................12
2. Pseudoparaphyses present; ascospores only 1-septate.................................3
2. Pseudoparaphyses present; ascospores more than 1-septate.........................7
3. Asci subglobose, ellipsoid-obclavate.. *Eumela*
3. Asci broadly cylindrical-clavate, saccate...4
4. Ascomata collapsed when dry; ascospores minute verrucose ... *Lasiostemma*
4. Ascomata not collapsed; ascospores 1-septate, non verrucose......................5
5. Ascospores 1-septate, green-olivaceous to brownish-olivaceous..... *Raciborskiomyces*
5. Ascospores 1-septate, hyaline...6
6. Ascospores ellipsoid-narrowly fusiform, 10-12.5 µm × 2.5 µm ..*Neocoleroa*
6. Ascospores ellipsoid-fusiform, 13-15(-17) × 4-5.5 µm...................*Epibryon*
7. Ascospores 3-septate..8
7. Ascospores more than 3-septate...11
8. Asci ellipsoid-obclavate, subglobose......................................*Keratosphaera*
8. Asci cylindrical-clavate 9
9. Ascospores pale brown ....*Nematostoma*
9. Ascospores hyaline 10
10. Ascospores narrowly fusiform-subclavate.............................*Aphanostigme*
10. Ascospores ellipsoid-subclavate .... *Phragmeriella*
11. Ascospores broadly fusiform, hyaline .... *Nematostigma*
11. Ascospores parallel, elongate fusiform, brown to reddish brown...... *Nematothecium*
12. Ascospores without septate.. *Myxophora*
12. Ascospores with septate 13
13. Ascospores dictyosporous..*Bryochiton*
13. Ascospores didymosporous, phragmosporous..14
14. Ascospores 1-septate...15
14. Ascospores more than 1-septate...21

15. Ascospores hyaline 16
15. Ascospores pale brown, light brown, brown to red brown 17
16. Ascospores narrowly oblong-ellipsoid .... *Episphaerella*
16. Ascospores ellipsoid-subfusiform..*Eudimeriolum*
17. Ascospores hyaline, brown to red brown, with gelatinous sheath .... *Phaeostigme*
17. Ascospores hyaline, brown, without gelatinous sheath 18
18. Ascospores broadly ellipsoid, rounded ends................................*Bryomyces*
18. Ascospores ellipsoid, obovoid 19
19. Ascomata with pedicel; ascospores olivaceous-brown ....*Pododimeria*
19. Ascomata without pedicel; ascospores light brown to brown 20
20 Ascospores oblong fusiform ....*Lizonia*
20. Ascospores narrow ellipsoid...*Phaeodimeriella*
21. Ascomata collapsed; ascospores elongate cylindrical-fusiform, hyaline .. *Ophiociliomyces*
21. Ascomata not collapsed, ascospores ellipsoid-fusiform, brown to red brown ..... *Toroa*



Note. (a)-(b) Ascomata. (c) Squash mount of ascoma throughout peridium, surrounded by setae (arrows) and light coloured ostiole. (d) Mycelium. (e) Pseudoparaphyses. (f)-(i) Asci at immature and mature states, with small ocular chamber (fissitunicate ascus in Fig. i). (j)-(m). Ascospores (strained by cotton blue in Fig. m). Scale bars: (a) = 5 mm, (b)-(c) = 100 μm, (d) = 50 μm, (f)-(i) = 20 μm, (j)-(m) = 10 μm

Figure 4.8 *Lasiostemma melioloides* (BPI, authentic specimen)

4.3.9 *Tubeufiaceae*

Tubeufiaceae Barr, Mycologia 71(5): 948 (1979), MycoBank: MB 81599

The family has recently been revised by Boonmee et al. (2011). It is part of the present study. The detail description of the fungal strains in this family was previously described in Chapter 2 (Section 2.3.2) and in Chapter 5 (Section 5.3.2).

Asexual state hyphomycetous, helicosporous. *Conidiophores* mononematous, macro to micronematous, erect or flexuous, septate, moderately to dark brown in colour. *Conidiogenous* cells holoblastic, mono- or polyblastic, integrated or discrete, terminal or intercalary. *Conidia* elongated, filiform to fusiform, curved, helicoid with varied number of coils, septate, sometimes dictyosporous, phragmosporous, hyaline to variedly coloured, smooth to verrucose, and as follows *Aquaphila, Chlamydotubeufia, Helicoma, Helicomyces, Helicosporium,* Monodictys-like*, Pendulispora, Peziotrichum, Tamhinispora* and *Xenosporium*.

Notes: The family *Tubeufiaceae* was introduced by Barr (1979) for saprobes and parasites with superficial, brightly, coloured ascomata, saccate asci, narrow, hyaline, multi-septate ascospores, and hyphomycetous asexual forms (Barr, 1979, 1980; Sivanesan, 1984; Kodsueb, Jeewon et al., 2006; Boonmee et al., 2012). Various genera have been assigned to the *Tubeufiaceae* ranging from four to six (Barr, 1979, 1987), 12 (Rossman, 1987), 23 (Eriksson, 2005) and 32 (Kirk et al., 2008). Molecular data, based on 28S rDNA, established the *Tubeufiaceae* as a well-supported family in the *Pleosporales* with sequences of eight taxa, comprising sexual and asexual morphs (Kodsueb, Jeewon et al., 2006). Kodsueb, Jeewon et al. (2006) accepted 18 genera although not all were examined. Boonmee et al. (2012) revisited the family and included sequences from fresh collections of the type species of the genera *Acanthostigma*, *Acanthophiobolus*, *Kamalomyces*, *Podonectria*, *Thaxteriella* and *Thaxteriellopsis* in their phylogenetic analyses of LSU. They maintained 21 genera, while some genera were excluded because of inconsistent morphology and lack of molecular data (*Allonecte*, *Letendraeopsis*, *Byssocallis*, *Taphrophila* and *Thaxterina*).

Based on combined ITS and LSU sequences with parsimony and Bayesian analyses, representatives of the *Tubeufiaceae* grouped in six well-supported clades. Currently, many genera are retained in *Tubeufiaceae* with distinctly different characters and molecular data are required to evaluate their placement. Asexual links to genera in the family have been shown by ITS and LSU sequences of 46 taxa (Tsui et al. 2006, 2007) resulting in the identification of nine well-supported clades. Based on recent morphological and molecular phylogenetic studies, we conclude that *Tubeufiaceae* comprises with 16 genera i.e. *Acanthophiobolus, Acanthostigma, Acanthostigmina*, *Aquaphila, Boerlagiomyces, Chaetosphaerulina, Chlamydotubeufia, Helicoma*, *Helicomyces*, *Helicosporium*, *Kamalomyces, Podonectria, Tamhinispora*, *Thaxteriella, Thaxteriellopsis* and *Tubeufia* (Boonmee et al., 2011; Rajeshkumar & Sharma, 2013; Chapter 5).

Family type: *Tubeufia* Penz. & Sacc., Malpighia 11(11-12): 517 (1898), MycoBank: MB 5635 (Fig. 4.9)

Notes: The genus *Tubeufia* was introduced by Penzig and Saccardo (1898) with *Tubeufia javanica* as the type and described from *Bambusa emarcidis* in Java, Indonesia. Currently 49 epithets are listed for the genus in Index Fungorum (2013). Other species are presumed to be related with typical *Tubeufia* based on similar morphological characters, such as superficial ascomata, some seated on a subiculum that are globose-subglobose to obclavate and brightly or darkly pigmented, asci and ascospores that are mostly narrow cylindrical-oblong with many septate, hyaline to pale brown. Besides, some *Tubeufia* species reproduce asexually in culture and are connected with asexual genera i.e. *Aquaphila*, *Helicoma, Helicomyces* or *Helicosporium* Most of these established links have been confirmed by cultural methods and phylogenetic relationships (Tsui et al., 2006, 2007; Promputtha & Miller, 2010; Sánchez & Bianchinotti, 2010; Sánchez, Miller & Bianchinotti, 2012; Boonmee et al., 2011).

Type species: *Tubeufia javanica* Penz. & Sacc., Malpighia 11(11-12): 517 (1898), MycoBank: MB 244755

Other genera included

Acanthostigma De Not., Sfer. Ital.: 85 (1863)

Type species: *Acanthostigma perpusillum* De Not., Sfer. Ital.: 207 (1863)

Acanthostigmina Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 118: 1499 [39 repr.] (1909)

Type species: *Acanthostigmina minuta* (Fuckel) Clem. & Shear, Gen. Fung. (Minneapolis): 270 (1931)

Acanthophiobolus Berl., Atti Congl. Bot. Intern. di Genova, 1892: 571 (1893) [1892]

Type species: *Acanthophiobolus helminthosporus* (Rehm) Berl., Die Pilze des Weinstockes, Vienna: 571 (1893) [1892]

Aquaphila Goh, K.D. Hyde & W.H. Ho, Mycol. Res. 102(5): 588 (1998)

Type species: *Aquaphila albicans* Goh, K.D. Hyde & W.H. Ho, Mycol. Res. 102(5): 588 (1998)

Boerlagiomyces Butzin, Willdenowia 8(1): 39 (1977)

Type species: *Boerlagiomyces velutinus* (Penz. & Sacc.) Butzin, Willdenowia 8(1): 39 (1977)

Chaetosphaerulina I. Hino, Bull. Miyazaki Coll. Agric. Forest. 10: 62 (1938)

Type species: *Chaetosphaerulina yasudai* I. Hino, Canad. J. Plant Sci. 10: 62 (1938)

Chlamydotubeufia Boonmee & K.D. Hyde, Fungal Diversity 51(1): 78 (2011)

Type species: *Chlamydotubeufia huaikangplaensis* Boonmee & K.D. Hyde, Fungal Diversity 51(1): 78 (2011)

Helicoma Corda, Icon. Fung. (Prague) 1: 15 (1837)

Type species: *Helicoma muelleri* Corda, Icon. Fung. (Prague) 1: 15 (1837)

Helicomyces Link, Mag. Gesell. naturf. Freunde, Berlin 3(1-2): 21 (1809)

Type species: *Helicomyces roseus* Link, Mag. Gesell. naturf. Freunde, Berlin 3(1-2): 21 (1809)

Helicosporium Nees, Syst. Pilze (Würzburg): 68 (1816) [1816-17]

Type species: *Helicosporium vegetum* Nees, Syst. Pilze (Würzburg): 68 (1816) [1816-17]

Kamalomyces R.K. Verma, N. Sharma & Soni, Forest Fungi of Central India: 196 (2008)

Type species: *Kamalomyces indicus* R.K. Verma, N. Sharma & Soni, Forest Fungi of Central India: 196 (2008)

Podonectria Petch, Trans. Br. mycol. *Soc*. 7(3): 146 (1921)

Type species: *Podonectria coccicola* (Ellis & Everh.) Petch, Trans. Br. Mycol. Soc. 7(3): 146 (1921)

Tamhinispora Rajeshkumar & Rahul Sharma, Mycosphere 4(2): 166 (2013)

Type species: *Tamhinispora indica* Rajeshkumar & Rahul Sharma, Mycosphere 4(2): 167 (2013)

Thaxteriella Petr., Annls mycol. 22(1/2): 63 (1924)

Type species: *Thaxteriella corticola* Petr., Annls Mycol. 22(1/2): 63 (1924)

Thaxteriellopsis Sivan., Panwar & S.J. Kaur, Kavaka 4: 39 (1977) [1976]

Type species: *Thaxteriellopsis lignicola* Sivan., Panwar & S.J. Kaur, Kavaka 4: 39 (1977) [1976]

Tubeufia Penz. & Sacc., Malpighia 11(11-12): 517 (1898)

Type species: *Tubeufia javanica* Penz. & Sacc., Malpighia 11(11-12): 517 (1898)

Key to genera of *Tubeufiaceae*

1. Sexual morphs present; ascomata with asci and ascospores..........................2

1. Asexual morphs present; both in natural habitat or in culture.....................12

2. Ascomata globose-subglobose, with setae/hyphae.......................................3

2. Ascomata subglobose, obovate, without setae/hyphae................................10

3. Ascomata covered by stiff setae, brown to dark brown, apically acute.........4

3. Ascomata covered by flexuous hairy hyphae 8

4. Ascospores narrowly filiform, spiral, longer than 100 µm .... *Acanthophiobolus*

4. Ascospores fusiform or clavate-fusiform, not spiral, shorter than 100 µm 5

5. Ascospores equally 5-septate...*Thaxteriellopsis*

5. Ascospores greater than 5-septate..6

6. Ascospores heavily guttulate ....*Chlamydotubeufia*

6. Ascospores non-guttulate...7

7. Ascospores hyaline, non mucilaginous sheath........................*Acanthostigma*

7. Ascospores hyaline to pale brown, thin mucilaginous sheath......... *Acanthostigmina*

8. Ascospores elongate-fusiform, less than 25-septate ....*Chaetosphaerulina*

8. Ascospores clavate-fusiform, cylindric-fusoid, greater than 25-septate........9

9. Ascospores muriform..*Boerlagiomyces*

9. Ascospores not muriform...*Kamalomyces*

10. Ascomata formed on scale insects ....*Podonectria*

10. Ascomata formed on decaying or rotting wood..11

11. Ascospores allantoid or worm-like, only 7-septate...................*Thaxteriella*

11. Ascospores elongated cylindrical-subfusiform, greater than 7-septate...... *Tubeufia*

12. Conidia dictyosporous, apically appendages, heavily pigmented..... *Tamhinispora*

12. Conidia not dictyosporous, non-appendages, hyaline, pale brown to brown 13

13. Conidia phragmosporous, fusoid to sickle-shaped, slightly curved....*Aquaphila*

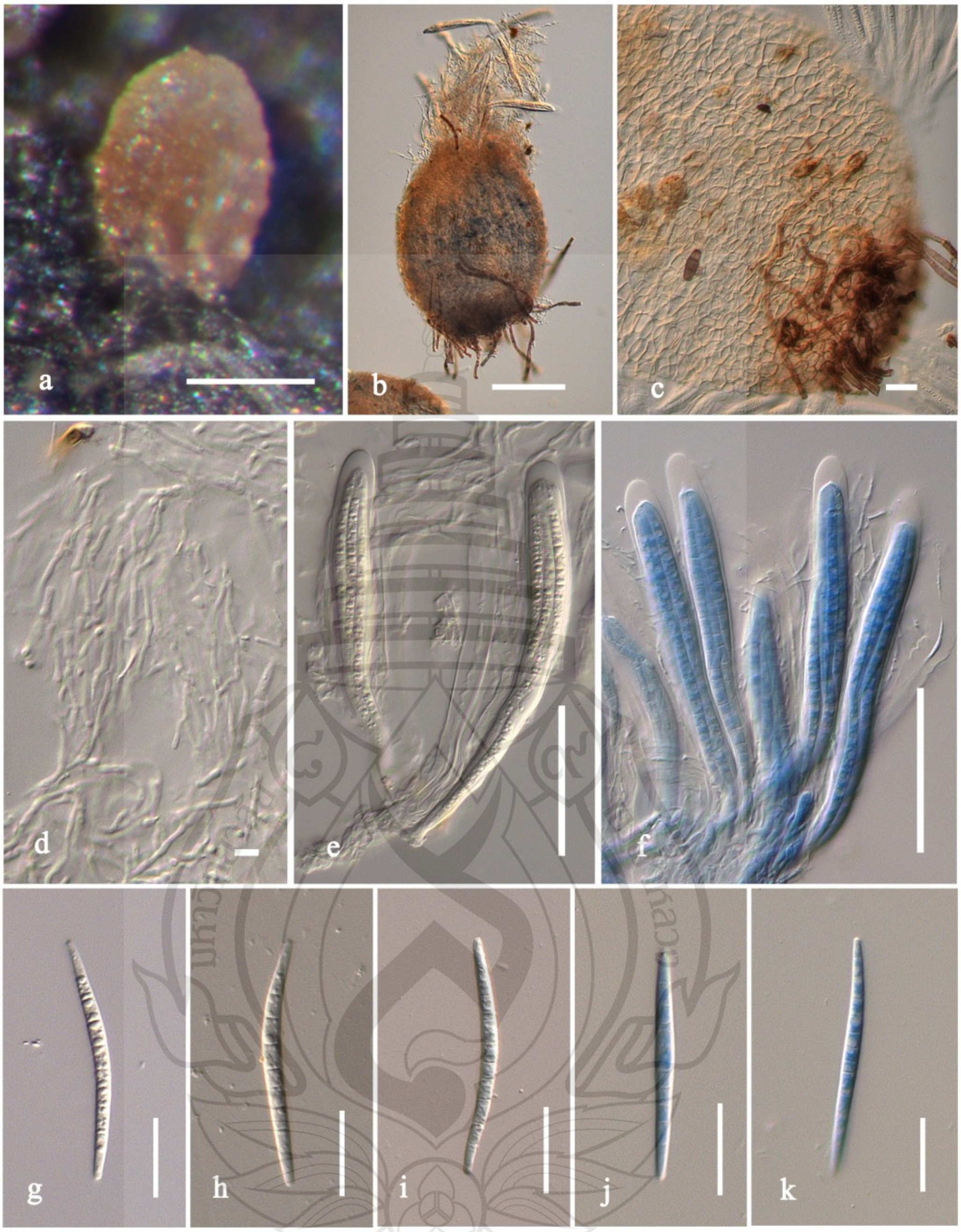
13. Conidia helicosporous..14

14. Conidiophores absent, conidia arising directly on hyphae ....*Helicomyces*

14. Conidiophores present, dark brown, many septate 15

15. Conidia coiled 1-1½ times, conidial filament wider than 6 µm ....*Helicoma*

15. Conidia coiled 3½-4½ times, conidial filament narrower than 6 µm ....*Helicosporium*



Note. (a) Ascoma seated on sparsely hyphae. (b)-(c) Squash mount of ascoma through peridium. (d) Pseudoparaphyses. (e)-(f) Asci. (g)-(k) Ascospores. Note asci and ascospores in blue were strained by cotton blue. Scale bars: (a)-(b) = 100 μm, (c) = 20 μm, (d) = 5 μm, (e)-(f) = 50 μm, (g)-(k) = 20 μm

Figure 4.9 *Tubeufia paludosa* (BPI, authentic specimen)

4.4 Conclusion

In this study, the generic types of nine families of Dothideomycetes, viz *Ascoporiaceae, Aulographaceae, Coccoideaceae, Cookellaceae, Kirschsteiniotheliaceae, Perisporiopsidaceae, Protoscyphaceae, Pseudoperisporiaceae* and *Tubeufiaceae* were examined using the herbarium type material. The type species of each family were determined in terms of morphology. Their detailed information was described and illustrated; these data are expected to be useful as references for future study. In addition, the identification key for these fungal species was proposed.

CHAPTER 5

Tubeufiales ord. nov. INTEGRATING SEXUAL AND ASEXUAL GENERIC NAMES

5.1 Introduction

The family *Tubeufiaceae* was established and accommodated in *Pleosporales* by Barr (1979, 1987). Most genera of *Tubeufiaceae* have uniloculate, superficial and pigmented (e.g. pale brown, brown, and dark brown to black), ascomata, and mostly multi-celled and hyaline ascospores. Many species of *Tubeufiaceae* such as *Aquaphila, Chlamydotubeufia*, *Helicoma, Helicomyces* and *Helicosporium* produce helicosporous asexual states (Boonmee et al., 2011; Tsui et al., 2006). With the integration of sexual and asexual names, it is necessary to determine which linked generic names have priority of application and thus should be used (Greuter et al., 2011; Hawksworth, 2011; McNeill, Turland, Monro & Lepschi, 2011; Taylor, 2011). There are several methods that are being used in traditional and modern mycology to accomplish taxonomic studies of fungi (Abarenkov et al., 2010; Berbee & Taylor, 2001; Brock, Döring & Bidartondo, 2009; Eberhardt, 2010; Figueiredo et al., 2010; Hibbett et al., 2007; 2011; Hyde & Zhang, 2008; Lücking, 2008; Lutzoni et al., 2004; Roe, Rice, Bromilow, Cooke & Sperling, 2010; Stajich et al., 2009). Traditionally, morphological characteristics were initially used to identify the taxa of *Tubeufiaceae*. Presently, molecular phylogenetic approaches revealed that existence of *Tubeufiaceae* forming a monophyletic clade in the Dothideomycetes (Schoch et al., 2006, 2009; Zhang et al., 2011). Sequencing of the conserved genes, mainly in the LSU (28S) and SSU (18S) rDNA regions, is used to analyze at familial and genera levels, and to study their evolutionary phylogenetic relationships (Boonmee et al., 2011; Tsui & Berbee, 2006).

The objective of the present study is to transfer the family *Tubeufiaceae* which has previously been classified in the order *Pleosporales* to a newly introduced order *Tubeufiales*. We also connect and accept the hitherto known genera of asexual and sexual morphs into a taxonomic framework in the family based on multigene phylogeny and morphology.

5.2 Materials and Methods

5.2.1 Examination of Herbarium Specimens

The generic types or authentic specimens of tubeufiaceous genera as follows *Acanthostigmella, Acanthostigmina, Chaetocrea, Helicoma, Helicomyces, Helicosporium, Malacaria,Paranectriella,Puttemansia, Rebentischia, Thaxteriella, Tubeufia* and *Uredinophila* were obtained from the repositories of U. S. National Fungus Collections (BPI), Farlow Reference Library and Herbarium of Cryptogamic Botany in Harvard University (FH), Kew Royal Botanic Gardens (K), and Naturhistorisches Museum Wien (W). Morphological details were examined and photomicrographs made using Carl Zeiss Microscopes, measurement confirmed using software of Micro Imaging GmbH. AxioVs40 V 4.8.2.0 (2006-2010). Morphologically microcharacters were detailed by description and illustration as described in Section 2.1.1, Chapter 2. The classification of *Tubeufiaceae* members is presently shown in Table 5.1.

5.2.2 Sample Fresh Collections

Fresh collections of *Tubeufiaceae* were made in northern Thailand (Chiang Mai and Chiang Rai). Newly collected specimens were characterised morphologically based on the protocol described in Chapter 2 (see Section 2.1.1). Single spore isolates were also carried out as previously shown in Section 2.2.2. The DNA experiment (i.e., DNA extraction, PCR, and DNA sequencing) were performed as described in Section 2.2.3. For phylogenetic analysis, TEF1-α and RPB2 gene sequences were also used in the phylogenetic analysis. The primers used were EF1-983F (GCY CCY GGH CAY CGT GAY TTY AT), EF1-2218R (AT GAC ACC RAC RGC RAC RGT YTG), fRPB2-5F (GAY GAY MGW GAT CAY TTY GG), and fRPB2-7cR (CCC ATR GCT TGY TTR CCC AT) (Liu, Whelen & Hall, 1999; Rehner & Buckley, 2005).

5.2.3 Phylogenetic Analysis

A BLAST search of new sequences were performed to verify identities of the closely related with sexual and asexual states, *Tubeufiaceae* in the GenBank database. Details of sequences are provided in Supplementary Table 5.2.

The phylogenetic analyses, provided two datasets for tubeufiaceous fungi. The first dataset, consists of multigene analysis of LSU, SSU, TEF1-α and RPB2, comprises 118 taxa including an outgroup, with 2847 characters to evaluate the taxonomic placement of *Tubeufiales* within the Dothideomycetes. The second dataset, was a combined multigene analysis of LSU, ITS, TEF1-α and RPB2, consisting of 82 taxa including an outgroup, with 2671 characters to focus on evolutionary relationships within members of the *Tubeufiaceae*.

Sequences and alignments were prepared by using the MAFFT v.7 web server (http://mafft.cber.jp/alignment/server/), manually improved and sequences concatenated were performed by BioEdit (Hall, 1999), for missing sequence positions are coded as "N". The construction of the maximum likelihood (ML) analysis using RAxML v. 7.2.8 (Stamatakis, Hoover, & Rougemont, 2008) as part of the "RAxML-HPC BlackBox on TG tool" performed at the CIPRES Science Gateway V. 3.3 (http://www.phylo.org/portal2/; Miller, Pfeiffer & Schwartz, 2010). Final ML search will be conducted under the GAMMA model. Executing 1000 rapid bootstrap inferences and thereafter a thorough ML search. Stopped rapid BS search after 300 replicates with MRE-based Bootstopping criterion. The RAxML analysis resulted in a best scoring likelihood tree selected with a final in value of -44888.616670 and -16007.915678 for the first run on datasets *Tubeufiales* and *Tubeufiaceae*, respectively. Phylogenetic trees were figured in Treeview (Page, 1996).

The Bayesian phylogeny was constructed with the program MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001) on CIPRES Science Gateway V. 3.3, using the parameter setting of 2 parallel runs, 4 chains, carried out for 4 000 000 generations, sample frequency every 1000 generations and all other parameters were left as default. The 50% majority rule consensus tree was constructed from the remaining trees in Treeview (Page, 1996).

Table 5.1 Various Classification of *Tubeufiaceae*

Barr (1979)	Barr (1980)	Rossman (1987)	Kirk et al. (2001)	Lumbsch & Huhndorf (2010)	Boonmee et al. (2011)	In this study
Letendraea	*Allonectria*	*Allonectria*	*Acanthophiobolus*	*Acanthostigma*	*Acanthostigma*	*Acanthophiobolus*
Melioliphila	*Boerlagiomyces*	*Boerlagiomyces*	*Acanthostigmella*	*Acanthophiobolus*	*Acanthophiobolus*	*Acanthostigma*
Podonectria	*Byssocallis*	*Byssocallis*	*Allonecte*	*Acanthostigmella*	*Acanthostigmella*	*Acanthostigmina*
Rebentischia	*Letendraea*	*Letendraea*	*Amphinectria*	*Allonecte*	*Amphinectria*	*Aquaphila*
Thaxteriella	*Melioliphila*	*Malacaria*	*Boerlagiomyces*	*Amphinectria*	*Aquaphila*	*Boerlagiomyces*
Tubeufia	*Paranectriella*	*Melioliphila*	*Borinquenia*	*Boerlagiomyces*	*Boerlagiomyces*	*Chaetosphaerulina*
	Podonectria	*Paranectriella*	*Byssocallis*	*Byssocallis*	*Chaetocrea*	*Chlamydotubeufia*
	Puttemansia	*Podonectria*	*Chaetocrea*	*Chaetocrea*	*Chaetosphaerulina*	*Helicoma*
	Rebentischia	*Puttemansia*	*Glaxoa*	*Chaetosphaerulina*	*Glaxoa*	*Helicomyces*
	Tubeufia	*Rebentischia*	*Letendraea*	*Glaxoa*	*Kamalomyces*	*Helicosporium*
		Tubeufia	*Letendraeopsis*	*Letendraeopsis*	*Malacaria*	*Kamalomyces*
		Uredinophila	*Malacaria*	*Malacaria*	*Melioliphila*	*Podonectria*
			Melioliphila	*Melioliphila*	*Paranectriella*	*Tamhinispora*
			Paranectriella	*Paranectriella*	*Podonectria*	*Thaxteriella*
			Podonectria	*Podonectria*	*Puttemansia*	*Thaxteriellopsis*
			Puttemansia	*Puttemansia*	*Rebentischia*	*Tubeufia*
			Rebentischia	*Rebentischia*	*Thaxteriella*	
			Taphrophila	*Taphrophila*	*Thaxteriellopsis*	
			Thaxterina	*Thaxteriella*	*Tubeufia*	
			Tubeufia	*Thaxteriellopsis*	*Uredinophila*	
			Uredinophila	*Thaxterina*		
				Tubeufia		
				Uredinophila		

Table 5.2 Fungal Taxa Used for Phylogenetic Analysis with GenBank Accession Numbers and New sequences of *Tubeufiaceae* from Thailand are marked by an asterisk (*)

Fungal isolates	Sources of culture	GenBank accession numbers				
		LSU	SSU	ITS	TEF1-α	RPB2
Acanthostigma chiangmaiensis	MFLUCC100125	JN865197	JN865185	JN865209	KF301560	–
Acanthostigma filiforme	ANM 101	GQ850495	–	–	–	–
Acanthostigma filiforme	ANM 514	GQ850494	–	GQ856146	–	–
*Acanthostigma fusiforme**	MFLUCC110510	KF301537	KF301546	KF301529	–	–
Acanthostigma minutum	ANM 238	GQ850487	–	–	–	–
Acanthostigma minutum	ANM 818	GQ850488	–	–	–	–
Acanthostigma minutum	ANM 880	GQ850486	–	–	–	–
Acanthostigma multiseptatum	ANM475	GQ850492	–	GQ856145	–	–
Acanthostigma multiseptatum	ANM665	GQ850493	–	GQ856144	–	–
Acanthostigma patagonica	BBB MVB573	JN127359	–	JN127358	–	–
Acanthostigma perpusillum	UAMH 7237	AY856892	AY856937	AY916492	–	–
Acanthostigma scopulum	ANM 95	GQ850490	–	–	–	–
Acanthostigma scopulum	ANM 386	GQ850489	–	–	–	–
Acanthostigma septoconstrictum	ANM 536.1	GQ850491	–	GQ856143	–	–
*Acanthostigma lignicola**	MFLUCC110378	KF301531	KF301539	KF301523	KF301552	KF301547
Acanthostigmina piniraiensis *	MFLUCC100116	KF301534	KF301542	KF301526	KF301555	–
Aliquandostipite khaoyaiensis	CBS118232	GU301796	AF201453	–	GU349048	FJ238360
Aquaphila albicans	BCC3520	DQ341102	DQ341091	DQ341098	–	–
Aquaphila albicans	BCC3543	DQ341101	DQ341093	DQ341096	–	–
Aureobasidium pullulans	CBS584.75	DQ470956	DQ471004	–	DQ471075	DQ470906
Bagnisiella examinans	CBS551.66	GU301803	GU296139	–	GU349056	GU371746

Table 5.2 (continued)

Fungal isolates	Sources of culture	GenBank accession numbers				
		LSU	**SSU**	**ITS**	**TEF1-α**	**RPB2**
Botryosphaeria dothidea	CBS115476	DQ678051	DQ677998	–	DQ767637	DQ677944
Botryosphaeria stevensii	CBS 431.82	DQ678064	DQ678012	–	DQ677914	DQ767644
Botryosphaeria tsugae	CBS418.64	DQ767655	AF271127	–	DQ677907	DQ677960
Capnodium coffeae	CBS147.52	DQ247800	DQ247808	–	DQ471089	DQ247788
Cercospora beticola	CBS116456	DQ678091	DQ678039	–	DQ677932	–
Chlamydotubeufia huaikangplaensis	MFLUCC100926	JN865198	JN865186	JN865210	–	–
Chlamydotubeufia khunkornensis	MFLUCC100117	JN865189	JN865177	JN865201	KF301565	–
Chlamydotubeufia khunkornensis	MFLUCC100118	JN865190	JN865178	JN865202	KF301564	–
*Chlamydotubeufia cf. huaikangplaensis**	MFLUCC110509	KF301535	KF301544	KF301527	–	–
*Chlamydotubeufia cf. huaikangplaensis**	MFLUCC110512	KF301536	KF301545	KF301528	KF301556	–
Cochliobolus heterostrophus	CBS 134.39	AY544645	AY544727	–	DQ497603	DQ247790
Conidioxyphium gardeniorum	CPC14327	GU301807	GU296143	–	GU349054	GU371743
Dothidea insculpta	CBS189.58	DQ247802	DQ247810	–	DQ471081	AF107800
Dothidea sambuci	DAOM231303	AY544681	AY544722	–	DQ497606	DQ522854
Dothiora cannabinae	CBS737.71	DQ470984	DQ479933	–	DQ471107	DQ470936
Elsinoe centrolobi	CBS222.50	DQ678094	DQ678041	–	DQ677934	–
Gibbera conferta	CBS191.53	GU301814	GU296150	–	GU349041	–
*Glonium circumserpens*2	CBS123343	FJ161200	FJ161160	–	FJ161108	FJ161126
Glonium stellatum	CBS207.34	FJ161179	FJ161140	–	FJ161095	–
Guignardia bidwellii	CBS237.48	DQ678085	DQ678034	–	–	DQ677983
Helicoma ambiens	UAMH 10533	AY856916	AY856955	AY916451	–	–
*Helicoma chiangraiense **	MFLUCC100115	JN865188	JN865176	JN865200	KF301551	–
Helicoma chlamydosporum	CBS160.69	AY856875	AY856923	–	–	–

Table 5.2 (continued)

Fungal isolates	Sources of culture	GenBank accession numbers				
		LSU	**SSU**	**ITS**	**TEF1-α**	**RPB2**
Helicoma conicodentatum	UAMH10534	AY856869	AY856918	AY916450	–	–
Helicoma dennisii	NBRC30667	AY856897	–	AY916455	–	–
Helicoma fagacearum *	MFLUCC110379	KF301532	KF301540	KF301524	KF301553	KF301548
Helicoma intermedium	ATCC22621	AY856912	–	–	–	–
Helicoma morganii	CBS281.54	AY856876	–	AY916468	–	–
Helicoma muelleri	CBS964.69	AY856877	GQ146459	AY916453	–	–
Helicoma palmigenum	NBRC32663	AY856898	–	AY916480	–	–
Helicoma pulchrum	MUCL39827	AY856872	–	AY916457	–	–
Helicoma siamense *	MFLUCC100120	JN865192	JN865180	JN865204	KF301558	KF301550
Helicoma vaccinii	CBS216.90	AY856879	AY856926	AY916486	–	–
Helicoma violaceum	CBS222.58	AY856880	–	AY916469	–	–
Helicomyces bellus	CBS113542	AY916088	–	AY916475	–	–
Helicomyces lilliputeus	NBRC32664	AY856899	AY856942	AY916483	–	–
Helicomyces roseus	BCC3381	AY787932	–	–	–	–
Helicomyces roseus	BCC8808	AY856910	–	AY916481	–	–
Helicomyces roseus	CBS283.51	DQ678083	DQ678032	–	DQ677928	DQ677981
Helicomyces torquatus	CBS189.95	AY856882	–	AY916472	–	–
Helicosporium abuense	CBS101688	AY916085	–	AY916470	–	–
Helicosporium aureum (*citreo-viride*)	NBRC7098	AY856894	–	AY916478	–	–
Helicosporium gracile	CBS284.54	AY916086	–	AY916485	–	–
Helicosporium griseum	CBS961.69	AY856884	–	AY916474	–	–
Helicosporium griseum	UAMH1694	AY856902	–	AY916473	–	–
Helicosporium guianense	CBS269.52	AY856893	AY856938	AY916487	–	–

Table 5.2 (continued)

Fungal isolates	Sources of culture	GenBank accession numbers				
		LSU	SSU	ITS	TEF1-α	RPB2
Helicosporium guianense	UAMH1699	AY856891	–	AY916479	–	–
Helicosporium indicum	CBS374.93	AY856885	–	AY916477	–	–
Helicosporium linderi	NBRC9207	AY856895	–	AY916454	–	–
Helicosporium lumbricoides	JCM9265	AY856889	–	AY916476	–	–
Helicosporium pallidum	CBS962.69	AY856886	AY856932	AY916460	–	–
Helicosporium pallidum	UAMH10535	AY856913	–	AY916462	–	–
Helicosporium panachaeum	CBS257.59	AY916087	–	AY916471	–	–
Helicosporium phragmitis	CBS271.52	AY856887	–	AY916461	–	–
Helicosporium talbotii	MUCL33010	AY856874	–	AY916465	–	–
Helicosporium vegetum	BCC3332	AY856907	–	AY916490	–	–
Helicosporium vegetum	BCC8125	AY856909	–	AY916491	–	–
Helicosporium vegetum	CBS941.72	AY856883	–	AY916488	–	–
Helicosporium vegetum	NBRC30345	AY856896	–	–	–	–
Hortaea werneckii	CBS100496	GU301817	GU296152	–	GU349050	GU371739
Hortaea werneckii	CBS708.76	GU301818	GU296153	–	GU349058	GU371747
Hysterium angustatum	CBS123334	FJ161207	FJ161167	–	FJ161111	FJ161129
*Hysterobrevium smilacis*1	CBS114601	FJ161174	FJ161135	–	FJ161091	FJ161114
Leptosphearia maculans	DAOM229267	DQ470946	DQ470993	–	DQ471062	DQ470894
Leptoxyphium fumago	CBS123.26	GU301831	GU296161	–	GU349051	GU371741
*Lophium mytilinum*2	CBS269.34	DQ678081	DQ678030	–	DQ677926	DQ677979
Macrophomina phaseolina	CBS227.33	DQ678088	DQ678037	–	DQ677929	DQ677986
Microthyrium microscopicum	CBS115976	GU301846	GU296175	–	GU349042	GU371734
Microxyphium aciculiforme	CBS892.73	GU301847	GU296176	–	GU349045	GU371736

Table 5.2 (continued)

Fungal isolates	Sources of culture	GenBank accession numbers				
		LSU	SSU	ITS	TEF1-α	RPB2
Microxyphium citri	CBS451.66	GU301848	GU296177	–	GU349039	GU371727
*Mycosphaerella graminicola*1	CBS292.38	DQ678084	DQ678033	–	–	DQ677982
Mycosphaerella punctiformis	CBS113265	DQ470968	DQ471017	–	DQ471092	DQ470920
Myriangium hispanicum	CBS247.33	GU301854	GU296180	–	GU349055	GU371744
Mytilinidion andinense	CBS123562	FJ161199	FJ161159	–	FJ161107	FJ161125
Mytilinidion mytilinellum	CBS303.34	FJ161184	FJ161144	–	FJ161100	FJ161119
Mytilinidion scolecosporum	CBS305.34	FJ161186	FJ161146	–	FJ161102	FJ161121
Neofusicoccum ribis	CBS115475	DQ678053	DQ678000	–	DQ677893	DQ677947
Ophiosphaerella herpotricha	CBS620.86	DQ678062	DQ678010	–	DQ677905	DQ677958
Phaeocryptopus nudus	CBS268.37	GU301856	GU296182	–	GU349034	–
Phaeosphaeria ammophilae	CBS114595	GU301859	GU296185	–	GU349035	GU371724
Phaeosphaeria avenaria	DAOM226215	AY544684	AY544725	–	DQ677885	DQ677941
Phaeosphaeria eustoma	CBS573.86	DQ678063	DQ678011	–	DQ677906	DQ677959
Phoma exigua	CBS431.74	EU754183	EU754084	–	GU349080	GU371780
Phoma herbarum	CBS276.37	DQ678066	DQ678014	–	DQ677909	DQ677962
Pleospora herbarum	CBS191.86	DQ247804	DQ247812	–	DQ471090	DQ247794
Psiloglonium araucanum	CBS112412	FJ161172	FJ161133	–	FJ161089	FJ161112
Psiloglonium simulans	CBS206.34	FJ161178	FJ161139	–	FJ161094	FJ161116
Pyrenophora phaeocomes	DAOM222769	DQ499596	DQ499595	–	DQ497607	DQ497614
Schismatomma decolorans	DUKE0047570	AY548815	AY548809	–	DQ883725	DQ883715
Sydowia polyspora	CBS116.29	DQ678058	DQ678005	–	DQ677899	DQ677953
Teratosphaeria associate	CBS112224	GU301874	GU296200	–	GU349025	GU371723
Thaxteriella helicoma	JCM2739	AY856888	AF201455	–	–	–

Table 5.2 (continued)

Fungal isolates	Sources of culture	GenBank accession numbers				
		LSU	SSU	ITS	TEF1-α	RPB2
Thaxteriella helicoma	UBC F13877	AY856917	AY916452	AY916452	–	–
Thaxteriella inthanonensis	MFLUCC110003	JN865187	JN865187	JN865211	–	–
Thaxteriellopsis lignicola	MFLUCC100121	JN865193	JN865181	JN865205	–	–
Thaxteriellopsis lignicola	MFLUCC100122	JN865194	JN865182	JN865206	KF301563	–
Thaxteriellopsis lignicola	MFLUCC100123	JN865195	JN865183	JN865207	KF301562	–
Thaxteriellopsis lignicola	MFLUCC100124	JN865196	JN865184	JN865208	KF301561	–
Tubeufia amazonensis	ATCC42524	AY856911	AY856951	AY916458	–	–
Tubeufia asiana	BCC3463	DQ341100	–	DQ341097	–	–
Tubeufia aurantiella	ANM718	GQ850485	–	GQ856140	–	–
Tubeufia cerea	CBS254.75	DQ470982	DQ471034	–	DQ471105	DQ470934
Tubeufia cerea	NBRC9014	AY856903	AY856947	AY916489	–	–
*Tubeufia chiangmaiensis**	MFLUCC110514	KF301538	KF301543	KF301530	KF301557	KF301549
Tubeufia cylindrothecia	BCC3559	AY849965	–	–	–	–
Tubeufia helicomyces	CBS245.49	DQ767654	DQ767649	–	DQ767638	DQ767643
Tubeufia helicomyces	CBS271.52	AY856887	AY856933	AY916461	–	–
Tubeufia helicomyces	MUCL15702	AY856873	AY856922	AY916459	–	–
Tubeufia khunkornensis	MFLUCC100119	JN865191	JN865179	JN865203	–	–
*Tubeufia miscanthi**	MFLUCC110375	KF301533	KF301541	KF301525	KF301554	–
Tubeufia paludosa	ANM953	GQ850483	GQ856139	–	–	–
Tubeufia paludosa	AR4206	DQ341103	DQ341094	DQ341095	–	–
Tubeufia paludosa	CBS120503	GU301877	GU296203	–	GU349024	GU371731
Tubeufia paludosa	CBS120503	GU301877	GU296203	–	GU349024	GU371731
Tubeufia paludosa	CBS245.49	–	GU566745	–	–	–

Table 5.2 (continued)

Fungal isolates	Sources of culture	GenBank accession numbers				
		LSU	SSU	ITS	TEF1-α	RPB2
Tubeufia paludosa	HKUCC9118	AY849966	–	–	–	–
Tyrannosorus pinicola	CBS124.88	DQ470974	DQ471025	–	DQ471098	DQ470928
*Venturia inaequalis*1	CBS594.70	GU301879	GU296205	–	GU349022	–
*Venturia inaequalis*2	CBS815.69	GU301878	GU296204	–	GU349023	–

Note. Abbreviations of isolates and culture collections: A.N. Miller: ANM; American Type Culture Collection, Virginia, U.S.A: ATCC; Bahía Blanca Biology Herbarium: BBB; BIOTEC Culture Collection, Bangkok, Thailand: BCC; Centralbureau voor Schimmel cultures, Utrecht, Netherlands: CBS; Duke University Herbarium, Durham, North Carolina, U.S.A: DUKE; The University of Hong Kong Culture Collection, Hong Kong, China: HKUCC; Culture Collection, International Fungal Research & Development Centre, Chinese Academy of Forestry, Kunming, China: IFRD; Japan Collection of Microorganisms: JCM; K. Tanaka: KT; Mae Fah Luang University Culture Collection, Thailand: MFLUCC; NITE Biological Resource Centre, Japan: NBRC; University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, Canada: UAMH

5.3 Results and Discussion

5.3.1 Phylogenetic Study

The tree in Fig. 5.1 resulted from a multigene analyses of 66 taxa in *Tubeufiaceae* and 52 taxa from Dothideomycetes rooted with species of *Schismatomma decolorans* as the outgroup. The distinct clade (named *Tubeufiales*) is clearly separated from other orders within the Dothideomycetes with strong-support (99% BS, 1.00 PP). We therefore introduce a new order *Tubeufiales* to accommodate this clade which is supported by molecular data and distinct morphology of cylindrical asci, elongated cylindrical or fusiform ascospores, and hyphomycetous asexual morphs with prominently helicoid conidia. The subclade of *Botryosphaeriales* is basal to this order and is separated with maximum-support (100% BS, 1.00 PP). The *Pleosporales* clade clustered with the orders *Hysteriales* and *Mytilinidiales* which is distant from *Tubeufiales*.

The tree in Fig. 5.2 resulted from a multi-gene phylogenetic analysis (LSU, ITS, TEF1 and RPB2) of a dataset comprising 82 taxa, rooted with *Botryosphaeria dothidea* and *B. stevensii* as the outgroup. The dendrogram separated into seven Clades (named arbitrarily A to G), in which we can also be distinguished based on morphological characters. Other segregates comprise subsets of morphologically inadequately distinct or known species and are orphaned clades which we leave unnamed. Clade A1 is the Clade with 99% BS and 1.00 PP support and incorporates *Tubeufia paludosa* which is believed to be an older name for the generic *T. javanica*. We have, however, made recent collections of the latter species and epitypify it below and show it to be distinct from, but related to *T. paludosa*.

Clade A1 also incorporates five species of *Tubeufia,* plus asexual forms in *Helicoma*, *Helicomyces* and *Helicosporium* and can be named *Tubeufia sensu stricto.* The only asexual state that can be definitively linked to *Tubeufia* is *Helicomyces*, as a sequence from the isolation of *H. roseus,* is included in the analysis and formed a sister group with *T. paludosa.* Although *Helicomyces* was introduced in 1809 and is an older name than *Tubeufia* (which was introduced in 1898), we prefer to maintain the name *Tubeufia* for the taxa placed in Clade A1 because 1) it is the basis of the family and ordinal name, and 2) the asexual genera are polyphyletic and thus cannot be used to represent the genus, and 3) the characteristics of *Tubeufia* species are probably better understood than *Helicomyces* species. Clade A2 comprises species of *Helicoma, Helicomyces, Helicosporium* and *Tubeufia*, that cluster with low bootstrap support. The species in this clade are not type strains and generally their identifications are not supported by drawings or type material and thus we cannot confirm the identifications; therefore this clade cannot be considered a distinct genus.

Clade B includes the type and other species of the genera *Aquaphila* and *Chlamydotubeufia,* which clustered with 83% BS, 0.98 PP support. We separate them into subclades B1 (*Aquaphila*) and B2 (*Chlamydotubeufia*). Clade B1 includes *Aquaphila* with its "*Tubeufia asiana*" sexual morph which is well-supported (100% BS and 1.00 PP). This Clade comprises species with shared sexual morphology namely, darkly pigmented ascomata covered by dark setae, cylindrical-clavate and apically thickened asci, and fusiform 6-7-septate ascospores. The asexual form (sickle-shaped conidia) and aquatic habitat in *Aquaphila* are distinct from species in *Chlamydotubeufia* which produce dictyochlamydospores (Tsui et al., 2007; Boonmee et al., 2011). Clade B2 includes *C. huaikangplaensis* and *C. khunkornensis*, an asexual form of *Chlamydotubeufia* and *Helicoma chlamydosporum* (Shearer, 1987; Boonmee et al., 2011). The genus has a helicoid form in *Helicoma chlamydosporum*, but also produces dictyochlamydospores. In this paper we synonymized *Tubeufia asiana* under *Aquaphila asiana* and *Helicoma chlamydosporum* under *Chlamydotubeufia chlamydosporum* (see taxonomic changes below).

Clade C comprises eight putatively named strains of various species in *Tubeufiaceae* with 93% BS, 1.00 PP support. This clade includes three strains of *Acanthostigmina* which is typified by *A. minutum*. We therefore treat Clade C as *Acanthostigmina sensu stricto*, however the relationship with other putatively named strains in Clade C i.e. *Acanthostigmina scopula, Acanthostigmina piniraiensis*, *Helicosporium aureum* and *Helicosporium guianense* needs further investigation.

Clade D is a well-supported clade (98% BS, 1.00 PP) representing *Helicoma* species, including an isolate of *H. muelleri,* the type species. Clade D includes sexual and asexual states of putatively named species in the genera *Helicoma*, *Helicosporium*, *Thaxteriella* and *Tubeufia*. We therefore treat Clade D as *Helicoma sensu stricto* and transfer *Thaxteriella helicoma* to *Helicoma helicoma* and *Thaxteriella inthanonensis* to *Helicoma inthanonense* (see taxonomic changes below). A new species named *Helicoma siamense* with a *Helicoma*-like asexual morph nested with the node of *Helicoma* species but with low support. An isolate wrongly named *T. paludosa* (HKUCC9118) produced a *Helicosporium*-like asexual state in culture and two species, *Tubeufia miscanthi* and *Helicoma khunkornense* (= *Tubeufia khunkornensis*) lacking asexual states in culture, are placed within *Helicoma* (94% BS and 1.00 PP support). A putatively named isolate of *Helicosporium linderi* also clustered in Clade D. Two new species *Helicoma chiangraiense* and *Helicoma fagacearum* nested within *Helicoma* with good support.

