



TAXONOMY AND PHYLOGENY OF MICROFUNGI ON GRASSES

ISHANI DILHARA GOONASEKARA

**DOCTOR OF PHILOSOPHY
IN
BIOSCIENCES**

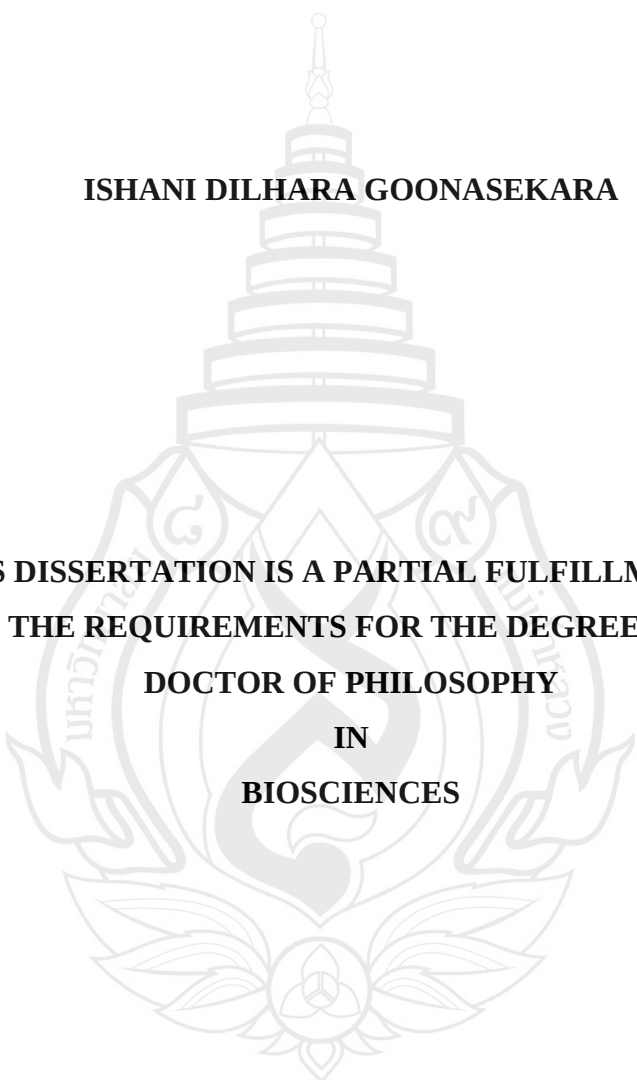
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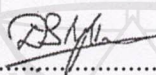
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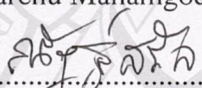
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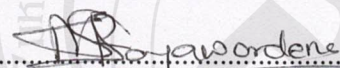
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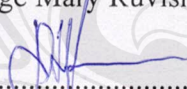
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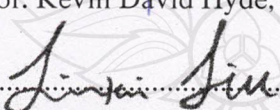
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ABSTRACT

Grasses are agriculturally, economically and ecologically dominant among plants. Their fungal diversity is fascinating but has been relatively poorly studied or restricted to a few important hosts or pathogens. Especially in tropical regions such as Thailand, there is a lack of updated compilations on fungal groups associated with grasses. Therefore, numerous grass inhabiting fungal species are awaiting discovery or re-study based on morphology and molecular phylogeny. As a basis for this study, a review and checklist with names of all microfungi occurring on grasses (excluding the bambusicolous fungi) in Thailand was compiled and published. The host plant(s) for each record and the availability of molecular data for the fungal species was given. In total, 172 species and 35 unidentified taxa were reported as follows: *Ascomycota*: 98 species and 28 unidentified, in 15 orders, 37 families and 68 genera; *Basidiomycota*: 73 species and 7 unidentified, in 8 orders, 8 families and 18 genera; *Chytridiomycota*: one identified species in *Physodermataceae* (*Physodermatales*). *Puccinia*, *Sporisorium*, *Tilletia* and *Ustilago* of the rusts and smuts, followed by *Bipolaris*/ *Cochliobolus*, *Curvularia*, *Cercospora*, *Colletotrichum* and *Fusarium* were the most commonly recorded genera. In this study, the collection, morpho-molecular identification and classification of saprobic microfungi from four selected grass hosts *Axonopus compressus*, *Imperata cylindrica*,

Thysanolaena latifolia and *Zea mays* was conducted. Over 350 samples were collected during January 2016–March 2021, mostly from northern Thailand (Chiang Mai, Chiang Rai, Lampang, Nan and Phayao provinces), as well as from several central and southern parts of the country. Mostly, the culms, leaves and sheaths of the grasses were observed. From the collections, taxa were recorded on 176 samples. Following the isolation, morphology study and molecular data analysis three new species were discovered. They are *Paraphaeosphaeria thysanolaenae* sp. nov. (*Didymosphaeriaceae*) isolated from *T. latifolia*, *Pestalotiopsis compressa* sp. nov. (*Sporocadaceae*) from *A. compressus* and *Clonostachys Chiangmaiensis* sp. nov. (*Bionectriaceae*) from *Z. mays*. Nine new host records were identified based on their morphology and phylogeny. Descriptions, illustrations and molecular data are provided for these species. *Cladosporium* was the most commonly occurring genus and was found on all hosts. More asexual morph species/ genera than sexual morphs were found. In *I. cylindrica* and *Z. mays* species were mostly found associated with leaves and sheaths rather than the culms, while in *T. latifolia* fungi almost always occurred on culms and occasionally on the inflorescence stalk. In *Axonopus compressus*, fungi were commonly found from all its parts, the leaves, sheaths and culms.