Clade E is a well-supported clade (100% BS, 1.00 PP) containing *Helicosporium vegetum* isolates, which we treat as *Helicosporium sensu stricto*. The clade also comprises strains of *Tubeufia cerea* and *Helicosporium guianense* located on the same branch with strong support. Therefore, *Tubeufia cerea* is synonymised with *Helicosporium cereum* (see taxonomic changes below). The putatively named *Acanthostigma patagonica* and *Helicoma vaccinii* also clustered together basally in Clade E with strong support (98% BS, 1.00 PP).

Clade F is a sister clade to Clade E (97% BS, 1.00 PP) and contains *Acanthostigma chiangmaiensis* and *A. perpusillum* which cluster with 98% BS, 1.00 PP support. We treat this clade as *Acanthostigma sensu stricto* as it contains the strain of *A. perpusillum*, representative as the type species of the genus.

Clade G is basal to all genera in *Tubeufiaceae* and is possibly the earliest evolving genus of the family. The clade contains four strains of *Thaxteriellopsis*, including the type strain, which cluster with strong support (86% BS, 1.00 PP). We therefore treat this as *Thaxteriellopsis sensu stricto*.

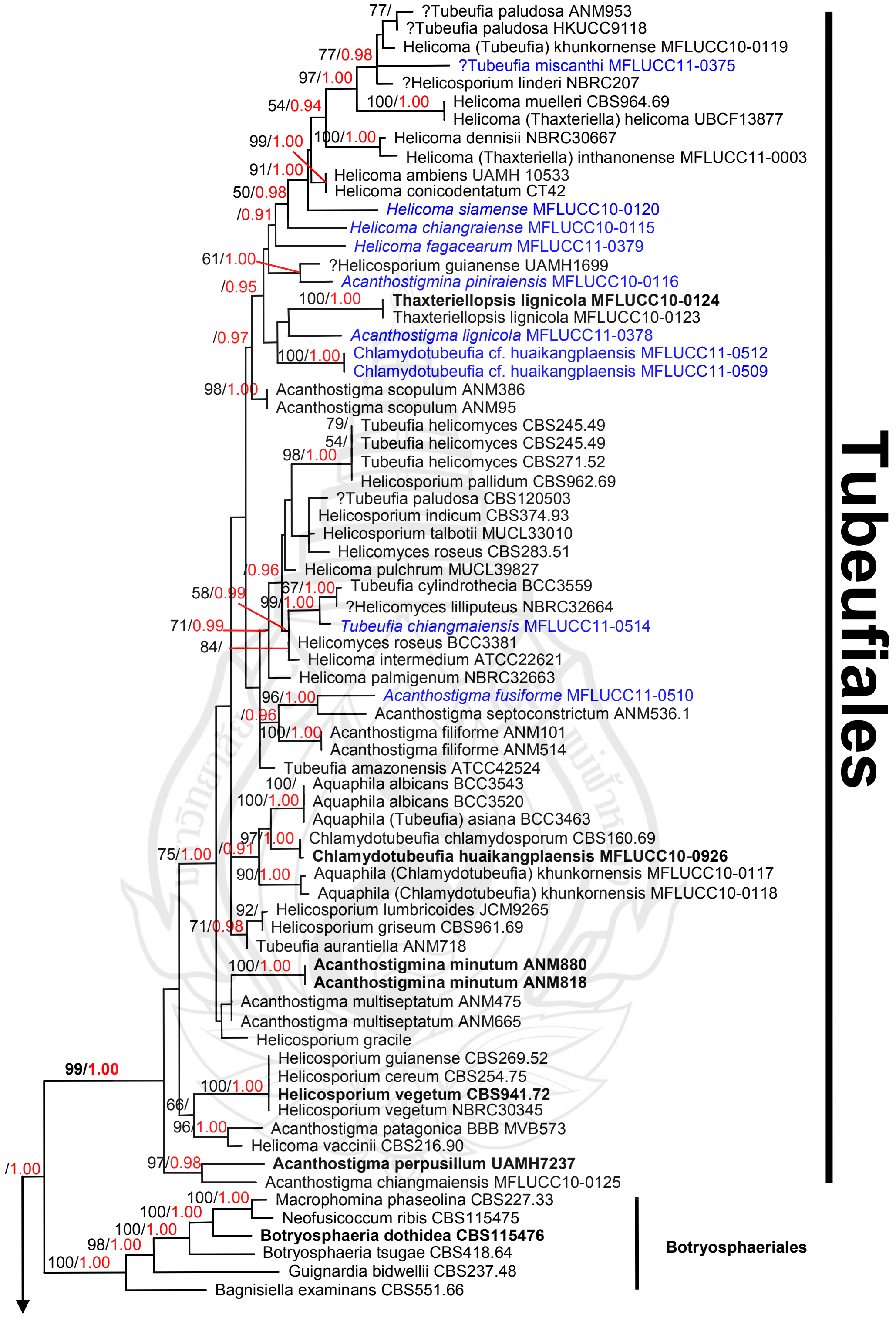
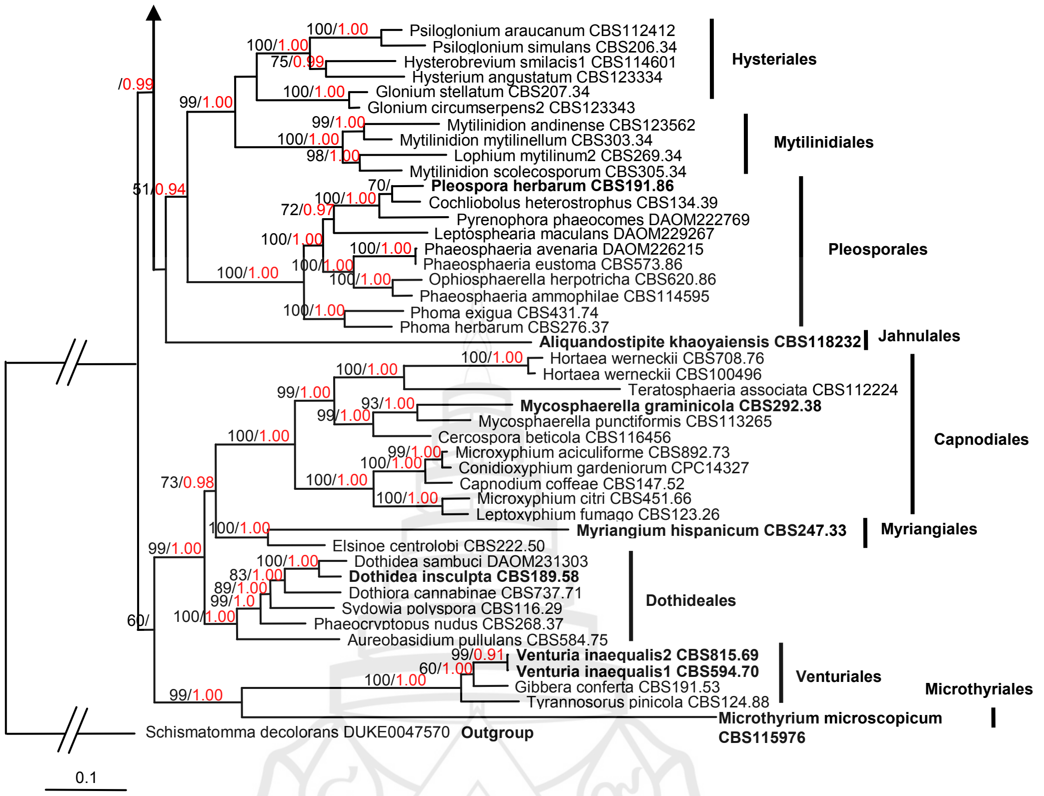


Figure 5.1 Phylogenetic Tree of *Tubeufiales* ord. nov., Based on RAxML Analysis



Note. RAxML tree is, generated from a data set of 118 taxa based on multi-gene analyses (LSU, SSU, TEF1 and RPB2) within orders of Dothideomycetes. The tree was rooted to species of *Arthoniomycetes*, and *Schismatomma decolorans.* The first set of numbers above the nodes are bootstrap (BS) from 1000 repetitions and the second position represented by Bayesian posterior probabilities (PP). The values of RAxML bootstrap ≥ 50% and Bayesian posterior probabilities ≥ 0.90 are shown above the branches. The placement of the new sequences are indicated in blue and new species in italics. Sequences of the type and epitype strains are highlighted in bold.

Figure 5.1 (continued)

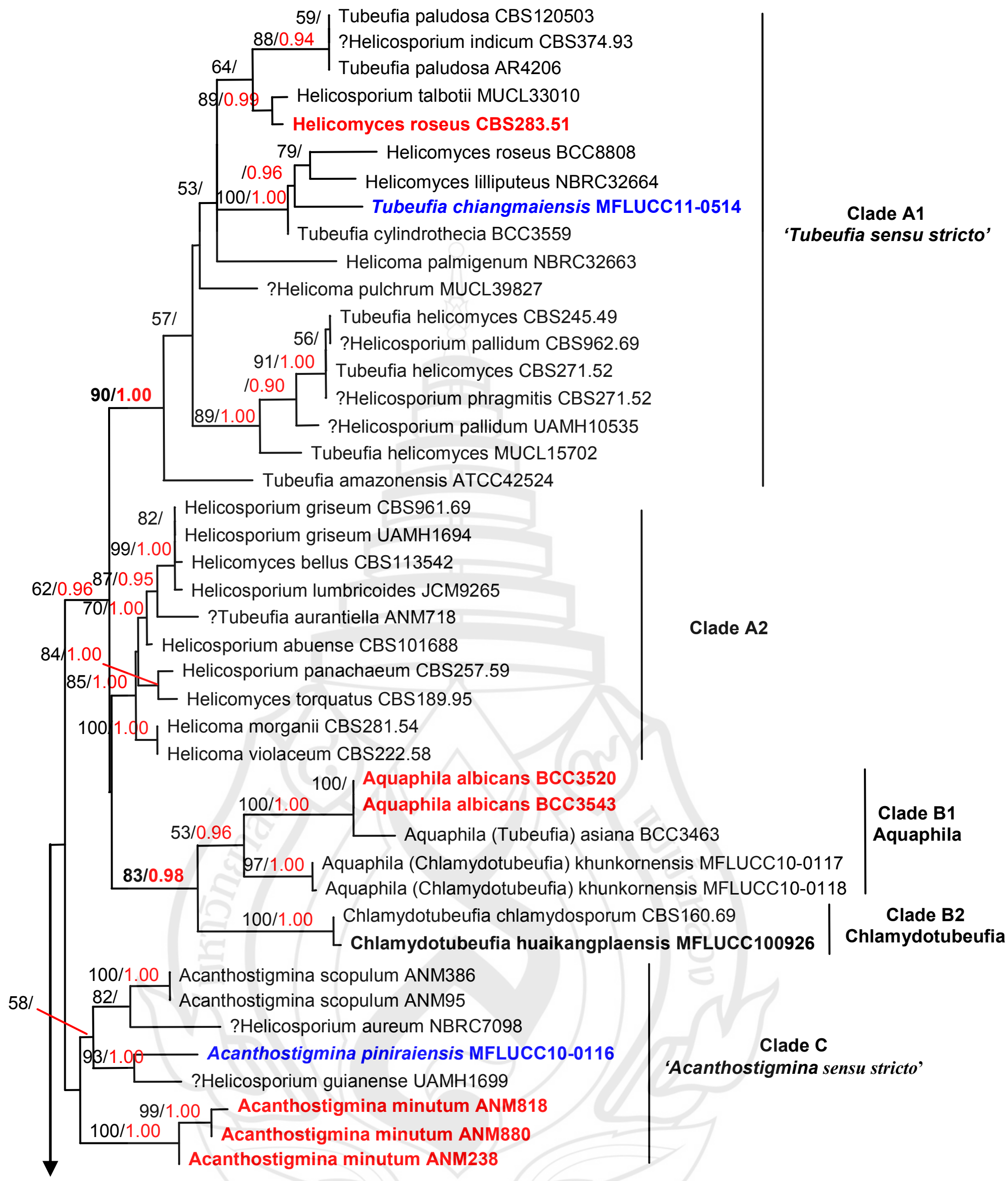
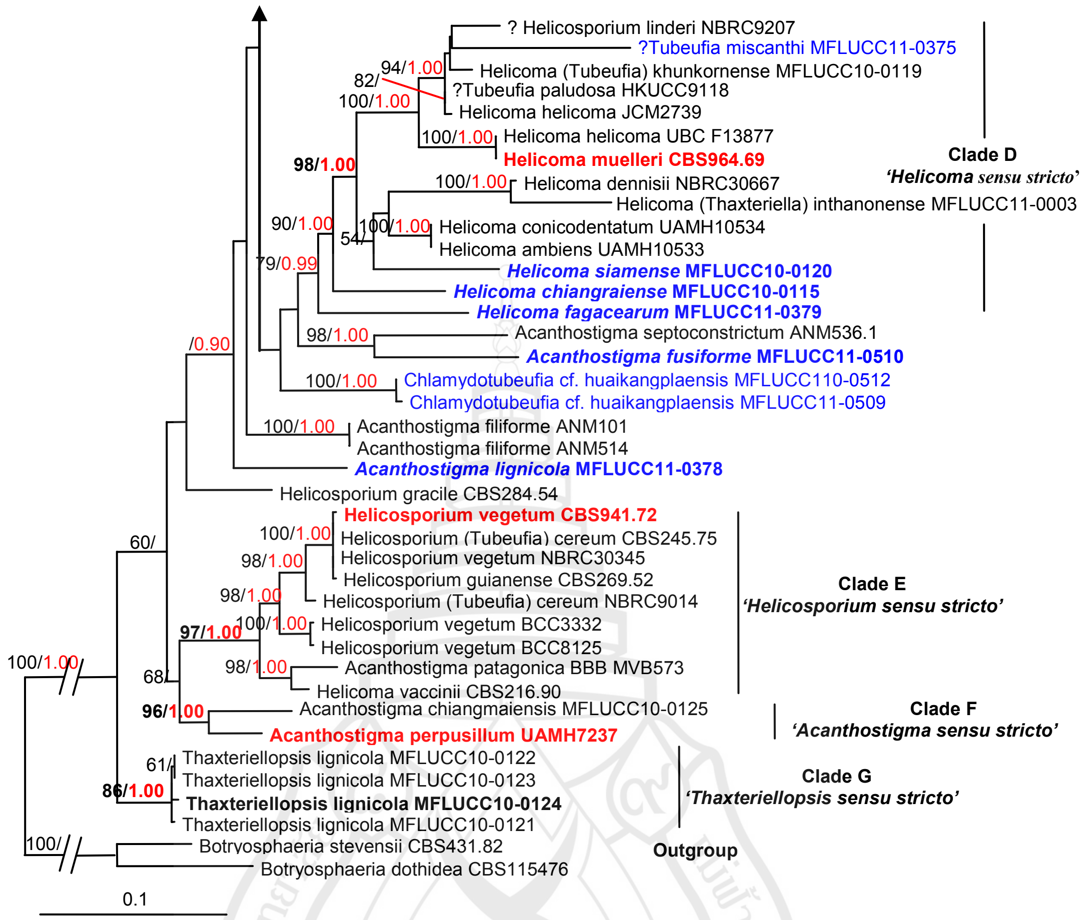


Figure 5.2 Phylogenetic Relationships of *Tubeufiaceae* and Its Members, Based on RAxML Analysis



Note. The phylogeny analysis was generated from a data set of 82 taxa based on multigene analyses (LSU, ITS, TEF1 and RPB2), and the tree was rooted to *Botryosphaeria dothidea* and *B. stevensii* The first set of numbers above the nodes are bootstrap (BS) from 1000 repetitions and the second position represented by Bayesian posterior probabilities (PP). The values of RAxML bootstrap ≥ 50% and Bayesian posterior probabilities ≥ 0.90 are shown above the branches. Ten newly sequenced taxa are indicated in blue and new species made in italics. Sequences of the type and epitype strains are indicated in black bold and the representative type species are indicated in red bold

Figure 5.2 (continued)

5.3.2 Taxonomy

Tubeufiales S. Boonmee, A. Y. Rossman & K. D. Hyde, ord. nov., MycoBank: MB804524

Saprobic or *parasitic,* common on decorticated or decaying woody and herbaceous substrates, less common on leaves, terrestrial habitats, widespread in temperate to tropical regions. *Ascomata* completely superficial, seated on subiculum, unilocular, globose-subglobose or clavate to obovate, coriaceous, solitary to gregarious, partially grouped, some translucent, light brown, brown to black, minutely papillate and with an ostiolate, some collapsing when dry, with radiating mycelium or appendages at the base, with or without setose or hairy appendages. *Peridium* somewhat thickened, mostly composed of cell of *textura angularis*, pale yellow, brow to dark brown or black in external layer. *Hamathecium* comprising numerous, filiform, branched, sometimes anastomosed, septate, hyaline pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, saccate or cylindrical-clavate, sometime broadly oblong-subclavate, lacking pedicel or with a distinct pedicel, without or with an apically rounded, distinct ocular chamber. *Ascospores* 2-3-seriate in the ascus, elongated, cylindric-subfusiform to narrowly oblong, tapering towards the narrow and subacute ends, distinctly multiseptate, hyaline to pale brown, smooth or minutely verrucose. *Asexual morphs* hyphomycetous; helicosporous, chlamydosporous and phragmosporous, with forms such as, *Aquaphila, Chlamydotubeufia, Helicoma, Helicomyces, Helicosporium, Monodictys*-like*, Pendulispora, Peziotrichum* and *Xenosporium*. Ordinal type: *Tubeufiaceae*

Tubeufiaceae M. E. Barr, Mycologia 71: 948 (1979), MycoBank: MB81599

The family has recently been revised by Boonmee et al. (2011). It is part of the present study. The detailed description of the fungal strains in this family was previously described in Chapter 2 (Section 2.3.2).

Morphologically, *Asexual state* hyphomycetous, helicosporous, *Conidiophores* mononematous, macro- to micronematous, erect or flexuous, septate, moderately to dark brown in colour. *Conidiogenous* cells holoblastic, mono- or polyblastic, integrated or discrete, terminal or intercalary. *Conidia* elongated, filiform to fusiform, curved, helicoid with varied number of coils, septate, sometimes dictyosporous, phragmosporous, hyaline to variedly coloured, smooth to verrucose, and as follows *Aquaphila, Chlamydotubeufia, Helicoma, Helicomyces, Helicosporium, Monodictys*-like*, Pendulispora, Peziotrichum* and *Xenosporium*.

Family type: *Tubeufia* Penz. & Sacc.

Tubeufia Penz. & Sacc., Malpighia 11(11-12): 517 (1898), MycoBank: MB5635

The detailed description of the generic type was previously described in Chapter 2 (Section 2.3.2).

Type species: *Tubeufia javanica* Penz. & Sacc., Malpighia 11(11-12): 517 (1898), MycoBank: MB244755

Synonyms

Tubeufia paludosa (P. Crouan & H. Crouan) Rossman, Mycologia 69(2): 383 (1977), MycoBank: MB325095 (Fig. 5.3)

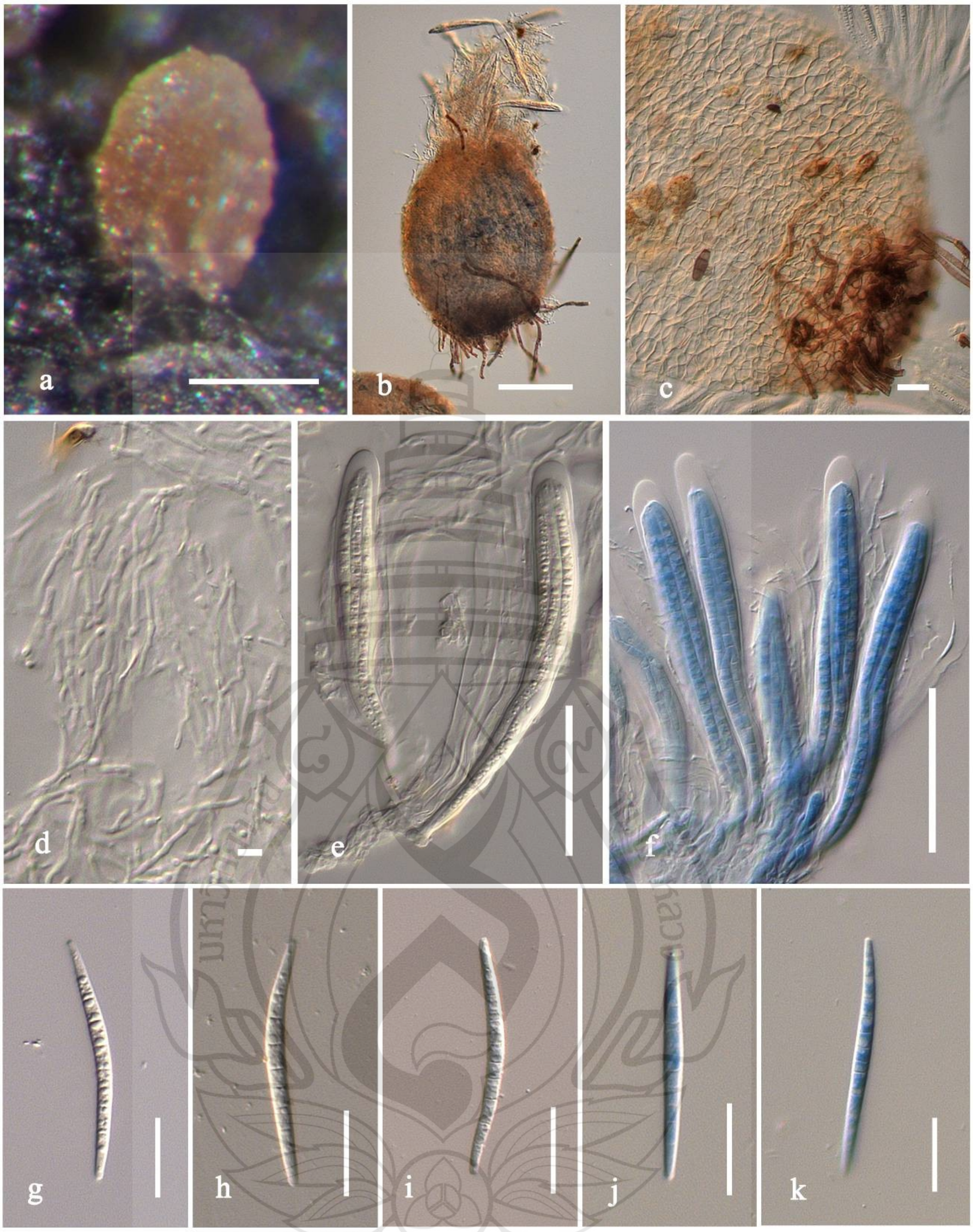
≡ *Nectria paludosa* P. Crouan & H. Crouan, Florule du Finistere p. 38. 1867

Saprobic on decorticated or decaying woody and herbaceous substrates, terrestrial habitats, widespread in temperate to tropical regions. *Ascomata* (267–)278–297(–312) µm high × 180–203 µm diam., completely superficial, seated on a subiculum, unilocular, globose-subglobose or clavate to obovate, coriaceous, solitary to gregarious, partially grouped, light brown to reddish brown, centrally ostiolate, translucent, perhaps collapsing when dry, with radiating mycelium or appendages at the base. *Peridium* composed of cell of *textura angularis*, with external layer pale yellow, brownish to brown, with inner layer hyaline and connected with membranous tissues. *Hamathecium* comprising numerous filiform 1–2 µm wide, septate, branched, anastomosed, hyaline, pseudoparaphyses. *Asci* 112–152(-158) × 12–16 µm ($\bar{x}$ = 128 × 14 µm, n = 20), 8-spored, bitunicate, fissitunicate, saccate or cylindric-clavate, apically rounded, with or without an ocular chamber, sessile or with a pedicel, sometimes as long as *ca*. 21–24(–33) µm. *Ascospores* (53–)59–73(–78) × 4–6 µm ($\bar{x}$ = 65 × 5 µm, n = 20), multiseriate and overlapping in the ascus, elongated, cylindric-subfusiform or narrowly oblong, tapering toward narrow and subacute ends, (9–)10–11(–13)-septate, not constricted at the median septum, hyaline, occasionally pale brown to light brown, guttulate when immature, smooth. *Asexual state* hyphomycetous, helicosporous, *Helicomyces*-like.

Material examined: USA, Tennessee, Blount Co., Great Smoky Mountains National Park, 25 mi W Gatlinburg, Cades Cove, Gregory Ridge Trail, alt. 1950 ft., 35°33'45"N 83°50'45"W, on wood rotten of unindentified angisoperm, collected by Rossman A.Y., 6 September 2005, BPI871087 (epitype designated here), ex-epitype culture: CBS 120503.

Notes: *Tubeufia paludosa* was originally described as *Nectria paludosa* Crouan & Crouan, Saccardo (1878) later transferred to Ophionectria (as *O. paludosa* Crouan & Crouan) Sacc., was finally transferred to *Tubeufia* (as *Tubeufia paludosa* (Crouan & Crouan) Rossman, on the basis of bitunicate asci by Rossman (1977). *Tubeufia paludosa* was also assigned to synonym with *T. javanica,* (Rossman, 1977). Barr (1980), however, thought *T. paludosa* is dissimilar to *T. javanica*, but accepted Rossman (1977) in synonymising *T. paludosa* with *T. javanica* in view of similar coloured ascomata and cylindrical ascospores. Yet, Index Fungorum is not depicting the synonymy of *T. paludosa* under *T. javanica* (http://www.indexfungorum.org/). The original descriptions of, *T. paludosa* (Crouan & Crouan) Rossman and *T. javanica* Penzig and Saccardo are similar in having ovoid, translucent, bright ascomata, filiform, cylindrical asci and filiform, hyaline and guttulate ascospores (Saccardo, 1883; Saccardo & Sydow, 1899). While, the illustrations of *Tubeufia javanica* in Penzig and Saccardo (1904) and *Tubeufia paludosa* in Samuels et al. (1979) and in Sivanesan (1984) differs in ascomata structures.

The demonstration of *Tubeufia paludosa* here is, morphologically similar with *T. javanica* as described by Penzig and Saccardo (1904) but it differing in shape of asci and ascospores (Fig. 5.3); further, asexual state is not found near the ascomata. Samuels et al. (1979) also examined and described *T. paludosa*, and considered the asexual morph found near the ascomata be possibly its asexual state, and named *Helicomyces*-like. Several authors (Promputtha & Miller, 2010; Schoch et al., 2009; Tsui & Berbee, 2006; Tsui et al., 2006, 2007), have investigated the molecular phylogeny of *T. paludosa*. The phylogenetic results suggest that *T. paludosa* is related with the asexual morphic genera *Helicomyces* and *Helicosporium*. We therefore epitypify the collection from BPI871087 as *T. paludosa* (CBS120503 and AR4206). According to the phylogenetic tree (Fig. 5.2) *T. paludosa* is related with asexual morph species *Helicosporium indicum* (Rao & D. Rao) with good support (88% BS, 0.94 PP). *Helicomyces roseus* Link clusters with *Helicosporium talbotii* Goos with 89% BS, 0.99 PP support and forming a subclade with *Tubeufia paludosa* with 64% bootstrap support (Clade A).



Note. (a)-(b) Ascomata. (c) Squash mount of ascoma through peridium. (d) Pseudoparaphyses. (e)-(f) Asci. (g)-(k) Ascospores. Note asci and ascospores in blue were strained by cotton blue. Scale bars: (a)-(b) = 100 μm, (c) = 20 μm, (d) = 5 μm, (e)-(f) = 50 μm, (g)-(k) = 20 μm

Figure 5.3 *Tubeufia paludosa* (BPI871087, CBS120503: epitype)

Tubeufia chiangmaiensis sp. nov. S. Boonmee & K. D. Hyde, MycoBank: MB804525 (Fig. 5.4)

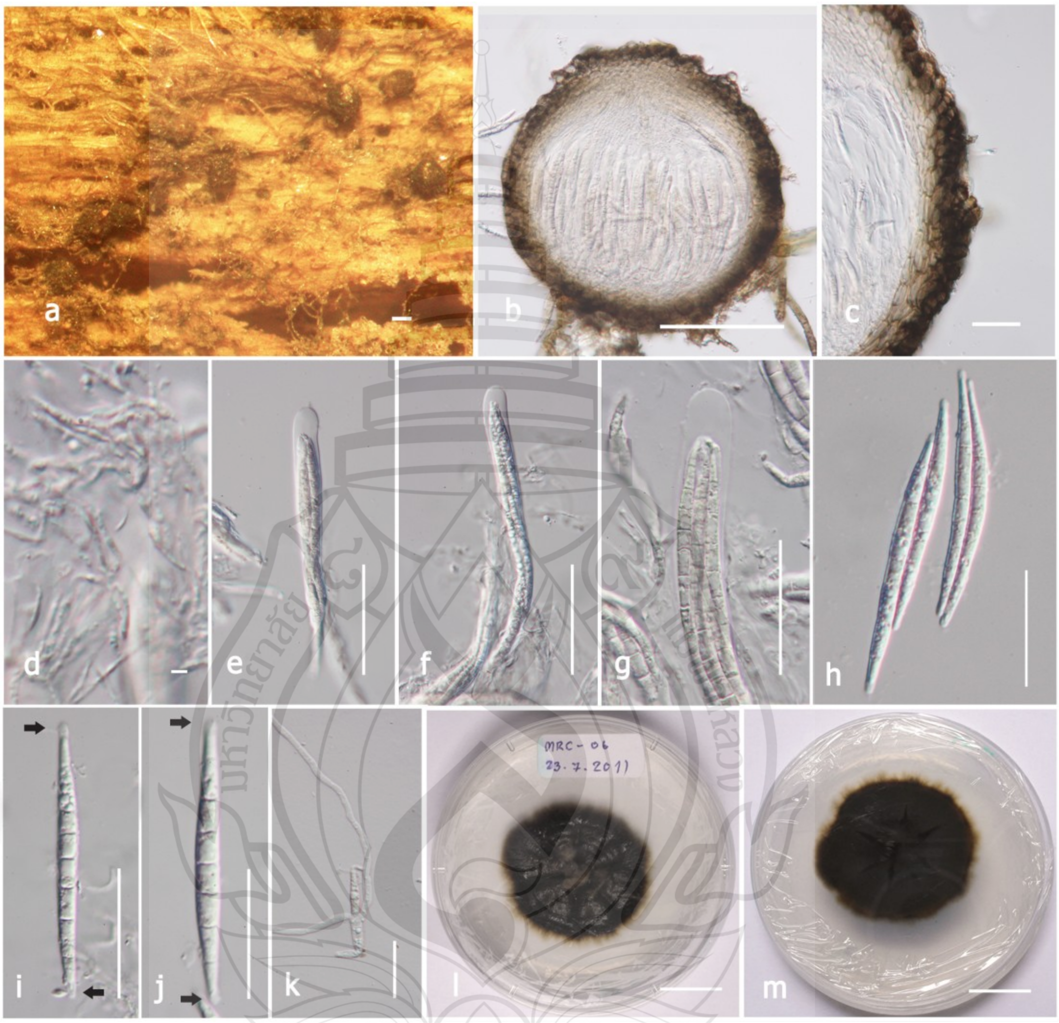
Habit saprobic on dead wood. *Ascomata* (200–)238–324 µm high × (180–) 227–269 µm diam. ($\bar{x}$ = 266 × 228 µm), superficial, globose-subglobose to ovate, solitary, scattered, dark brown with hyphae developing from ascomatal base onto substrate, slightly flat at the apex, brown to dark brown, ostiolate, collapsing when dry. *Peridium* 25–29 µm wide, comprising 4-5 layers, composed of brown to red brown cells *textura angularis*. *Hamathecium* comprising numerous filiform *ca.* 1–1.5 µm wide, hyaline, septate, branched, pseudoparaphyses. *Asci* (114.5–)120–137 × 11–14(–16) µm ($\bar{x}$ = 127 × 13 µm, n = 20), bitunicate, 8-spored, fissitunicate, elongated, cylindrical to slightly clavate, thick-walled and rounded at apex, with an ocular chamber, sessile. *Ascospores* 46–53(–55.5) × (3.5–)4–4.5 µm ($\bar{x}$ = 50 × 4 µm), 2–3-seriately overlapping in the ascus, cylindric-fusiform, tapering toward ends, with pad-like mucilage at both ends, straight to slightly curved, 7-septate, not constricted at any septum, hyaline, pale brown when maturity, smooth. *Asexual morph* unknown.

Cultural characteristics: Ascospores germinated on MEA within 12 hours and germ tubes produced from both ends. *Colonies* growing on MEA, reaching 5 mm in 1 week at 28^{o}C, mycelium partly superficial, partly immersed, slightly effuse, radially striated with fimbriate edge, dark-coloured, moist at surface view; asexual morph spores not formed within 30-60 days.

Material examined: THAILAND, Chiang Mai, Mae Taeng, Mushroom Research Center, N19°17.123′ E 98°44. 009′, elev. *ca* 900 m., on dead wood of an unidentified tree, 23 June 2011, Saranyaphat Boonmee, MRC-06: Holotype MFLU11–1149, ex-type culture: MFLUCC11– 0514 = BCC52386.

Notes: *Tubeufia chiangmaiensis* is consistent with morphological characters of the genus such as spherical ascomata with basal mycelium, brown-coloured, darkened when dry, fissitunicate, elongated, cylindrical to slightly clavate asci, cylindric-narrow fusiform, 7-transeptate, hyaline to pale brown ascospores, with pad-like mucilage at both ends and asexual state without conidia in culture. *Tubeufia cylindrothecia* has non translucent ascomata, and lacks a mucilaginous pad-like and smaller ascospores when compared to *Tubeufia chiangmaiensis* (Seaver, 1909). Also, *Tubeufia chiangmaiensis* differs from both *T. javanica* and *T. paludosa* in ascomatal and ascospore features

(Penzig & Saccardo, 1904; Rossman, 1977; Sivanesan, 1984; Fig. 5.3). Molecular phylogenetic results places *T. chiangmaiensis* clustering with asexual genus *Helicomyces* (*H. roseus* and *H. liliputeus*) by high value support (100 BS% and 100% PP) (Fig. 5.2).



Note. (a) Ascomata on substrate. (b) Section through ascoma. (c) Peridium. (d) Hamathecium and pseudoparaphyses. (e)-(g) Asci. (h)-(j) Ascospores (mucilaginous pad-like appendages, marked by arrows). (k) Germination of ascospore. (l)-(m) Colonies on MEA from surface and reverse. Scale bars: a-b = 100 µm, c, h-k = 30 mm. d = 5 µm, e-g = 50 µm, l-m = 10 mm

Figure 5.4 *Tubeufia chiangmaiensis* (MFLU11–1149, holotype)

Tubeufia miscanthi W.H. Hsieh, C. Y. Chen & Sivan. Rossman, Mycol. Res. 102: 234 (1978), MycoBank: MB443464 (Fig. 5.5)

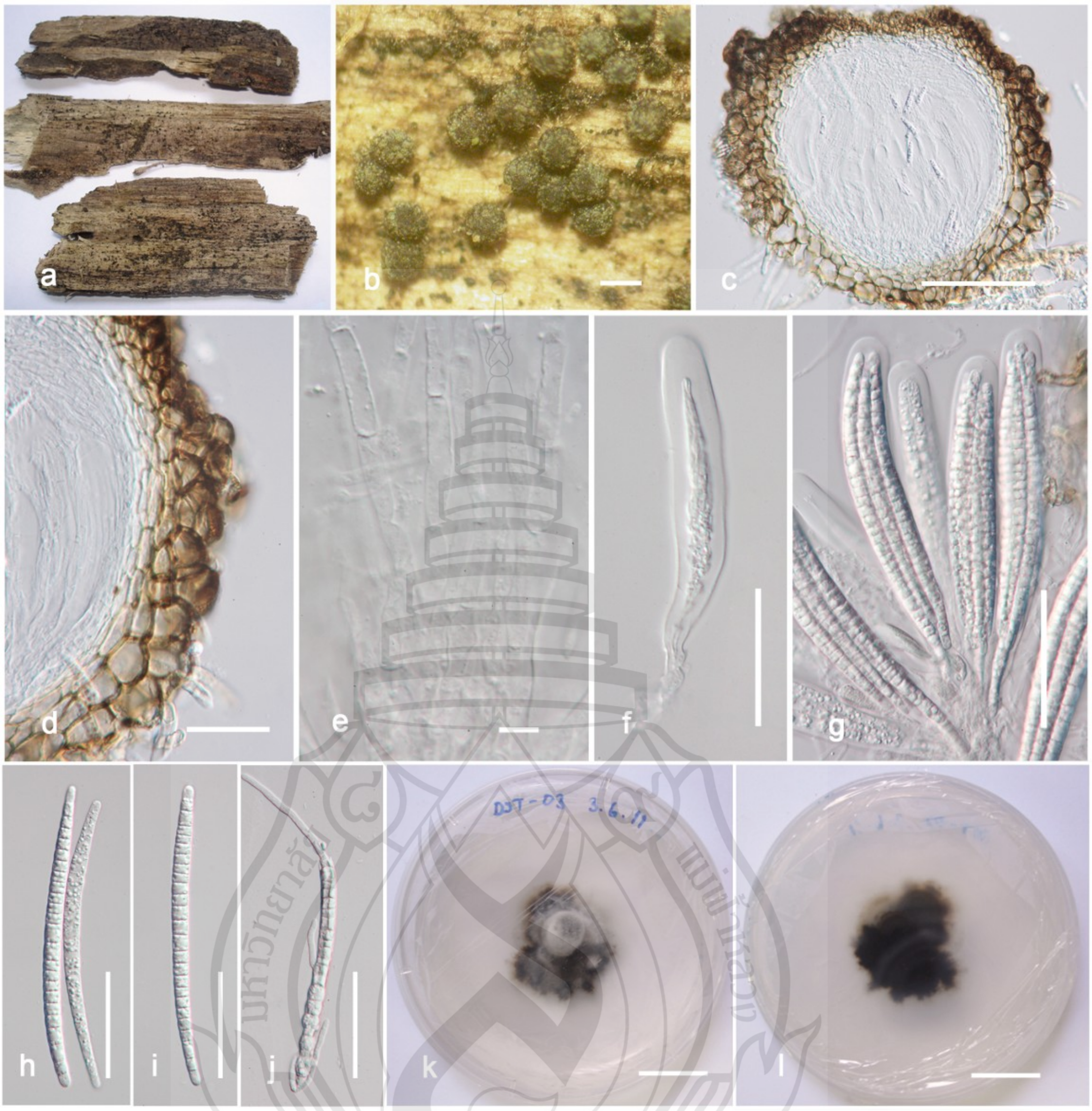
≡ *Taphrophila miscanthi* (W.H. Hsieh, Chi Y. Chen & Sivan.) Réblová & M.E. Barr, Sydowia 52(2): 283 (2000)

Habit saprobic on dead wood. *Ascomata* 285–358(–373) µm high × 299–339(–386.5) µm diam. ($\bar{x}$ = 330 × 334 µm), superficial, globose-subglobose to ovate, flat to slightly shrunken on the top, solitary, scattered, few hyphae developed from ascomatal base onto substrate, brown to dark brown when fresh, with a waxy covering on ascomal wall (under incident light), light brown when dry, darkened at the centre, ostiolate. *Peridium* 51–58 µm wide, comprising several layers of brown to red brown cells of *textura angularis*. *Hamathecium* comprising numerous long filiform, *ca*. 3–4 µm wide, hyaline, unbranched, septate, pseudoparaphyses. *Asci* (142–)154–188(–194) × 19–26(–30) µm ($\bar{x}$ = 172 × 24 µm, n = 20), bitunicate, 8-spored, cylindric-clavate, with a non-apparent ocular chamber, rounded at the apex, mostly sessile. *Ascospores* (122–)131–165(–187.5) × 7–10 µm ($\bar{x}$ = 147 × 9 µm, n = 20), multiseriately overlapping in the ascus, cylindric-clavate, elongated, tapering towards rounded ends, slightly curved, 19–23(–25) septate, slightly constricted at the septum, hyaline, with minutely globulate cells, smooth. *Asexual morph* unknown.

Cultural characteristics: Ascospores germinated on MEA within 12 hours and germ tubes produced from produced both ends. *Colonies* growing on MEA slowly, reaching less than 5 mm in 1 week at 28^{o}C, slightly effuse, undulate to fimbriate at the margin, dark, with white or light brown aerial mycelium, asexual morph spores not formed within 30-60 days.

Material examined: THAILAND, Chiang Mai, Muang, Huai Kok Ma, Doi Su Thep, N18°48.365' E98°54.522', 1015 m., on dead wood of an unidentified tree, 21 April 2011, Saranyaphat Boonmee, DST-03: Holotype MFLU11–0134, ex-type culture: MFLUCC11–0375 = BCC 52033.

Notes: This species is typical of *Tubeufia* with spherical, light-coloured ascomata, numerous pseudoparaphyses, cylindrical, elongated asci and cylindrical, many septate, hyaline ascospores. It is seemingly identical with an isolate of *Tubeufia miscanthi* from Taiwan (Hsieh, Chen & Sivanesan, 1998) and *T. paludosa* from Hong Kong (Kodsueb, Jeewon et al., 2006) in view of apparent global, light-coloured ascomata, thickened, septate pseudoparaphyses, cylindrical asci and many septate, hyaline ascospores. Recognising the presence of setae and pale brown ascomata *Tubeufia miscanthi* was transferred to *Taphrophila*, as *T. miscanthi* by Réblová and Barr (2000), as it had setae and pale brown ascomata. However, *Tubeufia miscanthi* is morphologically different from the type species *Taphrophila cornu-capreoli* (Scheuer, 1988; Hsieh et al., 1998). Accordingly *Taphrophila miscanthi* is referred to *Tubeufia* because of its morphological features and with molecular data that groups it with *Tubeufia* species and asexual species in *Helicoma* and *Helicosporium* in clade D (Fig. 5.2). Samuels et al. (1979), and Barr (1980) considered *T. miscanthi* to be identical to *T. paludosa* although *T. paludosa* isolate CBS120503 settled in Clade A, it is less similar from these isolates.



Note. (a)-(b) Ascomata on the substrate. (c) Section through ascoma. (d) Peridium. (e) Pseudoparaphyses. (f)-(g) Asci. (h)-(i) Ascospores. (j) Germinating of ascospore. (k)-(l) Colonies on MEA from surface and reverse. Scale bars: (a) = Substrate, (b)-(c) = 100 µm, (d) = 40 µm, (e) = 5 µm, (f)-(j) = 50 µm, (k)-(l) = 10 mm

Figure 5.5 *Tubeufia miscanthi* (MFLU11–0134, holotype)

Acanthostigma De Not., Sfer. Ital., 85 (1863), MycoBank: MB16

The detailed description of the generic type in this family was previously described in Chapter 2 (Section 2.3.2).

Type species: *Acanthostigma perpusillum* De Not., Sfer. Ital.: 207 (1863), MycoBank: MB204820

Notes: *Acanthostigma sensu stricto* is introduced on the bases of *Acanthostigma perpusillum* and the description given by De Notaris (1863). Réblová and Barr (2000) later monographed and accepted six species in *Acanthostigma*. Morphologically of all species are characterised by having dark brown to black ascomata covered by dark setae; ascospores usually fusiform, hyaline with well-developed septate. Multigene phylogenetic analysis indicated that several *Acanthostigma* epithets are polyphyletic and reside within the *Tubeufiaceae* (Boonmee et al., 2011; Promputtha & Miller, 2010; Sánchez et al., 2012).

We have tried to search out herbarium specimens of *Acanthostigma perpusillum* from several herbaria (e.g. GE, PAD, RO and TO), but none have been available for study. Réblová and Barr (2000) who reexamined, described and illustrated this species and Boonmee et al. (2011) made drawing's of it based on the description by Réblová and Barr (2000). Morphologically the species has markedly darkened setae and dark brown to black ascomata. Based on multigene analysis (LSU, ITS, TEF1 and RPB2) *A. perpusillum* and *A. chiangmaiensis* form a robust clade (Clade F) and a sister clade (Clade E) with *Helicosporium sensu stricto* (Fig. 5.2).

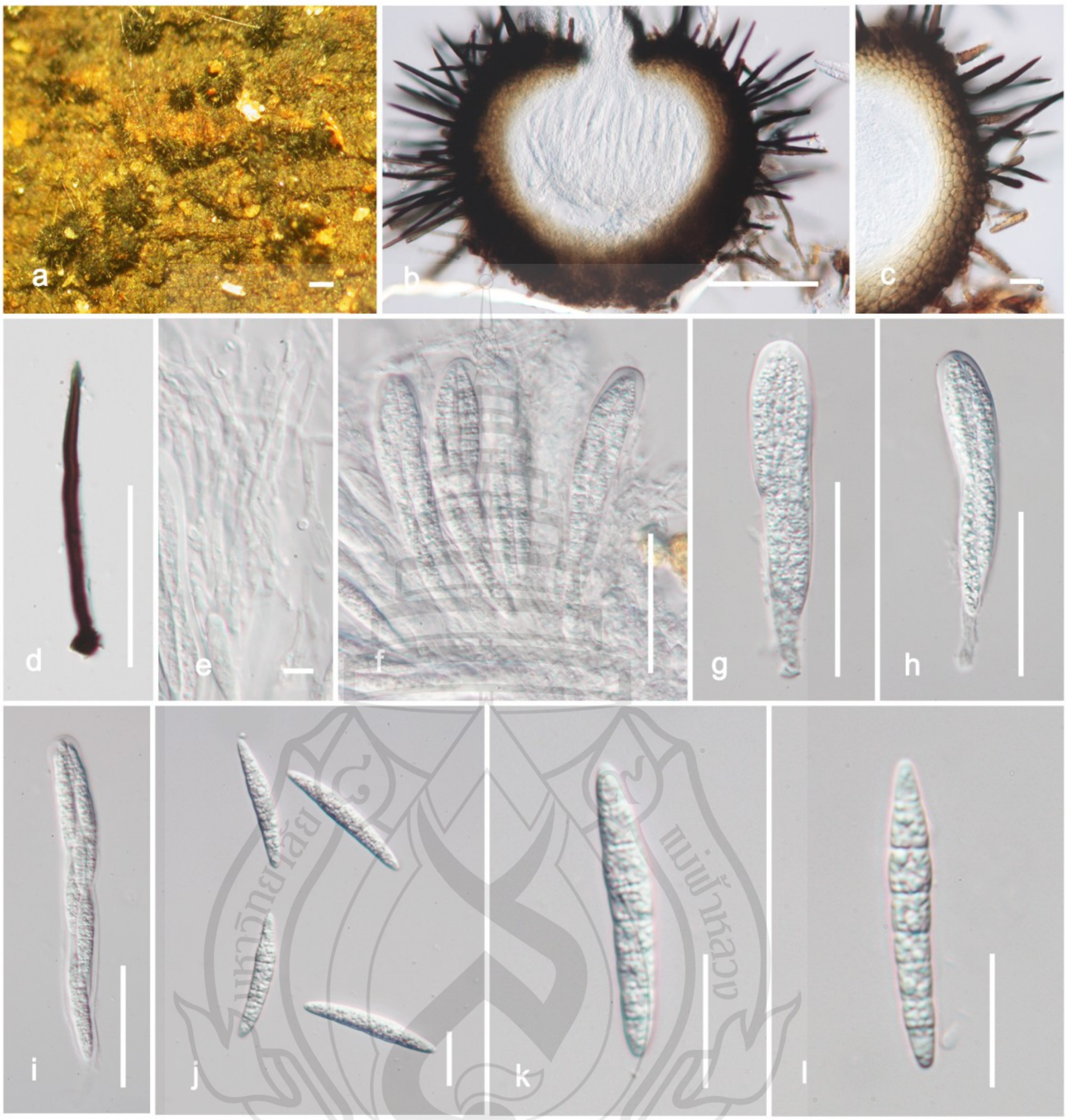
Acanthostigma lignicola sp. nov. S. Boonmee & K. D. Hyde, MycoBank: MB804551 (Figs. 5.6-5.7)

Habit saprobic on dead wood. *Ascomata* (216–)296–351 µm high × 266–340 µm diam. ($\bar{x}$ = 286 × 308 µm), superficial, solitary to clustered, scattered, globose to subglobose, dark brown to black, with setae all over the surface except base, ostiolate at the centre, setae stiff, tapering to acute tip at the apex, 56–77 µm long. *Peridium* 36–56 µm wide, composed of serveral layers of cells of *textura angularis*, with outer layer cells rather darkened and inner layer of cells pale brown to hyaline. *Hamathecium* numerous, filiform, hyaline, pseudoparaphyses, *ca.* 2 µm wide, branched, anastomosing. *Asci* (77–)80–106 × 15–19 µm ($\bar{x}$ = 93 × 17 µm, n = 20), bitunicate, 8-spored, fissitunicate, cylindrical-clavate or saccate, with rounded apex, ocular chamber not apparent, stalked and *ca.* 11 µm long. *Ascospores* (40–)45–53 × 5–8 µm ($\bar{x}$ = 47 × 7 µm, n = 20), 1-3-seriate overlapping in the ascus, fusiform, slightly curved, tapering toward ends, wider at supramedian, 6–7-septate, not constricted at the septum, hyaline, globular. *Asexual morph* hyphomycetous, helicosporous, *Helicoma*-like.

Cultural characteristics: Ascospores germinating on MEA within 12 hours. *Colonies* slow growing on MEA, attaining a diam. of less than 5 mm in 1 week at 28^{o}C, slightly convex, with an undulate edge, white or light brown and dark brown, white at the margin. *Mycelium* superficial, branched, septate, smooth, pale brown, with hyphae producing erect, septate conidiophores. *Conidiogenous cells* terminal, integrated, holoblastic, globose, sometimes subglobose to oval, golden-brown to olive-brown, 12–17.5 µm in diam. *Conidia* helicosporous, differentiating endogenously within the conidiogenous cells, released by breaking the wall of the conidiogenous cell, 12–17 µm diam. when coiled, filaments 4–7 µm wide, 5-septate, tapering towards rounded ends, not constricted at the septum, 1-coiled, hyaline, light-brown at maturity, mostly smooth, sometimes verrucose and slightly bulged at septa.

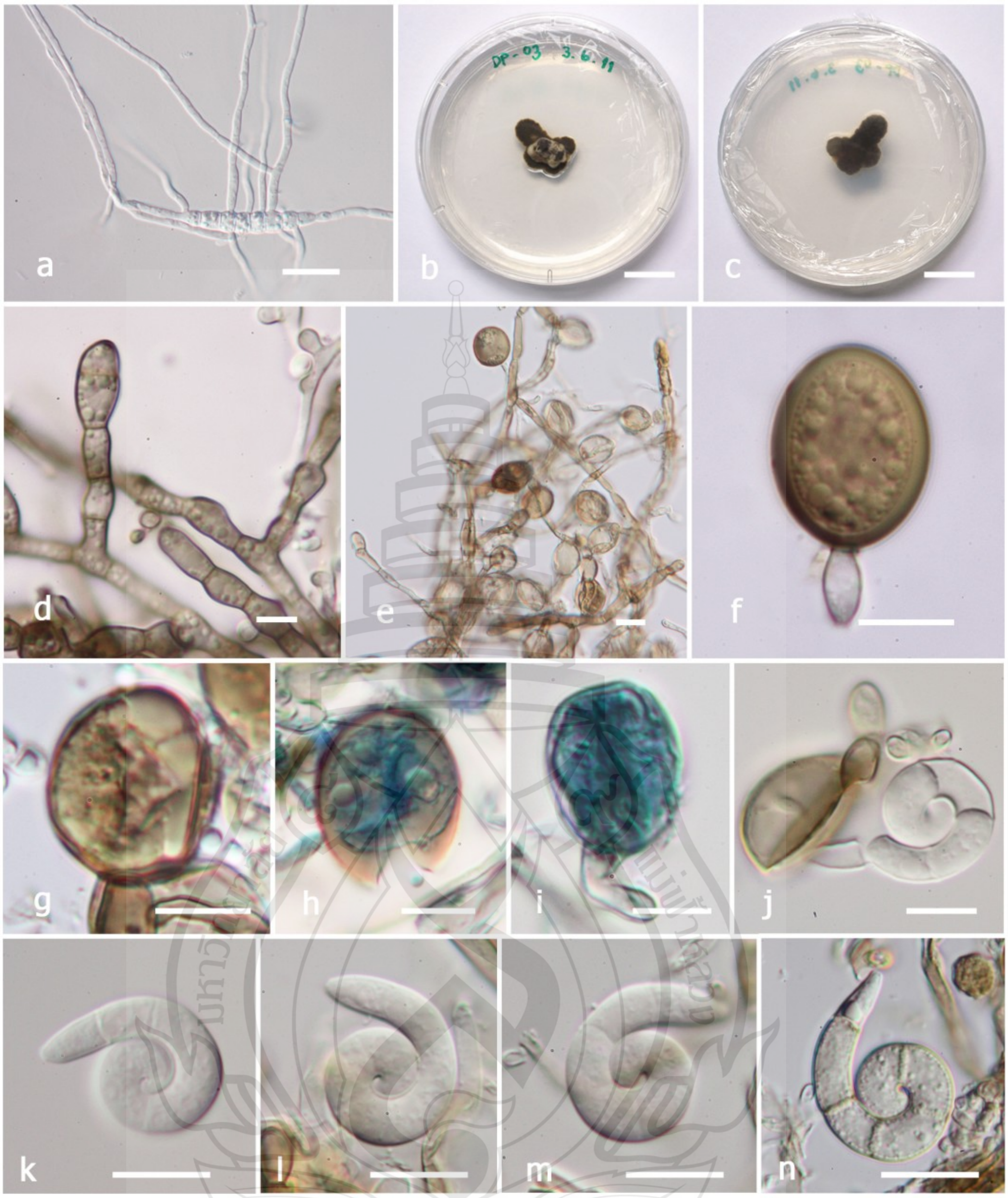
Material examined: THAILAND, Chiang Rai, Muang, Doi Pui, *ca*. 403-936 m., on dead wood of unidentified tree, 10 May 2011, Saranyaphat Boonmee, DP-03: Holotype MFLU11–0137), ex-type culture: MFLUCC11–0378 = BCC 52029.

Notes: The new species *Acanthostigma lignicola* is erected based on morphological consistency as described by Réblová and Barr (2000). However, *A. lignicola* differs from the type species *A. perpusillum* and other previously described species in the genus (Boonmee et al., 2011; Promputtha & Miller, 2010; Réblová & Barr, 2000; Sánchez et al., 2012) by its unique morphological features. Two species, *A. minutum* and *A. scopulum*, have already been transferred to the genus *Acanthostigmina* by Crane et al. (1998). *A. lignicola* comprises large ascomata (216–)296–351 µm high × 266–340 µm diam. and dense surface setae. The phylogenetic analysis based on multigene, classified *A. helicoma* to form an individual clade nested with the clade *Helicoma* with low support. Phylogenetically *A. lignicola* does not group with any other taxa, and produces an unique kind of conidiogenesis asexual state in culture that is *Helicoma*-like. The holoblastic conidium development is initiated at the tip of a conidiogenous cell by formation of a spherical, moderately dark brown cell within which the characteristic helicosporous conidium proper is endogenously differentiated. The helicoid conidium is released by break down of the outer thick-walled spherical case. Endogenous differentiation of the conidium is seen in most phialidic type conidiogenesis wherein the conidium primordial is differentiated enteroblastically within the phialide, but in most genera maturation of conidium is exogenous. In asexual morphic genera, such as, *Sporoschisma, Sporoschismopsis, Chalara* and *Fusichalara*, not only differentiation but also maturation of the conidium takes place within the venter of the philaide (Bhat, 2010). Certainly these are not similar to what is seen in the asexual morph *Acanthostigma.*



Note. (a) Ascomata on substrate. (b) Section through ascoma. (c) Peridium. (d) Single seta. (e) Pseudoparaphyses. (f)-(i) Asci. (j)-(l) Ascospores. Scale bars: (a)-(b) = 100 µm, (c), (j)-(l) = 20 µm, (d) = 40 µm, (e) = 5 µm, (f)-(i) = 50 µm

Figure 5.6 *Acanthostigma lignicola* (MFLU11–0137, holotype)



Note. Colonies on MEA. (a) Germinating ascospore. (b)-(c) Colonies on MEA from surface and reverse. (d)-(e) Mycelium and development of conidia in culture. (f) Conidiogenous cells. Note the formation of conidia in this stage. (g)-(j) Endogenous development of conidium. (j) Release of conidium. (k)-(n) Conidia. Scale bars: a = 20 µm, b-c, = 10 mm, d-n = 5 µm

Figure 5.7 Asexual State of *Acanthostigma lignicola* in Culture (MFLUCC11–0378, holotype)

Acanthostigma fusiforme sp. nov. S. Boonmee & K. D. Hyde, MycoBank: MB804552 (Figs. 5.8-5.9)

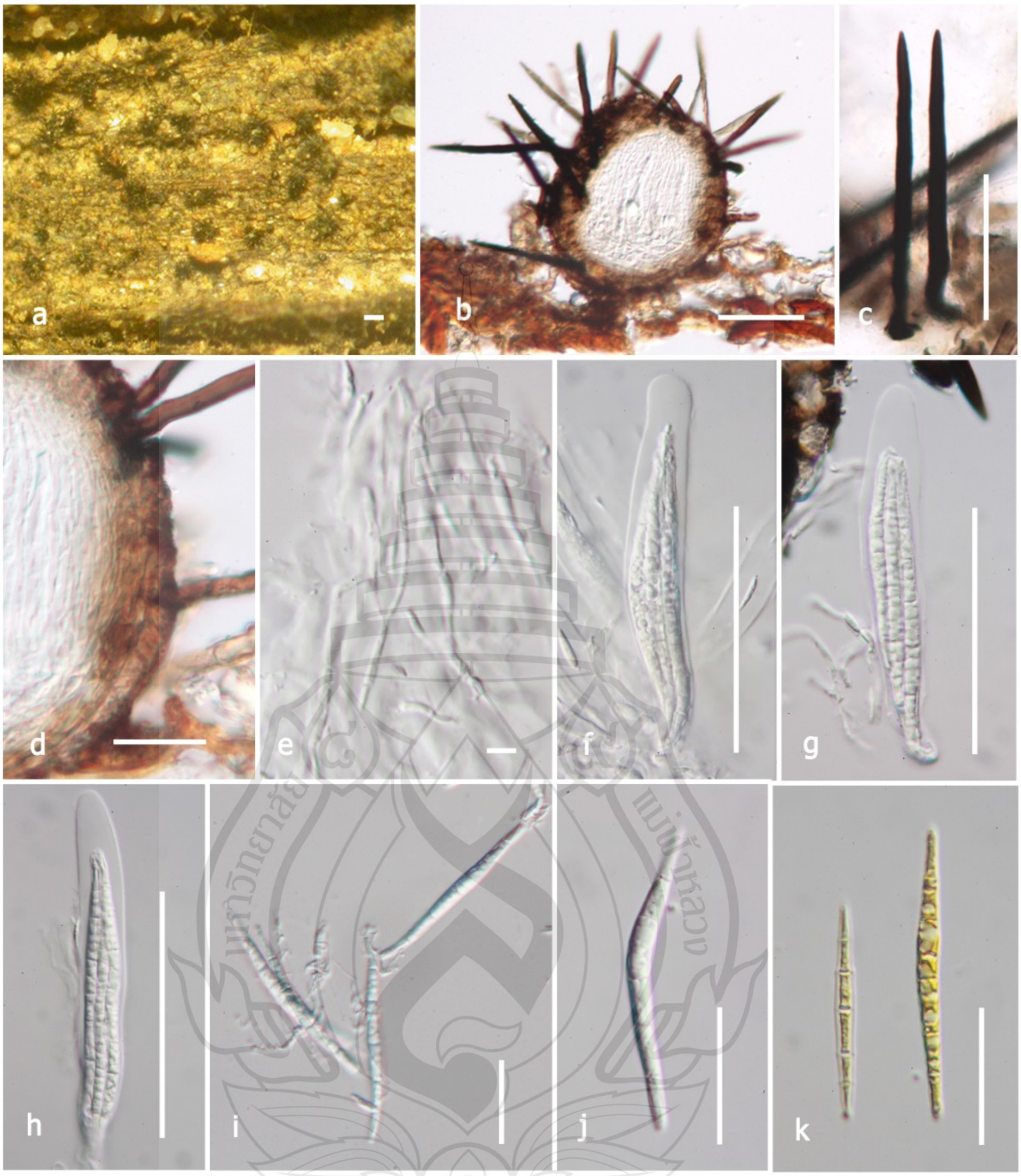
Habit saprobic on dead wood. *Ascomata* (111–)126–138 µm high × 98–100 (–125) µm diam. ($\bar{x}$ = 125 × 107 µm), superficial, globose to subglobose, solitary, scattered, red brown to dark brown to black, surrounded by black, with shining setae (27–)48–73 µm long and tapering towards acute tip, centrally ostiolate. *Peridium* 13–14 µm wide, composed of several layers of brown cells *textura angularis*. *Hamathecium* numerous filiform, hyaline, *ca.* 1.5–2 µm wide, septate, branched, septate, pseudoparaphyses. *Asci* 71–84 × 10–11(–12) µm ($\bar{x}$ = 79 × 11 µm, n = 20), bitunicate, 8-spored, cylindric-clavate, with thick and rounded apex, with a nonapparent ocular chamber, sessile. *Ascospores* (32–)40–48 × 3–4.5 µm ($\bar{x}$ = 43 × 4 µm, n = 20), multiseriately overlapping in the ascus, cylindrical, narrowly fusiform, tapering toward narrowly and subacute ends, straight to slightly curved, 5–7-septate, not constricted at the septum, hyaline, smooth. *Asexual morph* hyphomycetous, helicosporous, *Helicomyces*-like.

Cultural characteristics: Ascospores germinating on MEA within 8 hours and germ tubes produced at both ends. *Colonies* growing on MEA slowly, reaching less than 5 mm in 1 week at 28^{o}C, slightly raised-radially with lobate to entire edge, grayish to pale brown, laterally becoming dark brown. *Mycelium* developed on substrate, superficial, with hyaline to pale brown hyphae. *Conidiophores* micronematous, holoblastic, polyblastic, dentate on creeping hyphae, up to 5µm long, hyaline, smooth. *Conidia* helicosporous, (17–)23–30 µm diam. when coiled, conidial filaments 2 µm wide, coiled in 1–2½ dimensional times, tapering toward rounded ends, multiseptate, not constricted at the septum, hyaline, smooth.

Material examined: THAILAND, Chiang Mai, Mae Taeng, Mushroom Research Center, N19°17.123′ E 98°44. 009′, 900 m., on dead wood of unidentified tree, 23 June 2011, Saranyaphat Boonmee, MRC-03(T): Holotype MFLU11–1146, ex-type culture: MFLUCC11– 0510 = BCC 52383.

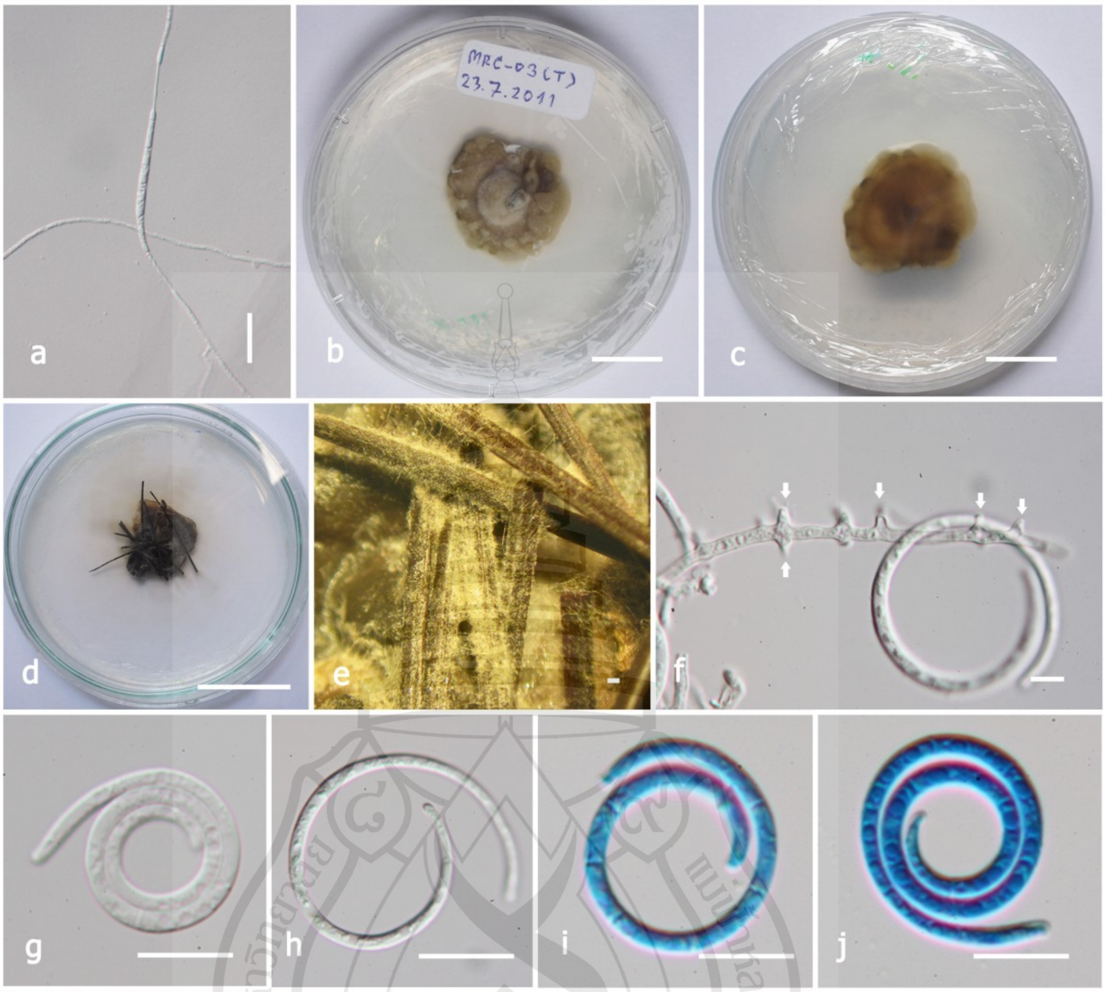
Notes: The new species *Acanthostigma fusiforme* belongs to the genus *Acanthostigma*. All species in *Acanthostigma* share the general features, such as spherical, dark ascomata, prominent dark setae, cylindric-clavate asci, conserved, fusiform and greater than 5-septate, hyaline ascospores (Boonmee et al., 2011; Chang, 2003;

Promputtha & Miller, 2010; Réblová & Barr, 2000; Sánchez et al., 2012). *Acanthostigma fusiforme* differs from all species of *Acanthostigma* in its small sized ascomata, cylindric-clavate, thickened apical asci and cylindric-narrowly fusiform ascospores. *Acanthostigma fusiforme* clusters with *Acanthostigma septoconstricum* in an individual clade with 98% BS and 1.00 PP support (Fig. 5.2, and basal Clade D. Both species are distinct differing in ascomata, ascus and ascospore size and shape. Additionally, *Acanthostigma fusiforme* produces endogenous helicoid conidial as asexual state in culture that is *Helicomyces*-like.



Note. (a) Ascomata on substrate. (b) Section through ascoma. (c) Setae. (d) Peridium. (e) Pseudoparaphyses. (f)-(h) Asci. (i)-(k) Ascospores. Note yellow strained by Melzer's reagent in Fig. k. Scale bars: (a)-(b) = 100 µm, (c), (f)-(h) = 50 µm, (d), (i)-(k) = 20 µm, (e) = 5 µm

Figure 5.8 *Acanthostigma fusiforme* (MFLU11–1146, holotype)



Note. Colonies on MEA. (a) Germinating ascospore. (b)-(c) Colonies on MEA from surface and reverse. (d)-(e) Testing asexual state on plant tissues. Note it could produce both conditions on the media with and/or without substrate. (f) Conidiophores formed on hyphae (arrows). (g)-(j) Conidia. Scale bars: (a) = 20 µm, (b)-(d) = 10 mm, (e)-(f) = 5 µm, (g)-(j) = 10 µm

Figure 5.9 Asexual State of *Acanthostigma fusiforme* in Culture (MFLUCC11–0510, holotype)

Acanthostigmina Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 118: 1499 [39 repr.] (1909), MycoBank: MB19

Habit saprobic on rotten wood, widespread in tropical regions. *Ascomata* superficial, solitary, scattered, some clustered, globose to subglobose, dark brown, with a central ostiole, surrounded by dark brown to black setae; setae (30–)56–84 (–105) µm long, tapering into an acute apex. *Peridium* thick, with several layers, composed of angular-subglobose cells, brown to red brown. *Hamathecium* comprising numerous filiform, hyaline and pale brown, branched, septate, pseudoparaphyses. *Asci* bitunicate, 8-spored, broadly cylindric-subclavate, thickened at the apex, without an ocular chamber, stipitate at the base, embedded in a gelatinous matrix. *Ascospores* 2–3-seriate in the ascus, elongated, cylindric-fusiform, tapering towards subrounded end, more than 7-septate, slightly constricted at the septum, hyaline young and pale brown with at maturity, smooth, some surrounded by a mucilaginous sheath, smooth. *Asexual morph* hyphomycetous, helicosporous, *Helicomyces*-like, *Helicosporium*-like.

Notes: The genus *Acanthostigmina* was introduced by von Höhnel (1909c) with *A. minuta* (Fuckel) Höhnel as the type species. Saccardo (1883) had earlier included this species in *Acanthostigma*, as *A. minutum* which is characterized by darkly pigmented ascomata covered by acute-stipped setae, bitunicate asci and hyaline, septate ascospores. Repetitive after von Höhnel (1909c) studied *A. minuta* it has been moved in and out of *Acanthostigma* by some authors (Barr, 1980; Crane et al., 1998; Réblová & Barr, 2000). Réblová and Barr (2000), however, treated *Acanthostigmina* as a synonym of *Acanthostigma*, after they studied *A. minutum* based on a material from France named as *A. scleracanthum*. They transferred two species, *A. hebridensis* and *A. trichella,* to the genus *Taphrophila* (Réblová & Barr, 2000). They also investigated the asexual state found on the specimen of *Tubeufia setosa* (synonymous) from herb. IMI, and found some *Helicomyces*-like helicoid conidia.

In this study, we reexamined *Acanthostigmina minutum*, as exsiccatae No. 1568, from Germany (Fig. 5.10), comprises of red brown ascomata, moderately stiff setae, broadly cylindric-subclavate, apically thickened asci and light-pigmented, 5-9-septate, hyaline to pale brown, ascospores with thin mucilaginous sheath. This collection differed from the description of *A. minutum* demonstrated by Réblová and Barr (2000) in which the ascospores were more than 10-septate, colourless and without mucilaginous a sheath whereas other characters they are rather similar. *Acanthostigma* (*A. perpusillum*) differed from *Acanthostigmina* (*A. minutum*) in the ascomata colour and size, density of setae and number of ascospore septa.

Molecular data indicates that three taxa of *A. minutum* from North America (ANM283, ANM818 and ANM880: not type sequences) formed an individual clade distant from the *A. perpusillum* clade (Promputtha & Miller, 2010). Sánchez and Bianchinotti (2010) later found a new collection in Argentina identified as *A. minutum*, which Sánchez et al. (2012) showed clustering with *A. perpusillum*. We also performed phylogenetic analysis with a multigene study, with *A. minutum* clustering in a sister clade of *A. scopulum*, and two asexual states of *Helicosporium* with 58% BS support (Fig. 5.2, Clade C).

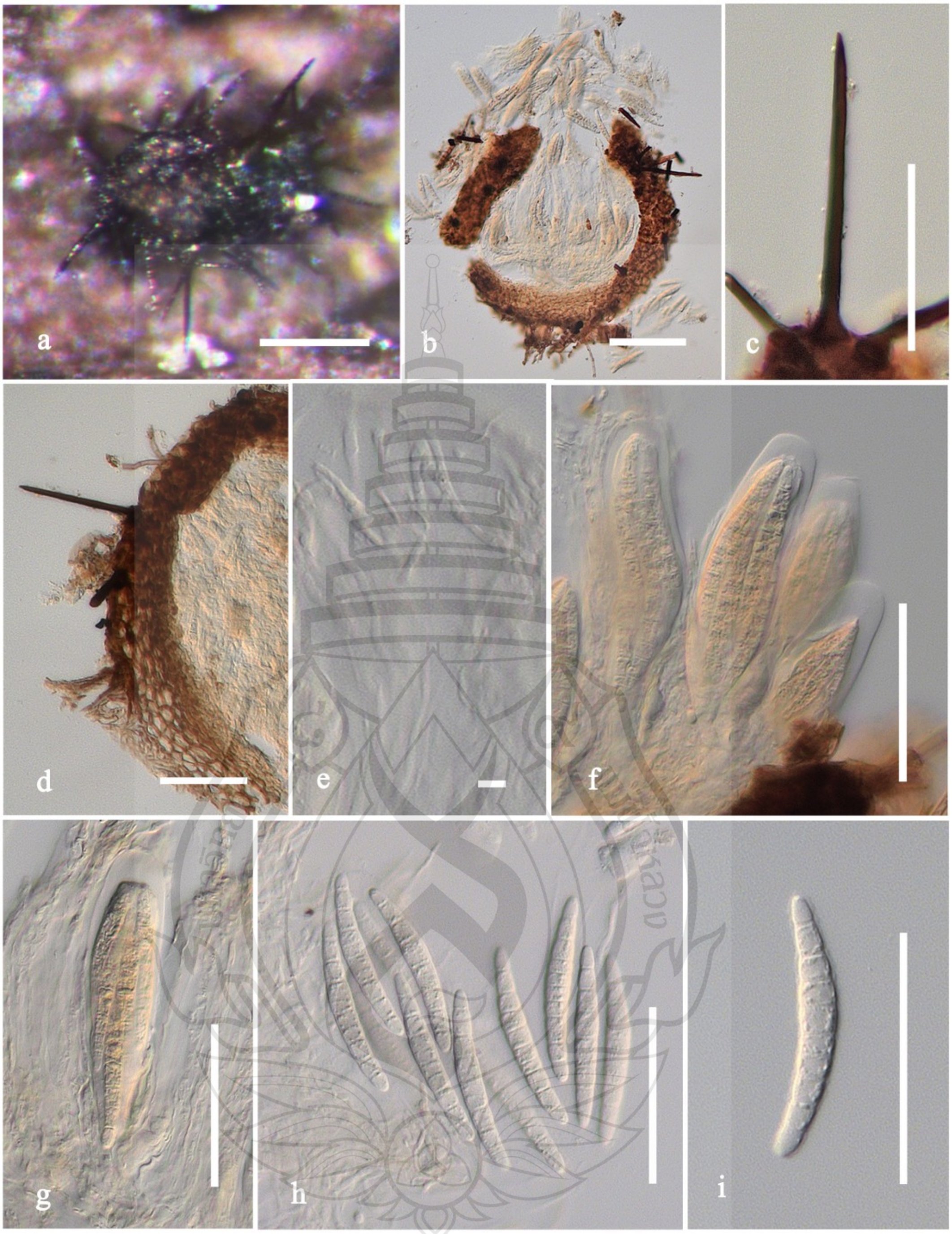
Type species: *Acanthostigmina minuta* (Fuckel) Clem. & Shear

Acanthostigmina minuta (Fuckel) Clem. & Shear, Gen. Fung. (Minneapolis): 270 (1931), MycoBank: MB431396 (Fig. 5.10)

≡ *Lasiosphaeria minuta* Fuckel, Jb. nassau. Ver. Naturk. 23-24: 148 (1870) [1869-70]

Habit saprobic on decaying wood rotten branch of *Fagus sylvatica*. *Ascomata* (211–)234–299 µm high × (216–)226–270 µm diam., superficial, solitary, scattered, some clustered, globose-subglobose, dark brown, central ostiole, surrounded by dark brown to black setae, (30–)56–84(–105) µm long, tapering toward an acute end at the apex. *Peridium* 29–34 µm wide, with several layers, composed of angular-subglobose cells, brown to red brown. *Hamathecium* comprising numerous filiform, *ca*. (1–)1.5–2 µm wide, hyaline and pale brown, septate, branched, pseudoparaphyses. *Asci* (73–)84–118 × 20–29.5 µm ($\bar{x}$ = 98 × 24 µm, n = 20), bitunicate, 8-spored, broadly cylindric-subclavate, thickened at the apex, without an ocular chamber, stipitate at the base, embedded in gelatinous matrix. *Ascospores* (42–)47–60 × 6–9 µm ($\bar{x}$ = 52 × 7 µm, n = 20), 2–3-seriate in the ascus, elongate-fusiform, tapering towards rounded ends, (6–)7–9-septate, broader at fourth and fifth cells from the apex, slightly constricted at the septum, hyaline and pale brown when age, some surrounded by a mucilaginous sheath, smooth. *Asexual morph* hyphomycetous, helicosporous, *Helicomyces*-like.

Material examined: GERMANY, Bavarian Alps Mts., Kampenwand, 1200 m., on decaying wood branch of *Fagus sylvatica*, June 1904, Rehm H., exsiccatae (Ascomyceten no. 1568) as *Acanthostigma minutum* (PAD), BPI 624355, authentic type specimen).



Note. (a) Ascomata. (b) Section through ascoma. (c) Setae. (d) Peridium. (e) Pseudoparaphyses. (f)-(g) Asci. (h)-(i) Ascospores. Scale bars: (a)-(b) = 200 µm, (c)-(d), (f)-(i) = 50 µm, (e) = 5 µm.

Figure 5.10 *Acanthostigmina minutum* (BPI 624355, authentic specimen)

Acanthostigmina piniraiensis sp. nov. S. Boonmee & K. D. Hyde, MycoBank: MB804553 (Figs. 5.11-5.12)

Habit saprobic on dead wood of *Pinus*. *Ascomata* 116–148.5 µm high × 108–138 µm diam. ($\bar{x}$ = 129.5 × 122 µm), superficial, globose to subglobose, black, solitary, scattered, surrounded black setae 39.2–72 × 5–7.5 µm, with setae tapering to an acute apex, with a not-so-apparent central ostiole. *Peridium* 12–18.5 µm wide, composed of several layers, with outer layer cells compressed and black, with inner layer comprising of *textura angularis* cells and brown. *Hamathecium* with numerous, filiform, hyaline, *ca*. 2 µm wide, septate, branched, pseudoparaphyses. *Asci* 131.5–183 × 14–18 µm ($\bar{x}$ = 150 × 16 µm), bitunicate, 8-spored, cylindrical, with thick and rounded apex, with an acute ocular chamber, stalked at the base. *Ascospores* 41.5–57 × 3–4 µm ($\bar{x}$ = 50.5 × 3 µm), multiseriate and overlapping in the ascus, long fusiform-cylindrical, straight to slightly curved, 7–8(–9)-septate, not constricted at any septum, hyaline, smooth. *Asexual morph* hyphomycetous, helicosporous, *Helicosporium*-like.

Cultural characteristics: Ascospores germinating on MEA within 12 hours and germ tubes produced at both ends. *Colonies* growing on MEA slowly, reaching less than 5 mm in 1 week at 28^{o}C, slightly convex, undulating to erose or dentate, with slightly radially striated with lobate edge, brown. *Mycelium* superficial, composed of branched, septate, smooth, hyaline, pale brown to brown hyphae. *Conidiophores* mononematous, erect, 3–5 µm long and 2–4 µm wide, smooth, pale brown to brown, smooth. *Conidiogenous cells* holoblastic, terminal or intercalary, dentate, smooth, with a thickened and truncate conidiogenous loci. *Conidia* helicosporous, (53–)65–92(–99) µm long, filaments 2 µm wide, 16–20(–25) µm diam. when coiled, curved and coiled in 1-dimensional time, elongate, tapering to narrowly and rounded ends, multiseptate, not constricted at the septum, hyaline, guttulate, smooth.

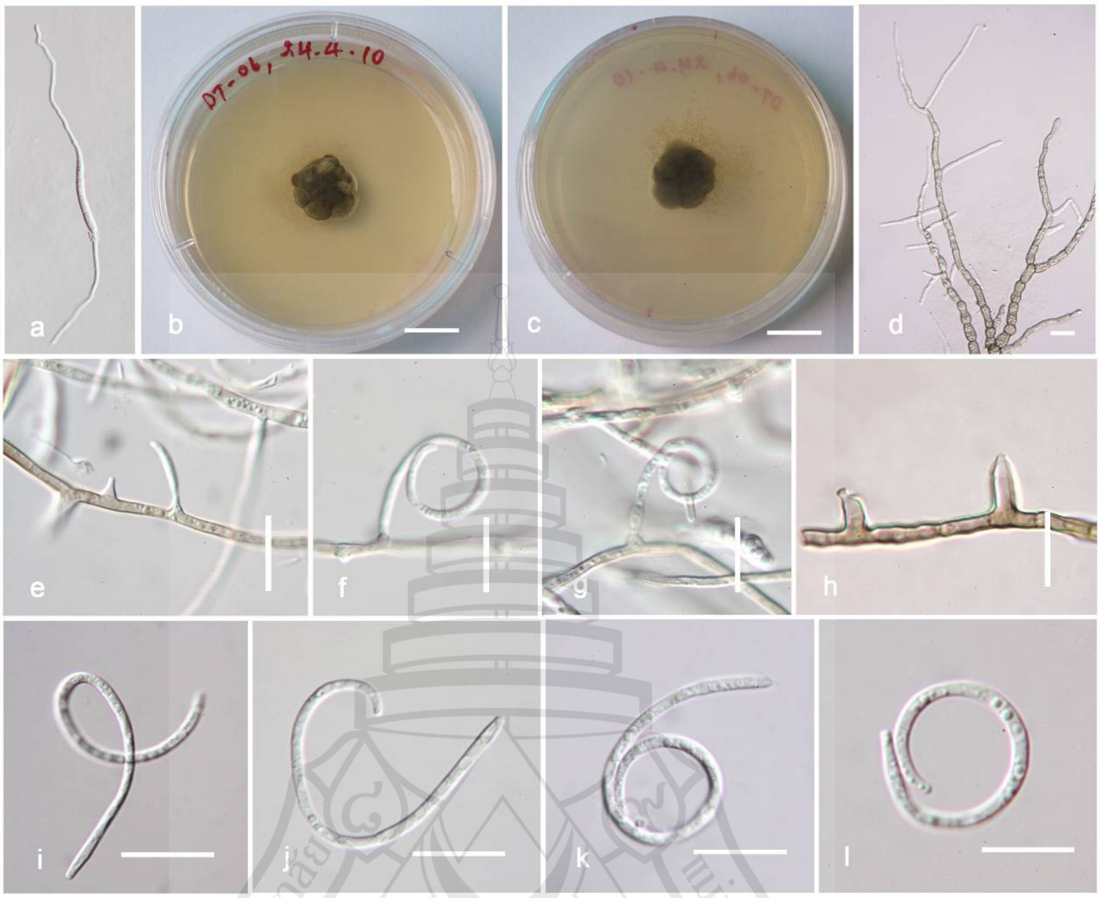
Material examined: THAILAND, Chiang Rai, Muang, Doi Tung, 1,509 m., on dead wood of *Pinus*, 6 November 2009, Saranyaphat Boonmee, DT-06: Holotype MFLU10-0049, ex-type culture: MFLUCC10–0116 = BCC 52036= IFRD 2196.)

Notes: With its well-developed setae on ascomata, cylindrical asci and oblong ascospores, the new species *Acanthostigmina piniraiensis* differs from all hitherto known species of *Acanthostigma* (Kodsueb et al., 2004; Réblová & Barr, 2000;

Sánchez & Bianchinotti, 2010; Sánchez et al., 2012) and *Acanthostigmina* (Promputtha & Miller, 2010; Réblová & Barr, 2000). *Acanthostigmina scopulum* and *A. piniraiensis* share some similar morphological features (Kodsueb et al., 2004; Promputtha & Miller, 2010; Réblová & Barr, 2000), they differ in the dimensions of the ascomata, asci and ascospores including the number septa, while *A. piniraiensis*, produced helicoid conidia in culture similar to *Helicosporium*-like. *Acanthostigmina piniraiensis* groups with *H. guianense* with high-support (93% BS, 1.00 PP), in a sister group to *A. scopulum* and *Helicosporium aureum* (Fig. 5.2, Clade C).

Note. (a) Ascomata superficial on substrate. Note ascomata surrounded by black setae. (b) Section through ascoma. (c) Peridium with dark setae. (d) Seta. (e) Pseudoparaphyses. (f)-(g) Asci. (h)-(m) Ascospores. Scale bars: (b) = 100 µm, (c)-(d), (h)-(m) = 20 µm, (e) = 5 µm, (f)-(g) = 50 µm

Figure 5.11 *Acanthostigmina piniraiensis* (MFLU10-0049, holotype)



Note. Colonies and asexual state on MEA culture. (a) Germinating ascospore. (b)-(c) Colonies on MEA at surface and reverse. Note dark brown colonies. (d) Arial mycelium on culture. (e)-(g) Conidiophores developing on hyphae. (h) Conidiogenous cells. (i)-(l) Conidia. Scale bars: (a)-(c) = 10 mm, (d)-(i) = 5 µm, (e)-(l) = 10 µm

Figure 5.12 Asexual State of *Acanthostigmina piniraiensis* in Culture (MFLUCC10–0116, holotype)

Boerlagiomyces Butzin., Willdenowia 8(1): 39 (1977), MycoBank: MB607

The briefly translated description and redrawn of *Boerlagiomyces velutinus* (as a previous named *Boerlagella velutina*) are provides based on the protologue of Penzig and Saccardo (1904) as follows: *Habit* saprobic on decaying wood. *Ascomata* superficial, with a subiculum, globose, scattered, with flexuous, hairy, black, velvety, septate setae, surrounded by black mycelium. *Peridium* composed of carbonaceous, multilayered walls. *Hamathecium* composed of filiform, thread-like, hyaline pseudoparaphyses. *Asci* 8-spored, bitunicate, cylindric-clavate, apically rounded, narrowing towards base, sessile or short-stalked. *Ascospores* 2–3-seriate in the ascus, cylindric-fusoid, gently curved, obtuse at both ends, multiseptate, muriform, not constricted at the septa, hyaline, smooth. *Asexual morph* unknown.

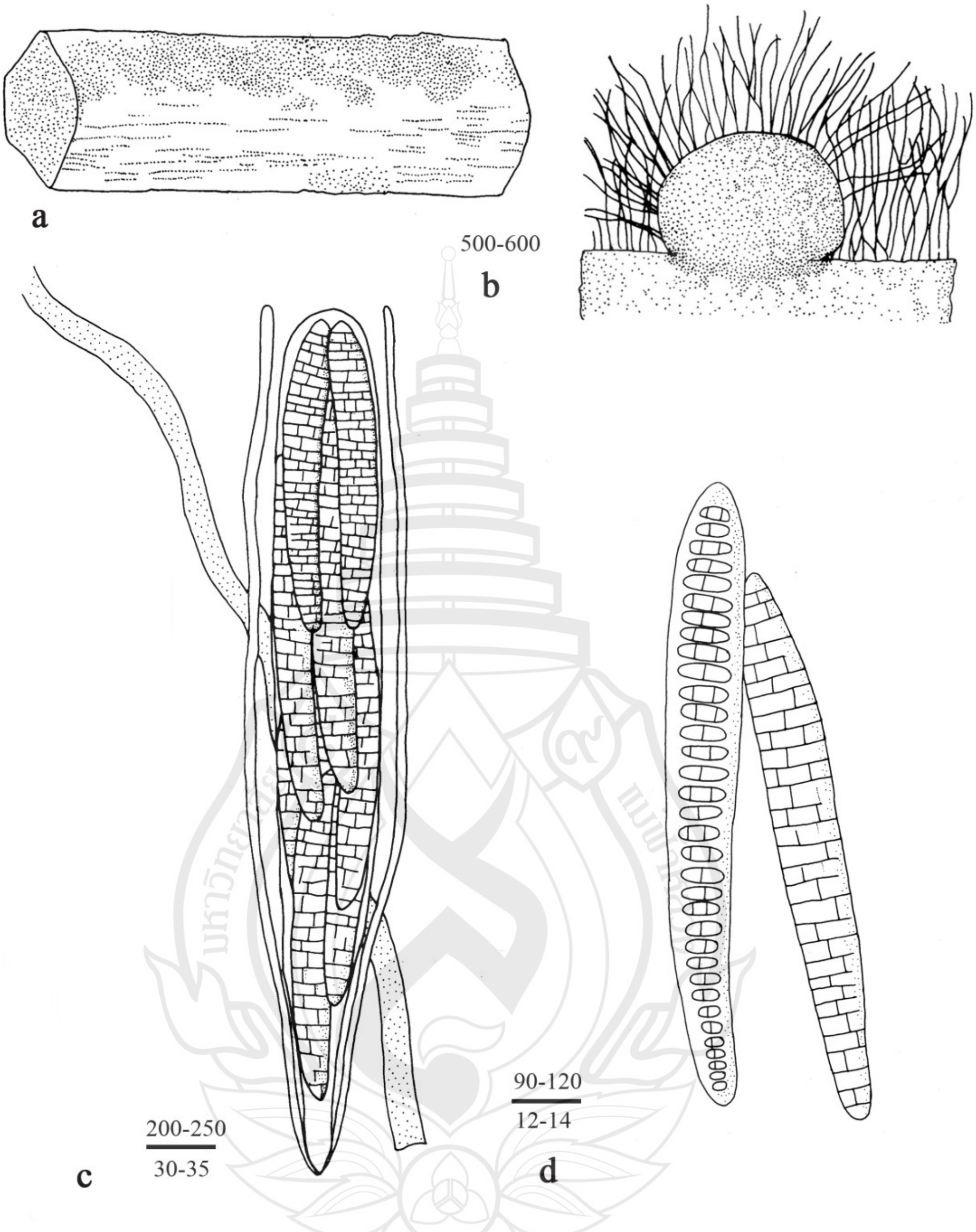
Type species: *Boerlagiomyces velutinus* (Penz. & Sacc.) Butzin

Boerlagiomyces velutinus (Penz. & Sacc.) Butzin, Willdenowia 8(1): 39 (1977), MycoBank: MB09608 (Fig. 5.13)

≡ *Boerlagella velutina* Penz. & Sacc., Malpighia 11(9-10): 404 (1897)

Herein, according to Penzig and Saccardo (1904) described: *Habit* saprobic on decaying wood or culms in petioles of *Plectocomia* sp. *Ascomata* 500–600 µm diam., superficial, dark brown, with black subiculum, globose to pyriform, scattered, ostiolate, with sparse, flexuous, black, septate, velvety, hyphal appendages, 210–250 × 3–6 µm, black, septate, surrounded black mycelium. *Peridium* composed of carbonaceous, multilayered walls. *Hamathecium* composed of filiform, thread-like, hyaline pseudoparaphyses, developing over the asci. *Asci* 200–250 × 30–35 µm, 8-spored, bitunicate, elongate, cylindric-clavate, apically rounded, narrowing towards base, sessile or short stalked. *Ascospores* 90–120 × 12–14 µm, 2–3-seriate in the ascus, cylindric-fusoid, gently curved, obtuse at both ends, muriform, 25–30-septate, not constricted at the septa hyaline, smooth. *Asexual morph* unknown.

Notes: The genus *Boerlagiomyces* was introduced by Butzin (1977), with *Boerlagiomyces velutinus* (Penz. & Sacc.) Butzin as the type species. It was accepted in the *Tubeufiaceae* by Barr (1980), on the basis of superficial, dark brown, soft ascomata, and well-developed hyphae (Penzig & Saccardo, 1904). Crane et al. (1998) monographed and accepted six species in the *Boerlagiomyces*. This genus presently comprises nine species epithets according to Index Fungorum. Molecular data are needed to confirms that all species in *Boerlagiomyces* are belong to *Tubeufiaceae*. While, *B. websteri* Shearer & J. L. Crane is differed from *B. velutinus*, molecular data places it with *Rhytisma acerinum* in *Rhytismataceae* (Kodsueb, Jeewon et al., 2006).



Note. Redrawn from Penzig & Saccardo (1904). (a)-(b) Substrate and ascoma, 500–600 μm diam. (c) Ascus, mycelium and pseudoparaphyses, ascus size: 200–250 μm × 30–35 μm (d) Ascospores, 90–120 μm × 12–14 μm

Figure 5.13 *Boerlagiomyces velutinus*

Chlamydotubeufia Boonmee & K. D. Hyde, Fungal Diversity 51(1): 78 (2011), MycoBank: MB563500

The detailed description of the generic type was previously described in Chapter 2 (Section 2.3.2).

Type species: *Chlamydotubeufia huaikangplaensis* Boonmee & K. D. Hyde

Chlamydotubeufia huaikangplaensis Boonmee & K. D. Hyde, Fungal Diversity 51(1): 78 (2011), MycoBank: MB563501

Chlamydotubeufia cf. huaikangplaensis Boonmee & K. D. Hyde, Fungal Diversity 51(1): 78 (2011) (Figs. 5.14-5.15)

Habit saprobic on dead wood. *Ascomata* 293.5–300 µm high × 271–340 µm diam. ($\bar{x}$ = 296 × 301 µm), superficial, solitary to clustered, scattered, globose to subglobose, black, surrounded by dark brown to black setae (37–)45–88 µm long and with tapering acute apex, centrally ostiolate. *Peridium* 47–51 µm wide, composed of serveral layers of cells of *textura angularis*, with dark brown to black cells. *Hamathecium* comprising numerous wide, filiform, *ca.* 3–3.5 µm wide, hyaline, septate, pseudoparaphyses. *Asci* (101–)103–110(–115) × 14–17 µm ($\bar{x}$ = 106 × 15 µm, n = 20), bitunicate, 8-spored, fissitunicate, cylindric-clavate, saccate, with rounded apex, with an ocular chamber, short-stalked. *Ascospores* (44–)50–56(–62.5) × 6–7 µm ($\bar{x}$ = 52 × 7 µm, n = 20), 2-seriately overlapping in the ascus, fusiform, slightly curved, tapering and rounded toward both ends, wider at median part, 7-septate, not constricted at the septa, hyaline, globular internally.

Cultural characteristics: Ascospores germinating on MEA within 12 hours with germ tubes produced from ascospores. *Colonies* growing on MEA slowly, reaching less than 5 mm in 1 week at 28^{o}C, flat to slightly effuse, sparsely hairy, with entire to fimbriate edge, dark. *Mycelium* superficial, with branched, septate, smooth, olivaceous brown, dark brown hyphae. *Conidiogenous cells* holoblastic, terminal or intercalary, micronematous, indistinguishable from hyphal cells. *Conidia* 67–88 µm long, 43–53 µm wide, dictyo-sporous, muriform, fusiform, wide in the middle, narrow at both ends, hyaline when young, dark brown to black at maturity, constricted at septa, seceding schizolytically from conidiogenous cells. *Asexual morph* hyphomycetous, helicosporous, dictyosporous, *Chlamydotubeufia*.

Material examined: THAILAND, Chiang Mai, Mae Taeng, Mushroom Research Center, N19°17.123′ E 98°44. 009′, 900 m., on dead wood of unidentified tree, 23 June 2011, Saranyaphat Boonmee, MRC-02: Holotype MFLU11–1145, ex-type culture: MFLUCC11–0509 = BCC 52381); MRC-05: Holotype MFLU11–1148, ex-type culture: MFLUCC11–0512 = BCC 52385.