Decaying grasses serve as an ideal substrate for fungi. Among the collections of grass litter from northern Thailand, several new records and species were discovered. *Dinemasporium pseudostrigosum* (*Chaetosphaeriaceae*) and *Robillarda africana* (*Sporocadaceae*) were collected for the first time in Thailand. It is also the first record of *R. africana* from grasses. *Coniothyrium Chiangmaiense* (*Coniothyriaceae*), *Epicoccum thailandicum* (*Didymellaceae*), *Neosetphoma poaceicola* and *Setophoma poaceicola* (*Phaeosphaeriaceae*) are described as new species. *Neosetphoma poaceicola* is the first sexual morph introduced in the genus, while *S. poaceicola* is the first *Setophoma* species discovered from Thailand. The first asexual morph of *Ophiosphaerella agrostidis* (*Phaeosphaeriaceae*) is described.

Phaeosphaeriaceae and *Pleosporaceae* are two families widely known to inhabit grasses and cereal crops as endophytes, saprobes and especially pathogens. *Parastagonospora* is an important genus in *Phaeosphaeriaceae* that includes pathogens causing leaf and glume blotch on cereal crops. In this study, a sexual morph species *P. elymi* isolated from dead stems of *Elymus repens* in Russia, and an asexual morph species *P. macrouniseptata* from dead stems of *Dactylis glomerata* in Italy is introduced. *Pyrenophora trichostoma* (*Pleosporaceae*) the causal agent of tan spot or yellow leaf spot on wheat was collected for the first time in Russia and is described. The species was recorded on two grass hosts, *Bothriochloa ischaemum* and *Bromopsis inermis*. The history of *P. trichostoma*, its distribution and impact as a pathogen, as well as its current status are discussed.

In addition to the main study of microfungi on grasses from Thailand, several fungal species of interest discovered outside this region or from non-grass hosts have been presented. These include the new taxa *Distoseptispora thysanolaenae* (*Distoseptisporaceae*), *Parabambusicola thysanolaenae* (*Parabambusicolaceae*), *Pseudocercospora tamarindi* (*Mycosphaerellaceae*), *Pseudotruncatella camporesii* (*Pseudotruncatellaceae*), *Seimatosporium quercina* (*Sporocadaceae*), and four new host and geographical records in *Sporocadaceae*.

Keywords: Checklist, Graminicolous Fungi, Poaceae, Saprobes, Thailand, Tropical Fungi

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ABBREVIATIONS AND SYMBOLS

cm	centimeter
com.	combination
diam.	diameter
e.g.	for example
et al.	and others
gen.	genus (singular) and genera (plural)
i.e.	that is
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 Poaceae

Grasses are annual, biennial, or perennial flowering plants which classify into the plant family Poaceae. They are usually herbaceous, and occurring in almost every habitat in the world (Watson, 1990; Kellogg, 2001; Sharp & Simon, 2002). Grasses play a crucial role in any ecological system, either as undergrowth or food for herbivours (the first members in the food cycle). They cover approximately 20% of the earth's land surface and are inclusive of various agriculturally, economically, and ecologically important plants (Wheeler et al., 1982; Gibson, 2009). Humans, as well as animals (especially herbivores), depend on grasses (as cereal crops) such as barley, maize, rice and wheat for food, while in some instances they are useful in making alcoholic beverages, for construction purposes, producing crystal sugar, and as an energy source (Kellogg, 2001; Gibson 2009). Natural grasslands are important for grazing wild and domestic herbivores and many grass species occur as weeds (Watson, 1990; Kellogg, 2001; Sharp & Simon, 2002; Dai et al., 2017; Thambugala et al., 2017), therefore they play an important role in forest ecosystems.

1.2 The Importance of Studying Microfungi on Grasses

Microfungi, or ascomycetes or their asexual morphs can occur on grasses as pathogens, endophytes or saprobes (Wong & Hyde, 2001; Manamgoda et al., 2011; Hyde et al., 2013; Quaedvlieg et al., 2013; Liu et al., 2015; Dai et al., 2017). They can be either *Dothideomycetes* or *Sordariomycetes*, although in most cases the asexual morphs of these fungi are known to create a wide economic impact as plant pathogens (Manamgoda et al., 2012; Quaedvlieg et al., 2013; Hyde et al. 2013, 2016;

Phookamsak et al., 2014; Liu et al., 2015; Thambugala et al., 2017). The fungal pathogens occurring on grasses have been studied by various authors with limit to specific cultivated crops (Lamprecht et al., 2011; Manamgoda et al., 2012, 2015; Damm et al., 2014; Bakhshi et al., 2015). As an example, diseases occurring on turfgrass have been published in a book (Smiley et al., 2005). Notable groups of fungi included in these studies are *Alternaria*, *Bipolaris*, *Curvularia*, and especially the asexual morph species of the family *Phaeosphaeriaceae*. The collection of smut fungi of the world published by Vánky (2012) includes an extensive record of the smut diseases occurring on grasses. Cercosporoid fungi occurring on *Poaceae* has been done by Braun et al. (2015). Various studies related to fungal endophytes, such as their effect in litter decomposition (Purahong & Hyde, 2011), have been conducted in the past several years. In recent studies, attempts have been made to introduce or resolve the classification of saprobic species belonging to the families *Apiosporaceae*, *Bambusicolaceae*, *Clavicipitaceae*, *Didymellaceae*, *Didymosphaeriaceae*, *Cainiaceae*, *Massarinaceae*, *Mycosphaerellaceae*, *Periconiaceae*, *Phaeosphaeriaceae*, *Pleosporaceae*, *Roussoellaceae* and *Sporormiaceae* that occur on various grass hosts (Liu et al., 2015; Li et al., 2016; Hyde et al., 2016; Maharachchikumbura et al., 2016; Wijayawardene et al., 2016; Thambugala et al., 2017). These numerous studies related to saprobic fungi indicate that they have a great diversity, however there is a lack of a thorough and collective inventory for Thailand or the South East Asian region in particular. For some introduced species, there is a lack of proper molecular analysis (Bhilabutra et al., 2010; Hyde et al., 2013; Thambugala et al., 2017). Therefore, the need for re-collection of fresh specimens of microfungi on grasses and a proper reassessment of their taxonomical placements, enabled by both morphological and molecular analyses remains.