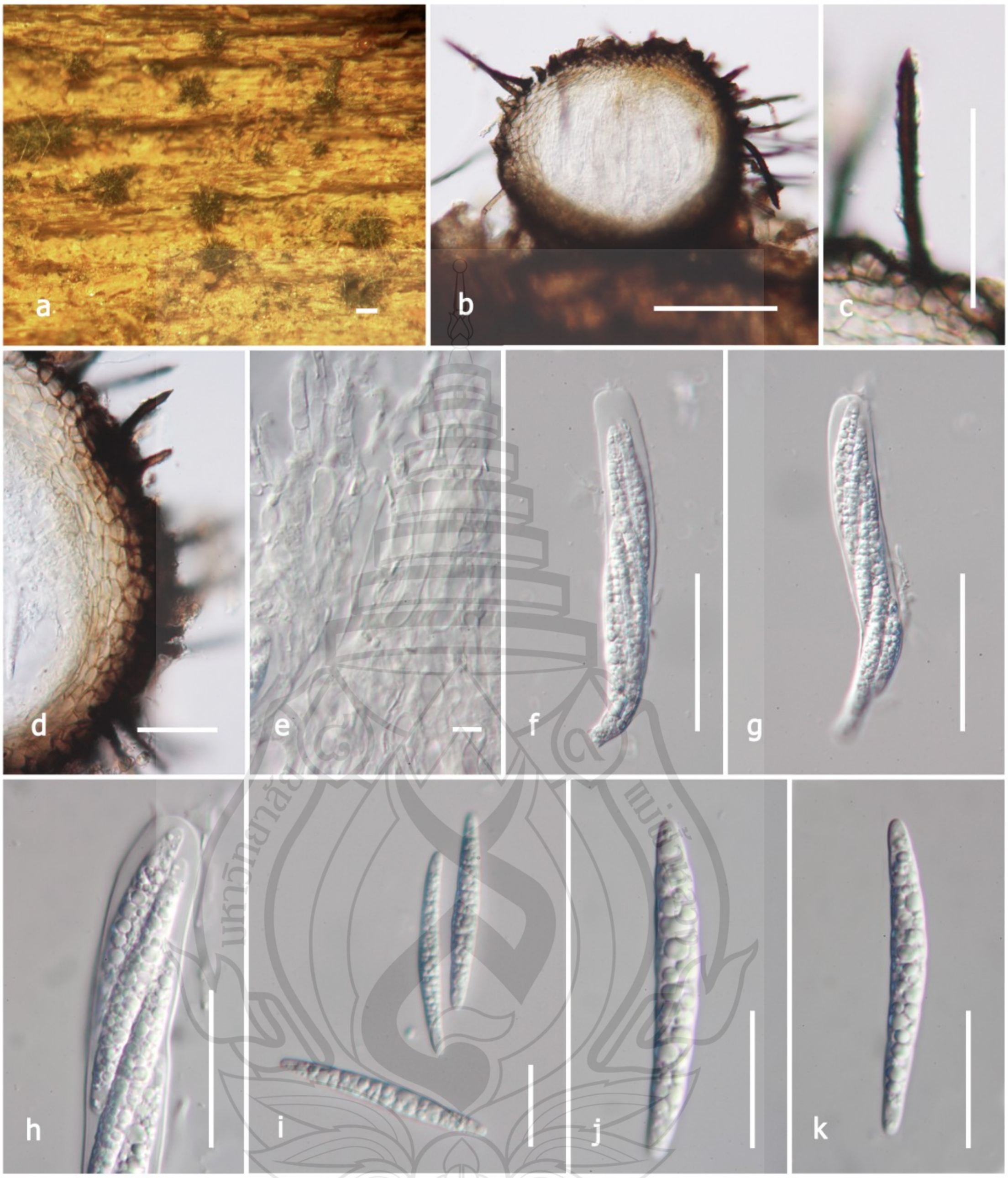
Notes: These two isolates are morphologically identical to *Chlamydotubeufia*, with the type species *C. huaikangplaensis* (Boonmee et al., 2011). However they do not cluster in the *Chlamydotubeufia* clade (Fig. 5.2, Clade B2), but group paraphyletically basally in the *Helicoma* clade.

Aquaphila asiana (Sivichai & K. M. Tsui) S. Boonmee & K. D. Hyde, comb. nov., MycoBank: MB804667

≡ *Tubeufia asiana* Sivichai & K. M. Tsui, Mycologia 99(6): 885 (2008) [2007]

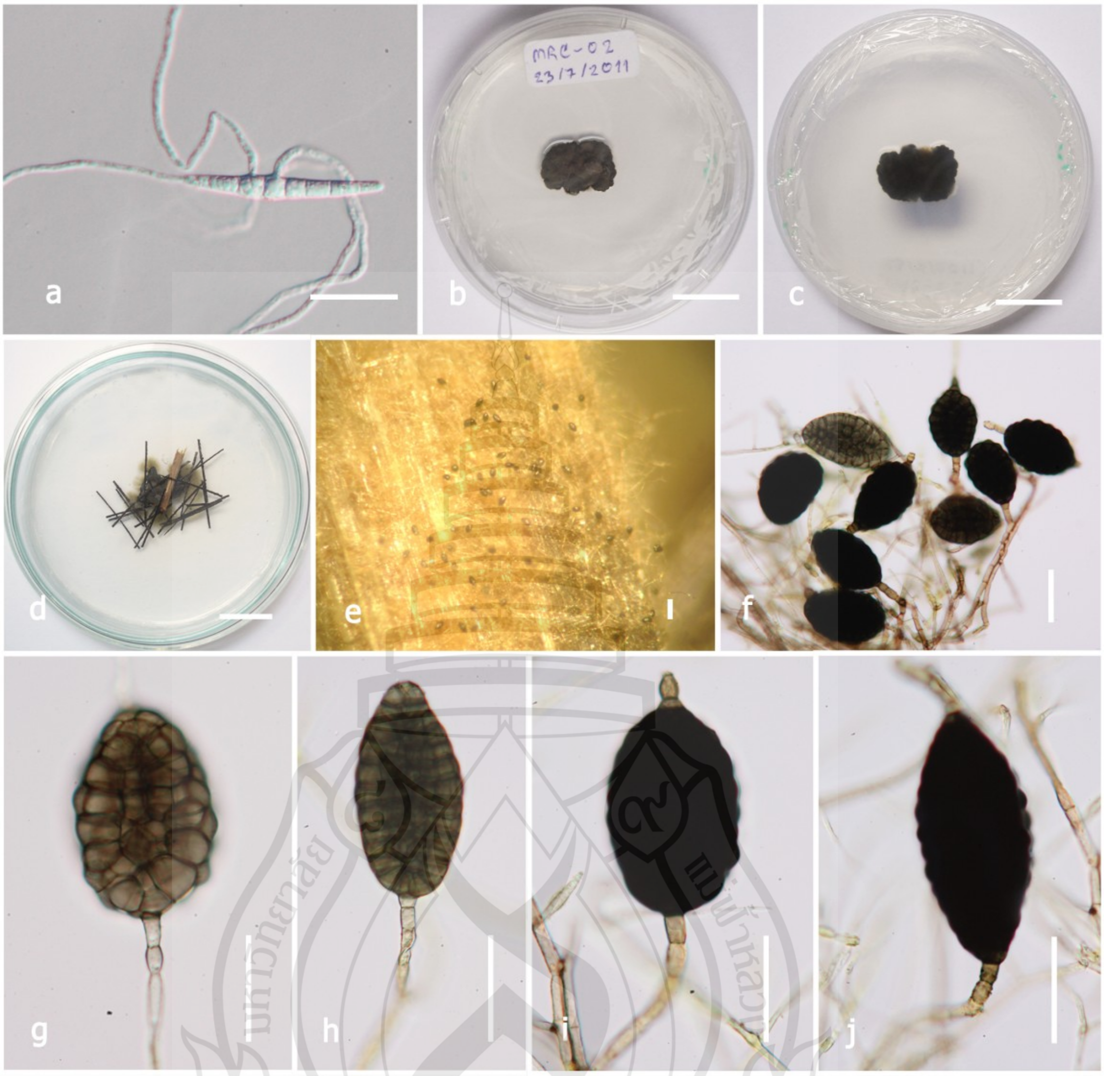
Aquaphila khunkornensis (Boonmee & K. D. Hyde) S. Boonmee & K. D. Hyde, comb. nov., MycoBank: MB804668

≡ *Chlamydotubeufia khunkornensis* Boonmee & K. D. Hyde, Fungal Diversity 51(1): 80 (2011)



Note. (a) Ascomata on substrate. (b) Section through ascoma. (c) Setae. (d) Peridium. (e) Pseudoparaphyses. (f)-(h) Asci. (i)-(k) Ascospores. Scale bars: (a)-(b) = 100 µm, (c),(f)-(h) = 50 µm, (d) = 40 µm, (e) = 5 µm, (i)-(k) = 20 µm

Figure 5.14 *Chlamydotubeufia cf. huaikangplaensis* (MFLU11–1145, holotype)



Note. Colonies on MEA. (a) Germinating ascospore. (b)-(c) Colonies on MEA from surface and reverse. Note dark brown colonies. (d) Anamorph testing on substrate. Note it could be produce both conditions on the medium, with and without substrate. (e) Anamorph occurring on plant tissues. (f)-(j) Conidia. Scale bars: (a) = 20 µm, (b)-(d) = 10 mm, (e)-(j) = 40 µm

Figure 5.15 Asexual State of *Chlamydotubeufia cf. huaikangplaensis* in Culture (MFLUCC11–0509, holotype)

Helicoma Corda, Icon. fung. (Prague) 1: 15 (1837), MycoBank: MB8473

Habit saprobic on woody substrate. *Mycelium* composed of partly immersed and partly superficial, pale brown, septate, branched hyphae. *Conidiophores* superficial, macronematous, crowded, erect, dark brown, septate, rarely branched, darkened and slightly constricted at the septum. *Conidiogenous cells* monoblastic to polyblastic, sometimes branched at the apex, brown to dark brown, smooth. *Conidia* tightly coiled 1–1½ times, conidial filament 6–9 µm wide, hyaline to pale brown, tapering toward flat end with a basal scar, septate, slightly constricted at the septum, smooth. *Sexual morph* ascomycetous, dothideomycetous, *Tubeufiaceae*, *Thaxteriella*, *Tubeufia*.

Type species: *Helicoma muelleri* Corda

Helicoma muelleri Corda, Icon. fung. (Prague) 1: 15 (1837), MycoBank: MB238937 (Fig. 5.16)

Habit saprobic on woody substrate. *Mycelium* composed of partly immersed and partly superficial, pale brown, septate, branched hyphae. *Conidiophores* superficial, macronematous, crowded, erect, dark brown, septate, rarely branched, darkened at the septum (43–)80–153.5 µm long × 7–10 µm wide ($\bar{x}$ = 106 × 8 µm, n = 20). *Conidiogenous cells* monoblastic to polyblastic, sometimes branched at the apex, brown to dark brown, smooth. *Conidia* (15–)16–19(–21) µm diam. and conidial filament 6–9 µm wide ($\bar{x}$ = 17 × 7 µm, n = 20), tightly coiled 1–1½ times, hyaline to pale brown, tapering toward flat end and with a basal scar 4–7(–8) µm wide, septate, slightly constricted at the septum, smooth.

Material examined: UNITED STATES, New Hampshire, Bartlett. Host: On dead wood (undermined substrate). Collected by Thaxter R., on April 1901, determined by Linder D., BPI 447569, not the type specimen.

Notes: The genus *Helicoma* was introduced by Corda (1837), with type species *H. muelleri* Corda. The genus is characterized by thick and dark brown, smooth, conidiophores, holoblastic conidiogenous cells and helicoid, hyaline, thick-walled, brown to dark brown conidia (Goos, 1986). Illustration presented here is based on authentic material *H. muelleri* on natural substrate. Various species of *Helicoma* are related with family species in *Tubeufiaceae* by association with nearby ascomata or found to produce in culture thus enabling investigation by molecular phylogenetic techniques (Boonmee et al., 2011; Tsui et al., 2006, 2007; Zhao et al., 2007). Herein, five species *Helicoma* are clustered in Clade D with high-supported (98% BS, 1.00 PP). Particularly, two sexual species *Thaxteriella helicoma* and *Thaxteriella inthanonensis* shared asexual *Helicoma*-like morphs and therefore, two new combinations are introduced to accommodate them in *Helicoma* i.e. *H. helicoma* and *H. inthanonensis*.

Helicoma khunkornense (Boonmee & K. D. Hyde) S. Boonmee & K. D. Hyde, comb. nov., MycoBank: MB804554

≡ *Tubeufia khunkornensis* Boonmee & K. D. Hyde, Fungal Diversity 51(1): 86 (2011)

Helicoma inthanonense (Boonmee & K. D. Hyde) S. Boonmee & K. D. Hyde, comb. nov., MycoBank: MB804555

≡ *Thaxteriella inthanonensis* Boonmee & K. D. Hyde, Fungal Diversity 51(1): 86 (2011)

Note. (a) Conidiophores with attached apical conidium on natural substrate. (b)-(c) Squash mount of conidiophores with conidial development at the apex (arrows). (d) Conidiophores with detached conidia and minute denticles (arrows). (e)-(g) Conidia. Scale bars: (a), (d)-(g) = 20 µm, (b)-(c) = 50 µm

Figure 5.16 *Helicoma muelleri* (BPI 447569)

Helicoma helicoma (W. Phillips & Plowr.) S. Boonmee & K. D. Hyde, comb. nov., MycoBank: MB804556 (Fig. 5.17)

≡ *Sphaeria helicoma* W. Phillips & Plowr., Grevillea, 6: 26 (1877)

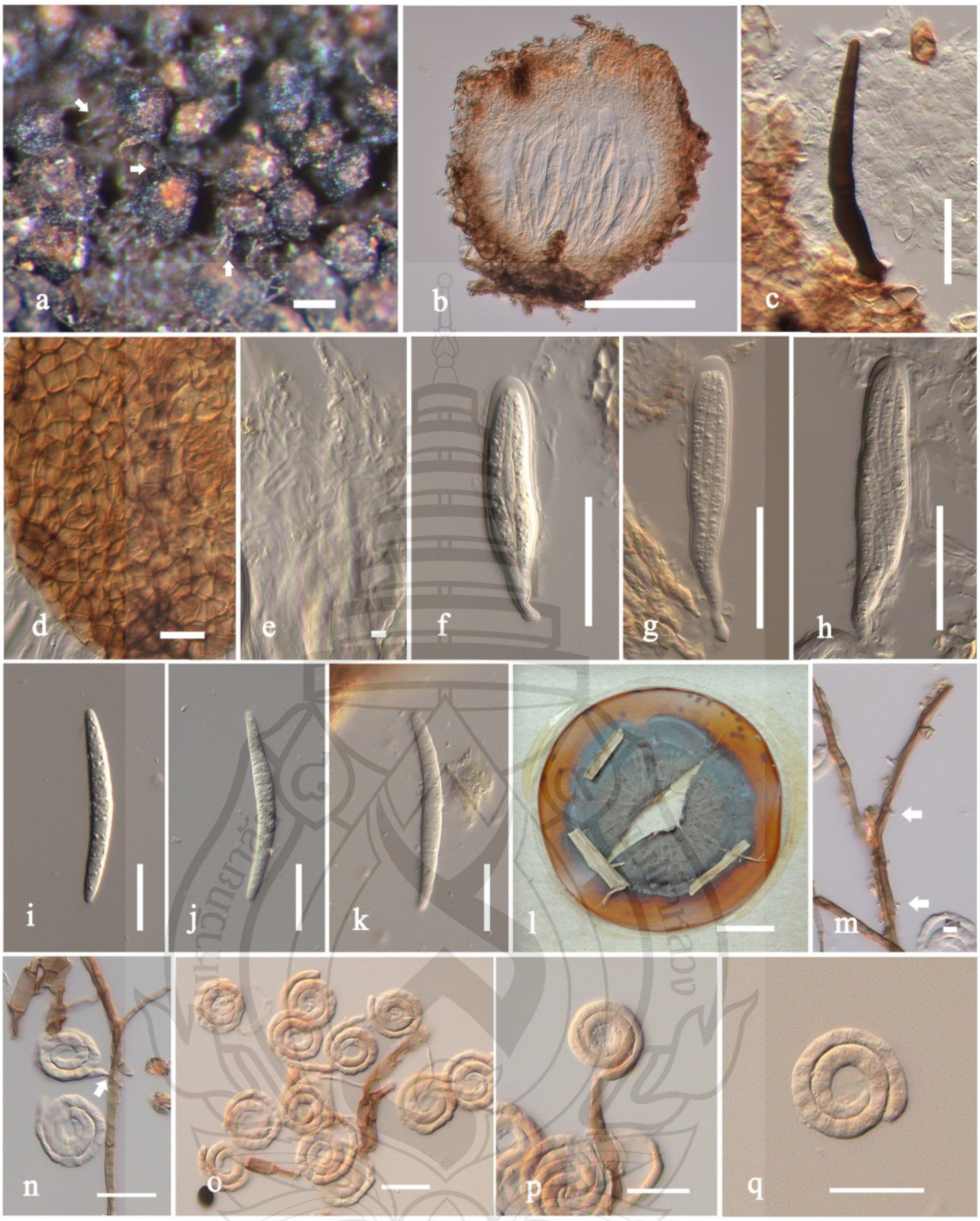
≡ *Thaxteriella helicoma* (W. Phillips & Plowr.) J. L. Crane, Shearer & M. E. Barr, Can. J. Bot. 76(4): 610 (1998)

Habit saprobic on leafy substrate. *Ascomata* (242–)303–340 µm high × (218–)238–263.5(–331.5) µm diam., superficial, subglobose, oval to obovoid, with softened texture, solitary, sometimes clustered, gregarious, dark brown, light brown on the top, with a central papillate ostiole, with sparse setae; setae 61–80 µm long, dark brown, tapering towards subacute apex, widest at the base, septate. *Peridium* 39–44 µm wide, composed of brown to red brown cells *textura angularis*. *Hamathecium* comprising numerous filiform pseudoparaphyses, 1–2 µm wide, hyaline, branched, septate. *Asci* (90–)96–135 × 15–20 µm ($\bar{x}$ = 112 × 19 µm, n = 20), bitunicate, 8-spored, elongate to cylindric-clavate, well-thickened at the apex, stalks (7–)20–31.5 µm long; partially of the asci length. *Ascospores* 42–61 × 5–8.5 µm ($\bar{x}$ = 53 × 6 µm, n = 20), 2–3-seriate in the ascus, elongate fusiform, subcylindrical, tapering towards subacute ends, (8–)9–10-septate, not constricted at the septum, hyaline, smooth. *Asexual morph* hyphomycetous, helicosporous, *Helicosporium*-like.

Cultural Characters (Based on dried culture): *Colonies* dark brown, superficial and partly immersed in the mycelium, with dark brown, septate, branched hyphae. *Conidiophores* macronematous, thick-walled, septate, branched, brown to moderately brown. *Conidiogenous cells* monoblastic to polyblastic, terminal or intercalary, dentate, brown to dark brown, darkened at the basal septum. *Conidia* helicoid, (17–)22–28 µm diam. and conidial filament 4–5 µm wide ($\bar{x}$ = 24 × 5 µm, n = 20), tightly coiled 2–3½ times, hyaline to pale brown, tapering toward flat end and scar, multiseptate, not constricted at the septum, smooth.

Material examined: BERMUDA, Devonshire Marsh, Devonshire Parish, on leaves of *Sabal bermudana*, collected by Korf R. P. and Rossman A. Y. (AR-BER 12), on 18 January 1980, determined by Rossman A. Y., BPI 1104599, dried culture: BER 12 80-4.

Notes: The species *Thaxteriella helicoma* was originally introduced in the genus *Sphaeria*, as *Sphaeria helicoma* W. Phillips & Plowr. by Phillips & Plowright (1877). Pirozynski (1972) redescribed and transferred the fungus to *Tubeufia* based on the asexual species of *Helicosporium*. Subsequently, Crane et al. (1998) transferred it to *Thaxteriella*, as *Thaxteriella helicoma* (W. Phillips & Plowr.) J. L. Crane, Shearer & M.E. Barr, because of the pigmented ascomata and peridial wall structure. In addition, *Thaxteriella helicoma* also produced asexual state as helicoid conidia in culture (Fig. 5.17). According to the present molecular phylogeny (Fig. 5.2), the isolates of *Thaxteriella helicoma* (JCM 2739 and UBC F13877) are included in Clade D, *Helicoma*, while the strain from UBC F13877 clustered with the type species *Helicoma muelleri* with highest support (100% BS, 1.00 PP). The placement of *Thaxteriella helicoma* into the *Helicoma,* as *Helicoma helicoma* herein, is well supported by asexual state in culture and molecular phylogenetic result.



Note. (a) Ascomata. (b) Section through ascoma. (c) Seta. (d) Peridial wall. (e) Pseudoparaphyses. (f)-(h) Asci. (i)-(k) Ascospores. (l) Colony on dried culture. (m) Mycelium. (n) Conidiophore (arrows). (o)-(q) Conidiophores with attached conidia. Scale bars: (a)-(c) = 100 µm, (c)-(d), (i)-(k), (n)-(q) = 20 µm, (f)-(h) = 50 µm, (e), (m) = 5 µm, (l) = 1 cm

Figure 5.17 *Helicoma helicoma* (BPI 1104599, dried culture: BER 12 80-4)

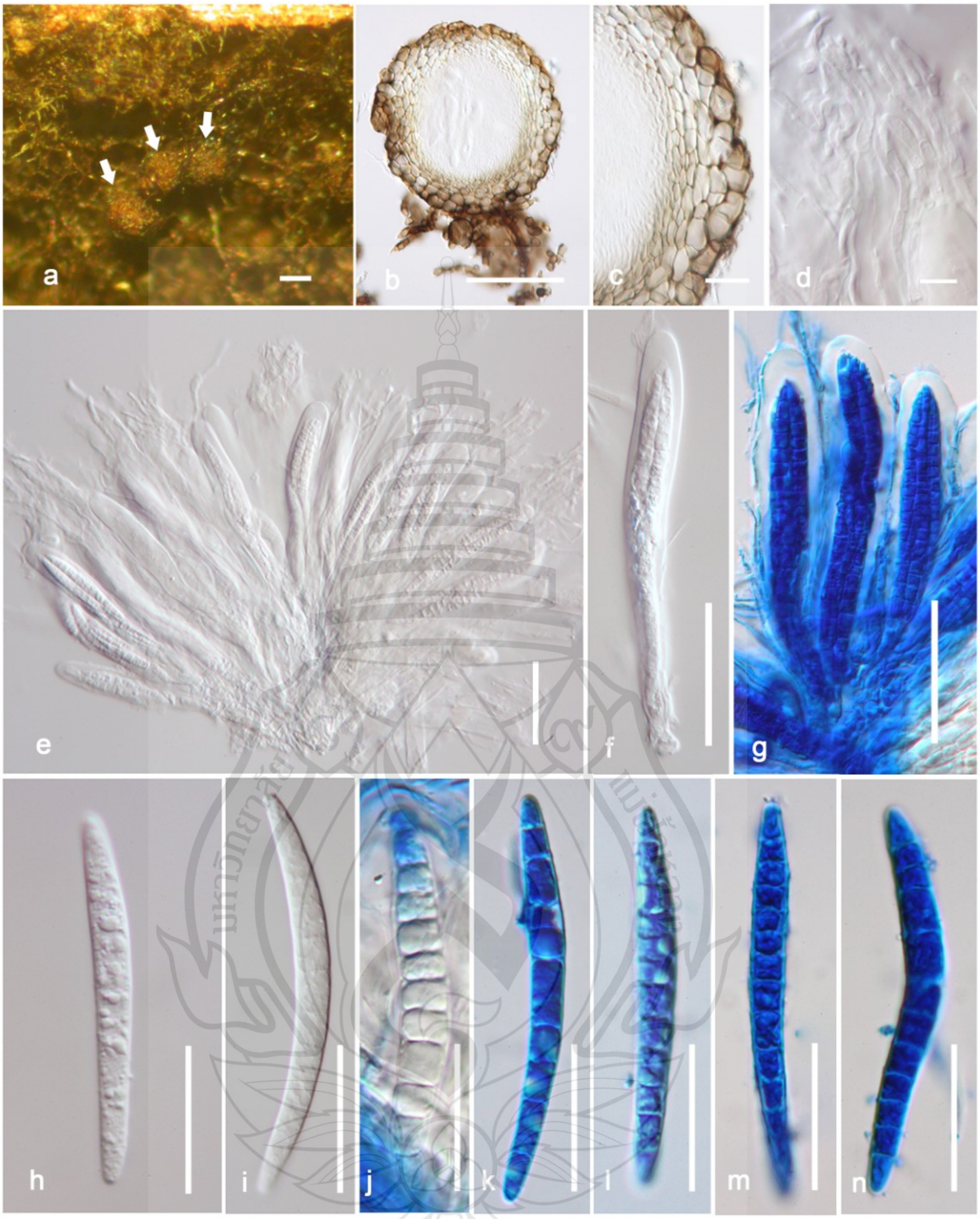
Helicoma siamense sp. nov. S. Boonmee & K. D. Hyde, MycoBank: MB804557 (Figs. 5.18-5.19)

Habit saprobic on dead wood. *Ascomata* 221.5–306 µm high × 180–268 µm diam. ($\bar{x}$ = 270 × 233 µm), superficial, with basal subiculum, solitary, scattered, globose to subglobose, reddish brown to dark brown, with a central ostiole, covered by brown mycelium, collapsing when dry. *Peridium* 30–48 µm wide, composed of several-layers, composed of cells *textura angularis*, with outer layer cells darkened and inner layer cells pale brown to hyaline. *Hamathecium* comprising numerous wide, filiform hyaline, pseudoparaphyses, branched, clearly septate. *Asci* (123–)127.5–139(–144) × (14–)17–20 µm ($\bar{x}$ = 133.5 × 18 µm, n = 20), bitunicate, 8-spored, cylindrical to clavate, with rounded apex, short-stalked. *Ascospores* (49.5–)59–64.5 (–68) × 5–7.5 µm ($\bar{x}$ = 62 × 6.5 µm, n = 20), 2–3-seriately overlapping in the ascus, cylindric-fusiform, tapering towards rounded ends, with wider supramedian part, slightly curved, 11–12-septate, not constricted at the septa, hyaline, smooth. *Asexual morph* hyphomycetous, helicosporous, *Helicoma*-like.

Cultural characteristics: Ascospores germinated on MEA within 24-36 hours and germ tubes produced from any cells. *Colonies* growing on MEA slowly, reaching to 6 mm in 1 week at 28^{o}C, flat, sparsely hairy, fimbriate, dark brown. *Mycelium* partly superficial, partly immersed, composed of branched, septate, smooth, pale brownish to reddish brown, red pigmented hyphal filaments. *Conidiophores* macronematous, 2–3 µm wide, erect, smooth, pale brown to brown, smooth. Conidiogenous cells holoblastic. *Conidia* helicosporous, 24–36 µm diam. when coiled, filaments 7–10 µm wide, coiled in 2–3 dimensional times, multiseptate, slightly constricted and darkened at septa, partly heavily pigmented, verrucose and rough-walled, brown to reddish brown, narrow at the base and wide at above half on uncoiling.

Material examined: THAILAND, Chiang Rai, Muang, Khun Korn Waterfall, N19°51-54' E 99°35.39', 671 m., on decaying wood of unidentified tree, 13 November 2009, Saranyaphat Boonmee, KK-09: Holotype MFLU10–0053, ex-type culture: MFLUCC10–0120 = BCC 52298= IFRD 2184.

Notes: This fungus is morphologically similar to *Tubeufia* and identified as a species in *Tubeufiaceae*. According to Barr (1980), *Tubeufiaceae* was established for those fungi having globose to subglobose, brownish to dark brown ascomata; bitunicate, cylindric-clavate asci, and elongated, fusiform, ellipsoidal, hyaline, multiseptate ascospores. However *Helicoma siamense* is different from *T. javanica* in size, shape and dimensions of ascomatal and ascospores. Besides, this species produced conidia as *Helicoma*-like which are multiseptate, helicoid and with coloured, verrucose, conidial filament. Phylogenetic study showed that *H. siamense* is clustering with *Helicoma* (*Thaxteriella*) *inthanonense* and three species of *Helicoma* with low-support. However, the morphological characteristics, both sexual and asexual, are distinct from all taxa in Clade D.



Note. (a) Ascomata (arrowed). (b) Section through ascoma. (c) Peridium. (d) Pseudoparaphyses embedded in a gelatinous matrix.(e)-(g) Asci. (h)-(n) Ascospores several-celled. Scale bars: (a)-(b) = 100 μm, (c), (h)-(n) = 20 μm, (d) = 5 μm, (e)-(g) = 50 μm

Figure 5.18 *Helicoma siamense* (MFLU10–0053, holotype)

Note. (a) Germinating ascospore. (b)-(c) Colonies on MEA at surface and reverse. Note black colonies. (d) Mycelium development in culture. (e)-(l) Conidiophores and conidia. Scale bars: (a), (g)-(l) = 20 µm, (b)-(c) = 10 mm, (d)-(f) = 5 µm

Figure 5.19 Asexual State of *Helicoma siamense* in Culture (MFLUCC10–0120, holotype)

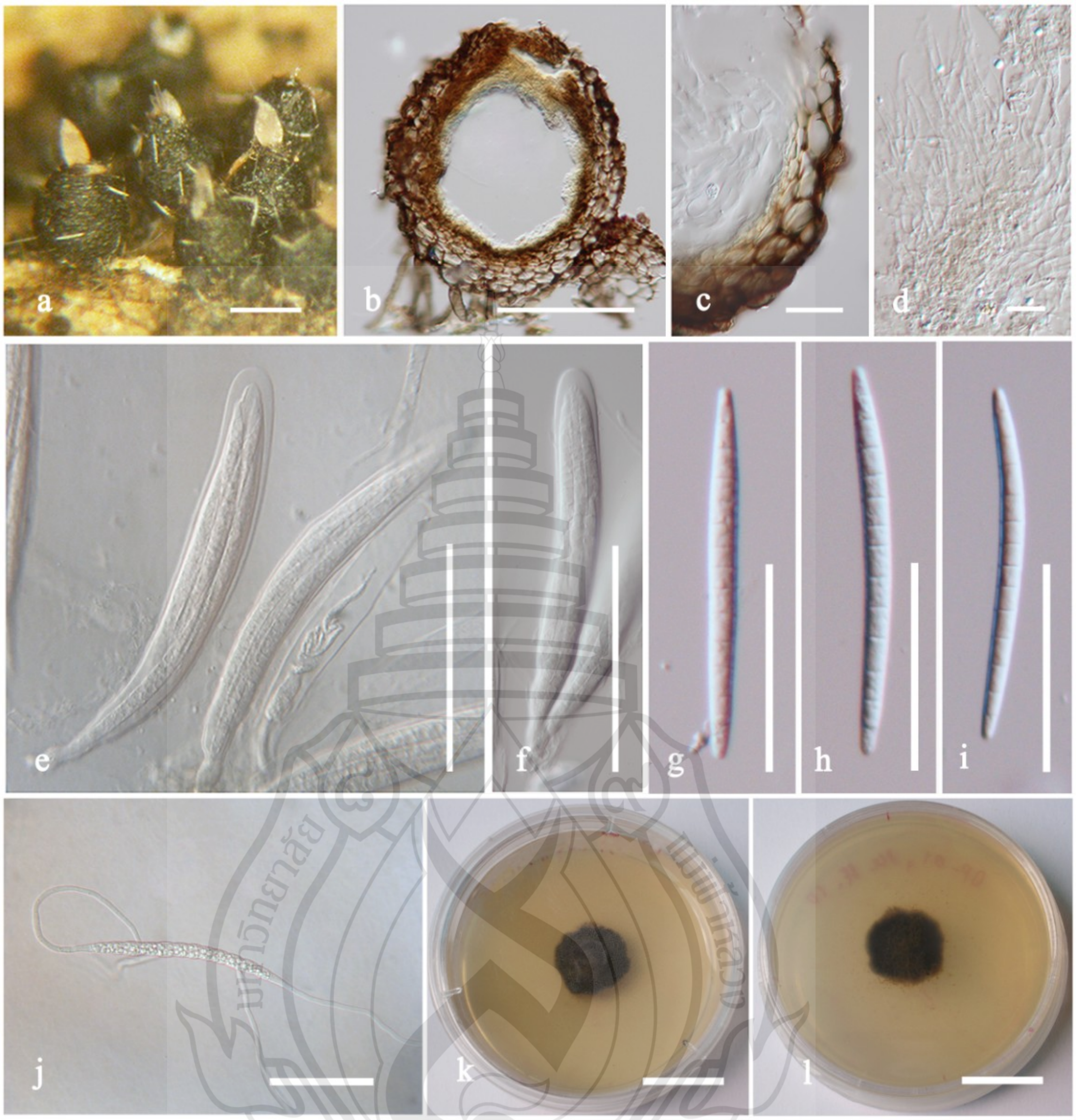
Helicoma chiangraiense sp. nov. S. Boonmee & K. D. Hyde, MycoBank: MB804558 (Fig. 5.20)

Habit saprobic on dead wood. *Ascomata* (183–)240.5–285 µm high × (189–) 241–296 µm wide. ($\bar{x}$ = 242.5 × 254 µm), superficial, subglobose, ellipsoidal-ovate, egg-shaped, with few hyphae developing from ascomatal base on substrate, solitary, scattered, dark brown to black, velvety, centrally ostiolate. White-colour on the tip of ascomata indicated oozing of ascospores in a mass. *Peridium* 28–34 µm wide, comprising 3–4 layers, composed of cells *textura angularis*; with inner layer of cells brown and outer layers dark brown. *Hamathecium* comprising numerous filiform, *ca*. 0.5–1 µm wide, hyaline, pseudoparaphyses. *Asci* (146–)153–179.5 × 18–23(–26) µm ($\bar{x}$ = 163.5 × 21 µm, n = 20), bitunicate, cylindrical, thickened and rounded apex, with a nonapparent ocular chamber, 8-spored, with basal stalk 14.5–24 µm long. *Ascospores* (63–)72–93.5 × 5–7.5 µm ($\bar{x}$ = 81 × 6 µm, n = 20), multiseriately overlapping in the ascus, cylindric-fusiform, with tapering and rounded ends, straight to slightly curved, 11–13-septate, not constricted at the septa, hyaline, smooth. *Asexual morph* unknown.

Cultural characteristics: Ascospores germinating on MEA within 12 hours and germ tubes produced at both ends. *Colonies* growing slowly on MEA, reaching less than 5 mm in 1 week at 28^{o}C, effuse, velvety, with a convex surface, with dark continuous margin, asexual morph spores not formed within 30-60 days.

Material examined: THAILAND, Chiang Rai, Muang, Doi Pui, *ca*. 403-936 m., on dead wood of *Fagaceae*, 15 August 2009, Saranyaphat Boonmee, DP-01: Holotype MFLU10–0048, ex-type culture: MFLUCC10–0115 = BCC 39624, 39625 = IFRD 2195.

Notes: *Helicoma chiangraiense* differs from all hitherto described *Tubeufia* species in having dark brown to black, shining, nonsetose ascomata with distinct ostiole which well exudes the ascospores. *Helicoma chiangraiense* clusters within the *Helicoma* clade though this species has no known asexual state.



Note. (a) Ascomata superficial on substrate. (b) Section through ascoma. (c) Peridium. (d) Pseudoparaphyses embedded in mucilaginous matrix. (e)-(f) Asci. (g)-(i) Ascospores. (j) Germinating ascospore. (k)-(l) Colonies on MEA from surface and reverse, colonies dark brown. Scale bars: (a)-(b), (e)-(f) = 100 μm, (c) = 20 μm, (d) = 5 μm, (g)-(j) = 50 μm, (k)-(l) = 10 mm

Figure 5.20 *Helicoma chiangraiense* (MFLU10–0048, holotype)

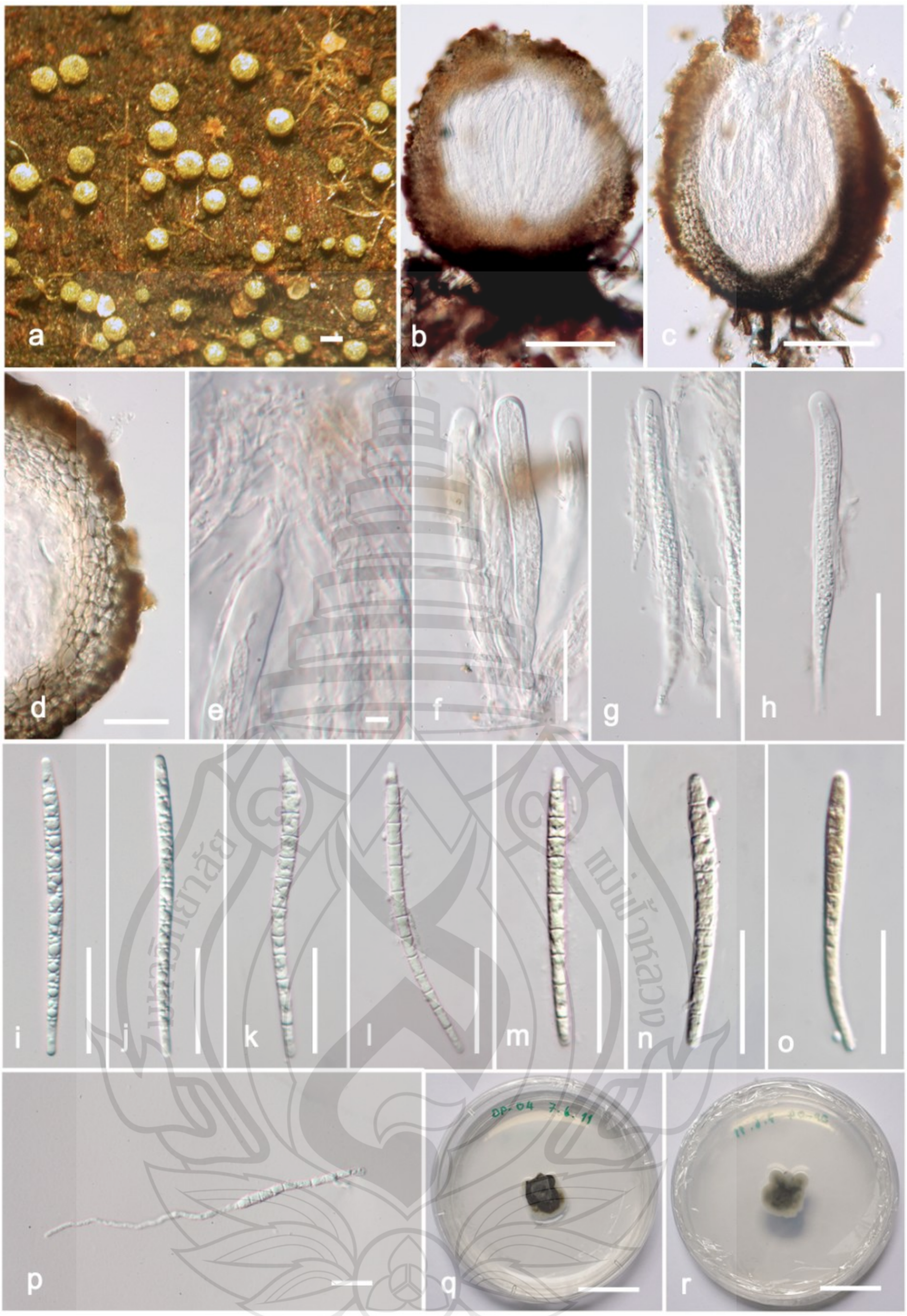
Helicoma fagacearum sp. nov. S. Boonmee & K. D. Hyde, MycoBank: MB804559 (Fig. 5.21)

Habit saprobic on dead wood. *Ascomata* 256–300(–324) µm high × 235–258(–265) µm wide ($\bar{x}$ = 282 × 251 µm), superficial, solitary, scattered, with a subiculum composed of dark brown hyphae developing from ascomatal base onto substrate, globose to subglobose, broadly ellipsoid-ovate, yellowish or brownish, light brown (white under incident light), centrally ostiolate, collapsing and brown when dry. *Peridium* 43–51 µm wide, comprising several layers of angular and prismatic cells, with inner layer of cells pale brown, with outer layer of cells reddish brown to brown. *Hamathecium* comprising numerous filiform, hyaline, *ca*. 1.5 µm wide, branched, septate, pseudoparaphyses. *Asci* (121–)134–160(–179) × 10–14 µm ($\bar{x}$ = 144 × 12 µm, n = 20), bitunicate, 8-spored, elongate-cylindrical, apically thickened when young, rounded at the apex, with a nonapparent ocular chamber, with a persistent pedicel. *Ascospores* (63–)71–86.5 × 4–6.5 µm ($\bar{x}$ = 78 × 5 µm, n = 20), 2–3-seriately overlapping in the ascus, cylindric-fusiform, tapering towards rounded ends, straight to slightly curved, 9–12(–13)-septate, not constricted at the septum, hyaline, pale brown or light brown at maturity, guttulate when young, smooth. *Asexual morph* unknown.

Cultural characteristics: Ascospores germinated on MEA within 12 hours and germ tubes produced at both ends. *Colonies* growing on MEA slowly, reaching less than 5 mm in 1 week at 28^{o}C, slightly convex on the surface, with undulated margin, white or light brown to dark brown, turning dark with age, asexual morph spores not formed within 30-60 days.

Material examined: THAILAND, Chiang Rai, Muang, Doi Pui, *ca*. 403-936 m., on dead wood of *Fagaceae*, 10 May 2011, Saranyaphat Boonmee, DP-04: Holotype MFLU11–0138, ex-type culture: MFLUCC11–0379 = BCC 52030.

Notes: *Helicoma fagacum* was found on wood belong to *Fagaceae* gathered in northern Thailand. Morphologically, this species is similar to *T. paludosa*, illustrated and described by several other (Kodsueb et al., 2004; Samuels et al., 1979; Sivanesan, 1984). However, *H. fagacearum* differs from *T. paludosa* by non-transparent ascomata (with yellowish white surface and brown peridium in section); and pale brown, non-mucilaginous ascospores. The asexual morph spores did not come in culture. Also *T. paludosa* is related with an asexual morph in the genus *Helicomyces* (Kodsueb et al., 2004; Samuels et al., 1979). Our phylogenetic study showed that *H. fagacearum* is nested within the *Helicoma* clade (Fig. 5.2, Clade D) and distantly placed from *T. paludosa*.



Note. (a) Ascomata. (b) Section through ascoma. (c) Peridium. (d) Pseudoparaphyses. (e)-(g) Asci. (h)-(j) Ascospores. (k) Germinating ascospore. (l)-(m) Colonies on MEA from surface and reverse. Scale bars: (a)-(b) = 100 µm, (c) = 40 µm, (d) = 5 µm, (e)-(g) = 50 µm, (h)-(k) = 20 µm, (l)-(m) =10 mm

Figure 5.21 *Helicoma fagacearum* (MFLU11–0138, holotype)

Helicomyces Link, Mag. Gesell. naturf. Freunde, Berlin 3(1-2): 21 (1809), MycoBank: MB237696

Habit saprobic on woody substrate. *Mycelium* composed of partly immersed and partly superficial, pale brown, septate, sparsely branched hyphae, with masses of crowded conidia. *Conidiophores* macronematous, erect, short, septate, thick-walled, pale to moderately dark brown. *Conidiogenous cells* monoblastic or polyblastic, terminal or intercalary, integrated, subhyaline, denticulate. *Conidia* loosely coiled 2½–4 times, becoming loosely uncoiled in water, conidial filament 4.5–6 µm wide, rounded at apical end, bright, hyaline to pale yellow, multiseptate, up to 56-septate, slightly constricted at the septum, smooth. *Sexual morph* ascomycetous, dothideomycetous, *Tubeufiaceae*, *Acanthostigma*, *Tubeufia*.

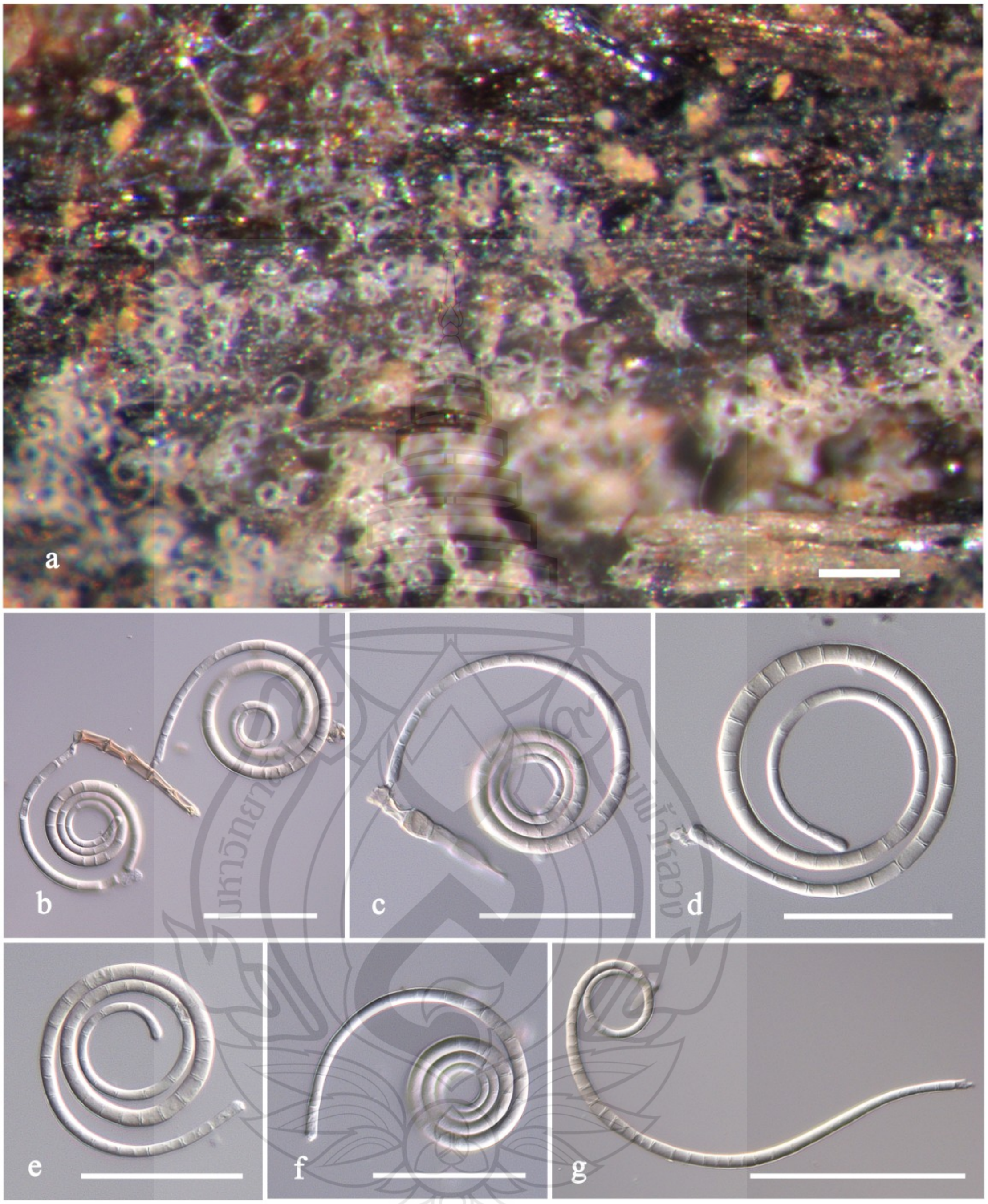
Type species: *Helicomyces roseus* Link

Helicomyces roseus Link, Mag. Gesell. naturf. Freunde, Berlin 3(1-2): 21 (1809), MycoBank: MB237696 (Fig. 5.22)

Habit saprobic on woody substrate. *Mycelium* composed of partly immersed and partly superficial, pale brown, septate, sparsely branched hyphae, with masses of crowded conidia. *Conidiophores* erect, 3–4 × 2–3 µm ($\bar{x}$ = 4 × 3 µm, n = 5), septate, thick-walled, pale to moderately dark brown, macronematous, short. *Conidiogenous cells* monoblastic or polyblastic, terminal or intercalary, integrated, subhyaline, denticulate. *Conidia* (40–)47–65(–75) µm diam. and conidial filament 4.5–6 µm wide ($\bar{x}$ = 54 × 6 µm, n = 20), with (298.5–)360–398 µm long, loosely coiled 2½–4 times, becoming loosely uncoiled in water, rounded at apical end, bright, hyaline to pale yellow, multiseptate, up to 56-septate, slightly constricted at the septum, smooth. *Sexual morph* ascomycetous, dothideomycetous, *Tubeufiaceae*, *Acanthostigma*, *Tubeufia*.

Material examined: UNITED STATES, North Carolina, Bearwallow Cove, on rotten wood, collected by Diehl W. W., on 4 January 1927, determined by Stevenson J. A., BPI 421361.

Notes: The genus *Helicomyces*, introduced by Link (1809), is characterized by helicoid conidia with slender, loosely coiled, multiseptate, hyaline, filament white to pinkish in mass, and typified by *H. roseus*. The illustration presented here demonstrated the formation of *Helicomyces roseus* on natural substrate. *Helicomyces roseus* was frequently found associated with *Tubeufia cylindrothecia*, which was first determined by Seaver and Waterston (1940), and later by Barr (1980). Tsui and Berbee (2006) and Tsui et al. (2006, 2007) have shown that *Helicomyces* is polyphyletic with sexual states in the genera *Acanthostigma* and *Tubeufia*.



Note. (a) Conidia arise directly from hyphal cells on natural substrate. (b)-(c) Conidiophores with attached conidia. (d)-(g) Conidia. Scale bars: (a), (g) = 100 µm, (b)-(f) = 50 µm

Figure 5.22 *Helicomyces roseus* (BPI 421361)

Helicosporium Nees, Syst. Pilze (Würzburg): 68 (1816) [1816-17], MycoBank: MB8484

Habit saprobic on leaves, bark, twigs, terrestrial or submerged in freshwater. *Conidiophores* arising directly on the substrate, from thick-walled, closely septate, repent hyphae, crowded or in fascicles, glistening, light coloured. *Conidiophores* macronematous, mononematous, setiferous, erect, septate, unbranched, dark brown, fertile in the middle, sterile and tapering towards narrow subacute at the apex, smooth. *Conidiogenous cells* polyblastic, intercalary, rarely terminal, with lateral minute denticles each with single conidium. *Conidia* coiled 3½–4½ times, tightly to loosely coiled, rounded at the apical end, truncate at the base, hyaline, 7–13-septate, slightly constricted at the septum, smooth. *Sexual morph* ascomycetous, dothideomycetous, *Tubeufiaceae*, *Acanthostigma*, *Tubeufia*.

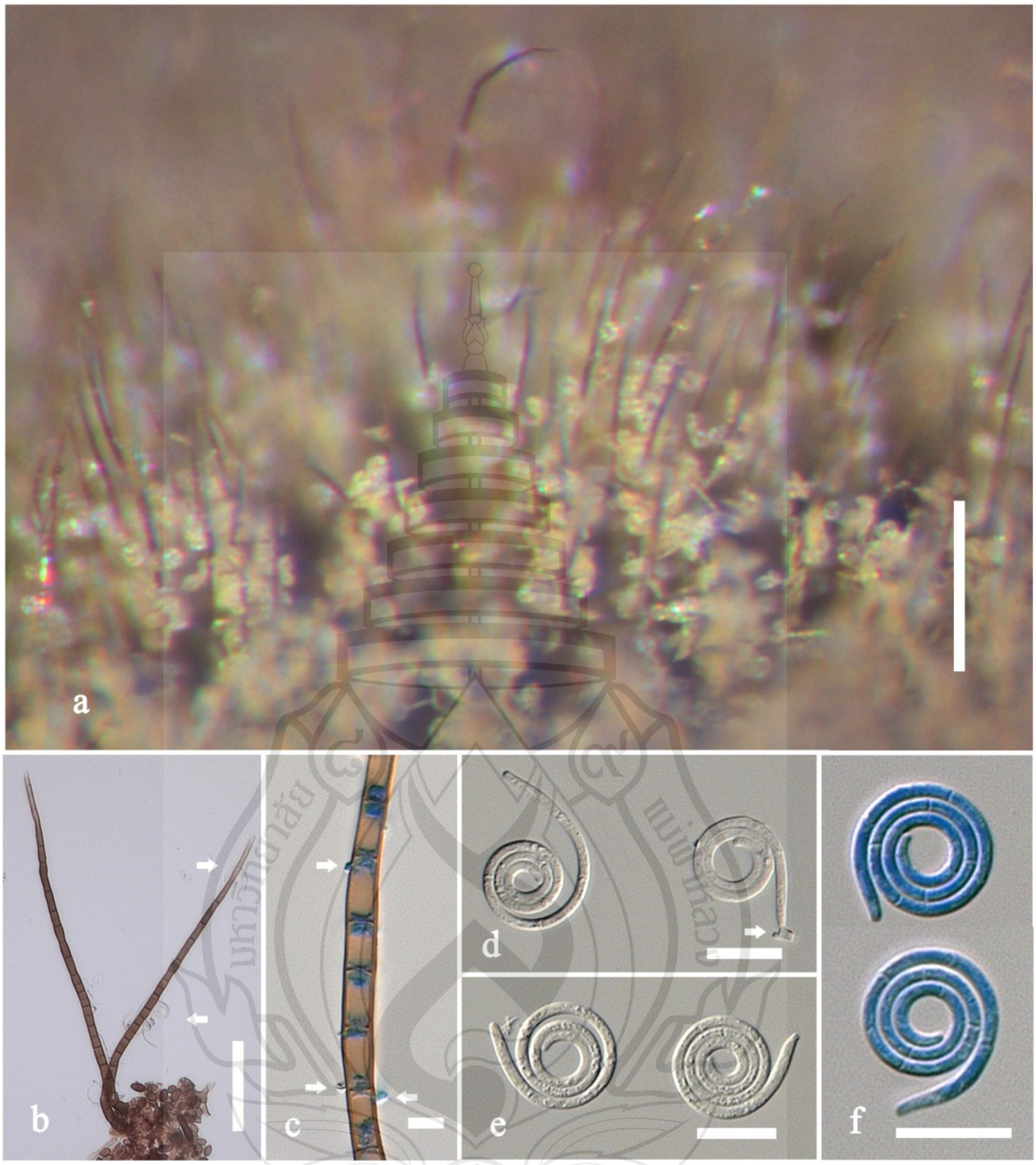
Type species: *Helicosporium vegetum* Nees

Helicosporium vegetum Nees, Syst. Pilze (Würzburg): 68 (1816) [1816-17], MycoBank: MB 145573 (Fig. 5.23)

Habit saprobic on leaves, bark, twigs, terrestrial or submerged in freshwater. *Conidiophores* arising directly on the substrate, from thick-walled, closely septate, repent hyphae, crowded or in fascicles, glistening, light colour. *Conidiophores* (98–) 107–220 µm long × 3–5 µm ($\bar{x}$ = 148 × 4 µm, n = 20), macronematous, mononematous, setiferous, erect, septate, unbranched, dark brown, fertile in the middle, sterile and tapering toward narrow subacute at the apex, smooth. *Conidiogenous cells* polyblastic, intercalary, rarely terminal, with lateral minute denticles each with single conidium. *Conidia* 10–15 µm diam. and conidial filament 1–2 µm wide ($\bar{x}$ = 13 × 1.5 µm, n = 20), coiled 3½–4½ times, tightly to loosely coiled, rounded at apical end, truncate at the base, hyaline, 7–13-septate, slightly constricted at the septum, smooth. *Sexual morph* ascomycetous, dothideomycetous, *Tubeufiaceae*, *Tubeufia*.

Material examined: UNITED STATES, Virginia, Falls Church, on rotten wood, collected by Shear C. L., on May 1936, determined by Stevenson J. A., BPI 447464.

Notes: *Helicosporium* was introduced on the basis of *Helicosporium vegetum* as characterized by Nees von Esenbeck, (1817). *Helicosporium* is polyphyletic within *Tubeufiaceae* and related with sexual states in the genera *Acanthostigma*, *Acanthostigmina* and *Tubeufia* (Fig. 5.2, Tsui & Berbee, 2006; Tsui et al., 2006). The type species, *H. vegetum*, is linked at the molecular level with the sexual state *Tubeufia cerea* (Berk. & M.A. Curtis) Höhn. (Fig. 5.2).



Note. (a) Conidiophores with conidia on natural substrate. (b)-(c) Conidiophores with minute denticles (arrows). (d)-(f) Conidia (strained by cotton blue in Fig. f). Scale bars: (a) = 100 μm, (b) = 50 μm, (c) = 5 μm, (d)-(f) = 10 μm

Figure 5.23 *Helicosporium vegetum* (BPI 447464)

Helicosporium cereum (Berk. & M. A. Curtis) S. Boonmee & K. D. Hyde, comb. nov., MycoBank: MB804560 (Fig. 5.24)

≡ *Sphaeria cerea* Berk. & M. A. Curtis, Grevillea 4(no. 31): 108 (1876)

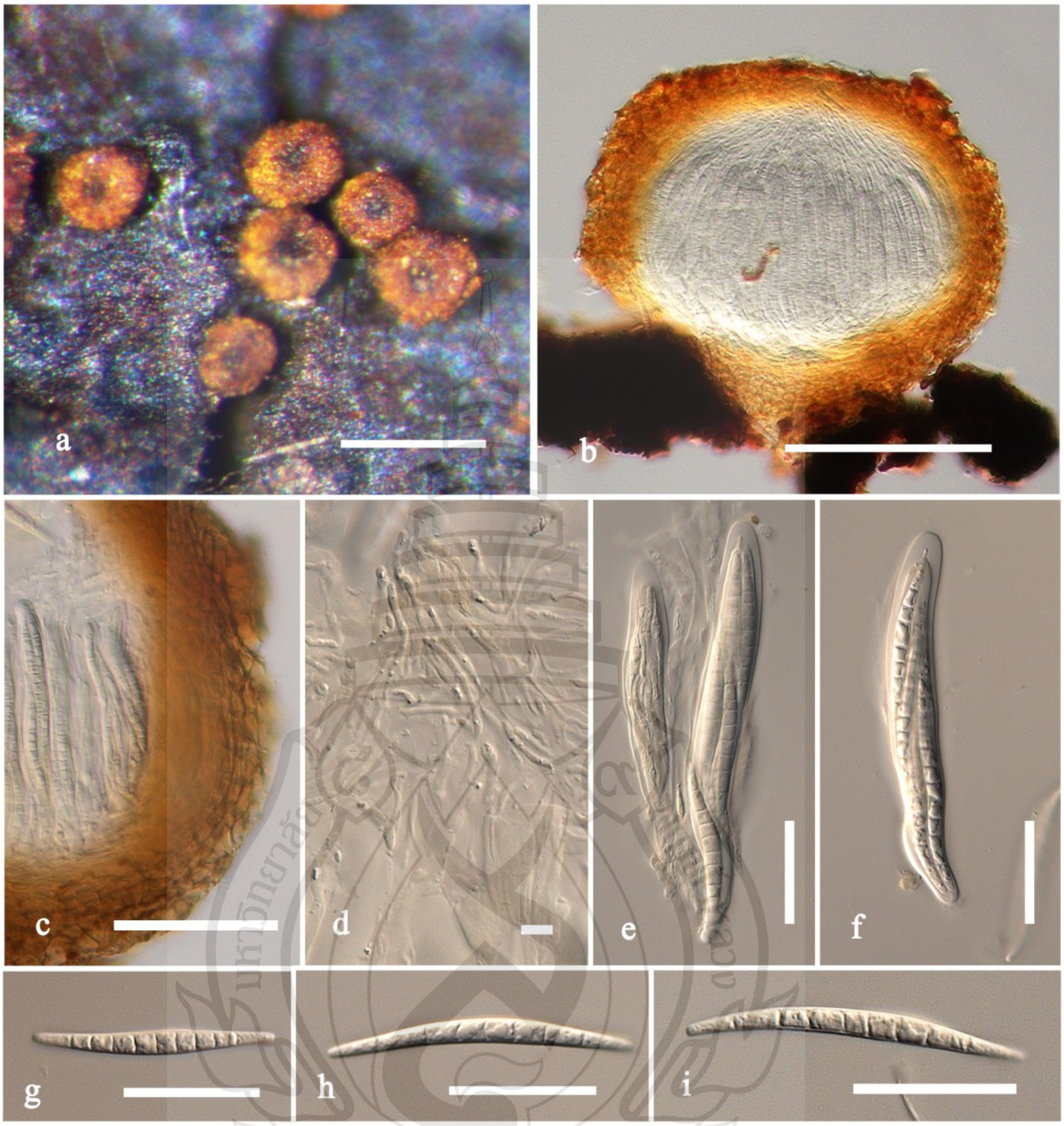
= *Tubeufia cerea* (Berk. & M. A. Curtis) Höhn., Sber. Akad. Wiss. Wien, Math-Naturw Kl., Abt. 1(128): 562 (1919)

Habit saprobic fungicolous, occurred on tromata of *Diatrype stigma*, *Diatrypaceae* (*Xylariales*), on decorticated or decaying wood, terrestrial habitats, widespread in temperate to tropical regions. *Ascomata* 180–211 high × 186–226.5 µm diam., superficial, solitary, scattered, globose-subglobose, bright yellow brown to yellow orange, collapsing when dry, darkened near the ostiole. *Peridium* 23–26 µm wide, several layers, composed of prismatica-angular cells, bright yellow. *Hamathecium* numerous filiform with 1–1.5 µm wide, hyaline, branched, septate, pseudoparaphyses. *Asci* 68–82(–92) × (7–)8–12 µm ($\bar{x}$ = 75 × 10 µm, n = 20), bitunicate, 8-spored, cylindric-clavate, thickened at the apex, with acute an ocular chamber, sessile. *Ascospores* 35–46 × 3–5 µm ($\bar{x}$ = 39 × 4 µm, n = 10), biseriate in the ascus, elongate-fusiform, tapering toward narrowly subacute both ends, 8-9 septate, slightly curved, hyaline, smooth. *Asexual morph* hyphomycetous, helicosporous, *Helicosporium*-like.

Material examined: UNITED STATES, Virginia, Shenandoah National Park, Beams Gap, occurred on tromata of *Diatrype stigma*, *Diatrypaceae*, (*Xylariales*), on dead wood, collected by Amy Y. Rossman, 1925; determined by Amy Y. Rossman, 19 June 1983, BPI 1107327, type not observed.

Notes: von Höhnel (1919) transferred species *Sphaeria cerea* Berk. & M.A. Curtis into the *Tubeufiaceae*, *Tubeufia*, as *Tubeufia cerea* (Berk. & M.A. Curtis) Höhn., based on bitunicate asci, and having narrow elongate, multiseptate ascospores. The species *T. cerea* is often found occurs on stromata of fungal species belonged to *Diatrypaceae* (*Xylariales*). Morphologically, *Tubeufia cerea* is distinguished from all species *Tubeufia* by having yellow brown to yellow orange, ascomata (Barr, 1980; Bigelow & Barr, 1963; Booth, 1964; Samuels et al., 1979;). Booth (1964) later, provided description and illustration of *Tubeufia cerea* based on the type specimen and accounted of this species with a list of synonyms. Additionally, the asexual state was also found near ascomata and identified to type species of the *Helicosporium, Helicosporium vegetum* Nees by Booth (1964), later by Samuels et al. (1979) and

Barr (1980). Presently, the molecular technicality was used to investigate *Tubeufia cerea* with its asexual morphs. The phylogenetic results suggest that *T. cerea* is connected with strains of type species *Helicosporium vegetum* with high-supported (Tsui & Berbee, 2006; Tsui et al., 2006). In this study, the isolates of *T. cerea* clustered with the strains of type species *H. vegetum* with high support (100% BS, 1.00 PP). *Tubeufia cerea* therefore, we transfer into *Helicosporium,* as *Helicosporium cereum* because this species is connected to the type species, *H. vegetum*, according to molecular phylogenetic result (Fig. 5.2).



Note. (a) Ascomata. (b) Section through ascoma. (c) Peridium. (d) Pseudoparaphyses. (e)-(f) Asci. (g)-(i) Ascospores. Scale bars: (a) = 200 µm, (b) = 100 µm, (c) = 50 µm, (d) = 5 µm, (e)-(i) = 20 µm

Figure 5.24 *Helicosporium cereum* (BPI 1107327)

Thaxteriella Petr., Annls mycol. 22(1/2): 63 (1924), MycoBank: MB5408

The detailed description of the generic type was previously described in Chapter 2 (Section 2.3.2).

Type species: *Thaxteriella corticola* Petr., Annls mycol. 22(1/2): 63 (1924), MycoBank: MB262097

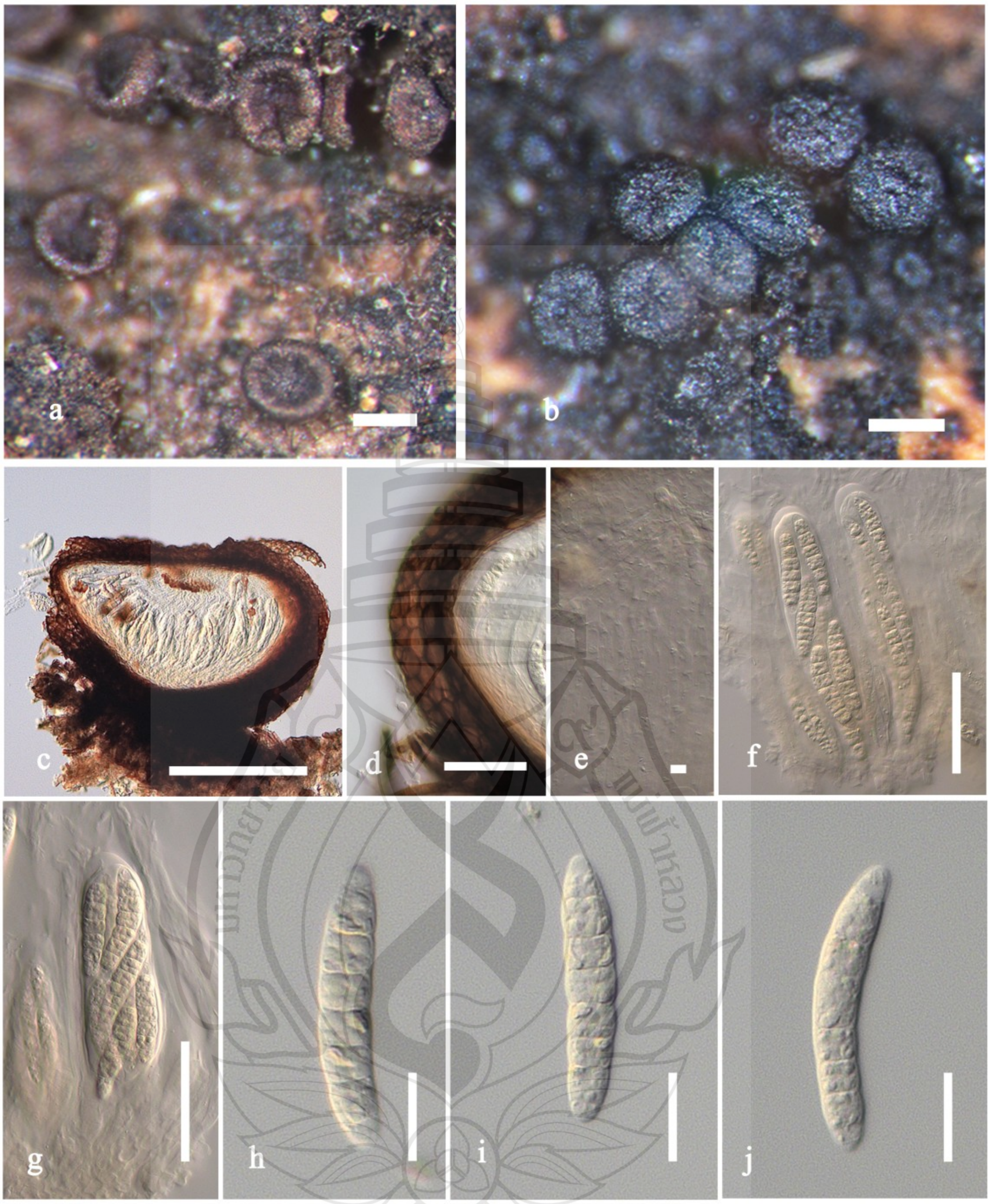
Thaxteriella pezizula (Berk. & M. A. Curtis) Petr., Sydowia 7(1-4): 110 (1953), MycoBank: MB119360 (Fig. 5.25)

≡ *Sphaeria pezizula* Berk. & M. A. Curtis 1876

Habit saprobic on woody substrate. *Ascomata* 236–374 × 365–422 µm diam., superficial, seated on mycelium, solitary to clustered, scattered on substrate, globose to subglobose, turbinate, dark brown to black, shiny, centrally ostiolate, collapsing on drying. *Peridium* 44.5–55 µm, several layers, somewhat thickened, composed of dark brown cells of *textura angularis*. *Pseudoparaphyses* 2–3.5 µm wide, filiform, septate, developing amongst asci, branched, septate. *Asci* (102–)113–146(–151) × 25–34 µm ($\bar{x}$ = 128 × 30 µm, n = 20), 8-spored, bitunicate, broadly cylindric-subclavate, thickened at the apex, shorted stalk. *Ascospores* 47–61 × 9.5–13 µm ($\bar{x}$ = 53 × 11 µm, n = 20), 2–3-seriate in the ascus, cylindrical to long fusiform, tapering towards rounded ends, 7-septate, straight or slightly curved, not constricted at the septum, hyaline to pale-yellowish. *Asexual morph* hyphomycetous, helicosporous, *Helicom*a-like.

Material examined: Unknown location, on dead wood of undetermined timber. Collector and Date: Unknown, BPI 800530, not the type specimen.

Notes: *Thaxteriella pezizula* was included in the genus *Thaxteriella* by Petrak (1953) based on morphological consistency with the described genus. Recognized by sphere or oblate spheroid, darkly pigmented, shiny, collapsing in the middle when dry, and worm-shaped ascospores (Barr, 1980; Berkeley, 1876; Linder, 1929; Sivanesan, 1984). In addition, Linder (1929) found that *Thaxteriella pezizula* concern with asexual morph know as hyphomycetous the genus *Helicoma* (i.e. *H. curtisii* and *H. muelleri*). However, fresh collections and phylogenetic study in these taxa are needed to confirm their relationships.



Note. (a)-(b) Ascomata (ascomata collapsing when dry in Fig. a). (c) Section through ascoma. (d) Peridium. (e) Pseudoparaphyses. (f)-(g) Asci. (h)-(j) Ascospores. Scale bars: (a)-(c) = 200 μm, (d) = 50 μm, (e) = 5 μm, (f)-(g) = 50 μm, (h)-(j) = 20 μm

Figure 5.25 *Thaxteriella pezizula* (BPI 800530)

5.3.3 Key to genera of *Tubeufiaceae*

1. Sexual morphs present; ascomata with asci and ascospores..........................2
1. Asexual morphs present; both in natural habitat or in culture.....................12
2. Ascomata globose-subglobose, with setae/hyphae.......................................3
2. Ascomata subglobose, ellipsoid, obovate, without setae/hyphae 10
3. Ascomata covered by stiff setae, brown to dark brown, apically acute.........4
3. Ascomata covered by flexuous hairy hyphae 8
4. Ascospores narrowly filiform, spiral, longer than 100 µm .... *Acanthophiobolus*
4. Ascospores fusiform or clavate-fusiform, not spiral, shorter than 100 µm 5
5. Ascospores equally 5-septate...*Thaxteriellopsis*
5. Ascospores greater than 5-septate...6
6. Ascospores heavily guttulate ....*Chlamydotubeufia*
6. Ascospores non-guttulate...7
7. Ascospores hyaline, non mucilaginous sheath........................*Acanthostigma*
7. Ascospores hyaline to pale brown, thin mucilaginous sheath....... *Acanthostigmina*
8. Ascospores elongate-fusiform, with less than 25 septa ....*Chaetosphaerulina*
8. Ascospores clavate-fusiform, cylindric-fusoid, with more than 25 septa..............9
9. Ascospores muriform...*Boerlagiomyces*
9. Ascospores not muriform..*Kamalomyces*
10. Ascomata formed on scale insects ....*Podonectria*
10. Ascomata formed on decaying or rotting wood..11
11. Ascospores allantoid or worm-like, 7-septate...........................*Thaxteriella*
11. Ascospores elongated cylindrical-subfusiform, with more than 7 septa...... *Tubeufia*
12. Conidia dictyosporous, apically appendaged, heavily pigmented ...... *Tamhinispora*
12. Conidia not dictyosporous, without appendages, hyaline, pale brown to brown 13
13. Conidia phragmosporous, fusoid to sickle-shaped, slightly curved......*Aquaphila*
13. Conidia helicosporous...14
14. Conidiophores absent, conidia arising directly on hyphae ....*Helicomyces*
14. Conidiophores present, dark brown, many septate 15
15. Conidia coiled 1-1½ times, conidial filament wider than 6 µm ....*Helicoma*
15. Conidia coiled 3½-4½ times, conidial filament narrower than 6 µm ....*Helicosporium*

5.3.4 Genera excluded from *Tubeufiaceae*, *Tubeufiales*

Acanthostigmella Höhn., Annls mycol. 3(4): 327 (1905), MycoBank: MB17

Saprobic on dead stems of grasses. *Ascomata* superficial, solitary, scattered, globose-subglobose, dark brown; with 23–74 µm long dark brown, apically acute, septate setae, darkened papilla and ostiole. *Peridium* thin-walled, composed of brown to red brown, 3–6.5 µm diam. cells of *textura angularis*. *Hamathecium* lacking pseudoparaphyses. *Asci* 8-spored, bitunicate, oblong, subclavate to broadly obovoid, with a blunt, wide and rounded pedicel, almost apedicellate. *Ascospores* 2–3-seriate in the ascus, ellipsoid, fusoid, ends rounded, 2–3-septate, constricted at the septum, yellow brown, smooth-walled. *Asexual state*: see under notes.

Type species: *Acanthostigmella genuflexa* Höhn., Annls mycol. 3(4): 327 (1905), MycoBank: MB207229 (Fig. 5.26)

≡ *Acanthostigma genuflexum* (Höhn.) Sacc. & Trotter, in Saccardo, Syll. fung. (Abellini) 22: 209 (1913)

≡ *Tubeufia genuflexa* (Höhn.) Arx & E. Müll., Stud. Mycol. 9: 83 (1975)

Saprobic on dead stems of grasses. *Ascomata* 20–79 µm high × 36.5–77 µm diam., superficial, solitary, scattered, globose-subglobose, dark brown; with surface setae, papillate and with darkened, 13–20 µm diam. ostiole at the tip, with setae 23–74 µm long, dark brown, apical acute, septate. *Peridium* thin-walled, *ca*. 4.5–5 µm wide, composed of brown to red brown cells 3–6.5 µm diam. of *textura angularis*. *Pseudoparaphyses* absent. *Asci* 19–23 × 8–11 µm ($\bar{x}$ = 21 × 9 µm, n = 10), 8-spored, bitunicate, oblong, subclavate to broadly obovoid, with a blunt, wide and rounded pedicel, almost apedicellate. *Ascospores* 7–11 × 2–3 µm ($\bar{x}$ = 9 × 3 µm, n = 10), 2–3-seriate in the ascus, ellipsoid, fusoid, rounded at both ends, 2-3-septate, constricted at the septum, yellow brown, smooth-walled. *Asexual state*: Unknown.

Material examined: AUSTRIA, Langenschönbichl, Tulln, von Höhnel, 3 June 1905 (FH, holotype, specimen not in good condition); UK, Suffolk, Halesworth (Canal side), on dead stem of *Phalaris arundinacea* (*Poaceae*), M.B. and J.P. Ellis, 21 June 1979 (IMI 252801).

Notes: *Acanthostigmella* was proposed by von Höhnel (1905) based on the type species *Acanthostigmella genuflexa*. We loaned the material from FH which was not in good condition and was devoid of any fungi. M.E. Barr had annotated the specimen as containing broken fragments bearing a few perithecia of a small spores species of *Gaeumannomyces.* Two slides were present and Barr (1977) obtained her description from these. The slides were not present in the material we loaned from FH. We therefore reverted to a more recent collection (IMI 252801) which is very similar to the species drawn from the FH type slides by Barr (1977) and is illustrated below. Morphologically, this specimen is rather different from all genera in *Tubeufiaceae* in having very small ascomata, a distinct papilla surrounded by setae, lack of pseudoparaphyses, broadly obovoid to fusoid asci, and 2-3-septate, yellow brown ascospores (Barr 1977; von Höhnel, 1905). Barr (1977, 1980) and Rossman (1987) suggested that *A. genuflexa* would be better accommodated in *Herpotrichiellaceae* (*Chaetothryriales*) rather than *Tubeufiaceae*. Untereiner et al. (1995) used molecular data from a putative strain of *Acanthostigmella brevispina* M.E. Barr & Rogerson from stromata of *Hypoxylon deustum* (Hoffm.) Grev., which placed the taxon in Dothideomycetes (Figures 22 and 23 see in Untereiner et al. 1995). Whether this species is related to *Acanthostigmella genuflexa* is debatable because of the presence of pseudoparaphyses, a hyphomycetous asexual state and fungicolous habitat. The placement of this genus therefore has to be Dothideomycetes *incertae sedis* until fresh collections are made and the species is sequenced. Presently, there are seven epithets listed in Index Fungorum.

The asexual states of *Acanthostigmella* are listed as hyphomycetous, helicosporous, dictyosporous, *Xenosporium*-like (Hyde et al., 2011). However no asexual state is linked to the generic type and therefore such linkages must be speculative for the genus.

Note. (a)-(b) Detailed of *Acanthostigmella genuflexa* specimen. (c) Ascomata on substrate. (b)-(d) Close up of ascomata with long setae. (e) Peridial wall. (f)-(g) Immature and mature asci. (h)-(j) Ascospores. Scale bars: (a) = 500 µm, (b) = 100 µm, (c)-(d) = 50 µm, (e)-(j) = 10 µm

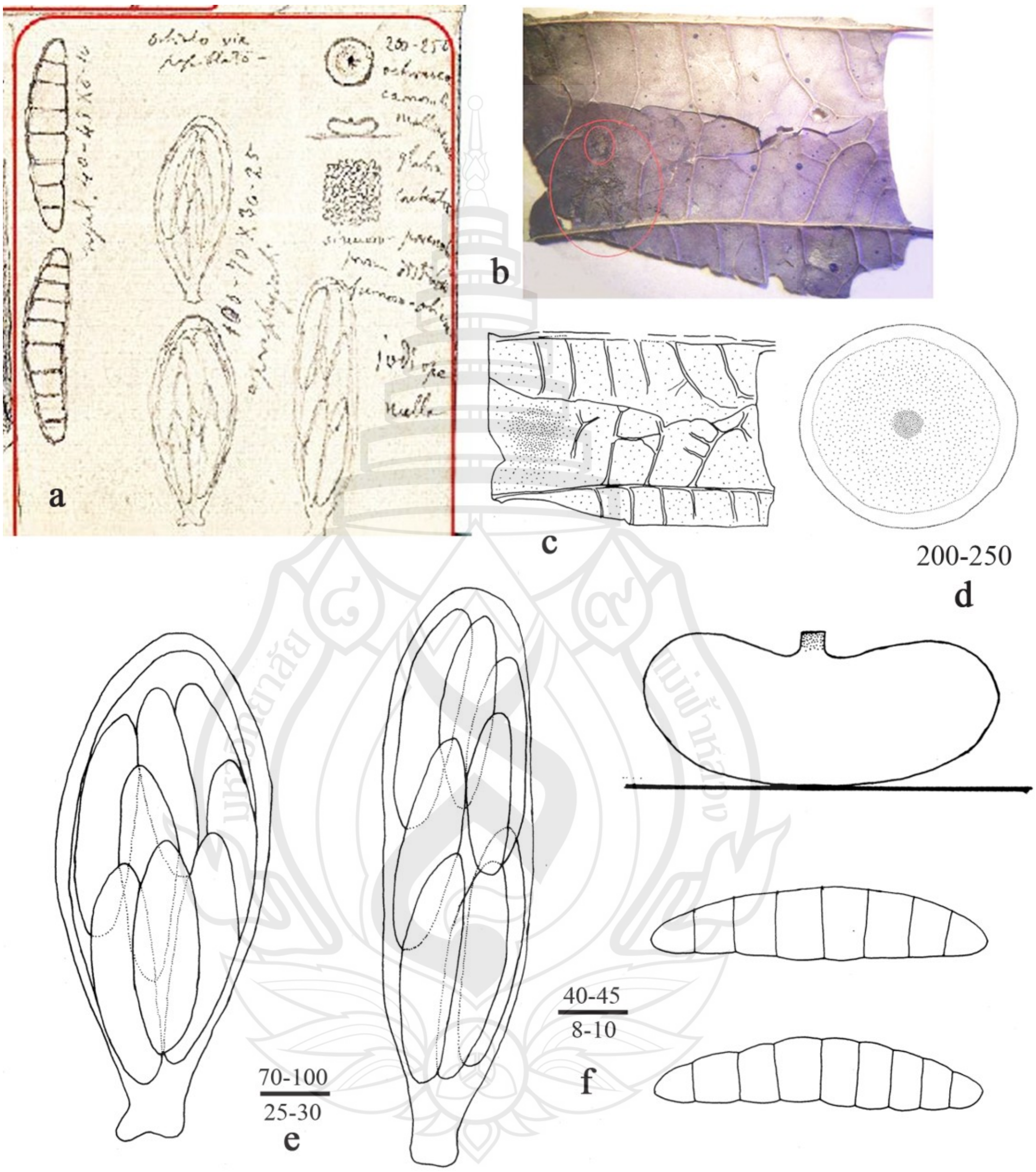
Figure 5.26 *Acanthostigmella genuflexa* (IMI 252801, holotype)

Amphinectria Speg., Boln Acad. nac. Cienc. Córdoba 26(2-4): 346 (1924), MycoBank: MB167

Type species: *Amphinectria portoricensis* Speg., Boln Acad. nac. Cienc. Córdoba 26(2-4): 346 (1924), MycoBank: MB254078 (Fig. 5.27)

Notes: *Amphinectria* was introduced by Spegazzini (1924) based on the type species *A. portoricensis*. Petrak (1951) examined the type specimen of *A. portoricensis* which has immature asci and concluded that the species is a lichen. Pirozynski (1977) later questionably synonymised *Amphinectria* with *Melioliphila*, while Rossman (1987) examined the type specimen of *A. portoricensis* and could not find any ascomata that resembled those described by Spegazzini and she remarked that the identity of this species is obscure. Rossman et al. (1999) placed *A. portoricensis* in *Tubeufiaceae* with uncertainty and remarked that until another specimen is located then *Amphinectria* is an ambiguous member. A second species in the genus, *Amphinectria erubescens*, was transferred to the genus *Hydropisphaera* (*Bionectriaceae*), based on its orange ascomata and unitunicate asci (Rossman et al., 1999). According to the original annotations of Spegazzini (LPS 13394) this genus and species is ambiguous and is unlike any other members of *Tubeufiaceae.* Therefore we treat *Amphinectria* as a doubtful genus.

We provide here a brief description of *A. portoricensis* based on the protologue of Spegazzini (1924), the original drawing in the herbarium packet and the rather depauperate type material. *A. portoricensis* develops on the leaf surface of *Comocladia glabra* (Schult.) Spreng. The subiculum is 5–10 mm diam., orbicular, slightly transparent, inconspicuous, loosely attached to the epidermis, with 2–3 µm diam., branching, radial hyphae. Ascomata are 200–250 µm diam., superficial, globose, solitary, yellow brown to brown (honey-coloured), membranous, developing at the centre of the subiculum, minutely papilla and ostiolate, collapsing when dry soften and lacking pseudoparaphyses. Asci are (70–)75–100 × (15–)25–30 µm, 8-spored, bitunicate, cylindrical-ellipsoid, bluntly rounded at the apex, with a short bifurcate pedicel. Ascospores are (32–)40–45 × (6–)8–10 µm, 2-seriate in the ascus, cylindrical-fusoid, tapering towards the sub-rounded ends, slightly inequilateral or curved, 7–9-septate, slightly constricted at the septum, hyaline, smooth, without gelatinous sheath. The asexual state is unknown.



Note. Redrawn from LPS 13394. (a)-(b). Material in LPS packet. (c)-(d). Substrate and ascoma. (e). Asci. (f). Ascospores

Figure 5.27 *Amphinectria portoricensis*

Chaetocrea Syd., Annls mycol. 25(1/2): 18 (1927), MycoBank: MB943

Fungicolous associated with black areas of fungi on leaves. *Ascomata* superficial, solitary or gregarious, globose-subglobose, membranous, white to light yellowish, covered by apically branched setae. *Peridium* comprising white-yellow cells of *textura angularis*. *Pseudoparaphyses* 2–2.5 µm wide, filiform, branched, septate. *Asci* bitunicate, 8-spored, elongate-clavate, cylindrical, short pedicellate, apically thickened, without an obvious ocular chamber. *Ascospores* fasciculate, filiform, tapering narrowly towards the lower end, with several eusepta, hyaline. *Asexual state*: Unknown.

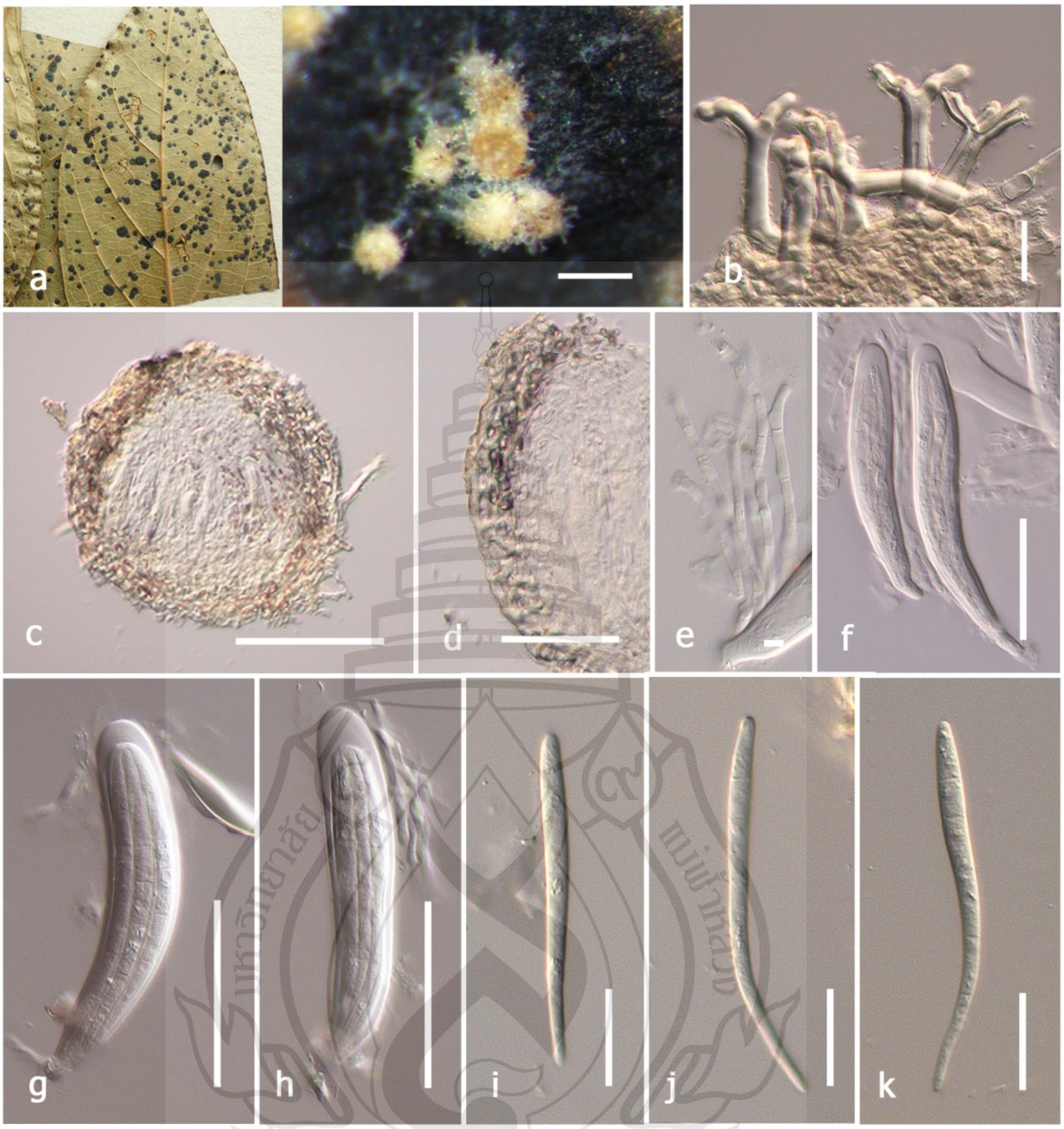
Type species: *Chaetocrea parasitica* Syd., Annls mycol. 25(1/2): 19 (1927), MycoBank: MB273588 (Fig. 5.28)

Fungicolous associated with black areas of *Cyclostomella disciformis* on leaves of *Nectandra sanguine*. *Ascomata* 161–187 µm high × 169–189 µm diam., superficial, solitary or gregarious, globose-subglobose, membranous, white to light yellowish, translucent or crystal-like, covered by 35–44(–48) µm long × 7–10 µm wide, apically branched, septate, pale yellow setae. *Peridium* 23–26 µm wide, 3–4 layers of white-yellow cells of *textura angularis*. *Pseudoparaphyses* 2–2.5 µm wide, filiform, developed over asci, hyaline, branched, septate. *Asci* 95–108(–121) × 17–21 µm ($\bar{x}$ = 102 × 19 µm, n = 10), 8-spored, bitunicate, elongate-clavate, cylindrical, short pedicellate, apically thickened, without an obvious ocular chamber. *Ascospores* (63–)71–83 × 5–6.5 µm ($\bar{x}$ = 76 × 6 µm, n = 10), fasciculate, filiform, tapering narrowly towards the lower end, (6–)7–9-euseptate, not constricted at the septum, hyaline, smooth-walled. *Asexual state*: Unknown.

Material examined: COSTA RICA, La Caja near San Jose, parasitic on *Cyclostomella disciformis* Pat. Growing on leaves of *Nectandra sanguine* Rottb, 4 January 1925 (No. 166), Acqu. 1978, No. 11007: F. Petrak Pilzherbarium (W, holotype).

Notes: *Chaetocrea* was introduced by Sydow (1927) for a single species *Chaetocrea parasitica* and placed in the *Hypocreaceae* as it had bright ascomata. *Chaetocrea parasitica* was described as fungicolous on blackened areas of the fungus *Cyclostomella disciformis* which parasitized leaves of *Nectandra sanguine*.

Rossman et al. (1999) studied genera in the *Hypocreaceae* (*Hypocreales*) and transferred *Chaetocrea*, based on *C. parasitica* to *Tubeufiaceae* based on its comprising of bitunicate asci, pseudoparaphyses and long ascospores. *Chaetocrea parasitica* however has predominantly branching and pale yellow setae, these are atypical of *Tubeufiaceae*. Therefore *Chaetocrea* is treated as a member of Dothideomycetes *incertae sedis* and needs recollection and sequencing.



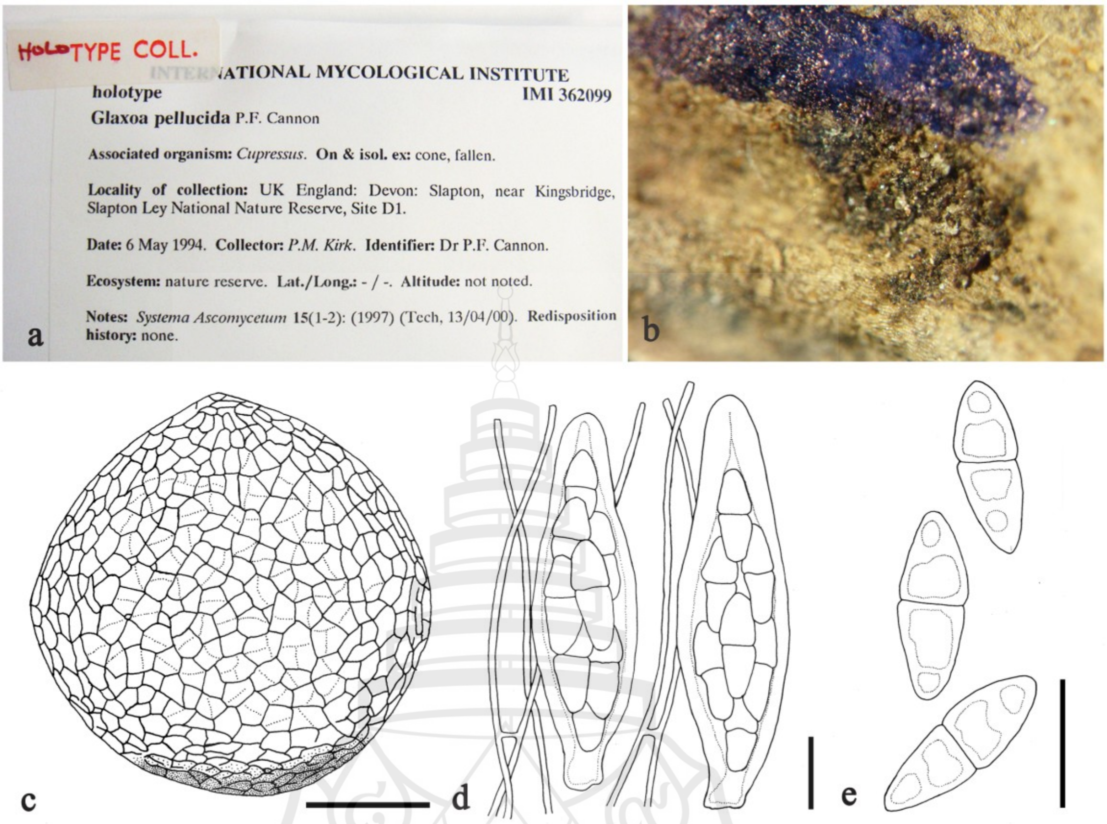
Note. (a) *Cyclostomella disciformis* on leaf and ascomata of *Chaetocrea parasitica* (right figure) on blackened fungus. (b) Apically branching setae. (c) Section through ascoma. (d) Peridium. (e) Pseudoparaphyses. (f)-(h) Asci. (i)-(k) Ascospores. Scale bars: (a) = 200 μm, (b), (i)-(k) = 20 μm, (c) = 100 μm, (d), (f)-(h) = 50 μm, (e) = 5 μm

Figure 5.28 *Chaetocrea parasitica* (W, holotype)

Glaxoa Cannon, Syst. Ascom. 15(1-2): 122 (1997), MycoBank: MB27769

Type species: *Glaxoa pellucida* P. F. Cannon, Syst. Ascom. 15(1-2): 122 (1997), MycoBank: MB436300 (Fig. 5.29)

Notes: *Glaxoa* is a monotypic genus, containing a single species *Glaxoa pellucida* (Cannon, 1997). Herein, we provide a drawing from Cannon (1997), because the type material of *G. pellucida* which we have examined (IMI 362099) is in poor condition. *Glaxoa pellucida* can clearly be distinguished from all genera in *Tubeufiaceae* by its 1-septate ascospores. Cannon (1997) placed *Glaxoa* in *Tubeufiaceae* because it was similar to *Letendraea*, but *Letendraea* is presently treated as a member of *Pleosporales* (Boonmee et al., 2011). *Glaxoa* is therefore placed in *Pleosporales incertae cedis* until the fresh collections are made and molecular sequences are available for study.



Note. Redrawn from Cannon (1997). (a) Herbarium packet. (b) Material of herb. K. (c) Ascoma. (d) Asci and interascal tissue. (e) Ascospores. Scale bars: (a)-(b) = detailed of *Glaxoa pullucida* specimen, (c) = 50 µm, (d)-(e) = 10 µm

Figure 5.29 *Glaxoa pullucida* (IMI362099, holotype)

Malacaria Syd., Annls mycol. 28(1/2): 69 (1930), MycoBank: MB2983

Parasitic on other fungi, associated with *Irenina glabra* (*Meliolaceae*); consist of superficial mycelium, branched and coloured, on leaves of *Coffea robusta*, pantropical. *Ascomata* superficially, solitary, globose-subglobose, light brown to orange brown, soften texture, membranous-walled, centrally ostiole, dark and collapsing when dry. *Peridium* composed of cells of *textura angularis*, 3-5 layers, light orange-celled. *Hamathecium* numerous filiform, hyaline, unbranched, septate, pseudoparaphyses. *Asci* bitunicate, 8-spored, oblong-subclavate, rounded at the apex, obtuse at the base.

Ascospores multiseriate, elongate-fusiform, slightly curved, 3–(-4)-septate, slightly constricted at septum, pale brown to light brown, smooth, apically subacute, continuous basal appendage, filiform or thread-like, hyaline. The asexual state is unknown.