Hence, this study aims to fulfill that gap by providing an insight into the fungal groups that occur on grasses based on their morphological as well as molecular characteristics. This is to expand the knowledge of the species or genera concerned. Samples were collected from four selected grass hosts *Axonopus compresses*, *Imperata cylindrica*, *Thysanolaena latifolia* and *Zea mays* commonly

found in Northern Thailand. While more emphasis is given to the above-mentioned hosts, the study also focuses on other interesting fungi occurring on grass litter in Thailand. In addition, microfungi occurring on several grass hosts from Italy and Russia will be studied in order to provide clear species descriptions for those with taxonomic confusions or lack of molecular. Several microfungi encountered during collections on non-grass hosts and outside Thailand, that are taxonomic novelties are presented as well. This is to provide a comprehensive overview of other taxon groups that requires attention in fungal taxonomy as well as to provide insight into their distribution in different climate zones.

1.3 Research Objectives

1.3.1 To collect, document and publish novel taxa of microfungi occurring on selected grass hosts in Thailand viz. *Axonopus compressus*, *Imperata cylindrica*, *Thysanolaena latifolia* and *Zea mays*.

1.3.2 To study interesting microfungi occurring on grasses from Italy and Russia.

1.3.3 To investigate phylogenetic relationships of saprobic microfungi on grasses and contribute to the understanding of their natural classification, host associations, sexual and asexual morphs.

1.3.4 To compare the fungal communities among the selected host species.

1.4 Research Contents

This thesis comprises seven chapters.

Chapter 1 provides a general introduction, which includes the background to Poaceae and the importance of grasses, the various fungal groups occurring on grasses that have been studied, the knowledge and data lacking with emphasis to Thailand, the research objectives and finally the contents.

Chapter 2 is based on a review of “The Role of Microfungi on Grasses”. Here, various aspects regarding grass inhabiting microfungi, their lifestyles, ecological and economic roles are included. Several important studies carried out by fungal taxonomists

in the study of microfungi on grasses are highlighted. A checklist of microfungi (excluding bambusicolous fungi) recorded on grasses for Thailand is provided. Different methods or applications in the identification and classification of microfungi, current trends, such as the incorporation of molecular approaches, challenges and threats to grass inhabiting fungi, and future perspectives are discussed.

Chapter 3 documents the taxonomic contributions to microfungi occurring on selected four grass hosts from northern Thailand, *Axonopus compressus*, *Imperata cylindrica*, *Thysanoloaena latifolia* and *Zea mays*. Various taxonomic novelties were identified based on a combined approach of morphological and phylogenetic analysis. They have been illustrated and described along with taxonomic notes. Phylogenetic analysis has been conducted and the results have been presented accordingly *Clonostachys Chiangmaiensis* on *Zea mays*, *Paraphaeosphaeria thysanolaenae* on *T. latifolia*, and *Pestalotiopsis compressa* on *A. compressus* are novel taxa, while six new records on *A. compressus*, three on *I. cylindrica*, seven on *T. latifolia* and five on *Z. mays* are presented.

Chapter 4 includes microfungi that have been studied from grass litter (host unknown) found in Thailand. This includes four new species viz. *Coniothyrium Chiangmaiense*, *Epicoccum thailandicum*, *Neosetophoma poaceicola*, *Setophoma poaceicola* and two new records for Thailand, *Dinemasporium pseudostrigosum* and *Robillarda Africana*.

Chapter 5 provides the taxonomy and phylogeny of some interesting microfungi occurring on grass hosts from Italy and Russia. Here, a new host record and geographical record (for Russia) of the pathogenic species *Pyrenophora trichostoma* is illustrated while a review including the history and current status of the species is given. *Parastagonospora elymi*, *Parastagonospora macrouniseptata* and *Septoriella camporesii* are introduced from grasses in these regions.

Chapter 6 is a collection of various interesting microfungi collected from Germany, Italy and Thailand. These include the new species *Pseudocercospora tamarindi* from Thailand, *Pseudotruncatella camporesii* from Italy, *Seimatosporium quercina* from Germany and new host or geographical records of *Pestalotiopsis verruculosa*, *Pseudopestalotiopsis kubahensis* and *Seiridium neocupressi* from Italy.

Chapter 7 which is the final chapter provides a general conclusion to this study, discusses the research advantages, drawbacks and suggestions for future work.



CHAPTER 2

REVIEW AND CHECKLIST OF MICROFUNGI ON GRASSES IN THAILAND (EXCLUDING BAMBUSICOLOUS FUNGI)

2.1 Introduction

Microfungi exist as saprobes, pathogens and endophytes on a wide range of grasses throughout the world. They have been studied for several decades but with emphasis on certain types of grasses especially cereal crops or those of economic value. Grasses are with no doubt a quintessential component of any ecosystem. Therefore, the fungi associated with them are of great taxonomical and economical value.

One of the most important roles played by grasses in any ecological system is providing undergrowth, which acts as a natural barrier against soil erosion. Some grasses exist as weeds. Natural and cultivated grasslands provide food and shelter for smaller animals while in agriculture and farming, they are used for grazing (Watson, 1990; Kellogg, 2001; Sharp & Simon, 2002; Dai et al., 2017; Thambugala et al., 2017; Goonasekara et al., 2018).

Grasses are one of the largest and agriculturally important group of plants comprising the family Poaceae (Gould, 1968; Chapman & Peat, 1992; Hyde et al., 2002). There are over 12,000 species belonging to 750-700 genera of which *Agrostis*, *Bambusa*, *Bromus*, *Chusquea*, *Digitaria*, *Eragrostis*, *Festuca*, *Panicum*, *Paspalum*, *Poa* and *Stipa* are considered the most species-rich genera (Hodkinson, 2018). They range from annuals, biennials and perennials (Watson, 1990; Kellogg, 2001, 2015; Sharp & Simon, 2002; Soreng et al., 2017; Hodkinson, 2018; The Plant List, 2021). Grasslands cover at least 40% of the earth's surface and are distributed worldwide (Wheeler et al., 1990; Gibson, 2008) across wetlands, forests and tundra (Biologyonline.com).

With a worldwide distribution, except in parts of Antarctica and Greenland, grasses cover at least 20% of the earth's surface (Wheeler et al., 1990; Gibson, 2009). Economically, *Poaceae* are the most important group of monocots, comprising a variety of plant species with great agricultural value (Encyclopedia of Life, 2018; The Plant List, 2018). Many humans depend on grasses, especially cereal crops such as barley, maize, rice and wheat for food, and for animals as fodder. They are useful in the production of alcoholic beverages, crystal sugar and paper, as an energy source or biofuel, in ornamental landscaping and various construction purposes, to name a few (Kellogg, 2001; Gibson, 2009; Thambugala et al., 2017; Encyclopedia of Life, 2018). As grasses play an important role in the reduction of soil erosion, they are always incorporated into biodiversity conservation and management procedures.

Grasses show various morphological characteristics and habits, some forming clumps, some single stemmed, some growing erect, slender and scrambling or climbing. The plants consist of these parts: rhizome, shoot, culm, culm lead, branch, leaf, inflorescence and fruit. For *Poaceae* species, the branching systems, rhizomes, presence or absence of bristles or hairs on the culms and culm sheaths, the morphological structure of inflorescences help to distinguish and identify genera and species (Kellogg, 2015; Hodkinson, 2018). They can be categorized as economically important (*Oryza*, *Sorghus*, *Triticum*, *Zea*), sugar crops (*Saccharum*, *Sorghum*), reeds (*Arundo*, *Phragmites*) and bamboo (*Bambusa*, *Phyllostachys*, Hodkinson et al., 2000).

Among living organisms, fungi are one of the most widespread and diverse (Crop, et al. 2021) occurring on grasses. An estimated number of 2.2-2.8 million fungal species is given although this amount can well be over 100 million (Douglas, 2016; Hawksworth & Lücking, 2017). At present about 148,000 fungal species have been described (Cheek et al., 2020) and this amount is likely to be higher, especially for fungi associated with grasses (Wong & Hyde, 2001; Hawksworth, 2001; O'Brien et al., 2005; Schmit & Mueller, 2007; Blackwell, 2011; Hawksworth & Lücking, 2017). Microfungi, or ascomycetes and their asexual morphs can occur on grasses as pathogens, endophytes or saprobes (Wong & Hyde, 2001; Manamgoda et al., 2011; Hyde et al., 2013; Quaedyvlieg et al., 2013; Liu et al., 2015b; Dai et al., 2017). This chapter highlights the various roles played by microfungi on grasses and the scope of and current approaches in studies related to grass inhabiting fungi. In addition, a checklist of

microfungi recorded on grasses in Thailand, excluding the bambusicolous fungi, is presented.

2.2 Materials and Methods

This review and checklist is based on published articles in journals, books, web-based resources such as reports on host plants, USDA database (Farr & Rossman, 2018), The Smut fungi of Thailand and Food and Agriculture Organization of the United Nations (FAO) websites, and graduate student theses. The checklist (Table 2.1) includes fungal species names, families and host species names or family. The fungal classification follows Wijayawardene et al. (2018). The names are used according to Index Fungorum (2018) and Wijayawardene et al. (2018). The host name given in the original citation is sometimes changed to be consistent with the current taxonomy based on The Plant List (<http://www.theplantlist.org>). Fungal genera and species are listed in alphabetical order. Species for which molecular data is available in GenBank are marked with an asterisk (*); it should be noted that among them some were introduced from hosts other than grasses or based only on morphology. In a few cases, neither the species nor a proper synonym was identified, therefore the species name is used as originally cited.

2.3 The Occurance of Microfungi on Grasses

Grasses can be found across tropical, subtropical, temperate regions (theplantlist.org) with some groups like bamboo occurring less in Europe, and hardly found in parts of Antarctica and Greenland. (Wheeler et al., 1990; Gibson, 2009). Therefore, grass fungi are ubiquitous. They can exist in various forms such as endophytes, pathogens, saprobes, yeasts (unicellular fungi), lichens (in association with algae or cyanobacteria), mycorrhizal fungi or bambusicolous fungi (associated with bamboo), on wild grasses in nature or forests, mountainous grasslands, domesticated turfgrass, marine grasses etc.