Type species: *Malacaria meliolicola* Syd., Annls mycol. 28(1/2): 69 (1930), MycoBank: MB120880 (Fig. 5.30)

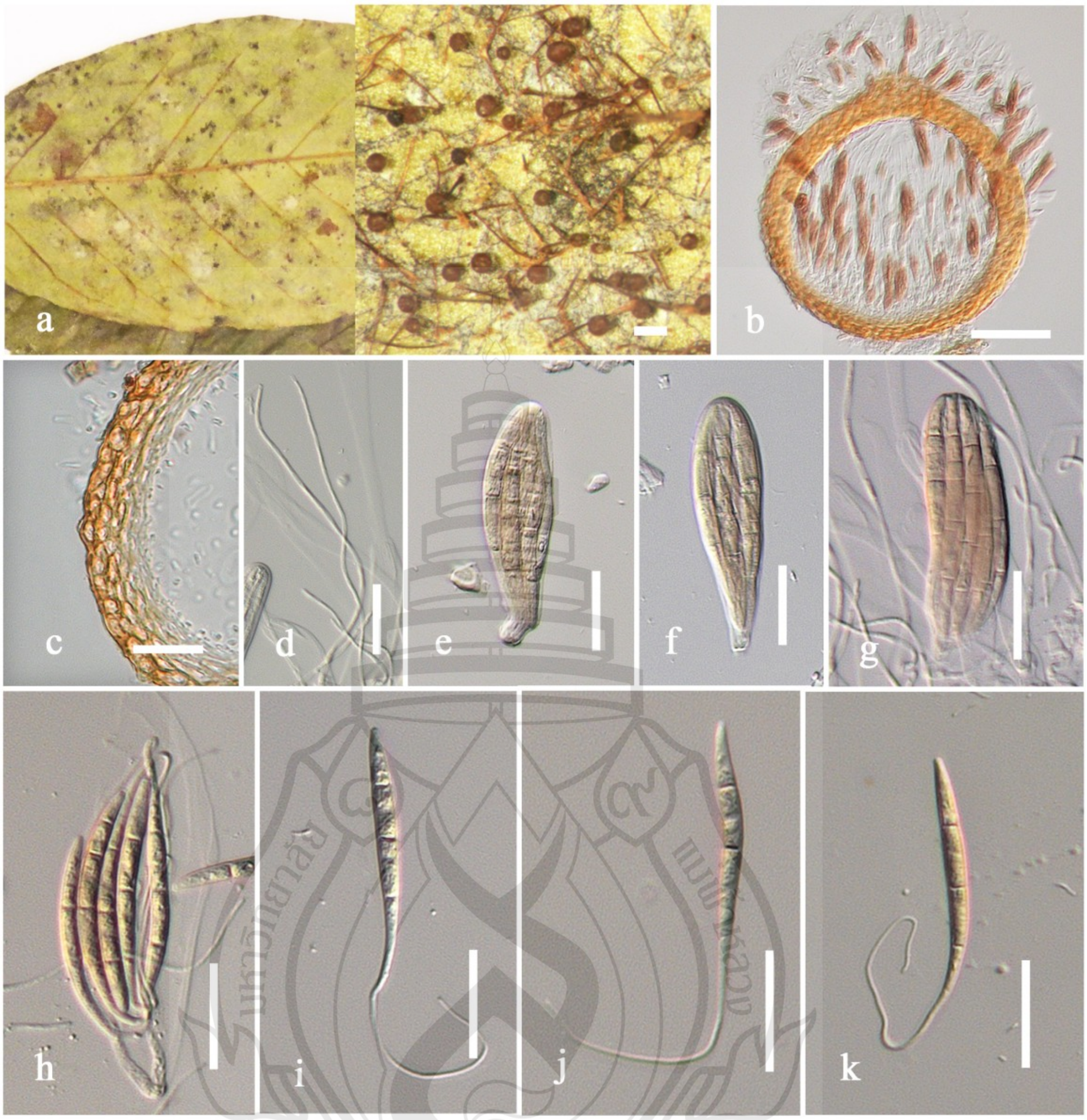
≡ *Paranectria flagellata* Hansf., Proc. Linn. Soc. London 153(1): 28 (1941)

≡ *Malacaria flagellata* (Hansf.) Hansf., Mycol. Pap. 15: 128 (1946)

Parasitic on other fungi, associated with *Irenina glabra* (Meliolaceae); consist of superficial mycelium, branched and coloured, on leaves of *Coffea robusta*, pantropical. *Ascomata* 144–170 µm high × 125–176 µm diam., superficially, solitary, globose-subglobose, light brown to orange brown, soften texture, membranous-walled, centrally ostiole, dark and collapsing when dry. *Peridium* 12–14 µm wide, composed of cells of *textura angularis*, 3-5-layers, light orange-celled. *Hamathecium* numerous filiform *ca*. 1–1.5 µm wide, hyaline, unbranched, septate, pseudoparaphyses. *Asci* 53–72.5 (–80) × 11–21 µm ($\bar{x}$ = 65 × 17 µm, n = 15), bitunicate, 8-spored, oblong-subclavate, rounded at the apex, obtuse at the base. *Ascospores* 42–51 × 4–5 µm ($\bar{x}$ = 46 × 4 µm, n = 10), multiseriate, elongate-fusiform, slightly curved, 3–(-4)-septate, slightly constricted at septum, pale brown to light brown, smooth, apically subacute, continuous basal appendage *ca*. 43–65 µm long, filiform or thread-like, hyaline. The asexual state is unknown.

Material examined: UGANDA, Kampala, elev. 4000 ft., on leaves of *Coffea robusta* and associated with *Irenina glabra* (Meliolaceae), coll. Hansford G.C., 1871, K (M): 177970, neotype of *Malacaria meliolicola*.

Notes: Sydow (1930) introduced the genus *Malacaria*, typified by *M. meliolicola* for species commonly associated with colonies of species in the family *Meliolaceae* growing on leaves. Presently, the genus comprises eight epithets (Index Fungorum). Rossman (1983) treated *M. flagellata* as a synonym of *M. meliolicola*, based on its similar morphology. She assigned a lectotype for *Paranectria flagellata* (= *Malacaria flagellata*) and since the type of *M. meliolicola* was lost she also assigned it as the neotype of this species (Rossman, 1987). *Malacaria meliolicola* was included in the *Tubeufiaceae* based on its soft ascomata, bitunicate asci and pseudoparaphyses. In this study, we reexamined on the lectotype specimen. *Malacaria meliolicola* has pale smoke-grey ascospores and unbranched septate pseudoparaphyses and is atypical of genera in *Tubeufiaceae*. The placement of *Malacaria* is therefore uncertain and the genus is treated as Dothideomycetes *incertae cedis*. New collections with sequence data are needed.



Note. (a) Appearance of fungus on black colonies of *Meliolaceae*. Orange coloured mycelia are those of the fungal parasite. (b) Section through ascoma. (c) Peridium comprising 3-5 layers of angular orange walled cells. (d) Pseudoparaphyses. (e)-(g). Asci. (h)-(k) Ascospores with long basal appendage. Scale bars: (a) = 500 µm, (b) = 50 µm, (c)-(k) = 20 µm

Figure 5.30 *Malacaria meliolicola* (K (M): 177970, neotype)

Melioliphila Speg., Boln Acad. nac. Cienc. Córdoba 26(2-4): 344 (1921), MycoBank: MB3109

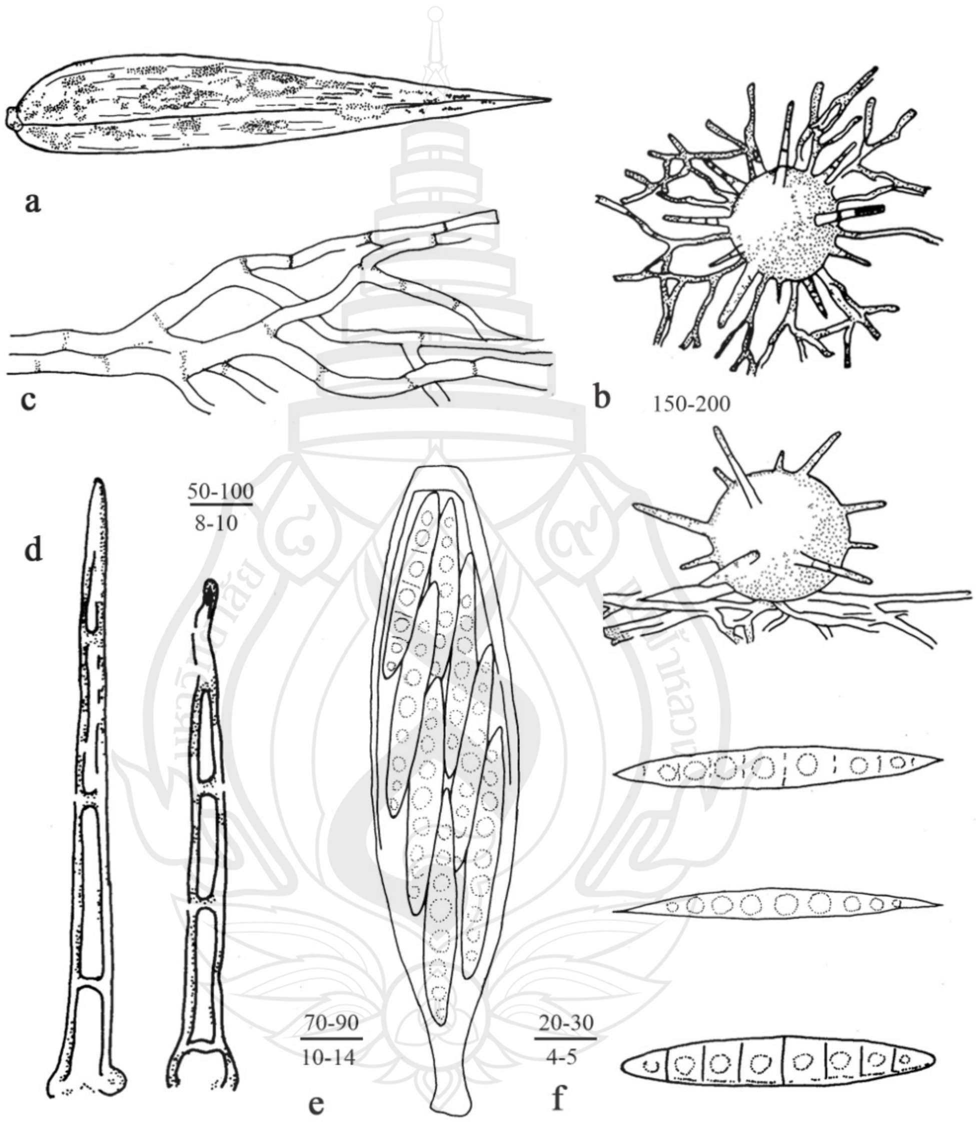
Type species: *Melioliphila graminicola* Speg., Boln Acad. nac. Cienc. Córdoba 26(2-4): 344 (1921), MycoBank: MB252431 (Fig. 5.31)

≡ *Calonectria graminicola* (Berk. & Broome) Wollenw., (1913)

Parasitic on with *Meliola panici* (*Meliolaceae*) growing on leaves of *Lasiacis divaricata* in Puerto Rico. *Ascomata* 150–200 µm diam., globose, developing on a poorly developed, yellowish to fleshy-pink, subiculum, covered by powder-like, white colonies, with sparsely radiating setae. *Setae* 50–100 × 8–10 µm, erect, narrowly tapering towards the apex, hyaline, septate. *Hamathecium* lacking pseudoparaphyses. *Asci* 70–90 × 10–14 µm, unitunicate or bitunicate, 8-spored, elliptical-fusoid, short pedicellate, somewhat flattened at the apex. *Ascospores* 30–40 × 4–5 µm, 2-seriate, narrowly-fusoid, initially with minute appendages at both ends, appendages absent when mature, later blunt at both ends, 7-9-guttulate present when immature, becoming 7–9-septate when mature, hyaline to pale smoky.

The brief description and drawing of *Melioliphila graminicola* is provided based on the protologue of Spegazzini (1924) and the drawing on the herbarium (LPS 1626 packet, www. cybertruffle.org.uk/spegazzini/eng/001626a).

Notes: *Melioliphila* was introduced by Spegazzini (1924) for mycoparasitic species associated with the *Meliola*, *Meliolaceae*. Initially, *Melioliphila* had been placed in the *Hypocreales* (Saccardo, 1972), but later it was transferred into "hypocreoid *Dothideales*" by Pirozynski (1977). Barr (1980) again transferred the *Melioliphila* to the *Tubeufiaceae* based on it being a mycoparasitic species, having bitunicate asci and elongate ascospores. Rossman (1979, 1987) reported that *M. graminicola* (F. Stev) Speg. (≡ *Calonectria graminicola* F. Stev.) is a synonym of *M. volutella* (Berk. & Broome) Rossman (≡ *Calonectria volutella*). *Melioliphila graminicola* was also placed in *Tubeufiaceae* by Rossman (1987) and Rossman et al. (1999). We could not loan the type of *M. graminicola* due to LPS policy. Paratype specimens of *M. graminicola* (BPI632019) examined were in poor condition. Therefore, we used the drawing of Spegazzini (1924) and drawing on original herbarium packet (www.cybertruffle.org.uk/spegazzini/eng/001626a) to illustrate *M. graminicola*. According to Spegazzini (1924), *M. graminicola* has colourless setae, lacks pseudoparaphyses, has elliptical-fusoid asci and fusoid ascospores and is atypical of genera in *Tubeufiaceae*. It is not clear whether the asci are unitunicate or bitunicate. Therefore, *Melioliphila* is treated as a doubtful genus.



Note. Redrawn from Spegazzini, 1924 and LPS 1626. (a) Substrate. (b)-(c) Ascoma and basal mycelium. (d) Setae. (e) Ascus. (f) Ascospores

Figure 5.31 *Melioliphila graminicola* (LPS 1626)

Paranectriella (P. Henn. ex Sacc & D. Sacc) Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 119: 899 [23 repr.] (1910), MycoBank: MB3708

Parasitic (or biotrophic) on leaves. *Ascostromata* superficial, solitary, scattered, white to light orange, fleshy, texture soft, surrounded by colourless, sparse hairs, multiloculate with ascomata arranged in a peripheral outer layer. *Ascomata* globose-subglobose, with individual ostiole. *Peridium* relatively thin, composed of light yellow cells of *textura angularis*. *Pseudoparaphyses* 1–2 µm wide, filiform, extending over asci, branched, septate and embedded in a gelatinous layer. *Asci* bitunicate, fissitunicate, 8-spored, saccate-cylindrical, short pedicellate, apically rounded with an indistinct ocular chamber. *Ascospores* 2-seriate in the ascus, ellipsoid-fusiform, trans-septate, with apical spine-like appendages. *Asexual state*: hyphomycetous, staurosporous, in *Araneomyces* and *Titaea*.

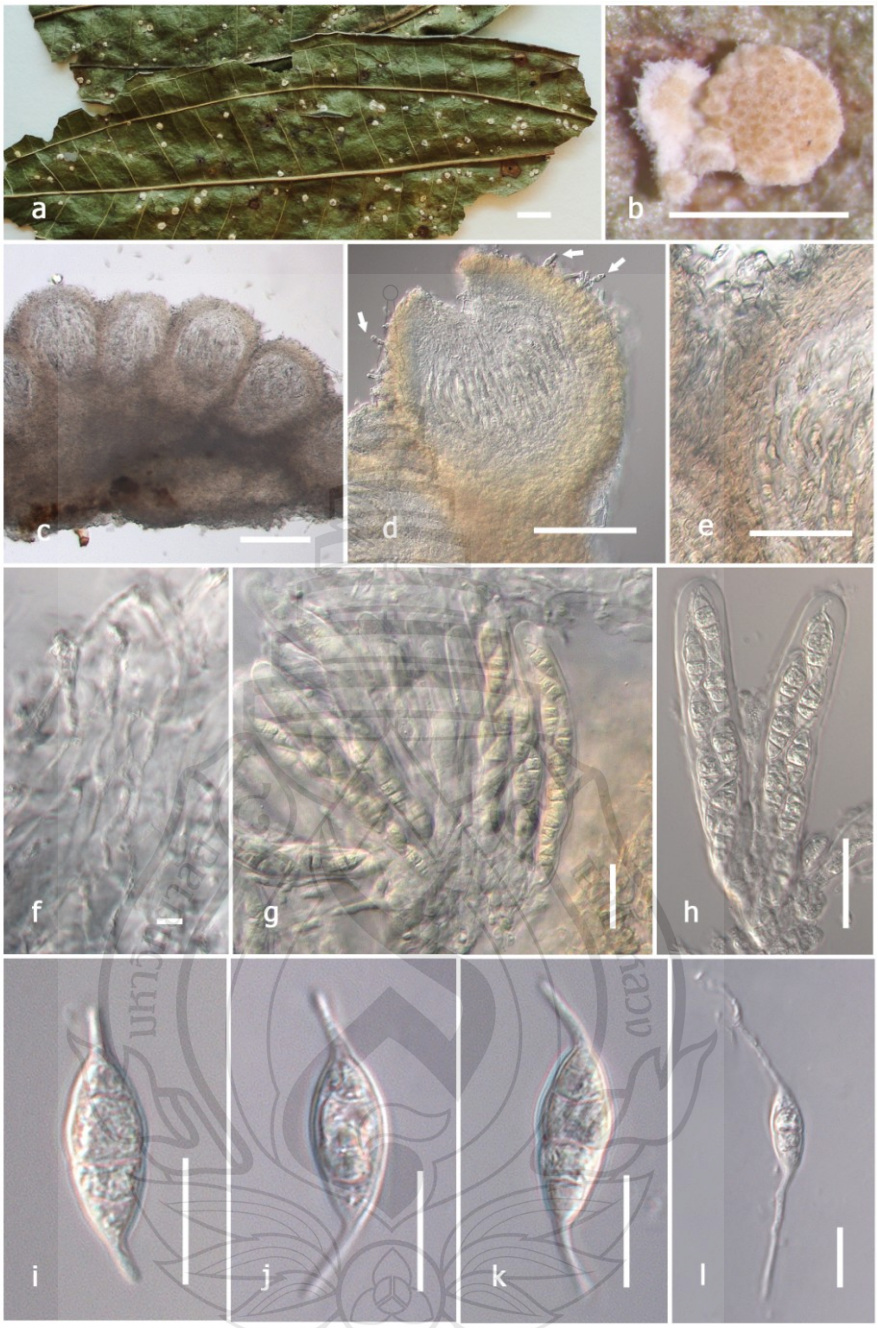
Type species: *Paranectriella juruana* (Henn.) Henn. ex Piroz., Kew Bull 31: 598 (1977), MycoBank: MB319198 (Fig. 5.32)

≡ *Paranectria juruana* Henn., Hedwigia 43(4): 245 (1904)

Parasitic (or biotrophic) on lower surface of leaves of *Miconia* sp. *Ascostromata* 0.7–0.8 mm diam., superficial, solitary, scattered, white to light orange, fleshy, texture soft, surrounded by colourless, sparse hairs, hairs 18–23 µm long × 4–6 µm wide, multiloculate with ascomata arrange in a peripheral outer layer. *Ascomata* 169–206(–240) µm high × (88–)99.5–131(–184) µm diam., globose-subglobose, with individual ostiole. *Peridium* 14–18 µm wide, composed of light yellow cells of *textura angularis*. *Pseudoparaphyses* 1–2 µm wide, hyaline, filiform, extending over asci, branched, septate and embedded in a gelatinous layer. *Asci* (63–)72–95 × 10–14 µm ($\bar{x}$ = 81 × 11 µm, n = 10), bitunicate, fissitunicate, 8-spored, saccate-cylindrical, short pedicellate, apically rounded with an indistinct ocular chamber, attached on gelatinous membrane. *Ascospores* 14–16 × 6–7 µm ($\bar{x}$ = 15 × 6 µm, n = 10), 2-seriate in the ascus, ellipsoid-fusiform, 3-septate, with apical spine-like appendages, appendages 4–7(–24) µm long (occasionally longer than 20 µm), hyaline, smooth. *Asexual state*: hyphomycetous, staurosporous in *Araneomyces* and *Titaea*.

Material examined: HAITI, Dept. de la Grand' anse, Massif de la Hotte, "Geffrard" 44 km south of Roseaux Road to Camp Perrin, alt. 780 m., 18°25'N 73°53'W, on leaves of *Miconia* sp., William R. Buck (9169), 14 November 1982, determined by A. Rossman (BPI 632134), type not observed.

Notes: The genus *Paranectriella* was first suggested as a subgenus of *Paranectria* by Hennings (1904c), which was validated by Saccardo, P. A. & Saccardo, D. (1905), while von Höhnel (1910) later raised *Paranectriella* to generic rank. Pirozynski (1977) confirmed the status of *Paranectriella* and described the type species, distinguishing *Paranectriella* from *Paranectria* as the former has bitunicate asci. Barr (1980) suggested that *Paranectriella* should be included in the *Tubeufiaceae* because of living tropical hyperparasites. Rossman (1987) accepted the genus *Paranectriella* in *Tubeufiaceae*, based on *P. juruana,* a parasitic spieces with bright white to yellow ascostromata containing many locules in a peripheral layer, bitunicate asci, numerous pseudoparaphyses and ascospores with polar spine-like appendages. The ascostromata comprises, hyaline, relatively loose mycelium and is intermediate between an ascostromata and a subiculum and this may cause consfusion due to differences in interpretation. The asexual states of *Paranectriella* which were found in association with *Paranectriella* are hyphomycetous, staurosporous, as named *Araneomyces* and *Titaea* (von Höhnel, 1909d; Hyde et al., 2011; Kirschner & Piepenbring, 2006; Saccardo, 1876). The description and illustration here is based on an authentic specimen of *Paranectriella juruana* (William R. Buck 9169, BPI 632134). Morphologically, *P. juruana* is characterized by bright ascostromata and ascospores with appendaged ends, and the genus has ten epithets as listed in Index Fungorum. These characters are atypical of genera in *Tubeufiaceae*, which have cylindrical ascospores and hyphomycetous, helicosporous asexual states. *Paranectriella* is placed in a new family *Paranectriellaceae* following this study.



Note. (a)-(b). Ascostromata. (c)-(d). Section through ascostroma. (e) Peridium of locules. (f) Pseudoparaphyses. (g)-(h) Bitunicate asci. (i)-(l) Ascospores with spine-like extension at each end. Scale bars: (a) = 5 mm, (b) = 1 mm, (c)-(d) = 100 µm, (e) = 50 µm, (g)-(h) = 20 µm, (f) = 5 µm, (i)-(l) = 10 µm

Figure 5.32 *Paranectriella juruana* (BPI 632134)

Puttemansia Henn., Hedwigia 41: 112 (1902), MycoBank: MB4562

Parasitic (or biotrophic) on lower surface of leaves of *Nectandra* sp. *Ascostromata* up to 1 mm diam., superficial, solitary, some grouped, pale yellow to vinaceous buff, stipitate, covered by hairy setae, white to hyaline, septate, multiloculate. *Ascomata* globose-subglobose, slightly flattened on the top, non-ostiole. *Peridium* composed of cell of *textura prismatica* to *oblonga*, with hyaline to light brownish cells. *Hamathecium* comprising numerous filiform, hyaline, anastomosing, branched, pseudoparaphyses, embedded on a gelatinous matrix. *Asci* bitunicate, 8-spored, oblong, elongate to cylindrical-clavate, rounded at the apex, obtused at the base. *Ascospores* 1–2-seriate in the ascus, fusiform, widest near the middle septum, with tapering towards narrow ends, 3-septate, slightly constricted at the septum, with continuous basal appendage, hyaline, smooth. *Asexual state*: hyphomycetous, staurosporous, *Guelichia*, *Tetranacrium*.

Type species: *Puttemansia lanosa* Henn., Hedwigia 41: 112 (1902), MycoBank: MB192912 (Fig. 5.33)

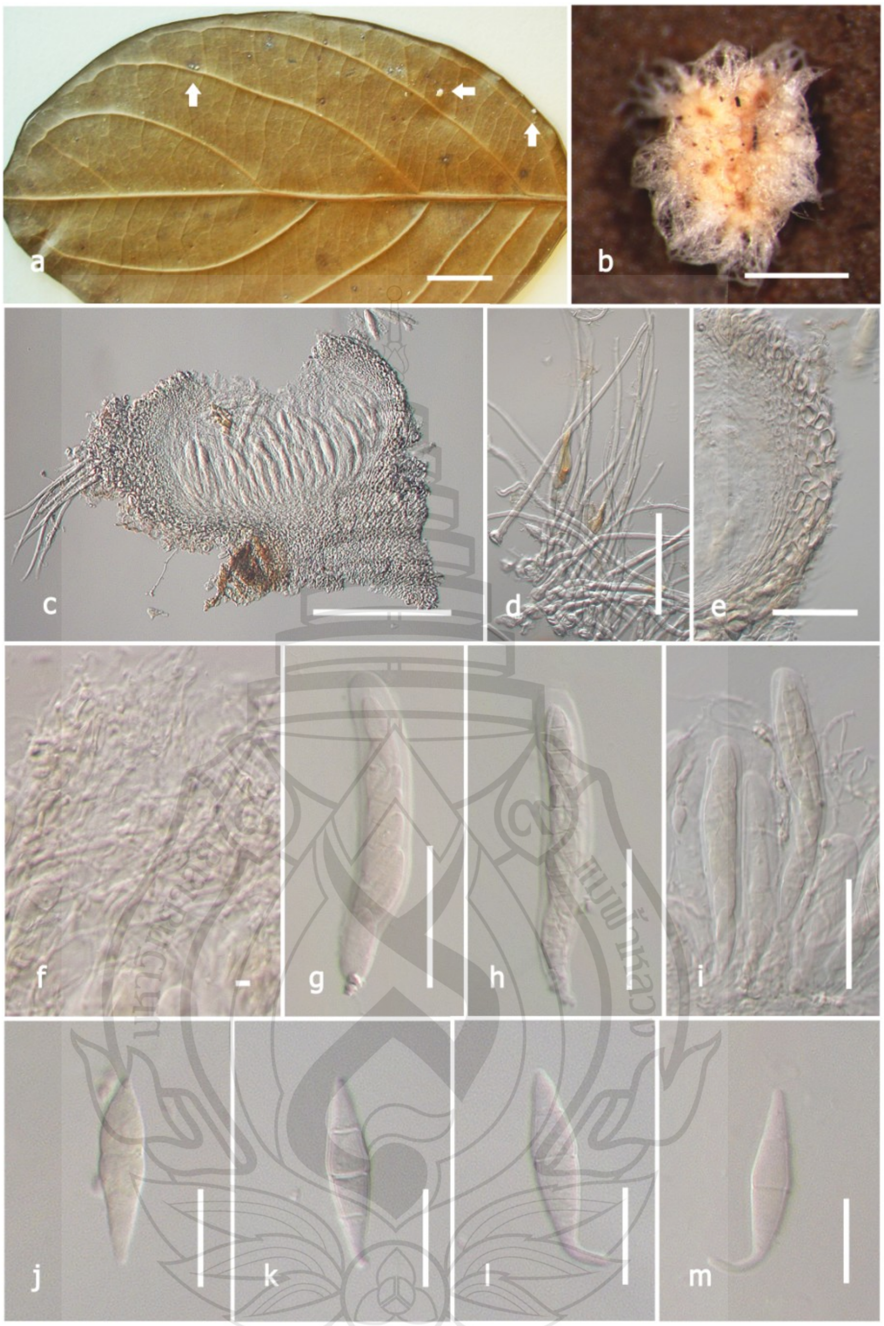
= *Guelichia paradoxa* Speg., Anal. Soc. cient. argent. 22(2): 174 (1886)

Parasitic (or biotrophic) on lower surface of leaves of *Nectandra* sp. *Ascostromata* up to 1 mm diam., superficial, solitary, some grouped, pale yellow to vinaceous buff, stipitate, covered by hairy setae with 200–386 µm long × 6–9 µm wide, white to hyaline, septate, multiloculate. *Ascomata* (244–)259–306(–567) µm high × (314.5–)371–421 µm diam. ($\bar{x}$ = 344 × 370 µm), globose-subglobose, slightly flattened on the top, non-ostiole. *Peridium* 64–73 µm wide, composed of cell of *textura prismatica* to *oblonga*, with hyaline to light brownish cells. *Hamathecium* comprising numerous filiform 1–2 µm wide, hyaline, anastomosing, branched, pseudoparaphyses, embedded on a gelatinous matrix. *Asci* (104–)112–133(–140) × 16.5–22 µm ($\bar{x}$ = 120 × 19 µm), bitunicate, 8-spored, oblong, elongate to cylindrical-clavate, rounded at the apex, obtused at the base. *Ascospores* (30–)38–50(–56) × 9–11 µm ($\bar{x}$ = 42 × 10 µm), 1–2-seriate in the ascus, fusiform, widest near the middle septum, with tapering towards narrow ends, 3-septate, slightly constricted at the septum, with continuous basal appendage, hyaline, smooth. *Asexual state*: hyphomycetous, staurosporous, *Guelichia*, *Tetracrium* (Hyde et al., 2011).

Material examined: COSTA RICA, San Pedro de San Ramon, on leaves of *Nectandra* sp., Alberto M. Brenes, on 8 October 1926, as *Fungi costaricensis* 157, (BPI 632856); type not examined.

Notes: Henning (1902) introduced the genus *Puttemansia* as a member of *Pezizaceae*. Clements and Shear (1931) and Rogerson (1970) placed *Puttemansia* in *Hyprcreacea* (*Hypocreales*), based on its atypical lichenicolous, hairy appendages and bright ascomata. Pirozynski (1977) and Rossman (1978) provided detailed descriptions of several species of *Puttemansia* and found that all species had bitunicate asci. Rossman (1978) suggested that *Puttemansia* was similar with *Podonectria* and *Tubeufia* in the *Tubeufiaceae* in having bright, fleshy ascomata, bitunicate asci and multiseptate ascospores (Barr, 1980). Rossman (1987) provided detailed descriptions of six species and accepted them in the *Tubeufiaceae*, whereas, *P. lanosa* Henn. was treated as a synonym of *Puttemansia albolanata* (Speg.) Höhn. The asexual states of *Puttemansia* are considered to be the hyphomycetous genera *Guelichia* Speg., *Tetranacrium* H.J. Huds. & B. Sutton and *Titaea* Saccardo, as they were commonly associated with *Puttemansia lanosa* (Barr, 1980; Hennings, 1902; Hyde et al., 2011; Petrak & Sydow, 1936; Rossman, 1987). The description and illustration herein, is from an authentic specimen of *Puttemansia lanosa* (BPI 632856) identified by Rossman. Morphologically features are cup-shaped ascomata, white hairy mycelium, and broadly fusiform appendaged ascospores, characters that are atypical of *Tubeufiaceae*. There are 25 species epithets listed for *Puttemansia* in Index Fungorum.

Puttemansia lanosa is similar to *Paranectriella juruana* as ascostromata are light coloured and ascospores are three spetate with appendages. They differ as ascostromata of *Puttemansia lanosa* contain far fewer ascomata (2-10 locules) and ascospores have a basal spine-like appendage. In *Paranectriella juruana* the ascostromata contain many ascomata (*ca*. 30), while ascospores have spine-like appendages at both ends. The genus *Puttemansia* therefore, is appropriated to place with the *Paranectriellaceae* based on similar morphology (Figs. 5.32-5.33)



Note. (a)-(b) Ascostromata. (c). Section through ascostroma. (d) Hairy mycelium of ascostromata. (e) Peridium. (f) Pseudoparaphyses. (g)-(i) Asci. (j)-(m) Ascospores with basal spine-like appendage. Scale bars: (a) = 5 mm, (b) = 500 μm, (c) = 200 μm, (d)-(e) = 100 μm, (g)-(i) = 50 μm, (f) = 5 μm, (j)-(m) = 20 μm

Figure 5.33 *Puttemansia albolanata* (BPI 632856)

Rebentischia P. Karst., Fungi Fenniae Exsiccati, Fasc. 9: no. 881 (1869), MycoBank: MB4658

Saprobic on decaying wood. *Ascomata* superficial, solitary or scattered, globose-subglobose, black, coriaceous, ostiole, some collapsed when dry. *Peridium* composed of cells of *textura angularis* layers of dark brown to black-walled cells, arranged in a *textura prismatica/angularis*. *Pseudoparaphyses* 1–2 µm wide, among asci, flexuous, hyaline, branched. *Asci* 8-spored, bitunicate, fissitunicate, cylindric-clavate, short pedicellate, apically thickened. *Ascospores* biseriate, elongate-obovoid, trans-septate, brownish to dark brown, light at the apex, base tapering with hyaline appendage. *Asexual state*: Unknown.

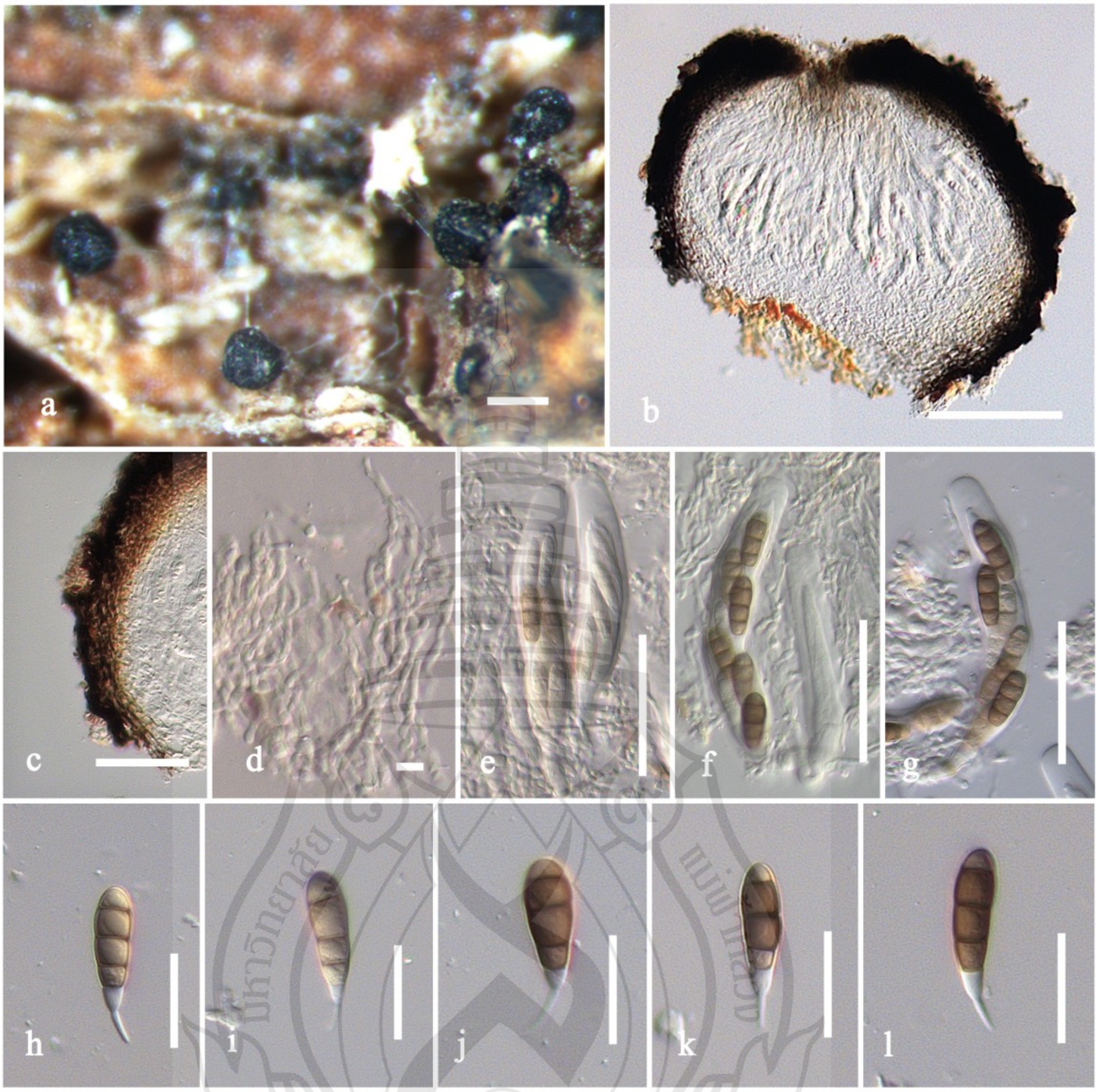
Type species: *Rebentischia pomiformis* P. Karst., Fungi Fenniae Exsiccati, Fasc. 9: no. 881 (1869), MycoBank: MB240368 (Fig. 5.34)

Saprobic on decaying wood of *Acer platanoides*. *Ascomata* 249–287 µm high × 270–296.5(–323) µm diam., superficial, solitary or scattered, globose-subglobose, black, coriaceous, ostiole, some collapsed when dry. *Peridium* 25–28 µm wide, composed of cells of *textura angularis* layers of dark brown to black-walled cells, arranged in a *textura prismatica/angularis*. *Pseudoparaphyses* 1–2 µm wide, among asci, flexuous, hyaline, branched. *Asci* (99–)106–126 × 15–20 µm ($\bar{x}$ = 112.5 × 17 µm, n = 10), 8-spored, bitunicate, fissitunicate, cylindric-clavate, short pedicellate, apically thickened. *Ascospores* 24–29(–35) × 6–10 µm ($\bar{x}$ = 27 × 8 µm, n = 20), biseriate, elongate-obovoid, 4-septate, slightly constricted at the septum, brownish to dark brown, light at the apex, base tapering with 4–8.5(–14) µm long narrow hyaline appendage, smooth-walled. *Asexual state*: Unknown.

Material examined: FINLAND, Abo., on wood of *Acer platanoides* L., P.A. Karsten, Finland Fungi 881, Farlow Herbarium, Harvard University, Herbarium of F. Theissen, FH, holotype.

Notes: Karsten (1869) introduced *Rebentischia* for the presently monotypic genus based on *Rebentischia pomiformis* which was first included with the *Sphaeriaceae*. *Rebentischia* has since been placed in several different families by several authors (Müller, 1950; von Arx & Müller, 1975; Saccardo, 1877, 1883).

Barr (1980) included *Rebentischia* in *Tubeufiaceae* based on its tropical saprobic habit, darkly pigmented ascomata, bitunicate asci, and elongated and multiseptate ascospores. In additional, Barr (1980) placed *Rebentischia pomiformis* in synonymy with *R. massalongi* as first suggested by von Arx and Müller (1975). Only two accepted species, *R. massalongi* (Mont.) Sacc. (= *R. pomiformis* P. Karst.) and *R. unicaudata* (Berk. & Broome) Sacc. were discussed by Barr (1980). Barr (1980) also considered that some coelomycetous taxa, such as *Asteromella*-like found near ascomata might be asexual state of *R. massalongi*. Presently, *Rebentischia* has 13 epithets listed in Index Fungorum. *Rebentischia pomiformis* which is listed as a distinct species in Index Fungorum and has darkened ascospores with a setiform basal appendage and appears more typical of *Pleosporales.* The genus is therefore included in *Pleosporales incertae cedis* until fresh collections and molecular data are available for phylogenetic study.



Note. (a) Scattered ascomata on host. (b) Section through ascoma. (c) Peridium. (d) Wide flexuous pseudoparaphyses. (e)-(g) Immature and mature asci. (h)-(l) Ascospores with basal appendage. Scale bars: (a) = 200 μm, (b) = 100 μm, (c), (e)-(g) = 50 μm, (d) = 5 μm, (h)-(l) = 10 μm

Figure 5.34 *Rebentischia pomiformis* (FH, holotype)

Uredinophila Rossman, Mycol. Pap. 157: 43 (1987), MycoBank: MB25148

Parasitic on *Pucciniales* on leaves. *Ascomata* superficial on substrate and surrounded by rust fungi, developing on a subiculum, globose to subglobose, solitary, scattered, translucent, yellow to orange, soft, apex with hyaline hairs. *Peridium* composed of hyaline or pale yellow, cells arranged in a *textura angularis*. *Pseudoparaphyses* numerous, 1–2 µm wide, cylindrical, branched, raised above asci, embedded in a gelatinous matrix. *Asci* bitunicate, 8-spored, cylindrical-clavate, with short knob-like pedicel, apex rounded. *Ascospores* multiseriate and helically coiled, filiform or fusiform, narrowly elongate, tapering to a narrow rounded base, trans-septate, hyaline. *Asexual state*: Unknown.

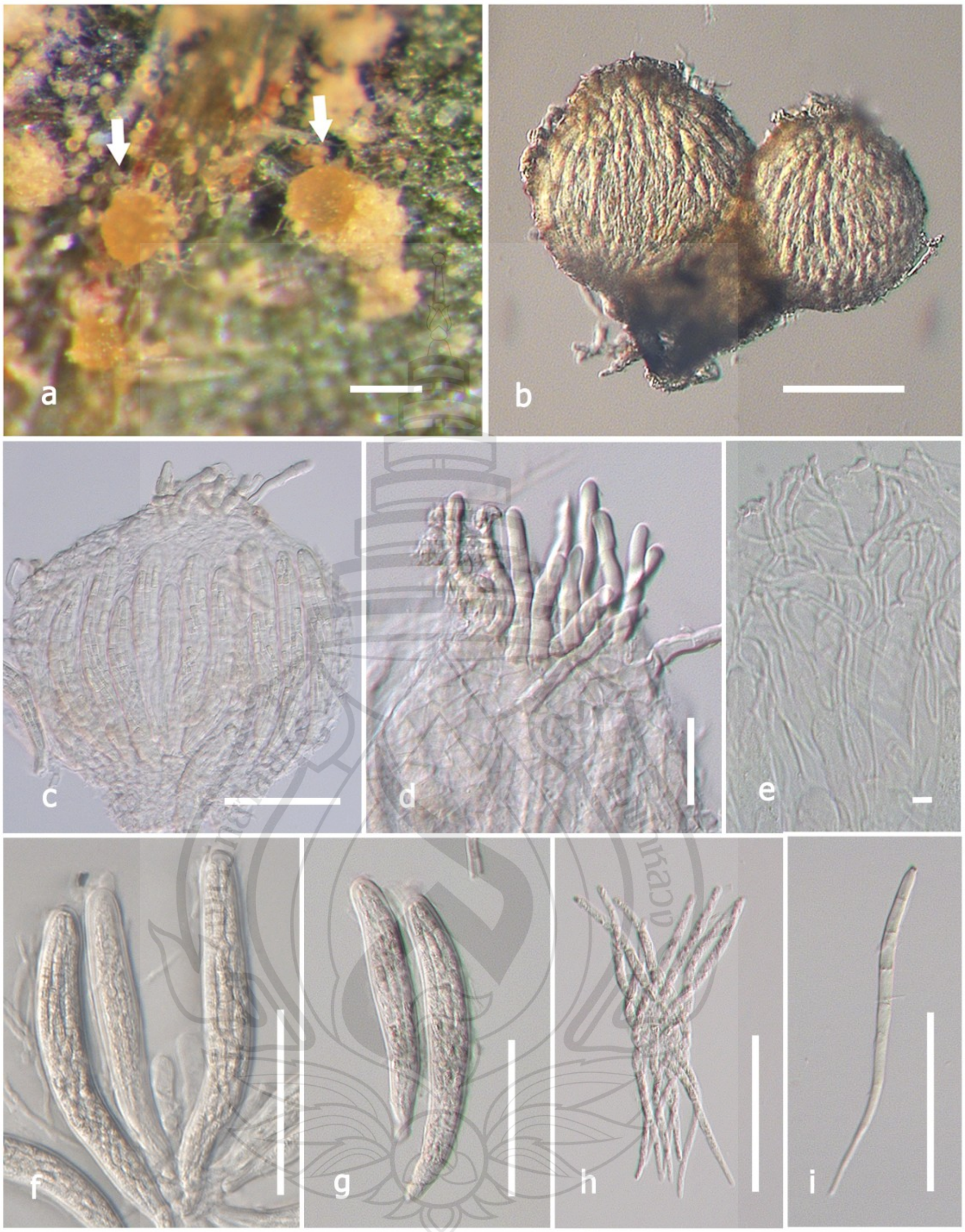
Notes: Rossman (1987) introduced the genus *Uredinophila* for two mycoparasitic fungi associated with rusts, i.e. *U. erinacea* (Rehm) Rossman and *U. tropicalis* (Speg.) Rossman. The *Uredinophila* was excluded from the *Ophionectria* based on its bitunicate asci and spirally elongated ascospores (Rossman, 1977); the genus was placed in the *Tubeufiaceae* by Rossman (1987) who provided key, descriptions and illustrations of the two accepted. We reexamined an authentic specimen of *U. tropicalis* (BPI 632877) listed by Rossman (1987) which has characters that are atypical of *Tubeufiaceae*. Therefore, *Uredinophila* is placed in Dothideomycetes *incertae sedis*.

Type species: *Uredinophila tropicalis* (Speg.) Rossman, Mycol. Pap. 157: 45 (1987), MycoBank: MB130896 (Fig. 5.35)

≡ *Ophionectria tropicalis* Speg., Anal. Soc. cient. argent. 16(5): 242 (1883)

Parasitic on *Desmella superficialis* (*Pucciniales*) on leaves of *Dryopteris tetragona*. *Ascomata* (122–)134.5–176(–196) µm high × (111–)141–167(–196) µm diam. ($\bar{x}$ = 156 × 156 µm), superficial on substrate and surrounded by rust fungi, developing on a subiculum, globose to subglobose, solitary, scattered, translucent, yellow to orange, soft, apex with (16–)21.5–35(–39) µm long, hyaline hairs which are rounded at the ends and lack septa. *Peridium* 9.5–13 µm wide, composed of hyaline or pale yellow, 6–9 µm wide, cells arranged in a *textura angularis*. *Pseudoparaphyses* numerous, 1–2 µm wide, cylindrical, cellular, hyaline, branched, raised above asci, embedded in a gelatinous matrix. *Asci* (85–)92.5–115 × 12–15 µm ($\bar{x}$ = 99 × 13 µm), bitunicate, 8-spored, cylindrical-clavate, with short knob-like pedicel, apex rounded. *Ascospores* (96–) 107.5–113 × 3.5–5 µm ($\bar{x}$ = 103 × 4 µm), multiseriate and helically coiled, filiform or fusiform, narrowly elongate, apex rounded, tapering to a narrow rounded base, with 7 or more septa, not constricted at the septa, hyaline, smooth-walled. *Asexual state*: Unknown.

Material examined: VENEZUELA, Caguita, near Pueto La Cruz, fungicolous on uredosoris of the fern rust *Desmella superficialis*, on leaves of *Dryopteris tetragona*, H. Sydow, 27 December 1927, Sydow 840 (BPI 632877, issued as *Ophionectria tropicalis*).



Note. (a) Ascomata associated with a rust. (b)-(c) Squash mount of ascomata. (d) Close up of setae and peridial wall. (e) Pseudoparaphyses. (f)-(g) Asci. (h)-(i) Ascospores. Scale bars: (a) = 100 µm, (c), (f)-(i) = 50 µm, (d) = 20 µm, (e) = 5 µm

Figure 5.35 *Uredinophila tropicalis* (BPI 632877, issued as *Ophionectria tropicalis*)

5.4 Conclusion

An attempt was made, in this paper, to reexamine 17 type specimens and analyze molecular sequence data from ten new collections and 56 GenBank strains of *Tubeufiaceae.* Based on the results obtained, a new order *Tubeufiales* is introduced to accommodate this group of fungi which are distinct from *Pleosporales*, in the Dothideomycetes. Besides, we examined and incorporated sexual and asexual states of genera in *Tubeufiales* to provide a modern treatment based on single names for related asexual and sexual states. Presently accepted genera include *Acanthophiobolus, Acanthostigma, Acanthostigmina*, *Aquaphila, Boerlagiomyces, Chaetosphaerulina, Chlamydotubeufia, Helicoma*, *Helicomyces*, *Helicosporium*, *Kamalomyces, Podonectria, Tamhinispora, Thaxteriella, Thaxteriellopsis* and *Tubeufia*. *Acanthostigmella, Amphinectria*, *Chaetocrea*, *Glaxoa*, *Malacaria*, *Melioliphila*, *Paranectriella*, *Puttemansia*, *Rebentischia* and *Uredinophila* are excluded from *Tubeufiaceae* and new placements are suggested. The new species *Acanthostigma lignicola, A. fusiforme, Acanthostigmina piniraiensis, Helicoma chiangraiense, H. fagacearum, H. siamense* and *Tubeufia chiangmaiensis* are introduced with descriptions and illustrations and discussed in comparison to related taxa. *Aquaphila asiana* (≡ *Tubuefia asiana*), *Aquaphila khunkornensis* (≡ *Chlamydotubeufia khunkornensis*), *Helicoma khunkornense* (≡ *Tubeufia khunkornensis*), *Helicoma helicoma* (≡ *Thaxteriella helicoma*), *Helicoma inthanonense* (≡ *Thaxteriella inthanonensis*) and *Helicosporium cereum* (≡ *Tubeufia cerea*) are the new combinations declared. In this study a multigene (LSU, ITS, TEF1-α, RPB2) analysis was used to resolve species and genera within the *Tubeufiaceae*. Seven clades are clearly recognised. Clade A represents the genus *Tubeufia sensu stricto*, comprising 18 sexual and asexual isolates and is well-supported (90% BS, 1.00 PP). Clade B clusters into two well-supported subclades, i.e. Clade B1 *Aquaphila* and Clade B2 *Chlamydotubeufia*. Clade C is *Acanthostigmina sensu stricto*, comprising 8 sexual and asexual isolates. Clade D represents the genus *Helicoma sensu stricto* and is strongly supported (98% BS and 1.00 PP support). Clade E is *Helicosporium sensu stricto* and is also strongly supported (97% BS and 1.00 PP support). Clade F represents *Acanthostigma sensu stricto*, and comprises two well-supported species while Clade G comprises a single lineage (86% BS and 1.00 PP support), representing the genus *Thaxteriellopsis sensu stricto*.

CHAPTER 6

OVERALL CONCLUSIONS

6.1 Diversity of Dothideomycetes On Woody Litter in Northern Thailand

This is the first study of the diversity of Dothideomycetes on woody litter in terrestrial forests in northern Thailand. The sampling sites were chosen randomly in various localities of Chiang Mai, Chiang Rai, Lam Pang and Phrae. Previous studies in tropical forests have yielded high diversity of saprobic fungi, including Dothideomycetes (Bhilabutra, McKenzie, Hyde & Lumyong, 2010; Hawksworth, 2004; Kodsueb, McKenzie, Lumyong & Hyde, 2008; Wang et al., 2008). A frequency of saprobic Dothideomycetes can be found in the *Botryosphaeriaceae, Tubeufiaceae* and few in *Kirschsteiniotheliaceae*, and other families (Boonmee et al., 2011, 2012; Liu et al., 2011, 2012; Wu et al., 2011). Identification criteria are based on morphology and molecular phylogenetic analysis.

6.2 *Kirschsteiniotheliaceae*

Kirschsteiniothelia species are common on decaying wood during the rainy season in Chiang Mai and Chiang Rai. Detailed records concerning the *Kirschsteiniotheliaceae* focused on morphological descriptions of new species and the use of molecular phylogeny in explaining their relationships with other Dothideomycetes. Two new species of *Kirschsteiniothelia* were published. Currently, *Kirschsteiniothelia* comprises 18 species, while *K. elaterascus* is transferred to the genus *Morosphaeria* and *K. maritima* transferred to the new genus *Halokirschsteiniothelia* as a result of this study.

Future collections of *Kirschsteiniotheliaceae* are needed from different regions of Thailand. These collections should be isolated, sequenced and analyzed, to establish relationships with other Dothideomycetes using sequence analysis.

6.3 *Tubeufiaceae*

Investigation of *Tubeufiaceae* on woody litter was carried out during 2009-2011. Tubeufiaceous species can be recognized in nature by brightly pigmented, fleshy, superficial ascomata, with or without setae. This study provides a contribution to the knowledge of *Tubeufiaceae* and validates the taxonomy of the *Tubeufiaceae* based on herbarium type specimens, fresh collections and molecular phylogenetic classification. The fungal taxa are described and illustrated in Chapter 2, comprising of *Acanthostigma, Acanthophiobolus, Allonecte, Byssocallis, Chaetosphaerulina, Chlamydotubeufia, Letendraeopsis, Podonectria, Taphrophila, Thaxteriella, Thaxteriellopsis, Thaxterina* and *Tubeufia*. Sexual-asexual connections are confirmed by culture and phylogeny. Five new species, one new genus and one epitypification are presented in this study. Six genera of *Tubeufiaceae* are excluded due to different morphological characteristics. *Chlamydotubeufia* and *Thaxteriellopsis* species were commonly found in northern Thailand. Pigment in media was produced by isolates of *Tubeufia khunkornensis*. Further studies should concentrate on secondary metabolites from this family, which can be applied for agricultural and biotechnological uses.

6.4 *Tubeufiales*

The Tubeufiales was focused based on the *Tubeufiaceae*, and represents a new order separated from Pleosporales. Multigene phylogenetic analysis encompassing all members of Tubeufiaceae, plus other Dothideomycetes showed Tubeufiales to be a monophyletic lineage. Taxonomic and diversity research indicated that *Acanthostigma*, *Chlamydotubeufia* and *Tubeufia* were commonly found from different habitats in northern Thailand. The chlamydosporous and helicosporous asexual states were frequently formed in culture. The asexual states *Aquaphila, Helicoma, Helicomyces* and *Helicosporium* are accepted as well resolved genera of *Tubeufiaceae* in this study. Further collections of *Tubeufiaceae* are needed from different parts of Thailand and other tropical and temperate countries to understand their taxonomy and ecology.

6.5 Families of Dothideomycetes

Dothideomycetes are grouped into 15 orders based on molecular data (This study, Boehm, Mugambi et al., 2009; Boonmee et al. in prep; Crous et al., 2009; Minter et al., 2007; Mugambi & Huhndorf, 2009; Nelsen et al., 2009; Ruibal et al., 2009; Schoch et al., 2009; Shearer et al., 2009; Suetrong et al., 2009; Tanaka et al., 2009; Wu et al., 2011; Zhang et al., 2011, 2012). Most families in this class are however, classified by morphological features (Barr 1987, 1990; Eriksson, 1981; Luttrell, 1955; von Arx & Müller, 1975). In this study, nine families of Dothideomycetes are described and discussed using morphological traits, except for *Kirschsteiniotheliaceae* and *Tubeufiaceae* which are also resolved based on molecular phylogenetic studies. Many families of Dothideomycetes need fresh collections and molecular data to resolve their taxonomic status.

6.6 Significance and Publications Resulting from This Thesis

During this study, I participated in more than 30 field trips to collect saprobic fungi on woody litter, and collected more than 150 specimens and examined them for fungi. I found more than 80 interesting specimens of saprobic fungi belonging to Dothideomycetes and studied these in more detail. Finally, 51 specimens were identified belonging to Dothideomycetes, including two species of *Kirschsteiniotheliaceae*, 14 species of *Tubeufiaceae* and several other species in the Dothideomycetes. I isolated 54 strains from my collections and sequenced five genes of all 51 isolates, with 97 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.

Comparative morphology and molecular phylogenetic analysis resolved two new species of *Kirschsteiniotheliaceae* and twelve new species of *Tubeufiaceae*. This finding also provides an understanding of the relationships between sexual and asexual morphs of species in *Kirschsteiniotheliaceae* and *Tubeufiaceae*.

During this study I also examined herbaria materials of 73 genera in nine families of Dothideomycetes and these data are presently in preparation in a monograph of the families of Dothideomycetes. The results of my study have been published in two papers in international journals as first author and six publications as co-author, and significantly improve the understanding of Dothideomycetes. As a result, it will be useful for future researchers who study and the saprobic families, *Kirschsteiniotheliaceae* and *Tubeufiaceae* (*Tubeufiales*) of Dothideomycetes.

List of publications

Boonmee, S., Zhang, Y., Chomnunti, P., Chukeatirote, E., Tsui, C. K. M., Bahkali, A. H., & Hyde, K. D. (2011). Revision of lignicolous *Tubeufiaceae* based on morphological reexamination and phylogenetic analysis. **Fungal Divers.**, **51**(1), 63–102.

Boonmee, S., Hyde, K. D., KoKo, T. W., Chukeatirote, E., Chen, H., Cai, L., McKenzie, E. H. C., Jones, E. B. G., Kodsueb, R., & Hassan, B. A. (2012). Two new *Kirschsteiniothelia* species with *Dendryphiopsis* anamorphs cluster in *Kirschsteiniotheliaceae* fam. nov. **Mycologia, 104,** 698–714.

Boonmee, S., Rossman, A. Y., Liu, J. K., Crous, P. W., Bhat, J. D., Chukeatirote, E., Jones, E. B. G. & Hyde, K. D. (2013). *Tubeufiales*, ord. nov., integrating sexual and asexual generic names. (in prep.)

Wu, H. X., Schoch, C. L., Boonmee, S., Bahkali, A. H., Chomnunti, P. & Hyde, K. D. (2011). A reappraisal of *Microthyriaceae*. **Fungal Divers., 51**(1), 189–248.

Liu, J. K., Phookamsak, R., Jones, E. B. G., Zhang, Y., KoKo, T. W., Hu, H. L., Boonmee, S., Doilom, M., Chukeatirote, E., Bahkali, A. H., Wang, Y. & Hyde, K. D. (2011). *Astrosphaeriella* is polyphyletic, with species in *Fissuroma* gen. nov., and *Neoastrosphaeriella* gen. nov. **Fungal Divers., 51,** 135–154.

Liu, J. K., Phookamsak, R., Doilom, M., Wiki, S., Mei, L. Y., Ariyawansha, H., Boonmee, S., Chomnunti, P., Dai, D. Q., Bhat, D. J., Romero, A. I., Xhuang, W. Y., Monkai, J., Jones, E. B. G., Chukeatirote, E., KoKo, T. W., Zhoa, Y. C., Wang, Y. & Hyde, K. D. (2012). Towards a natural classification of Botryosphaeriales. **Fungal Divers., 57**(1), 149–210.

Monkai, J., Liu, J. K., Boonmee, S., Chomnunti, P., Chukeatirote, E., Jones, E. B. G., Wang, Y. & Hyde, K. D. (2013). *Planistromellaceae* (Botryosphaeriales). **Cryptogam., Mycol., 34**(1), 45–77.

REFERENCES

REFERENCES

Abarenkov, K., Tedersoo, L., Nilsson, R. H., Veldre, V., Parmasto, E., Prous, M., Aan, A., Ots, M., Kurina, O., Ostonen, I., Jõgeva, J., Halapuu, S., Põldmaa, K., Toots, M., Truu, J., Larsson, K. H. & Kõljalg, U. (2010). PlutoF–a Web Based Workbench for Ecological and Taxonomic Research, with an Online Implementation for Fungal ITS Sequences. **Evol. Bioinf., 6,** 189–196.

Alexopoulos, C. J., Mims, C. W. & Blackwell, M. (1996). **Introductory Mycology** (4th ed.). New York: Wiley.

Aptroot, A., Lücking, R., Sipman, H. J. M., Umaña, L. & Chaves, J. L. (2008). Pyrenocarpous lichens with bitunicate asci: a first assessment of the lichen biodiversity inventory in Costa Rica. **Biblioth. Lichenol., 97,** 1–162.

Ariyawansa, H. A., Jones, E. B. G., Suetrong, S., Alias, S. A., Kang, J. C. & Hyde, K. D. (2013). *Halojulellaceae* a new family of the order *Pleosporales*. **Phytotaxa** (in press).

Arnaud, G. (1915). Notes mycologiques (G. *Isaria* et *Parodiopsis*). **Bull. Soc. Mycol. Fr., 31**(1), 20–23.

Arnaud, G. (1918). Les *Astérinées*. **Ann. Ecole. Nat. Agric. Montpellier nouv. Série., 16,** 1–288.

Arnaud, G. (1920). La famille des *Parodiellinacées* (Pyrénomycètes). **C. R. Acad. Se. Paris, T., 170,** 202–204.

Arnaud, G. (1921). Les *Astérinées*: II. Études sur les champignons (*Parodiellinacées*, inclus *Erysiphées*). **Ann. Épiphyt., 7,** 1–115.

Arnaud, G. (1925). Les *Asterinees*. IVe Partie. (Études sur la Systématique des champignons *Pyrénomycetes*). **Ann. Sci. Nat. Bot. Illus., 7,** 643–723.

Barr, M. E. (1968). The *Venturiaceae* in North America. **Can. J. Bot., 46**(6), 799–864.

Barr, M. E. (1975). The genus *Ostreichnion*. **Mycotaxon, 3,** 81–88.

Barr, M. E. (1976). Perspectives in the ascomycotina. **Mem. New York Bot. Gard., 28**(1), 1–8.

Barr, M. E. (1977). *Acanthostigmella* (Herpotrichiellaceae). **Mycotaxon, 6,** 17–23.

Barr, M. E. (1979). A classification of *Loculoascomycetes*. **Mycologia, 71,** 935–957.

Barr, M. E. (1980). On the family *Tubeufiaceae* (Pleosporales). **Mycotaxon, 12,** 137–167.

Barr, M. E. (1983). The ascomycete connection. **Mycologia, 75**(1), 1–13.

Barr, M. E. (1987). **Prodomus to class *Loculoascomycetes***. Massachusetts: Newell.

Barr, M. E. (1990). Prodromus to nonlichenized, pyrenomycetous members of class Hymenoascomycetes. **Mycotaxon, 39,** 43–184.

Barr, M. E. (1997). Notes on some 'dimeriaceous' fungi. **Mycotaxon, 64,** 149–171.

Barr, M. E. & Huhndorf S. M. (2001). *Loculoascomycetes*. In: McLaughlin D. J., McLaughlin E. G. and Lemke P. A. (eds.). **The Mycota, Systematics and Evolution Part A, vol. VII** (pp. 283–305). New York: Springer Berlin Heidelberg.

Bhat, D. J. (2010). **Fascinating Microfungi (Hyphomycetes) of Western Ghats, India.** Goa: Broadway Book Centre.

Batista, A. C. (1959). *Monografia* dos fungos *Micropeltaceae*. **Publ. Inst. Micol. Univ. Recife, 56,** 1–519.

Batista, A. C. (1960). *Lembopodia*, *Yamamotoa* e *Peresiopsis*, novos gêneros de *Asterinaceae*. **Publ. IMUR Recife, 291,** 1–35.

Batista, A. C., Maia, H. S. & Farr, M. L. (1958). Novos generos e novasespecies de fungos *Asterinaceae*. **Revta Biol., Lisb., 1**(3–4), 287–298.

Berbee, M. L. & Taylor, J. W. (2001). Fungal molecular evolution: gene trees and geologic time. In: McLaughlin, D., McLaughlin, E., Lemke, P. (eds). **The Mycota systematics and evolution** (pp. 229–245). Berlin: Springer-Verlag.

Berbee, M. L. (1996). *Loculoascomycetes* origins and evolution of filamentous ascomycete morphology based on 18S rRNA gene sequence data. **Mol. Biol. Evol., 13**(3), 462–470.

Berkeley, M. J. (1845). Decades of fungi. Decades III-VII. Australian fungi. **London J. Bot., 4,** 42–73.

Berkeley, M. J. (1876). Notices of North American fungi. **Grevillea, 4**(31), 105–106.

Berlese, A. N. (1893). Descrizione di alcuni nuovi generi di pirenomiceti. **Reprint from Atti del Congresso Botanico Internazionale, 1892,** 567–576.

Berlese, A. N. (1894). Icones fungorum omnium hucusque cognitorum. **Biblioth Mycol., 16,** 1–243

Bhilabutra, W., McKenzie, E. H. C., Hyde, K. D. & Lumyong, S. (2010). Fungi on the grasses, *Thysanolaena latifolia* and *Saccharum spontaneum*, in northern Thailand. **Mycosphere, 1**(4), 301–314.

Bigelow, H. E. & Barr, M. E. (1963). Contribution to the fungus flora of northeastern North America III. **Rhodora, 65,** 289–309.

Boehm, E. W. A., Mugambi, G. K., Miller, A. N., Huhndorf, S. M., Marincowitz, S., Spatafora, J. W. & Schoch, C. L. (2009). A molecular phylogenetic reappraisal of the *Hysteriaceae*, *Mytilinidiaceae* and *Gloniaceae* (*Pleosporomycetidae*, *Dothideomycetes*) with keys to world species. **Stud. Mycol., 64,** 49–83.

Boehm, E. W. A., Schoch, C. L. & Spatafora, J. W. (2009). On the evolution of the *Hysteriaceae* and *Mytilinidiaceae* (Pleosporomycetidae, Dothideomycetes, Ascomycota) using four nuclear genes. **Mycol. Res., 113,** 461–479.

Boonmee, S., Zhang, Y., Chomnunti, P., Chukeatirote, E., Tsui, C. K. M., Bahkali, A. H. & Hyde, K. D. (2011). Revision of lignicolous *Tubeufiaceae* based on morphological reexamination and phylogenetic analysis. **Fungal Divers., 51,** 63–102.

Boonmee, S., Hyde, K. D., KoKo, T. W., Chukeatirote, E., Chen, H., Cai, L., McKenzie, E. H. C., Jones, E. B. G., Kodsueb, R. & Hassan, B. A. (2012). Two new *Kirschsteiniothelia* species with *Dendryphiopsis* anamorphs cluster in *Kirschsteiniotheliaceae* fam. nov. **Mycologia, 104**(3), 698–714.

Booth, C. (1964). Studies of *Pyrenomycetes*: VII. **Mycol. Pap., 94,** 1–16.

Bose, S. K. (1961). Studies on *Massarina* Sacc. and related genera. **Phytopathol. Z., 41,** 151–213.

Brandriff, H. (1936). The development of the ascocarp of *Acrospermum compressum*. **Mycologia, 28,** 228–235.

Brock, P. M., Döring, H. & Bidartondo, M. I. (2009). How to know unknown fungi: the role of a herbarium. **New Phytol., 181,** 719–724.

Butzin, F. (1977). *Boerlagiomyces*, ein neuer Name für die *Pilzgattung Boerlagella*. **Willdenowia, 8,** 37–40.

Campbell, J., Ferrer, A., Raja, H. A., Sivichai, S. & Shearer, C. A. (2007). Phylogenetic relationships among taxa in the *Jahnulales* inferred from 18S and 28S nuclear ribosomal DNA sequences. **Can. J. Bot., 85,** 873–882.

Cai, L., Hyde, K. D. & Tsui, C. K. M. (2006). **Genera of Freshwater Fungi.** Fungal Diversity Research Series 18. Hong Kong: Fungal Diversity press.

Cai, L., Hyde, K. D., Taylor, P. W. J., Weir, B. S., Waller, J., Abang, M. M., Zhang, J. Z., Yang, Y. L., Phoulivong, S., Liu, Z. Y., Prihastuti, H., Shivas, R. G., McKenzie, E. H. C., & Johnston, P. R. (2009). A polyphasic approach for studying Colletotrichum. **Fungal Divers., 39,** 183–204.

Cannon, P. F. (1997). Two new genera of Ascomycota, and other new or interesting fungi from Slapton Ley National Nature Reserve and its environs. **Syst. Ascom., 15,** 121–138.

Cannon, P. F. & Kirk, P. M. (2007). **Fungal Families of the World.** Wallingford: CABI.

Chang, H. S. (2003). *Tubeufia dactylariae* sp. nov. and *Acanthostigma scopulum*, a new record of Taiwan. **Bot. Bull. Acad. Sin., 44,** 253–256.

Chaverri, P. & Gazis, R. O. (2010). *Perisporiopsis lateritia*, a new species on decaying leaves of *Hevea* spp. from the Amazon basin in Peru. **Mycotaxon, 113,** 163–169.

Chen, C. Y. & Hsieh, W. H. (2004). On the type species of the genus *Kirschsteiniothelia*, *K. aethiops*. **Sydowia, 56,** 229–236.

Chen, C. Y., Wang, C. L. & Huang, J. W. (2006). Two new species of *Kirschsteiniothelia* from Taiwan. **Mycotaxon, 98,** 153–158.

Choi, Y. W., Hyde, K. D. & Ho, W. H. (1999). Single spore isolation of fungi. **Fungal Divers., 3,** 29–38.

Chomnunti, P., Schoch, C. L., Aguirre–Hudson, B., KoKo, T. W., Hongsanan, S., Jones, E. B. G., Kodsueb, R., Chukeatirote, E., Bahkali, A. H. & Hyde, K. D. (2011). *Capnodiaceae*. **Fungal Divers., 51,** 103–134.

Clements, F. E. & Shear, C. L. (1931). **Genera of Fungi. edn 2. i–vii, 58 plates.** USA, New York: H.W. Wilson Company.

Corda, A. C. J. (1837). Icones fungorum hucusque cognitorum. **J. G. Calve, Prague, 1,** 1–32.

Crane, J. L. (1971). Illinois fungi. 1. *Chaetosticta*. **Can. J. Bot., 49**(1), 31–34.

Crane, J. L., Shearer, C. A. & Barr, M. E. (1998). A revision of *Boerlagiomyces* with notes and a key to the saprobic genera of *Tubeufiaceae*. **Can. J. Bot., 76,** 602–612.

Crous, P. W., Braun U. & Groenewald, J. Z. (2007). *Mycosphaerella* is polyphyletic. **Stud. Mycol., 58,** 1–3.

Crous, P. W., Gams, W., Stalpers, J. A., Robert, V. & Steghuis, G. (2004). MycoBank: an online initiative to launch mycology into the 21st century. **Stud. Mycol., 50,** 19–22.

Crous, P. W., Slippers, B., Wingfield, M. J., Rheeder, J., Marasas, W. F. O., Philips A. J. L., Alves, A., Burgess, T. I., Barber, P. A. & Groenewald, J. Z. (2006). Phylogenetic lineages in the *Botryosphaeriaceae*. **Stud. Mycol., 55,** 235–253.

Crous, P. W., Schoch, C. L., Hyde, K. D., Wood, A. R., Gueidan, C., de Hoog, G. S. & Groenewald, J. Z. (2009). Phylogenetic lineages in the *Capnodiales*. **Stud. Mycol., 64,** 17–47.

de Gruyter, J. D., Aveskamp, M. M., Woudenberg, J. H. C., Verkley, G. J. M., Groenewald, J. Z. & Crous, P. W. (2009). Molecular phylogeny of *Phoma* and allied anamorph genera: towards a reclassification of the *Phoma* complex. **Mycol. Res., 113,** 508–519.

de Gruyter, J., Woudenberg, J. H., Aveskamp, M. M., Verkley, G. J., Groenewald, J. Z. & Crous, P. W. (2010). Systematic reappraisal of species in *Phoma* section *Paraphoma, Pyrenochaeta and Pleurophoma*. **Mycologia, 102**(5), 1066–1081.

De Notaris, G. (1863). **Sfériacei Italici.** Centuria I. Fasc. 2–3. Genova, 90 pp., 120 pl.

del Prado, R., Schmitt, I., Kautz, S., Palice, Z., Lücking, R. & Lumbsch, H. T. (2006). Molecular data place *Trypetheliaceae* in Dothideomycetes. **Mycol. Res., 110,** 511–520.

Eberhardt, U. (2010). A constructive step towards selecting a DNA barcode for fungi. **New Phytol., 187,** 266–268.

Ellis, M. B. (1971). **Dematiaceous hyphomycetes** (pp. 1-608). Kew: Commonwealth Mycological Institute.

Ellis, J. P. (1980). The genus *Morenoina* in Britain. **Trans. Br. Mycol. Soc., 74,** 297–307.

Eriksson, O. E. (1981). The families of the bitunicate ascomycetes. **Opera Bot., 60,** 1–220.

Eriksson, O. E. (1982). Outline of the ascomycetes-1982. **Mycotaxon, 15,** 203–248.

Eriksson, O. E. & Hawksworth, D. L. (1986) Outline of the Ascomycetes. **Syst. Ascom., 5,** 185–324.

Eriksson, O. E. (2005). Outline of Ascomycota, The present classification contains all accepted genera and higher taxa above the generic level in phylum Ascomycota. **Myconet, 11,** 1–113.

Eriksson, O. E. & Hawksworth, D. L. (1991). Outline of the Ascomycetes – 1990. **Syst. Ascom., 9,** 39–271.

Eriksson, O. E. & Winka, K. (1997). Supraordinal taxa of Ascomycota. **Myconet, 1,** 1–16.

Farr, M. L. (1979). The didymosporous dimeriaceous fungi described on *Asteraceae*. **Mycologia, 71,** 243–803.

Figueira, D. & Barata, M. (2007). Marine fungi from two sandy beaches in Portugal. **Mycologia, 99,** 20–23.

Figueiredo, J. G., Goulin, E. H., Tanaka, F., Stringari, D., Kava-Cordeiro, V., Galli-Terasawa, L. V., Staats C. C., Schrankand, A. & Glienke, C. (2010). Agrobacterium tumefaciens-mediated transformation of *Guignardia citricarpa*. **J. Microbiol. Methods., 80,** 143–147.

Fries, E. M. (1818). Observationes mycologicae. Gerh. Bonnier, **Copenhagen, 2,** 347–351.

George, M. (1895). **British fungus–flora.** A classified text–book of mycology. London: G. Bell and sons.

Goos, R. D. (1986). A review of the anamorph genus *Helicoma*. **Mycologia, 78,** 744–761.

Greuter, W., Garrity, G., Hawksworth, D. L., Jahn, R., Kirk, P. M., Knapp, S., McNeill, J., Michel, E., Patterson, D. J., Pyle, R., Tindall, B. J. (2011). Draft BioCode (2011): principles and rules regulating the naming of organisms. **Bionomina, 3,** 26–44.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. **Nucl. Acids. Symp. Ser., 41,** 95–98.

Hansford, G. C. (1945). Contributions towards the fungus flora of Uganda. VII. New records and revisions. **Proc. Linn. Soc. Lond., 157,** 37–38.

Hansford, G. C. (1946). The foliicolous Ascomycetes, their parasites and associated fungi. **Mycol. Pap., 15,** 1–240.

Hawksworth, D. L. (1985a). *Kirschsteiniothelia* a new genus for the *Microthelia incrustans*–group (Dothideales). **Bot. J. Linn. Soc., 91,** 181–202.

Hawksworth, D. L. (1985b). A redisposition of the species referred to the ascomycete genus *Microthelia*. **Bull. Br. Mus. Bot., 14,** 43–181.

Hawksworth, D. L. (1977). Taxonomic and biological observations on the genus *Lichenoconium* (Sphaeropsidales). **Persoonia, 9,** 159–198.

Hawksworth, D. L. (2004). Fungal diversity and its implications for genetic resource collections. **Stud. Mycol., 50,** 9–18.

Hawksworth, D. L. (2011). Naming *Aspergillus* species: progress towards one name for each species. **Med. Mycol., 49,** 70–76.

Hawksworth, D. L. & Eriksson, O. (1986). The names of accepted orders of ascomycetes. **Syst. Ascom., 5,** 175–184.

Hawksworth, D. L., Kirk, P. M., Sutton, B. C. & Pegler, D. N. (1995). **Ainsworth & Bisby's Dictionary of the 8th eds**. Wallingford: CABI.

Hennings, P. C. (1895). Fungi Goyazenses. **Hedwigia, 34,** 88–116.

Hennings, P. C. (1900). Fungi japonica. **Bot. Jahrb. Syst. Pflanzengesch. Pflanzengeogr., 28,** 259–280.

Hennings, P. C. (1902). Fungi S. Paulenses I. a cl. Puttemans collecti. **Hedwigia, 41,** 104–118.

Hennings, P. C. (1904a). Fungi fluminenses a cl. E. Ule collecti. **Hedwigia, 43,** 78–95.

Hennings, P. C. (1904b). Einige neue Pilze aus Japan. **Hedwigia, 43,** 143–144.

Hennings, P. C. (1904c). Fungi Amazonici a cl. Ernesto Ule collecti II. **Hedwigia, 43,** 242–273.

Hernandez, C. I. B., Heredia, G., Arias, R. M., Portales, J. M. & Ruiz, R. F. C. (2008). Saprotrophic anamorphic fungi from Tabasco State. III. **Rev. Mex. Mic., 28,** 25–39.

Hibbett, D. S., Binder, M., Bischoff, J. F., Blackwell, M., Cannon, P. F., Eriksson, O. E., Huhndorf, S., James, S. T., Kirk, P. M., Lucking, R., Lumbsch, H. T., Lutzoni, F., Matheny, P. B., Mclaughlin, D. J., Powell, M. J., Redhed, S., Schoch, C. L., Spatafora, J. F., Stalpers, J. A., Vilgalys, R., Aime, M. C., Aptroot, A. A., Bauer, R., Begerow, D., Benny, G. L., Castelbury, L. A., Crous, P. W., Dai, Y. C., Gams, W., Geiser, D. M., Griffith, G. W., Gueidan, C., Hawksworth, D. L., Hestmark, G., Hosaka, K., Humber, R. A., Hyde, K. D., Ironisde, J. E., Koljag, U., Kurtzman, C. P., Larsson, K. H., Lichtwardt, R., Longcore, J., Miadlikowska, J., Miller, A., Moncalvo, J. M., Mozley–Standridge, S., Oberwinkler, F., Parmasto, E., Reeb, V., Rogers, J. D., Roux, C., Ryvarden, L., Sampaio, J. P., Schüßler, A., Sugiyama, J., Thorn, R. G., Tibell, L., Untereiner, W. A., Walker, C., Wang, Z., Weir, A., Weiss, M., White, M. M., Winka, K., Yao, Y. J. & Zhang, N. (2007). A higher-level phylogenetic classification of the fungi. **Mycol. Res., 111,** 509–547.

Hibbett, D. S., Ohman, A., Glotzer, D., Nuhn, M., Kirk, P. M. & Nilsson, R. H. (2011). Progress in molecular and morphological taxon discovery in Fungi and options for formal classification of environmental sequences. **Fungal Biol. Rev., 25,** 38–47.

Hino, I. (1938). Illustrationes fungorum bambusicolorum. **Bull. Miyazaki Coll. Agric. Forest., 10,** 55–64.

Hofmann, T. A., Kirschner, R. & Piepenbring, M. (2010). Phylogenetic relationships and new records of *Asterinaceae* (Dothideomycetes) from Panama. **Fungal Divers., 43,** 39–53.

Hosagoudar, V. B. (2012). *Asterinales* of India. **Mycosphere, 2**(5), 617–852.

Hsieh, W. H., Chen, C. Y. & Sivanesan, A. (1998). Six new ascomycetes from Taiwan. **Mycol. Res., 102**(2), 228–234.

Huelsenbeck, J. P. & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. **Bioinformatics, 17,** 754–755.

Hughes, S. J. (1953). Conidiophores, conidia, and classification. **Can. J. Bot., 31,** 577–659.

Hughes, S. J. (1976). Sooty moulds. **Mycologia, 68**(4), 693–820.

Hyde, K. D., Chomnunti. P., Crous, P., Groenewald, J., Damm, U., KoKo, T. W., Shivas, R., Summerell, B. & Tan, Y. (2010). A case for re-inventory of Australia's plant pathogens. **Persoonia, 25,** 50–60.

Hyde, K. D., Jones, E. B. G., Leaño, E., Ponting, A. D. & Vrijmoed, L. L. P. (1998). Role of fungi in marine ecosystems. **Biod. Cons., 7,** 1147–1161.

Hyde, K. D., McKenzie, E. H. C. & KoKo, T. W. (2011). Towards incorporating anamorphic fungi in a natural classification-checklist and notes for 2010. **Mycosphere, 2,** 1–88.

Hyde, K. D. & Zhang, Y. (2008). Epitypification: should we epitypify? **J. Zhejiang Uni. Sci. B., 9,** 842–846.

Inácio, C. A. & Cannon, P. F. (2008). **The genera of the *Parmulariaceae*.** CBS Biodiversity Series vol. 8. Utrecht, Netherlands: CBS Fungal Biodiversity Centre.

Inderbitzin, P., Landvik, S., Abdel-Wahab, A. & Berbee, M. L. (2001). *Aliquandostipitaceae*, a new family for two new tropical ascomycetes with unusually wide hyphae and dimorphic ascomata. **Am. J. Bot., 88,** 52–61.

Jones, E. B. G., Wong, S. W., Sivichai, S., Au, D. W. T & Hywel-Jones, N. L. (1999). Lignicolous freshwater Ascomycota from Thailand: *Micropeltopsis quinquecladiopsis* sp. nov. **Mycol. Res., 103,** 729–735.

Kar, A. K. & Maity, M. K. (1971). Leaf–inhabiting pyrenomycetes of West Bengal (India). **Mycologia, 63,** 1024–1029.

Karsten, P. (1869). Fungi Fenniae Exsiccati, Fasc. 9: no. 881. **Mycol. Fenn., 2,** 1–250.

Kirk, P. M., Cannon, P. F., David, J. C. & Stalpers, J. A. (2001). **Ainsworth & Bisby's dictionary of the fungi** (9th ed.). Wallingford: CABI.

Kirk, P. M., Cannon, P. F., Minter, D. W. & Stalpers, J. A. (2008). **Ainsworth & Bisby's dictionary of the fungi** (10th ed.). Wallingford: CABI.

Kirschner, R. & Piepenbring, M. (2006). A new fungicolous species of *Titaea* and new reports of *Bahusaganda indica* and *Exosporium ampullaceum* (hyphomycetes) from tropical rainforests in Panama. **Fungal Divers., 21,** 93–103.

Kirschner, R., Araúz, V., Herbst, F., Hofmann, T. A., Ix, S., Nozon, T. & Piepenbring, M. (2010). A new species of *Puttemansia* (Pleosporales) and new records of further Ascomycota from Panama. **Sydowia, 62**(2), 225–241.

Kishi, K. (1998). **Plant Diseases in Japan.** Tokyo: Zenkoku Noson Kyoiku Kyokai.

Kobayashi, T., Katsumoto, K., Abiko, K., Abe, Y. & Kakishima, M. (1992). **Illustrated Genera of Plant Pathogenic Fungi in Japan.** Tokyo: Zenkoku Noson Kyoiku Kyokai.

Kodsueb, R., Dhanasekaran, V., Aptroot, A., Lumyong, S., McKenzie, E. H. C., Hyde, K. D. & Jeewon, R. (2006). The family *Pleosporaceae*: intergeneric relationships and phylogenetic perspectives based on sequence analyses of partial 28S rDNA. **Mycologia, 98,** 571–583.

Kodsueb, R., Jeewon, R., Vijaykrishna, D., Mckenzie, E. H. C., Lumyong, P., Lumyong, S. & Hyde, K. D. (2006). Systematic revision of *Tubeufiaceae* based on morphological and molecular data. **Fungal Divers., 21,** 105–130.

Kodsueb, R., Lumyong, S., Lumyong, P., McKenzie, E. H. C., Ho, W. H. & Hyde, K. D. (2004). *Acanthostigma* and *Tubeufia* species, including *T. claspisphaeria* sp. nov., from submerged wood in Hong Kong. **Mycologia, 96,** 667–674.

Kodsueb, R., McKenzie, E. H. C., Lumyong, S. & Hyde, K. D. (2008). Fungal succession on woody litter of *Magnolia liliifera* (Magnoliaceae). **Fungal Divers., 30,** 55–72.

Kohlmeyer, J. & Kohlmeyer, E. (1979). **Marine Mycology: The Higher Fungi.** New York: Academic Press.

KoKo, T. W., Stephenson, S. L., Bahkali, A. H. & Hyde, K. D. (2011). From morphology to molecular biology: can we use sequence data to identify fungal endophytes? **Fungal Divers., 50,** 113–120.

Kutorga, E. & Hawksworth, D. L. (1997). A reassessment of the genera referred to the family *Patellariaceae* (*Ascomycota*). **Syst. Ascom., 15,** 1–110.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. & Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. **Bioinformatics, 23,** 2947–2948.

Li, Y., Wu, H., Chen, H. & Hyde, K. D. (2011). Morphological studies in *Dothideomyctes*: *Elsinoe* (*Elsinoaceae*), *Butleria*, and three excluded genera. **Mycotaxon, 115,** 507–520.

Libert, M. A. (1834). Plantae Cryptogamae quas in Arduenna collegit. **Fasc. 3,** 201–300.

Liew, E. C. Y., Aptroot, A. & Hyde, K. D. (2000). Phylogenetic significance of the Pseudoparaphyses in Loculoascomycete Taxonomy. **Mol. Phyl. Evol., 16**(3), 392–402.

Lindau, G. (1896). *Hysteriineae*. In: Engler & Prantl, Naturliche Pflanzenfamilien. **I. Teil. I. Abteilung., 1**, 265–278.

Lindau, G. (1897). *Pyrenomycetineae, Laboulbeniineae*. **Engler Prantl. Nat. Pflanzenfamilien., Teil. I (Leipzig), 1**(1), 321-505.

Linder, D. H. (1929). A monograph of the helicosporous fungi. **Ann. Mo. Bot. Gard., 16,** 227–338.

Lindemuth, R., Wirtz, N. & Lumbsch, H. T. (2001). Phylogenetic analysis of nuclear and mitochondrial DNA sequences supports the view that loculoascomycetes (Ascomycota) are not monophyletic. **Mycol. Res., 105,** 1176–1181.

Link, H. F. (1809). Observationes in ordines plantarum naturales. **Dissertatio I. Mag. Ges. naturf. Fr. Berl., 3,** 3–42.

Liu, Y. J., Whelen, S. & Hall, B. D. (1999). Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. **Mol. Biol. Evol., 16**(12), 1799–808.

Liu, Y. X., Hyde, K. D., Ariyawansa, H. A., Li, W. J., Zhou, D. Q., Yang, Y. L., Chen, Y. M. & Liu, Z. Y. (2013). *Shiraiaceae*, new family of Pleosporales (Dothideomycetes, Ascomycota). **Phytotaxa, 103**(1), 51–60.

Liu, J. K., Phookamsak, R., Doilom, M., Wiki, S., Mei, L. Y., Ariyawansha, H., Boonmee, S., Chomnunti, P., Dai, D. Q., Bhat, D. J., Romero, A. I., Xhuang, W. Y., Monkai, J., Jones, E. B. G., Chukeatirote, E., KoKo, T. W., Zhoa, Y. C., Wang, Y. & Hyde, K. D. (2012). Towards a natural classification of Botryosphaeriales. **Fungal Divers., 57**(1), 149–210.

Liu, J. K., Phookamsak, R., Jones, E. B. G., Zhang, Y., KoKo, T. W., Hu, H. L., Boonmee, S., Doilom, M., Chukeatirote, E., Bahkali, A. H., Wang, Y. & Hyde, K. D. (2011). *Astrosphaeriella* is polyphyletic, with species in *Fissuroma* gen. nov., and *Neoastrosphaeriella* gen. nov. **Fungal Divers., 51,** 135–154.

Lücking, R. (2008). Taxonomy: a discipline on the brink of extinction: are DNA barcode scanners the future of biodiversity research? **Arch. Sci., 61,** 75–88.

Lumbsch, H. T. & Huhndorf, S. M. (2010). Myconet Volume 14. Part one. Outline of Ascomycota-2009. Part Two. Notes on Ascomycete Systematics. Nos. 4751–5113. **Fieldiana Life Earth Sci., 1,** 1–64.

Lumbsch, H. T. & Lindemuth, R. (2001). Major lineages of Dothideomycetes (Ascomycota) inferred from SSU and LSU rDNA sequences. **Mycol. Res., 105,** 901–908.

Luttrell, E. S. (1951). Taxonomy of Pyrenomycetes. **Univ. Missouri Stud., 24**(3), 1–120.

Luttrell, E. S. (1955). The ascostromatic ascomycetes. **Mycologia, 47,** 511–532.

Luttrell, E. S. (1965). Paraphysoids, pseudoparaphyses, and apical paraphyses. **Trans. Br. Mycol. Soc, 48**(1), 135–144.

Luttrell, E. S. (1973). Loculoascomycetes. In: Ainsworth G. C., Sparrow F. K. and Sussman A. S. **The fungi. An advanced treatise.** New York: Academic Press.

Luttrell, E. S. (1975). Centrum development in *Didymosphaeria sadasivanii* (*Pleosporales*). **Am. J. Bot., 62,** 186–190.

Luttrell, E. S. (1981). The Pyrenomycete centrum–Loculoascomycetes. In Reynolds, D. R. (ed.). **Ascomycete Systematics: The Luttrellian Concept.** New York: Springer-Verlag.

Lutzoni, F., Kauff, F., Cox, C. J., McLaughlin, D., Celio, G., Dentinger, B., Padamsee, M., Hibbett, D., James, T. Y., Baloch, E., Grube, M., Reeb, V., Hofstetter, V., Schoch, C. L., Arnold, A. E., Miadlikowska, J., Spatafora, J., Johnson, D., Hambleton, S., Crockett, M., Shoemaker, R., Sung, G. H., Lücking, R., Lumbsch, T., O'Donnell, K., Binder, M., Diederich, P., Ertz, D., Gueidan, C., Hansen, K., Harris, R. C., Hosaka, K., Lim, Y. W., Matheny, B., Nishida, H., Pfister, D., Rogers, J., Rossman, A. Y., Schmitt, I., Sipman, H., Stone, J., Sugiyama, J., Yahr, R. & Vilgalys, R. (2004). Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. **Am. J. Bot., 91,** 1446–1480.

Marincowitz, S., Crous, P. W., Groenewald, J. Z. & Wingfield, M. J. (2008). **Microfungi occurring on Proteaceae in the Fynbos.** Utrecht, Netherlands: CBS Fungal Biodiversity Centre.

Massee, G., (1895). **British Fungus Flora, A Classified Textbook of Mycology 4: 538**. London: George Bell & Sons.

Matsushima, T. (1975). **Icones microfungorum a Matsushima lectorum.** Osaka: Nippon Printing Publishing Co.

McNeill, J., Turland, N. J., Monro, A. M. & Lepschi, B. J. (2011). XVIII International Botanical Congress: Preliminary mail vote and report of Congress action on nomenclature proposals. **Taxon, 60,** 1507–1520.

Meier, C. L., Rapp, J., Bowers, R. M., Silman, M. & Fierer, N. (2010). Fungal growth on a common wood substrate across a tropical elevation gradient: Temperature sensitivity, community composition, and potential for above-ground decomposition. **Soil Biol. Biochem.,** 1–8.

Miller, J. H. (1938). Studies in the development of two *Myriangium* species and the systematic position of the order Myriangiales. **Mycologia, 2**(30), 158–181.

Miller, M. A., Pfeiffer, W. & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. **In Proceedings of the Gateway Computing Environments Workshop (GCE)** (pp. 1–8). New Orleans, LA. CA: San Diego Supercomput. Center.

Minter, D. W., Peredo, H. L. & Watson, A. (2007). *Acrospermum chilense* sp. nov. from Chile and the *Acrospermales* ord. nov. Bol. Soc. **Argent. Bot., 42**(1-2), 107–112.

Monkai, J., Liu, J. K., Boonmee, S., Chomnunti, P., Chukeatirote, E., Jones, E. B. G., Wang, Y. & Hyde, K. D. (2013). *Planistromellaceae* (Botryosphaeriales). **Crypto., Mycol.**, **34**(1), 45–77.

Mugambi, G. K. & Huhndorf, S. M. (2009). Molecular phylogenetics of *Pleosporales*: *Melanommataceae* and *Lophiostomataceae* recircumscribed (Plesporomycetidae, Dothideomycetes, Ascomycota). **Stud. Mycol., 64,** 103–121.

Müller, E. (1950). Die schweizerischen Arten der Gattung Leptosphaeria und ihere Verwandten. **Sydowia, 4,** 185–319.

Müller, E. & von Arx, J. A. (1950). Einige Aspeckte zur Systematik pseudospharialer Ascomyceten. **Ber. Schweiz Bot. Ges., 60,** 329–397.

Müller, E. & Sanwal, B. D. (1954). Über die Gattungen *Microcyclus* Sacc., *Coccoidella* v. Höhn., *Coccodothis* Theiss. et Syd. und *Coccodothella* Theiss. et Syd. **Sydowia, 8**(1–6), 231–244.

Müller, E. & von Arx, J. A. (1962). Die Gattungen der didymosporen Pyrenomyceten. **Beitr. Kryptogamenfl. Schweiz, 11**(2), 1–922.

Nannfeldt, J. A. (1932). Studien über die Morphologie und Systematik der nichtlichenisierten inoperculaten Discomyceten. **Nova Acta Regiae Soc. Sci. Upsal. Ser. IV, 8**(2), 1–3688.

Nees von Esenbeck, C. D. G. (1817). System der Pilze und Schwämme. **Würtzburg,** 1–334.

Nelsen, M. P., Lücking, R., Grube, M., Mbatchou, J. S., Muggia, L., Plata, E. R. & Lumbsch, H. T. (2009). Unraveling the phylogenetic relationships of lichenised fungi in Dothideomyceta. **Stud. Mycol., 64,** 135–144.

Nelsen, M. P., Plata, E. R., Andrew, C. J., Lücking, R. & Lumbsch, H. T. (2011). Phylogenetic diversity of trentepohlialean algae associated with lichen-forming fungi. **J. Phycol., 47,** 282–290.

Nordén, B., Sunhede, S. & Larsson, E. (2005). New species of *Moristroma* (Ascomycetes) and phylogenetic position of the genus. **Mycol. Prog., 4,** 325–332.

Nylander, J. A. A. (2004). **MrModeltest 2.3. Program distributed by the author.** Sweden: Uppsala University.

Ohm, R. A., Feau, N., Henrissat, B., Schoch, C. L., Horwitz, B. A., Barry, K. W., Condon, B. J., Copeland, A. C., Dhillon, B., Glaser, F., Hesse, C. N., Kosti, I., LaButti, K., Lindquist, E. A., Lucas, S., Salamov, A. A., Bradshaw, R. E., Ciuffetti, L., Hamelin, R. C., Kema, G. H., Lawrence, C., Scott, J. A., Spatafora, J. W., Turgeon, B. G., de Wit, P. J., Zhong, S., Goodwin, S. B. & Grigoriev, I. V. (2012). Diverse Lifestyles and Strategies of Plant Pathogenesis Encoded in the Genomes of Eighteen Dothideomycetes Fungi. **PLOS Pathog., 8**(12), 1–26.

Page, R. D. M. (1996). TreeView: an application to display phylogenetic trees on personal computers. **Comput. Appl. Biosci., 12**(4), 357–358.

Pang, K. L., Abdel-Wahab, M. A., Sivichai, S., El-Sharouney, H. M. & Jones E. B. G. (2002). *Jahnulales* (Dothideomycetes, Ascomycota): a new order of lignicolous freshwater ascomycetes. **Mycol. Res., 106,** 1031–1042.

Penzig, A. J. O. & Saccardo, P. A. (1898). Diagnoses fungorum novorum in insula Java collectorum. Ser. II. **Malpighia, 11,** 491–530.

Penzig, O. & Saccardo, P. A. (1904). **Icones fungorum javanicorum.** Leiden: Brill Publishers.

Pereira, O. L. & Barreto, R. W. (2003). *Anhellia verruco–scopiformans* sp. nov. (Myriangiales) associated to scaby brooms of Croton migrans in Brazil. **Fungal Diver., 12,** 155–159.

Petch, T. (1921). Studies in entomogenous fungi. 1. The nectriae parasitic on scale insects. **Trans. Br. Mycol. Soc., 7,** 89–167.

Petrak, F. (1924). Mykologische Notizen VII. **Annls. Mycol., 22,** 1–182.

Petrak, F. (1951). Ergebnisse einer Revision der Grundtypen verschiedener Gattungen der Askomyzeten und Fungi imperfecti. **Sydowia, 26,** 169–198.

Petrak, F. (1953). Ein Beitrag zur Pilzflora Floridas. **Sydowia, 7,** 103–132.

Petrak, F. & Sydow, H. (1936). Kritisch-systematische Originaluntersuchungen tiber Pyrenomyzeten, *Sphaeropsideen* und *Melanconieen*. VII. **Annls. Mycol., 21**(5–6), 349–384.

Phillips, A. J. L., Alves, A., Pennycook, S. R., Johnston, P. R., Ramaley, A., Akulov, A. & Crous, P. W. (2008). Resolving the phylogenetic and taxonomic status of dark–spored teleomorph genera in the *Botryosphaeriaceae*. **Persoonia, 21,** 29–55.

Phillips, W. & Plowright, C. B. (1877). New and rare British fungi. **Grevillea, 6**(37), 22–29.

Pirozynski, K. A. (1972). Microfungi of Tanzania. I. Miscellaneous Fungi on Oil Palm. II. New Hyphomycetes. **Mycol. Pap., 12,** 1–64.