Various geographical factors including pH, shade, humidity, moisture, soil nutrients composition, time of the year and other weather related variables can affect the type of microfungi associated with grass hosts. Host related factors such as the age of the plant, height, stem and leaf composition, can influence host specificity or tissue specificity of microfungal groups.

2.4 The Fungal-Host Relationship

Mutualistic interactions between plants and microbes initiated 400-460 Million years ago (Mya) from when glomalean spores (*Glomerales*) were recorded. This is before the origin of grasses which took place about 350-400 (Redecker et al., 2000; Prasad et al., 2011) during the Gondwanan era. However, few studies have investigated the co-evolution of fungal groups and grasses. The association of *Epichloë* (*Clavicipitaceae*) within the Pooideae has been studied, and they are known to have a symbiotic relationship but it is not necessarily host-specific (Schardl et al., 2005; Saikkonen et al., 2016). There have been several studies conducted to show the co-divergence of endophytes and grass species as well as the host jumps (Jackson, 2004; Schardl et al., 2005, 2008). A study conducted by Schardl et al. (2008) on the *Epichloë* species and their host plants, investigated the host-endophyte co-divergence. As a result, a general interest in this fungal group and related genera in *Clavicipitaceae* especially with regard to their molecular data analysis was initiated (Leuchtmann et al., 2014; Schardl et al., 2014; Chen et al., 2015).

2.5 The Effect of Pathogenic Fungi on Grasses

Grasses are often affected by pathogenic fungi. Most commonly found microfungi occurring on grasses are either *Dothideomycetes* or *Sordariomycetes* in *Ascomycota*. In many cases the asexual morphs of these fungi have a wide economic impact as plant pathogens (Manamgoda et al., 2012a, b, 2013; Quaedvlieg et al., 2013; Hyde et al., 2013, 2016; Phookamsak et al., 2014a; Liu et al., 2015b; Thambugala et al., 2017). The pathogenic microfungi on grasses have been studied by

various authors with limit to specific hosts, especially those associated with *Zea mays* and other cereal crops (Lamprecht et al., 2011; Manamgoda et al., 2012a, 2015; Damm et al., 2014; Bakhshi et al., 2015). Some of the commonly occurring pathogenic fungi include *Alternaria*, *Bipolaris*, *Cercospora*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Pleospora*, *Pseudocercospora*, *Pyrenophora*, *Pythium*, asexual morphs of *Phaeosphaeriaceae*, rusts and smuts (Vánky, 2012; Manamgoda et al., 2012a, b, 2015; Phookamsak et al., 2014, 2019; Damm et al., 2014; Crous et al., 2013, 2016). Vánky (2012) listed the smut fungi of the world which included an extensive record of the smut diseases on grasses. Braun et al. (2015) provided a study of Cercosporoid fungi occurring on Poaceae.

Just as pathogenic fungi that incur huge losses in crop yield and quality, some fungi can also affect the cattle or animals that feed on pasture lands. One such example is the fungus *Pseudopithomyces chartarum* which causes facial eczema disease in sheep (Brook, 1963).

2.6 The Ecological Role Played by Endophytic Fungi

Many plants contain endophytic fungi and grasses are no exception (Müller & Krauss, 2005; Su et al., 2010). The study of endophytic fungi for the discovery of novel compounds has fascinated many researchers throughout the past decades. One of the main reasons being that some of these compounds have mass potential in other fields such as biotechnology, pharmacy, medicine, agriculture and various industrial applications (Hyde & Soyong, 2008; Mitchell et al., 2008, 2009; Xu et al., 2010).

The presence of endophytes is considered a defense mechanism against herbivory, as the grasses become less prone to attack by insects and most cattle will avoid feeding on them (Clay, 1988). Endophytic fungi produce substances that have the ability to improve the tolerance of its host plants to various biotic as well as abiotic stresses (Bacon et al., 1997; Malinowski et al., 2000; Rodriguez et al., 2004; Martin & Dombrowski, 2015). A good example is *Epichloë* sp., a member of the *Clavicipitaceae* group of fungi which infects grasses. This fungus and its relationship with host plants has been studied extensively due to its impact on agriculture.

Epichloë sp., produces alkaloids and other metabolites that benefit the plants by providing resistance to insects (Siegel et al., 1989; Clement et al., 1994), grubs (Rostás et al., 2015) and nematodes (West, 1988; Kimmons, 1990).

Another advantage is that these compounds also help in enhancing nutrient uptake (Malinowski et al., 2000; Murphy et al., 2015), increasing plant biomass and disease tolerance, and increasing resistance to droughts and flooding (Clay, 1988; Clay et al., 1993; Saikonen et al., 1999; Gilbert, 2012). Fungal genera such as *Acremonium*, *Balsania*, *Claviceps*, *Epichloë* and *Myriogenospora* can produce alkaloid compounds causing cattle-poisoning, ergot poisoning, intoxication, “rye-grass staggers” and narcosis in grazing cattle, leading to economic losses (Clay, 1988). It is considered a defense mechanism, making grasses less favourable for feeding. Other endophytic fungi investigated for their interesting metabolic activities associated with grass hosts include *Curvularia protuberata*, *Fusarium culmorum* and *Piriformospora indica* (Verma et al., 1998) to name a few. *Piriformospora indica* is considered an important organism as biocontrol and bio-fertilizer in barley (Murphy et al., 2014). The effect of fungal endophytes towards litter decomposition has been studied by Purahong and Hyde (2011).

2.7 The Study of Saprobic Microfungi: Challenges and Limitations

In recent studies, attempts have been made to introduce or resolve the classification of saprobic species that occur on various grass hosts belonging to the families *Apiosporaceae*, *Bambusicolaceae*, *Cainiaceae*, *Clavicipitaceae*, *Didymellaceae*, *Didymosphaeriaceae*, *Massarinaceae*, *Mycosphaerellaceae*, *Periconiaceae*, *Phaeosphaeriaceae*, *Pleosporaceae*, *Roussoellaceae* and *Sporormiaceae* (Liu et al., 2015b; Li et al., 2016; Hyde et al., 2016; Maharachchikumbura et al., 2016; Wijayawardene et al., 2016; Thambugala et al., 2017).

Among microfungi associated with grasses, the taxonomic placement of several genera or groups has at times, been problematic. One common issue is the overlapping morphological features among close members of a group, such as what can be observed in “Cercosporoid” fungi (Braun et al., 2015; Liu et al., 2015). There

is sometimes the co-occurrence of many taxa on the same leaf lesion. Any morphological similarities therefore may only add to the confusion.

Several studies have provided evidence to suggest that certain fungi are host specific or host recurrent (Zhou & Hyde, 2001; McKenzie et al., 2002; Santana et al., 2005), or at least occur frequently on certain hosts (Yanna et al., 2001). However, this could be less likely in the case of saprobic fungi (Zhou & Hyde, 2001). While some previously described species defined as “host-specific” have now been found to be associated with multiple hosts. This is all due to the recent advances in taxonomic studies on grass hosts incorporating molecular data (Hyde et al., 2013, 2016; Phookamsak et al., 2014; Ariyawansa et al., 2015; Li et al., 2016; Thambugala et al., 2017). Most of the significant plant pathogens like *Alternaria*, *Bipolaris*, *Fusarium*, *Parastagonospora*, *Pseudoseptoria* occur on grass hosts (Manamgoda et al., 2011, 2015; Dean et al., 2012; Quaedylied et al., 2013; Thambugala et al., 2017) and cause heavy crop losses worldwide are not easy to identify based on their morphology alone. Therefore, in order for a proper disease management to take place, these pathogens should be accurately identified at either their sexual or asexual morph phase.

With the increasing amount of novel species being introduced, a proper classification based on solid morphological as well as molecular evidence is necessary. This is especially lacking for microfungi on grass hosts in the tropical region as they have been mostly confined to specific hosts (Bhilabutra et al., 2010; Manamgoda et al., 2011, 2012; Thambugala et al., 2017).

2.8 The Study of Microfungi on Grasses in Thailand

In Thailand, there has been a keen interest in the study of microfungi related to many important hosts ranging from bamboo, banana, *Castanopsis* sp., *Dracaena* sp., ginger, *Magnoliaceae*, palms, *Pandanus* spp. and *Shorea* sp. (Lumyong et al., 2000; Photita et al., 2001; Bussaban et al., 2001, 2004; Promputha et al., 2002, 2004; Jones et al., 2004; Pinnoi et al., 2006; Aptroot et al., 2007; Pinruan et al., 2007; Aung et al., 2008; Duong et al., 2008; Kodsueb et al., 2008; Thongkantha et al., 2008;

Osono et al., 2009; Fournier et al., 2010). However, there is a need of a thorough inventory with details of species that have occurred on grasses, that is accessible to the public and researchers. As a necessity in fungal taxonomy at present, the availability of DNA sequence data will enable accurate species delimitation, fill in the gaps of knowledge in fungal classification and provide a strong backbone tree for fungal groups of interest. This will be possible with the re-collection and re-examination of fungal species from well-known as well as unexplored areas such as Thailand or the south-east Asian region in particular.

2.8.1 The Checklist of Microfungi on Grasses in Thailand (Excluding Bambusicolous Fungi)

The numerous fungal taxonomy studies associated with grasslands or grasses indicate there is a great amount of diversity in them. However, there is a lack of a thorough and collective inventory for Thailand or the south-east Asian region. For many species, there is a lack of molecular data (Bhilabutra et al., 2010; Hyde et al., 2013; Thambugala et al., 2017), especially for type specimens. As recent approaches in fungal taxonomy and classification involve both morphological and molecular analyses, there remains a need for re-collection and a reassessment of certain fungi. A checklist (Table 2.1) was prepared with the aim that it will be a useful guide for the study of microfungi in Thailand. The host plant(s) from which the fungi were recorded is given, so as to provide an understanding of their host distribution in Thailand. Species, for which molecular data is available in GenBank are indicated. The list does not include microfungi recorded from bamboo.