Pirozynski, K. A. (1977). Notes on hyperparasitic *Sphaeriales*, Hypocreales and 'hypocreoid Dothideales'. **Kew Bull., 31,** 595–610.

Promputtha, I. & Miller, A. N. (2010). Three new species of *Acanthostigma* (*Tubeufiaceae*, Dothideomycetes) from Great Smoky Mountains National Park. **Mycologia, 102,** 574–587.

Raja, H. A. & Shearer, C. A. (2006). *Jahnula* species from North and Central America, including three new species. **Mycologia, 98,** 312–332.

Raja, H. A. & Shearer, C. A. (2008). Freshwater ascomycetes: new and noteworthy species from aquatic habitats in Florida. **Mycologia, 100**(3), 467–489.

Rajeshkumar, K. C. & Sharma, R. (2013). *Tamhinispora* a new genus belongs to family *Tubeufiaceae* from the Western Ghats, India based on morphology and phylogenetic analysis. **Mycosphere, 4**(2), 165–174.

Rannala, B. & Yang, Z. (1996). Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. **J. Mol. Evol., 43,** 304–311.

Réblová, M. & Barr, M. (2000). The genus *Acanthostigma* (*Tubeufiaceae*, Pleosporales). **Sydowia, 52,** 258–285.

Rehm, H. (1896). Ascomyceten: *Hysteriaceen* und *Discomyceten*. In Rabenhorst's L (ed). **Kryptogamen–Flora von Deutschland, Oesterreich und der Schweiz** (2nd ed.). Leipzig: Eduard Kummer.

Rehner, S. A. & Buckley, E. (2005). A *Beauveria* phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs. **Mycologia, 97**(1), 84–98.

Reynolds, D. R. (1989). The Bitunicate Ascus Paradigm. **Bot. Rev., 55**(1), 1–52.

Robert, V., Stegehuis, G. & Stalpers, J. (2005). **The MycoBank engine and related databases**. Retrieved April, 2011, from www.mycobank.org/

Roe, A. D., Rice, A. V., Bromilow, S. E., Cooke, J. E. & Sperling, F. A. (2010). Multilocus species identification and fungal DNA barcoding: insights from blue stain fungal symbionts of the mountain pine beetle. **Mol. Ecol. Resour., 10**(6), 946–59.

Rodrigues, K. F. & Samuels, G. J. (1994). *Letendraeopsis palmarum*, a new genus and species of loculoascomycetes. **Mycologia, 86,** 254–258.

Rogerson, C. T. (1970). The Hypocrealean fungi (Ascomycetes, Hypocreales). **Mycologia, 62,** 865–910.

Romero, A. I. & Samuels, G. J. (1991). Studies on xylophilous fungi from Argentina: VI. Ascomycotina on *Eucalyptus viminalis* (Myrtaceae). **Sydowia, 43,** 228–248.

Ronquist, F. & Huelsenbeck, J. P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. **Bioinformatics, 19,** 1572–1574.

Rossman, A. Y. (1977). The genus *Ophionectria* (Euascomycetes, Hypocreales). **Mycologia, 69,** 355–391.

Rossman, A. Y. (1978). *Podonectria*, a genus in the Pleosporales on scale insects. **Mycotaxon, 7,** 163–182.

Rossman, A. Y. (1979). A preliminary account of the taxa described in *Calonectria*. **Mycotaxon, 8,** 485–558.

Rossman, A. Y. (1983). The phragmosporous species of *Nectria* and related genera. **Mycol. Pap., 150,** 1–164.

Rossman, A. Y. (1987). The *Tubeufiaceae* and similar Loculoascomycetes. **Mycol. Pap., 157,** 1–71.

Rossman, A. Y., Samuels, G. J., Rogerson, C. T. & Lowen, R. (1999). Genera of *Bionectriaceae*, *Hypocreaceae* and *Nectriaceae* (Hypocreales, Ascomycetes). **Stud. Mycol., 42,** 1–248.

Ruibal, C., Gueidan, C., Selbmann, L., Gorbushina, A. A., Crous, P. W., Groenewald, J. Z., Muggia, L., Grube, M., Isola, D., Schoch, C. L., Staley, J. T., Lutzoni, F. & de Hoog, G. S. (2009). Phylogeny of rock-inhabiting fungi related to Dothideomycetes. **Stud. Mycol., 64,** 123–133.

Saccardo, P. A. (1876). Fungi veneli novi vel critici. Nuov. Giorn. **Bot. Ital., Ser. 5, 8,** 181–211.

Saccardo, P. A. (1877). Fungi venetino vive criticive Mycologiae Venetae addendi. **Michelia, 1**(1), 1–72.

Saccardo, P. A. (1878). Fungi Veneti novi vel critici vel mycologiae Venetae addendi. Ser. IX. **Michelia, 1**(4), 361–445.

Saccardo, P. A. (1883). Sylloge Fungorum. **Patavii Italy, 2,** 1–815.

Saccardo, P. A. (1886). Omnium hucusque cognitorum. **Syll. Fung., 4,** 507–508.

Saccardo, P. A. (1972). Supplementum Universale Pars XI. **Syll. Fung., 26,** 224.

Saccardo, P. A. & Saccardo, D. (1905). Supplementum universale. Pars VI. Hymenomycetae–Laboulbeniomycetae. **Syll. Fung., 17,** 860.

Saccardo, P. A. & Sydow, P. (1899). Sylloge Fungorum. **14**(1), 1–724.

Saccardo, P. A. & Sydow, P. (1902). Supplementum Universale, Pars V. **Syll. Fung., 16,** 1–1291.

Saccardo, P. A. & Trotter, A. (1913). Supplementum Universale Pars IX. **Syll. Fung., 22,** 1–1612.

Samuels, G. J. & Müller, E. (1978). Life history studies of Brazilian Ascomycetes 2. **Sydowia, 31,** 137–141.

Samuels, G. J. & Romero, A. I. (1991). Ascoporia (Fungi, Loculoascomycetes), a new genus from Para. **Bolm. Mus. Paraense Emílio Goeldi Sér. Bot., 7,** 263–268.

Samuels, G. J., Rossman, A. Y. & Müller, E. (1979). Life history studies of Brazilian Ascomycetes 6. Three species of *Tubeufia* with, respectively, dictyosporouspycnidial and helicosporous anamorphs. **Sydowia, 31,** 180–192.

Sánchez, R. M. & Bianchinotti, M. V. (2010). New records in the *Tubeufiaceae* from Andean Patagonian forests of Argentina. **Mycotaxon, 111,** 131–141.

Sánchez, R. M., Miller, A. N. & Bianchinotti, M. V. (2012). A new species of *Acanthostigma* (*Tubeufiaceae*, Dothideomycetes) from the southern hemisphere. **Mycologia, 104**(1), 223–231.

Sawada, K. (1959). Descriptive catalogue of Taiwan (Formosan) fungi. **Part XI Special Publ. Coll. Agric. Natl. Taiwan Univ., 8,** 54.

Scheuer, C. (1988). Ascomyceten auf *Cyperaceen* und *Juncaceen* im Ostalpen-raum. **Biblioth. Mycol., 123,** 1–124.

Scheuer, C. (1991). *Taphrophila* (Dothideales: *Tubeufiaceae*) and two species of *Tubeufia* with dark setae. **Mycol. Res., 95,** 811–816.

Schoch, C. L., Crous, P. W., Groenewald, J., Barres, B., Boehm, E., Degruyter, J., De Hoog, G., Dixon, L. J., Fournier, J., Grube, M., Gueidan, C., Harada, Y., Hatakeyama, Hirayama, K., Hosoya, T., Hyde, K., Jones, E., Kohlmeyer, J., Lucking, R., Lumbsch, H., Lutzoni, F., Marvanova, L., Mbatchou, J., Miller, A., Mugambi, G., Muggia, L., Nelson, M., Nelson, P., Owensby, C., Phongpaichit, S., Pointing, S., Pujade-Renaud, V., Raja, H., Rivas Plata, E., Robbertse, B., Ruibal, C., Sakayaroj, J., Sano, T., Selbmann, L., Shearer, C., Shirouzu, T., Slippers, B., Suetrong, S., Tanaka, K., Volkmann-Kohlmeyer, B., Wood, A., Woudenberg, J., Yonezawa, H., Zhang, Y. & Spatafora, J. (2009). A class-wide phylogenetic assessment of Dothideomycetes. **Stud. Mycol., 64,** 1–15.

Schoch, C. L., Shoemaker, R. A., Seifert, K. A., Hambleton, S., Spatafora, J. W. & Crous, P. W. (2006). A multigene phylogeny of the Dothideomycetes using four nuclear loci. **Mycologia, 98**(6), 1041–1052.

Seaver, F. J. (1909). The Hypocreales of North America - I. **Mycologia, 1**(2), 41–76.

Seaver, F. J. & Chardón, C. E. (1926). Botany of Porto Rico and the Virgin Islands. Mycology. **Scient. Surv. P. Rico, 1,** 40–41.

Seaver, F. J. & Waterston, J. M. (1940). Contribution to the mycoflora of Bermuda-I. **Mycologia, 32,** 388–407.

Seephueak, P., Phongpaichit, S., Hyde, K. D. & Petcharat, V. (2011). Diversity of saprobic fungi on decaying branch litter of the rubber tree (*Hevea brasiliensis*). **Mycosphere, 2**(4), 307–330.

Seifert, K. A., Morgan-Jones, G., Gams, W. & Kendrick, B. (2011). **The Genera of Hyphomycetes.** Utrecht, Netherlands: CBS Fungal Biodiversity Centre.

Shearer, C. A. (1987). *Helicoma chlamydosporum*, a new hyphomycete from submerged wood in Panama. **Mycologia, 79,** 468–472.

Shearer, C. A. (1993). A new species of *Kirschsteiniothelia* (Pleosporales) with an unusual fissitunicate ascus. **Mycologia, 85,** 963–969.

Shearer, C. A., Raja, H. A., Miller, A. N., Nelson, P., Tanaka, K., Hirayama, K., Marvanova, L., Hyde, K. D. & Zhang, Y. (2009). The molecular phylogeny of freshwater Dothideomycetes. **Stud. Mycol., 64,** 145–153.

Sherwood, M. A. (1977). The ostropalean fungi. **Mycotaxon, 5**(1): 1–277.

Sierra, D. L. (2006). Contribución al estudio de los Ascomicetes bitunicados de Cataluña. **Acta Bot. Barc., 50,** 5–434.

Sivanesan, A. (1981). *Balladynopsis*, *Balladynocallia* and *Alina*. **Mycol. Pap., 146,** 1–38.

Sivanesan, A. (1984). **The bitunicate Ascomycetes and their anamorphs.** Vaduz: J. Cramer.

Sivanesan, A., Panwar, K. & Kaur, S. J. (1976). *Thaxteriellopsis lignicola*, gen et sp nov, a new Loculoascomycete from India. **Kavaka, 4,** 39–42.

Sivanesan, A., Rajak, R. C. & Gupta, R. C. (1988). *Thaxterina*, a new tubeufiaceous genus with multispored asci from India. **Trans. Br. Mycol. Soc., 90,** 662–665.

Spegazzini, C. L. (1924). Algunos honguitos portoriqueños. **Bol. Acad. Nac. Ci., 26,** 335–368.

Stajich, J. E., Berbee, M. L., Blackwell, M., Hibbett, D. S., James, T. Y., Spatafora, J. W. & Taylor, J. W. (2009). The Fungi. **Curr. Bio., 19,** 840–845.

Stamatakis, A., Hoover, P. & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML Web Servers. **Syst. Biol., 57,** 758–771.

Starbäck, K. (1899). The *Myriangiales* Bihag Sv. **Vet. Akad. Handl,. 25,** 3–37.

Stenroos, S., Laukka, T., Huhtinen, S., Döbbeler, P., Myllys, L., Syrjänen, K. & Hyvönen, J. (2010). Multiple origins of symbioses between ascomycetes and bryophytes suggested by a five–gene phylogeny. **Cladistics 26,** 281–300.

Stevens, F. L. (1917). Porto Rican fungi, old and new. **Trans. Ill. Acad. Sci., 10,** 162–218.

Subramanian, C. & Sekar, G. (1982). *Thaxteriellopsis lignicola* and its *Moorella* anamorph. **Proc. Indian Acad. (Plant Sci.), 91,** 1–7.

Suetrong, S., Hyde, K. D., Ahang, Y., Bahkali, A. B. & Jones, E. B. G. (2011). *Trematosphaeriaceae* fam. nov. (Dothideomycetes, Ascomycota). **Crypto. Mycol., 32,** 343–358.

Suetrong, S., Sakayaroj, J., Phongpaichit, S., & Jones, E. B. (2010). Morphological and molecular characteristics of a poorly known marine ascomycete, *Manglicola guatemalensis* (Jahnulales: Pezizomycotina; Dothideomycetes, Incertae sedis): new lineage of marine ascomycetes. **Mycologia, 102**(1), 83–92.

Suetrong, S., Schoch, C. L., Spatafora, J. W., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Sakayaroj, J., Phongpaichit, S., Tanaka, K., Hirayama, K. & Jones, E. B. G. (2009). Molecular systematics of the marine *Dothideomycetes*. **Stud. Mycol., 64,** 155–173.

Swofford, D. L. (2002). **PAUP: Phylogenetic Analysis Using Parsimony (* and Others Methods), version 4.0 b10.** Sunderland, MA: Sinauer Associates.

Sydow, H. (1925). Fungi in itinere costaricensi collecti. Pars prima. **Annls. Mycol., 23**(3-6), 308–429.

Sydow, H. (1927). Fungi in itinere costaricense collecti. **Annls. Mycol., 25**(1–2), 1–160.

Sydow, H. (1930). Fungi venezuelani. **Annls. Mycol., 28**(1–2), 29–224.

Sydow, H. (1939). Fungi Venezuelani. **Annls Mycol., 28,** 29–224.

Tanaka, K., Hirayama, K., Yonezawa, H., Hatakeyama, S., Harada, Y., Sano, T., Shirouzu, T. & Hosoya, T. (2009). Molecular taxonomy of bambusicolous fungi: *Tetraplosphaeriaceae*, a new pleosporalean family with Tetraploa–like anamorphs. **Stud. Mycol., 64,** 175–209.

Taylor, J. W. (2011). One Fungus = One Name: DNA and fungal nomenclature twenty years after PCR. **IMA Fungus, 2,** 113–120.

Theissen, F. & Sydow, H. (1918). Vorentwurfe zu den Pseudosphaeriales. **Annls Mycol., 16,** 1–34.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequnce weighting, position specific gap penalties and weith matrix choice. **Nucleic Acids Res., 22**(22), 4673–4680.

Tsui, C. K. M. & Berbee, M. L. (2006). Phylogenetic relationships and convergence of helicosporous fungi inferred from ribosomal DNA sequences. **Mol. Phyl. Evol., 39,** 587–597.

Tsui, C. K. M., Sivichai, S. & Berbee, M. L. (2006). Molecular systematics of *Helicoma*, *Helicomyces* and *Helicosporium* and their teleomorphs inferred from rDNA sequences. **Mycologia, 98,** 94–104.

Tsui, C. K. M., Sivichai, S., Rossman, A. Y. & Berbee, M. L. (2007). *Tubeufia asiana*, the teleomorph of *Aquaphila albicans* in the *Tubeufiaceae*, Pleosporales, based on cultural and molecular data. **Mycologia, 99,** 884–894.

Untereiner, W. A., Straus, N. A. & Malloch, D. (1995). A molecular-morphotaxonomic approach to the systematics of the *Herpotrichiellaceae* and allied black yeasts. **Mycol. Res., 99,** 897–913.

Vasilyeva, L.N. & Stephenson, S.L. (2010). The problems of traditional and phylogenetic taxonomy of fungi. **Mycosphere, 1,** 45–51.

Vilgalys, R. & Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. **J. Bacteriol., 172,** 4238–4246.

Voglmayr, H. & Jaklitsch, W. M. (2011). Molecular data reveal high host specificity in the phylogenetically isolated genus *Massaria* (Ascomycota, *Massariaceae*). **Fungal Divers., 46,** 133–170.

von Arx, J. A. (1963). Die Gattungen der Myriangiales. **Persoonia, 2**(4), 421–475.

von Arx, J. A. & Müller, E. (1975). A re–evaluation of the bitunicate Ascomycetes with keys to families and genera. **Stud. Mycol., 9,** 1–159.

von Höhnel, F. (1905). Mycologische Fragmente. LXXVII-XCVII. **Annls. Mycol., 3**(4), 323–339.

von Höhnel, F. (1909a). Fragmente zur Mykologie (VI. Mitteilung, Nr. 182 bis 288). **Sitzb. Akad. Wiss. Wien Kl., 118,** 354–359.

von Höhnel, F. (1909b). Fragmente zur Mykologie: VII. Mitteilung (Nr. 289 bis 353). **Sitzb. Akad. Wiss. Wien Kl., 118,** 847–848.

von Höhnel, F. (1909c). Fragmente zur Mykologie: IX. Mitteilung (Nr. 407 bis 467). **Sitzb. Akad. Wiss. Wien Kl., 118,** 1461–1552.

von Höhnel, F. (1909d). Fragmente zur Mykologie 297. Über *Paranectria stromaticola* P. Henn. **Sitzb. Akad. Wiss. Wien Kl., 119,** 823–824.

von Höhnel, F. (1910). Fragmente zur Mykologie: X. Mitteilung (Nr. 468 bis 526). **Sitzb. Akad. Wiss. Wien Kl., 119,** 393–473.

von Höhnel, F. (1911). Fragmente zur Mykologie (XIII. Mitteilung, Nr. 642 bis 718). **Sber. Akad. Wiss. Wien Math. Naturw. Kl., 120,** 379–384.

von Höhnel, F. (1919). Fragmente zur Mykologie. XXII Mitteilungen, nr. 1092 bis 1153. **Sber. Akad. Wiss. Wien Math. Naturw. Kl., 1**(128), 549–634.

Vrijmoed, L. L. P. (2000). Isolation and culture of higher filamentous fungi. In Marine Mycology–A Practical Approach (eds K. D. Hyde & S. B. Pointing). **Fungal Divers. Res. Ser., 1,** 1–20.

Walker, J. (1980). *Gaeumannomyces*, *Linocarpon*, *Ophiobolus* and several other genera of scolecospored ascomycetes and *phialophora* conidial states, with a note on hyphopodia. **Mycotaxon, 11,** 1–129.

Wang, Y. Z., Aptroot, A. & Hyde, K. D. (2004). Revision of the Ascomycete genus *Amphisphaeria*. **Fungal Divers., 13,** 1–180.

Wang, H. K., Hyde, K. D., Soytong, K. & Lin, F. C. (2008). Fungal diversity on fallen leaves of *Ficus* in northern Thailand. **J. Zhejiang Univ-SC. B., 9**(10), 835–841.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. (eds), **PCR protocols: a guide to methods and applications** (pp. 315–322). London: Academic Press.

Wijayawardene, D. N. N., Mckenzie, E. H. C. & Hyde, K. D. (2012). Towards incorporating anamorphic fungi in a natural classification–checklist and notes for 2011. **Mycosphere, 3**(2), 157–228.

Woronichin, N. N. (1925). Über die Capnodiales. **Annls. Mycol., 23,** 174–178.

Wu, H. X., Schoch, C. L., Boonmee, S., Bahkali, A. H., Chomnunti, P. & Hyde, K. D. (2011). A reappraisal of *Microthyriaceae*. **Fungal Divers., 51,** 189–248.

Zhang, Y., Crous, P. W., Schoch, C. L., Bahkali, A.H., Guo, L. D. & Hyde, K. D. (2011). A molecular, morphological and ecological re-appraisal of *Venturiales*-a new order of *Dothideomycetes*. **Fungal Divers., 51**(1), 249–277.

Zhang, Y., Crous, P. W., Schoch, C. L. & Hyde, K. D. (2012). *Pleosporales*. **Fungal Divers., 52,** 1–845 225.

Zhang, Y., Fournier, J., Crous, P. W., Pointing, S. B. & Hyde, K. D. (2009). Phylogenetic and morphological assessment of two new species of *Amniculicola* and their allies (Pleosporales). **Persoonia 23,** 48–54.

Zhang, Y., Schoch, C. L., Fournier, J., Crous, P. W., de Gruyter, J., Woudenberg, J. H. C., Hirayama, K., Tanaka, K., Pointing, S. B., Spatafora, J. W. & Hyde, K. D. (2009). Multi–locus phylogeny of *Pleosporales*: a taxonomic, ecological and evolutionary re–evaluation. **Stud. Mycol, 64,** 85–102.

Zhao, G., Liu, X. & Wu, W. (2007). Helicosporous hyphomycetes from China. **Fungal Divers., 26,** 313–524.

APPENDIX

APPENDIX A

CHEMICAL REAGENTS AND MEDIA

1. India ink used for observing mucilaginous sheath or appendage of ascospores in some species

2. Potassium hydroxide (KOH) used in the rehydration of dried specimens. 5% aqueous solution

3. Lactoglycerol used for maintaining semipermanent slides

Lactic acid	10 ml
Glycerol	10 ml
Distilled water	10 ml

Mix 10 ml lactic acid, 10 ml glycerol, and add 10 ml distilled water

4. Lactophenol-Cotton Blue used to highlight fungal structures for viewing with the compound light microscope. Cotton blue is the most popular stain for observe pseudoparaphyses, septate or ascus walled. This is gives excellent clarity and is also suitable for most fungal groups

Phenol (crystals) 20 g

Lactic acid 16 ml

Glycerol 31 ml

Dissolve phenol in distilled water, add lactic acid, glycerol and 0.05 g of Poirrier's (cotton) blue or acid fuchsin

5. Melzer's Reagent used for identification of ascomycete fungi. Amyloid reaction of asci changed to blue or heavily purple colours

Chloral hydrate 100 g

Potassium iodide 5 g

Iodine 1.5 g

Distilled water 100 ml

6. Water Agar (WA) used for single spore isolation of fungi

Agar 15 g

Dissolve 15 g agar in distilled water then mixed and add volume to 1000 ml of water. Heath until dissolved and autoclave at 121^{o}C for 15 minutes

7. Malt Extract Agar (MEA) used for fungal cultivation

Agar 15 g

Peptone 0.78 g

Glycerol 2.35 g

Dextrin 2.75 g

Maltose, Technical 12.75 g

Suspend 33.6 g of malt extract agar in distilled water and mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the power and bring volume to 1000 ml. Autoclave at 121^{o}C for 15 minutes.

APPENDIX B

FRESH COLLECTIONS OF DOTHIDEOMYCETES FROM NORTHERN THAILAND

Table B1 Fresh Collections of Dothideomycetes from Northern Thailand

No	MFLU Code	MFLUCC Code	IFRDCC Code	BCC Code	Family	Substrate	Host
1	MFLU10-0025	10-0095	2191	35848	Melanommataceae	Dead bamboos	Bamboos
2	MFLU10-0028	10-0098	2174	38861	Botryosphaeriaceae	Dried climbs	*Entada* sp. (Vines)
3	MFLU10-0059	10-0126	2182	38862	Microthyriaceae	Dried branch	Unidentified
4	MFLU10-0038	10-0107	2170	38863	Pleosporaceae	Dead stem	Unidentified
5	MFLU10-0026	10-0096	2169	38870	Melanommataceae	Dead wood	Unidentified
6	MFLU10-0036	10-0105	2179	52293	Kirschsteiniotheliaceae	Decaying wood	Unidentified
7	MFLU10-0050	10-0117		39642	Tubeufiaceae	Dead wood	Unidentified
8	MFLU10-0048	10-0115	2195	39624,	Tubeufiaceae	Dead wood	Unidentified
9	MFLU10-0063	10-0130	2168	39914	Bitunicate	Dead wood	*Leucaena leucocephala* (Lamk.) de Wit
10	MFLU10-0057	10-0124	2197	52463	Tubeufiaceae	Dead wood	*Zizyphus mauritiana* Lamk./Jujube
11	MFLU10-0032	10-0102	2183	52150	Mauritiana sp.	Dried branch	Unidentified
12	MFLU10-0058	10-0125	2198	52464	Tubeufiaceae	Dead wood	Unidentified
13	MFLU10-0030	10-0100	2178		Bitunicate	Dead wood	Unidentified
14	MFLU10-0061	10-0128	2200		Bitunicate	Dead wood	Pinus

Table B1 (continued)

No	MFLU Code	MFLUCC Code	IFRDCC Code	BCC Code	Family	Substrate	Host
15	MFLU10-0062	10-0129	2185	52149	Bitunicate	Dead wood	Unidentified
16	MFLU10-0037	10-0106	2181	52380	Kirschsteiniotheliaceae	Dried branch	Unidentified
17	MFLU10-0042	10-0111	2172	52028	Myiocopron sp.	Dried branch	*Castanopsis indica*
18	MFLU10-0027	10-0097	2190	52378	Melanommataceae	Dead wood	Unidentified
19	MFLU10-0060	10-0127	2199		Bitunicate	Dried branch	Unidentified
20	MFLU10-0051	10-0118	2186	52295	Tubeufiaceae	Dead wood	*Aleurites moluccana*: Candlenut tree
21	MFLU10-0049	10-0116	2196	52036	Tubeufiaceae	Dead wood	Pinus
22	MFLU10-0064	10-0131	2188	52296	Bitunicate	Dead wood	Unidentified
23	MFLU10-0052	10-0119	2180	52297	Tubeufiaceae	Dead wood	Unidentified
24	MFLU10-0053	10-0120	2184	52298	Tubeufiaceae	Dead wood	Unidentified
25	MFLU10-0054	10-0121	2167	52299	Tubeufiaceae	Dried bark	Unidentified
26	MFLU10-0029	10-0099	2176	reject	Bitunicate	Dead wood	Unidentified
27	MFLU10-0055	10-0122	2175	52328	Tubeufiaceae	Dead wood	Unidentified
28	MFLU10-0031	10-0101	2187	52329	Bitunicate	Dried branch	Unidentified
29	MFLU10-0056	10-0123	2177	52330	Tubeufiaceae	Dried bark	Unidentified
30	MFLU10-0972	10-0926		52144	Tubeufiaceae	Dead wood	Pinus
31	MFLU10-0973	10-0927		52145	Bitunicate	Dead wood	Dicotyledons
32	MFLU10-0974	10-0928		52146	Bitunicate	Dead wood	Dicotyledons
33	MFLU10-0975	10-0930		52147	Bitunicate	Dried bark	Unidentified
34	MFLU10-0971	10-0929		52151	Melanommataceae	Dried branch	Unidentified
35	MFLU11-0001	11-0001		reject	Pleosporaceae	Dead wood	Unidentified
36	MFLU11-0002	11-0002		52152	Bitunicate	Dried branch	Unidentified

Table B1 (continued)

No	MFLU Code	MFLUCC Code	IFRDCC Code	BCC Code	Family	Substrate	Host
37	MFLU11-0003	11-0003		52153	Tubeufiaceae	Dried bark	Unidentified
38	MFLU11-0004	11-0004		52154	Bitunicate	Dried bark	Unidentified
39	MFLU11 0133	11-0374		52032	Bitunicate	Dead wood	Unidentified
40	MFLU11 0134	11-0375		52033	Tubeufiaceae	Dead wood	Unidentified
41	MFLU11 0135	11-0376		52034	Bitunicate	Dead wood	Unidentified
42	MFLU11 0136	11-0377		52035	Bitunicate	Dead wood	Unidentified
43	MFLU11 0137	11-0378		52029	Tubeufiaceae	Dead wood	Unidentified
44	MFLU11 0138	11-0379		52030	Tubeufiaceae	Dead wood	Unidentified
45	MFLU11 0139	11-0380		52031	Bitunicate	Dead wood	Unidentified
46	MFLU11-1145	11-0509		52381	Tubeufiaceae	Dead wood	Unidentified
47	MFLU11-1146 (T)	11-0510		52383	Tubeufiaceae	Dried bark	Unidentified
48	MFLU11-1147	11-0511		52384	Bitunicate	Dead wood	Unidentified
49	MFLU11-1148	11-0512		52385	Tubeufiaceae	Dead wood	Unidentified
50	MFLU11-1146 (A)	11-0513		52382	Tubeufiaceae	Dead wood	Unidentified
51	MFLU11-1149	11-0514		52386	Tubeufiaceae	Dead wood	Unidentified

APPENDIX C

LIST OF HERBARIUM SPECIMENS OF DOTHIDEOMYCETES

Table C1 List of Herbarium Specimens of Dothideomycetes

Families	Genera	Type species
Ascoporiaceae Kutorga & D. Hawksw. (1997)	*Pseudosolidum* Lloyd	*Pseudosolidum solidum* (Berk. & M.A. Curtis) Lloyd (1923)
Aulographaceae P.M. Kirk, P.F. Cannon & J.C. David (2001)	*Aulographum* Lib.	*Aulographum hederae* Lib. (1834)
	Polyclypeolina Bat. & I.H. Lima ex Bat.	*Polyclypeolina brideliae* (Hansf.) Bat. (1959)
Coccoideaceae Sacc. & D. Sacc. (1905)	*Coccoidea* P. Henn.	*Coccoidea quercicola* Henn. & Shirai (1900)
	Coccoidella Höhn.	*Coccoidella scutula* (Berk. & M.A. Curtis) Höhn. (1909)
Cookellaceae Höhn. ex Sacc. & Trotter (1913)	*Cookella* Sacc.	*Cookella microscopica* Sacc. (1878)
	Pycnoderma Syd.	*Pycnoderma bambusinum* Syd. & P. Syd. (1914)
	Uleomyces P. Henn.	*Uleomyces parasiticus* Henn. (1895)
Parodiopsidaceae Toro (1952)	*Alina* Racib.	*Alina jasmini* Racib. (1909)
	Balladyna Racib.	*Balladyna gardeniae* Racib. (1900)

Table C1 (continued)

Families	Genera	Type species
Parodiopsidaceae Toro (1952)	*Balladynocallia* Bat.	*Balladynocallia glabra* (Hansf.) Bat. (1965)
	Balladynopsis Theiss. & Syd.	*Balladynopsis philippinensis* Syd. (1918)
	Chevalieropsis G. Arnaud	*Chevalieropsis ctenotricha* (Pat. & Har.) G. Arnaud (1923)
	Cleistosphaera Syd. & P. Syd.	*Cleistosphaera macrostegia* Syd. & P. Syd. (1916)
	Dimeriella Speg.	*Dimeriella hirtula* Speg. (1908)
	Dimerium Syd. & P. Syd.	*Dimerium* (Sacc. & P. Syd.) Sacc. & D. Sacc., (1904)
	Dysrhynchis Clem.	*Dysrhynchis pulchella* (Sacc.) Clem. (1931)
	Hyalomeliolina F. Stevens	*Hyalomeliolina guianensis* F. Stevens (1923)
	Leptomeliola Höhn.	*Leptomeliola hyalospora* (Lév.) Höhn. (1919)
	Neoparodia Petr. & Cif.	*Neoparodia ekmanii* Petr. & Cif. (1932)
	Ophiomeliola Starb.	*Ophiomeliola lindmanii* Starbäck (1899)
	Ophioparodia Petr. & Cif.	*Ophioparodia pulchra* Petr. & Cif. (1932)
	Parodiellina P. Henn. ex G. Arnaud	*Parodiellina manaosensis* (Henn.) G. Arnaud (1918)
	Perisporiopsis Henn. 1904	*Perisporiopsis struthanthi* Henn. (1904)
	Pilgeriella P. Henn.	*Pilgeriella perisporioides* Henn. (1900)
	Scolionema Theiss. & Syd.	*Scolionema palmarum* (Kunze) Theiss. & Syd. (1918)
	Stomatogene Theiss.	*Stomatogene agaves* (Ellis & Everh.) Theiss. (1918)
Protoscyphaceae Kutorga & D. Hawksw. (1997)	*Protoscypha* Syd.	*Protoscypha pulla* Syd. (1925)

Table C1 (continued)

Families	Genera	Type species
Pseudoperisporiaceae Toro (1926)	*Aphanostigme* Syd.	*Aphanostigme solani* Syd. (1926)
	Bryochiton Döbbeler & Poelt	*Bryochiton monascus* Döbbeler & Poelt (1978)
	Bryomyces Döbbeler	*Bryomyces scapaniae* Döbbeler (1978)
	Epibryon Döbbeler	*Epibryon plagiochilae* (Gonz. Frag.) Döbbeler (1978)
	Episphaerella Petr.	*Episphaerella manihotis* (Henn.) Petr. (1924)
	Eudimeriolum Speg.	*Eudimeriolum elegans* Speg. (1912)
	Eumela Syd.	*Eumela chiococcae* Syd. (1925)
	Keratosphaera H.P. Upadhyay	*Keratosphaera batistae* H.B.P. Upadhyay (1964)
	Lasiostemma Theiss.	*Lasiostemma melioloides* (Berk. & Ravenel) Theiss., Syd. & P. Syd. (1917)
	Lizonia (Ces. & De Not.) De Not.	*Lizonia empirigonia* (Auersw.) De Not. (1863)
	Myxophora Döbbeler & Poelt	*Myxophora amerospora* Döbbeler & Poelt (1978)
	Nematostigma Syd. & P. Syd.	*Nematostigma obducens* Syd. & P. Syd. (1913)
	Nematostoma Syd. & P. Syd.	*Nematostoma artemisiae* Syd. & P. Syd. (1914)
	Nematothecium Syd. & P. Syd.	*Nematothecium vinosum* Syd. & P. Syd. (1912)
	Neocoleroa Petr.	*Neocoleroa sibirica* Petr. (1934)
	Ophiciliomyces Bat. IH.Lima	*Ophiociliomyces bauhiniae* Bat. & I.H. Lima (1955)
	Phaeodimeriella Speg.	*Phaeodimeriella occulta* (Racib.) Speg. (1908)
	Phaeostigme Syd. & P. Syd.	*Phaeostigme picea* (Berk. & M.A. Curtis) Syd. & P. Syd. (1917)
	Phragmeriella Hansf.	*Phragmeriella ireninae* Hansf. (1946)

Table C1 (continued)

Families	Genera	Type species
Pseudoperisporiaceae Toro (1926)	*Pododimeria* E. Müll.	*Pododimeria gallica* E. Müll. (1959)
	Raciborskiomyces Siemaszko	*Raciborskiomyces polonicus* Siemaszko (1925)
	Toroa Syd.	*Toroa dimerosporioides* (Speg.) Syd. [as 'dimerosporoides'] (1926)
Tubeufiaceae M.E. Barr (1979)	*Acanthostigma* De Not.	*Acanthostigma perpusillum* De Not. (1863)
	Acanthophiobolus Berl.	*Acanthophiobolus helminthosporus* (Rehm) Berl. (1893)
	Acanthostigmella Höhn	*Acanthostigmella genuflexa* Höhn. (1905)
	Allonecte Syd.	*Allonecte lagerheimii* (Pat.) Syd. (1939)
	Amphinectria Speg. (K. A. Pirozynski)	*Amphinectria portoricensis* Speg. (1924)
	Boerlagiomyces Butzin	*Boerlagiomyces velutinu*s (Penz. & Sacc.) Butzin (1977)
	Byssocallis Syd.	*Byssocallis phoebes* Syd. (1927)
	Chaetocrea Syd.	*Chaetocrea parasitica* Syd. (1927)
	Chaetosphaerulina I. Hino	*Chaetosphaerulina yasudai* I. Hino (1938)
	Glaxoa P. Cannon	*Glaxoa pellucida* P.F. Cannon (1997)
	Kamalomyces R.K. Verma, N. Sharma & Soni	*Kamalomyces indicus* R.K. Verma, N. Sharma & Soni 2008
	Letendraeopsis K.F. Rodriguez & Samuels	*Letendraeopsis palmarum* K.F. Rodrigues & Samuels (1994)
	Malacaria Syd.	*Malacaria meliolicola* Syd. (1930)
	Melioliphila Speg.	*Melioliphila graminicola* (F. Stevens) Speg. (1924)
	Paranectriella (P. Henn. & Sacc.) Höhn.	*Paranectriella juruana* (Henn.) Höhn. (1910)
	Podonectria Petch	*Podonectria coccicola* (Ellis & Everh.) Petch (1921)
	Puttemansia P. Henn.	*Puttemansia lanosa* Henn. (1902)

Table C1 (continued)

Families	Genera	Type species
Tubeufiaceae M.E. Barr (1979)	*Rebentischia* P. Karst.	*Rebentischia pomiformis* P. Karst. (1869)
	Taphrophila Scheuer	*Taphrophila cornu-capreoli* Scheuer (1988)
	Thaxteriella Petr.	*Thaxteriella corticola* Petr. (1924)
	Thaxteriellopsis Sivan., Panwar & S.J. Kaur	*Thaxteriellopsis lignicola* Sivan., Panwar & S.J. Kaur (1977)
	Thaxterina Sivan., Rajak & R.C. Gupta	*Thaxterina multispora* Sivan., R.C. Rajak & R.C. Gupta (1988)
	Tubeufia Penz. & Sacc.	*Tubeufia javanica* Penz. & Sacc. (1898)
	Uredinophila Rossman	*Uredinophila tropicalis* (Speg.) Rossman (1987)

APPENDIX D

ABSTRACT PRESENTED AT CONFERENCE AND PUBLICATIONS

Taxonomy, phylogenetics and diversity of *Dothideomycetes* in Thailand

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The *Dothideomycetes* in Thailand are poorly studied. In fact, the sooty moulds (*Capnodiales*) have hardly been researched. In this project we are studying the sooty moulds on mostly living leaves as well as other *Dothideomycetes* on decaying leaves and wood in northern Thailand. At the same time we have loaned types of *Dothideomycetes* genera from herbaria and are redescribing these fungi. This is important as the *Dothideomycetes* are generally poorly known and are not well documented. We have made numerous forays and have more than 20 collections of *Dothideomycetes* species. We are in the process of identifying these collections and have isolated many. The isolation of these fungi is not easy as often more than one taxa is mixed with others. We have identified several genera, i.e., *Astrosphaeriella* sp., *Guignadia* sp., *Botryosphaeria* sp. and *Phragmocapnias* sp., and are starting to carry out molecular work on the isolates.

วงศ์วานวิวัฒนาการของเชื้อรา Tubeufiaceae และ Capnodiales ในภาคเหนือของประเทศไทย

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ทำการเก็บตัวอย่างราดำในกลุ่ม Capnodiales และ ราแซบโพรบกลุ่ม Tubeufiaceae ซึ่งอยู่ในกลุ่มราโดทิดีโอมายซีสต์ จากใบพืชสดและเศษซากไม้ ในภาคเหนือของประเทศไทย ราดำจะมีลักษณะเป็นเส้นใยสีดำปกคลุมผิวใบของพืชแต่ไม่เข้าทำลายพืช มักพบราจำพวกนี้อาศัยร่วมกับพวกเพลี้ยอ่อน หรือเพลี้ยแป้ง สำหรับรากลุ่มแซบโพรบ Tubeufiaceae จะมีการสร้าง fruiting bodies แบบ pseudothecial ซึ่งมีตั้งแต่สีน้ำตาลเข้มถึงสีดำและพบได้ทั่วไปบนพื้นผิวของเศษซากไม้ ในการศึกษาในครั้งนี้ได้เก็บรวบรวมตัวอย่างราดำได้ 16 สายพันธุ์ และรากลุ่มแซบโพรบ Tubeufiaceae จำนวน 11 สายพันธุ์ ทำการจัดจำแนกตามลักษณะทางสัณฐานวิทยา และการวิเคราะห์ข้อมูลในระดับ phylogenetics จาก 3 ยีนคือ 5.8S rDNA (ITS), 18S rDNA (SSU), 28S rDNA (LSU) โดยทำการวิเคราะห์แบบ Maximum Parsimony และ Bayesian Inference ผลการวิเคราะห์พบว่าราดำมีความสัมพันธ์กับราในสกุล *Capnodium*, *Conidioxyphium*, *Leptoxyphium* และ *Microxyphium* ในขณะที่ราแซบโพรบกลุ่ม Tubeufiaceae มีความสัมพันธ์กับราในสกุล *Acanthostigma*, *Thaxteriella* และ *Tubeufia* ตามลำดับ อย่างไรก็ตามจากลักษณะทางสัณฐานวิทยาที่ปรากฎออกมาพบว่าความแตกต่างระหว่างราสกุลเหล่านี้ยังไม่ค่อยชัดเจน เชื้อราบาง taxa ที่พบจากการศึกษานี้เป็นเชื้อชนิดใหม่ที่ยังไม่มีการรายงานมาก่อน และในขณะนี้อยู่ระหว่างการเขียนบทความวิจัย เพื่อตีพิมพ์เผยแพร่ ทั้งนี้การวิเคราะห์วงศ์วานวิวัฒนาการของเชื้อรากลุ่ม Tubeufiaceae และการเปรียบเทียบสัณฐานวิทยาของเชื้อราที่พบกับสายพันธุ์ต้นแบบของเชื้อราในสกุล *Acanthostigma*, *Thaxteriella* และ*Tubeufia* จะส่งผลในการจัดกลุ่มใหม่ของเชื้อในวงศ์นี้ให้ถูกต้องยิ่งขึ้น

O-BD004
Biodiversity of saprotrophic Dothideomycetes in northern Thailand

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This study was carried out to investigate diversity of saprotrophic Dothideomycetes on decaying wood in terrestrial forests of northern, Thailand. The specific character distinguishing Dothideomycetes are the bitunicate ascus and includes the family Tubeufiaceae. This is a large group of saprotrophs with a cosmopolitan distribution involved in nutrient cycling. Taxa have superficial fruiting bodies which are dark brown to black globose to subglobose and often form on a subiculum. Some genera have species with setose ascomata. Ascospores are usually cylindrical, cylindric-fusiform, fusiform or fusiform to clavate and are most hyaline or greyish and only have transverse septa. They are commonly found in subtropical or tropical forests on dead or decaying of wood, branches, twigs, and culms. Collections were examined and fungi isolated and morphological characters described and illustrated. Taxa were identified using morphological characters were possible. DNA sequence analysis of 5.8S rDNA (ITS), 18S rDNA (SSU), 28S rDNA (LSU) genes was used to determine the phylogenetic analysis of the evolutionary relationships among these fungi.

Keywords: bitunicate ascus, decomposer fungi, topical forests

MSA 2012: Abstracts

Poster Session 2
Day: Monday, 16 July 2012
Location: Omni Hotel Ballroom C
Time: 2100-2200
P34: Tropical species of Tubeufiaceae (Dothideomycetes) in northern Thailand

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Abstract

Fifteen species of Tubeufiaceae were found on decaying wood in northern Thailand. Genera in this family are characterized by pseudothecial ascomata that are superficial, light brown, dark brown to black, often smooth. The bitunicate asci are cylindrical to broadly clavate and contain eight ascospores that are filiform, cylindrical to narrowly fusiform, tapering towards to rounded to sub-acute ends, multiseptate, hyaline, and pale yellow or brown. Some of these fungi produce a hyphomycetous asexual state in culture such as *Chlamydotubeufia khunkornensis* with chlamydospores, *Thaxteriella inthanonensis* with helicoma-like conidia, and *Thaxteriellopsis lignicola* with helicomyces-like conidia. The relationship between sexual and asexual states is poorly known especially for species from Thailand. Several of these taxa appear to be new to science.

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Revision of lignicolous *Tubeufiaceae* based on morphological reexamination and phylogenetic analysis

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Abstract In this paper we revisit the family *Tubeufiaceae* with notes on genera that we have re-examined where possible. Generic type specimens of *Acanthophiobolus, Kamalomyces, Podonectria, Thaxteriella* and *Thaxteriellopsis* were re-examined, described and illustrated and shown to belong to *Tubeufiaceae*. Notes are provided on *Acanthostigma, Chaetosphaerulina, Thaxterina* and *Tubeufia*, which are retained in *Tubeufiaceae*; however, we were unable to locate the types of these genera during the time frame of this study. *Allonecte* is excluded from the *Tubeufiaceae*, as the ascospores are fusiform-ellipsoidal, grey-brown and 1-septate and the asci are cylindrical, all of which are features more typical of *Pleosporaceae*, where it is transferred. *Byssocallis* has yellow to orange ascomata and clavate ascospores which is atypical of *Tubeufiaceae*. Thus its taxonomic status needs to be reevaluated. *Lentendraeopsis* has an endophytic habit, cylindro-clavate asci and two-celled ascospores more typical of *Pleosporales*, where it is transferred. *Taphrophila* has small ascomata, a thin peridium, branching setae around the apex of the ascomata, clavate to saccate asci and lacks pseudoparaphyses. These are features atypical of the *Tubeufiaceae*, and *Taphrophila* should be placed in the Dothideomycetes *incertae cedis.* Twelve new collections of *Tubeufiaceae* from Thailand were isolated, and their DNA was extracted. The sequence data of LSU, SSU and ITS rDNA were amplified and analyzed using parsimony and likelihood methods. The results of phylogenetic analysis was used to establish the inter-generic relationships in *Tubeufiaceae. Thaxteriellopsis lignicola,* epitypified in this investigation, is a sister taxon in the family *Tubeufiaceae* based on phylogenetic analysis of rRNA sequence data. *Chlamydotubeufia* is introduced as a new genus based on the production of dictyochlamydosporous anamorphs, including two new species. Three new species, one each in *Acanthostigma*, *Tubeufia* and *Thaxteriella* are also described and illustrated. The phylogenetic placement of these genera is also discussed.

Keywords Anamorph · *Aquaphila* · Dothideomycetes · Helicosporous · Molecular phylogeny · Woody litter fungi

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Two new *Kirschsteiniothelia* species with *Dendryphiopsis* anamorphs cluster in *Kirschsteiniotheliaceae* fam. nov.

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Abstract: Two new *Kirschsteiniothelia* species are proposed in this study; both were collected on decaying wood from Chiang Mai and Chiang Rai provinces in northern Thailand. The taxa were isolated and the morphological characters are described and illustrated. ITS, LSU and SSU combined sequence analysis showed taxa of *Kirschsteiniothelia* separating into three lineages: (i) *K. elaterascus* grouped within Morosphaeriaceae (Pleosporales); (ii) *K. maritima* clustered with *Mytilinidion* spp. as a sister group in the Mytilinidiaceae clade; and (iii) the two new *Kirschsteiniothelia* species, which produce *Dendryphiopsis* anamorphs in culture, clustered with *K. aethiops* (the generic type) and the anamorph *D. atra.* The new family Kirschsteiniotheliaceae is introduced to accommodate taxa grouping with *K. aethiops. K. elaterascus* is transferred to *Morosphaeria* (Morosphaeriaceae) and a new genus *Halokirschteiniothelia* is introduced to accommodate *K. maritima* (Mytilinidiaceae).

Key words: Dothideomycetes, Kirschsteiniotheliaceae, new species, phylogeny

Introduction

Kirschsteiniothelia was introduced by Hawksworth (1985a) and is represented by the type *K. aethiops* based on *Sphaeria aethiops* Berk. & M.A. Curtis. The genus is characterized by superficial to semi-immersed, hemispherical or subglobose, dark brown to black ascomata, cylindrical-clavate asci that develop among numerous pseudoparaphyses, and mostly one-septate (in some species two-septate), ellipsoidal, dark-brown ascospores; there are presently 18 species recorded in *Index Fungorum* and seven estimated species in Kirk et al. (2008).

Kirschsteiniothelia D. Hawksw. is a genus of the Dothideomycetes (Hawksworth 1985a), although its ordinal and familial placements are uncertain and it is currently classified as Dothideomycetes incertae sedis in *Index Fungorum* and in Lumbsch and Huhndorf (2010). In MycoBank (Crous et al. 2004, Robert et al. 2005) this genus is placed in the Pleosporaceae, while its known hyphomycete anamorphs are referred to the Pleomassariaceae (Hyde et al. 2011). Thus placement of the genus is uncertain. Schoch et al. (2006) analyzed molecular data for *K. aethiops*, which did not cluster close to Pleosporaceae, and it was suggested that this genus should be transferred to a separate family. In a recent molecular phylogenetic analysis of the Dothideomycetes *K. elaterascus* Shearer clustered in the same clade as *Morosphaeria* (Morosphaeriaceae) while *K. maritma* (Linder) D. Hawksw. clustered in the Mytiliniadiaceae clade, as a sister group of *Mytilinidion* (Schoch et al. 2009, Suetrong et al. 2009).

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CURRICULUM VITAE

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