Table 2.1 Checklist of microfungi on grasses (excluding bambusicolous fungi) in Thailand

Species	Family	Host	Reference
<i>ASCOMYCOTA</i>			
<i>Alfaria terrestris</i> L. Lombard & Crous	<i>Stachybotryaceae</i>	on grass litter	Hyde et al. (2018)
* <i>Alternaria padwickii</i> (Ganguly) M.B. Ellis	<i>Pleosporaceae</i>	<i>Oryza sativa</i>	Giatgong (1980) as <i>Trichoconis padwickii</i> , Richardson (1990) as <i>Trichoconiella padwickii</i>

Table 2.1 (continued)

Species	Family	Host	Reference
* <i>Ascochyta sorghi</i> Sacc.	<i>Didymellaceae</i>	<i>Sorghum</i> sp.	Richardson (1990)
<i>Aspergillus</i> sp.	<i>Aspergillaceae</i>	<i>Sorghum vulgare</i>	Giatgong (1980)
<i>Astrosphaeriella exorrhiza</i> Boise	<i>Astrosphaeriellaceae</i>	<i>Thysanolaena maxima</i>	Phookamsak et al. (2015, 2017)
* <i>Astrosphaeriella thysanolaenae</i> Phook. & K.D. Hyde	<i>Astrosphaeriellaceae</i>	<i>Thysanolaena maxima</i>	Phookamsak et al. (2015, 2017)
<i>Balansia</i> sp.	<i>Clavicipitaceae</i>	<i>Panicum trigonum</i>	Giatgong (1980), Lenne (1990)
* <i>Bipolaris brachiariae</i> Y. Marin, Senwanna & Crous	<i>Pleosporaceae</i>	<i>Brachiaria mutica</i>	Marin-Felix et al. (2017)
* <i>Bipolaris coffeana</i> Sivan.	<i>Pleosporaceae</i>	<i>Digitaria</i> sp.	Manamgoda et al. (2014)
* <i>Bipolaris maydis</i> (Y. Nisik. & C. Miyake) Shoemaker	<i>Pleosporaceae</i>	<i>Zea mays</i>	Giatgong (1980) as <i>Helminthosporium maydis</i> , Richardson (1990) as <i>Cochliobolus heterostrophus</i> , Marin-Felix et al. (2017)
* <i>Bipolaris oryzae</i> (Breda de Haan) Shoemaker	<i>Pleosporaceae</i>	<i>Oryza sativa</i>	Giatgong (1980) as <i>Cochliobolus miyabeanus</i> , Giatgong (1980) as <i>Helminthosporium oryzae</i> , Richardson (1990) as <i>C. miyabeanus</i> , Manamgoda et al. (2012a, 2014), Jeon et al. (2015), Xiao et al. (2015), Khemmuk et al. (2016), Tan et al. (2016), Zhang et al. (2017), Marin-Felix et al. (2017)
* <i>Bipolaris sacchari</i> (E.J. Butler) Shoemaker	<i>Pleosporaceae</i>	<i>Saccharum officinale</i> , <i>Saccharum officinarum</i>	Giatgong (1980) as <i>Helminthosporium sacchari</i>
* <i>Bipolaris sorokiniana</i> Shoemaker	<i>Pleosporaceae</i>	<i>Hordeum vulgare</i> , <i>Triticum aestivum</i> , <i>Triticum vulgare</i>	Richardson (1990) as <i>Cochliobolus sativus</i> Marin-Felix et al. (2017) Giatgong (1980) as <i>Helminthosporium sativum</i>

Table 2.1 (continued)

Species	Family	Host	Reference
<i>Bipolaris</i> sp.	<i>Pleosporaceae</i>	<i>Oryza sativa</i>	Manamgoda et al. (2012a)
<i>Bipolaris stenospila</i> (Drechsler) Shoemaker	<i>Pleosporaceae</i>	<i>Saccharum officinarum</i>	Giatgong 1980 as <i>Cochliobolus stenospilus</i>
<i>Blumeria</i> sp.	<i>Erysiphaceae</i>	<i>Triticum vulgare</i>	Giatgong (1980) as <i>Oidium</i> sp.
<i>Botryosphaeria</i> sp.	<i>Botryosphaeriaceae</i>	<i>Sorghum halepense</i>	Giatgong (1980)
<i>Brachysporium</i> sp.	<i>Trichosphaeriaceae</i>	<i>Oryza sativa</i>	Giatgong (1980)
<i>Capnodium</i> sp.	<i>Capnodiaceae</i>	<i>Saccharum officinarum</i>	Giatgong (1980)
* <i>Catenulocercospora fusimaculans</i> (G.F. Atk.) C. Nakash., Videira & Crous	<i>Mycosphaerellaceae</i>	<i>Agrostis</i> sp., <i>Beckeropsis</i> sp., <i>Brachiaria</i> sp., <i>Chasmopodium</i> sp., <i>Digitaria</i> sp., <i>Echinochloa</i> sp., <i>Eleusine</i> sp., <i>Entolasia</i> sp., <i>Ichnanthus</i> sp., <i>Leptoloma</i> sp., <i>Oplismenus</i> sp., <i>Panicum</i> sp., <i>Paspalidium</i> sp., <i>Rottboellia</i> sp., <i>Sorghum</i> sp., <i>Stenotaphrum</i> sp., <i>Urochloa</i> sp., <i>Zea</i> sp., <i>Panicum maximum</i>	Phengsintham et al. (2012), Phengsintham et al. (2013) as <i>Passalora fusimaculans</i> Phengsintham et al. (2012)
* <i>Ceratocystis paradoxa</i> (Dade) C. Moreau	<i>Ceratocystidaceae</i>	<i>Saccharum officinarum</i>	Lenne (1990) as <i>Cercospora fusimaculans</i> Giatgong (1980) as <i>Thielaviopsis paradoxa</i>
<i>Cercospora barretoana</i> (U. Braun & Crous) U. Braun & Crous	<i>Mycosphaerellaceae</i>	<i>Echinochloa esculenta</i>	Phengsintham et al. (2013) as <i>Passalora barretoana</i>
<i>Cercospora longipes</i> E.J. Butler	<i>Mycosphaerellaceae</i>	<i>Saccharum officinarum</i>	Giatgong (1980)
* <i>Cercospora sorghi</i> Ellis & Everh.	<i>Mycosphaerellaceae</i>	<i>Sorghum vulgare</i>	Giatgong (1980)
<i>Cercospora</i> sp.	<i>Mycosphaerellaceae</i>	<i>Saccharum officinarum</i>	Giatgong (1980)

Table 2.1 (continued)

Species	Family	Host	Reference
<i>Cladosporium</i> sp.	<i>Cladosporiaceae</i>	<i>Digitaria</i> sp. <i>Paspalum dilatatum</i>	Giatgong (1980) Giatgong (1980), Lenne (1990)
* <i>Claviceps africana</i> Freder., Mantle & De Milliano	<i>Clavicipitaceae</i>	<i>Sorghum bicolor</i>	Bandyopadhyay et al. (1998), Pazoutova et al. (2004), Pazoutova & Frederickson (2005)
* <i>Colletotrichum endophyticum</i> Manamgoda, Udayanga, L. Cai & K.D. Hyde	<i>Glomerellaceae</i>	<i>Pennisetum purpureum</i>	Manamgoda et al. (2013), Sharma and Shenoy (2014), Liu et al. (2015a), Diao et al. (2017)
* <i>Colletotrichum falcatum</i> Went	<i>Glomerellaceae</i>	<i>Saccharum officinarum</i>	Giatgong (1980), Giatgong (1980) as <i>Physalospora tucumanensis</i> , Crouch (2014)
* <i>Colletotrichum graminicola</i> (Ces.) G.W. Wilson	<i>Glomerellaceae</i>	<i>Rottboellia cochinchinensis</i>	Sherriff et al. (1995)
		<i>Sorghum sudanense</i> , <i>Sorghum vulgare</i> , <i>Zea mays</i>	Giatgong (1980)
* <i>Colletotrichum siamense</i> Prihast., L. Cai & K.D. Hyde	<i>Glomerellaceae</i>	<i>Cymbopogon citratus</i>	Munir et al. (2016)
<i>Colletotrichum</i> sp.	<i>Glomerellaceae</i>	<i>Digitaria</i> sp., <i>Sorghum vulgare</i>	Giatgong (1980)
* <i>Coniothyrium Chiangmaiense</i> Goonas., Thambug. & K.D. Hyde	<i>Coniothyriaceae</i>	on grass litter	Thambugala et al. (2017)
* <i>Curvularia alcornii</i> Manamgoda, L. Cai & K.D. Hyde	<i>Pleosporaceae</i>	<i>Panicum</i> sp.	Manamgoda et al. (2012a, b)
		<i>Pennisetum clandestinum</i>	Manamgoda et al. (2012a)
		<i>Zea mays</i>	Manamgoda et al. (2012a, b), Khemmuk et al. (2016)
		<i>Zea</i> sp.	Manamgoda et al. (2015)

Table 2.1 (continued)

Species	Family	Host	Reference
* <i>Curvularia asiatica</i> Manamgoda, L. Cai & K.D. Hyde	<i>Pleosporaceae</i>	<i>Oryza sativa</i> , <i>Saccharum</i> <i>officinarum</i> <i>Panicum</i> sp.	Manamgoda et al. (2012a, b, 2015) Manamgoda et al. (2012a, b, 2015), Jeon et al. (2015), Khemmuk et al. (2016), Zhang et al. (2018)
* <i>Curvularia</i> <i>chiangmaiensis</i> Y. Marín, Senwanna & Crous	<i>Pleosporaceae</i>	<i>Zea mays</i>	Marin-Felix et al. (2017)
* <i>Curvularia</i> <i>dactyloctenicola</i> Y. Marín, Senwanna & Crous	<i>Pleosporaceae</i>	<i>Dactyloctenium</i> <i>aegyptium</i>	Marin-Felix et al. (2017)
* <i>Curvularia hawaiiensis</i> (Bugnic. ex M.B. Ellis) Manamgoda, L. Cai & K.D. Hyde	<i>Pleosporaceae</i>	<i>Panicum</i> sp.	Manamgoda et al. (2012a, 2015)
* <i>Curvularia hominis</i> K.C. Cunha, Madrid, Gené & Cano	<i>Pleosporaceae</i>	<i>Echinochloa</i> sp., <i>Oryza</i> <i>sativa</i>	Manamgoda et al. (2015)
* <i>Curvularia lunata</i> (Wakker) Boedijn	<i>Pleosporaceae</i>	<i>Oryza sativa</i> <i>Panicum</i> sp. <i>Sorghum</i> <i>vulgare</i> <i>Zea mays</i>	Giatgong (1980), Richardson (1990) as <i>Cochliobolus lunatus</i> , Manamgoda et al. (2012a), Xiao et al. (2015) Manamgoda et al. (2012) Giatgong (1980) Giatgong (1980), Ariyawansa et al. (2015a), Manamgoda et al. (2015)
* <i>Curvularia nodosa</i> Y. Marín, Cheew. & Crous	<i>Pleosporaceae</i>	<i>Brachiaria</i> <i>reptans</i> <i>Chloris</i> <i>barbata</i> <i>Digitaria</i> <i>ciliaris</i>	Marin-Felix et al. (2017) Marin-Felix et al. (2017) Marin-Felix et al. (2017)
* <i>Curvularia pallescens</i> Boedijn	<i>Pleosporaceae</i>	<i>Oryza sativa</i>	Sivanesan (1987) as <i>Cochliobolus pallescens</i>
* <i>Curvularia</i> <i>pseudobrachyspora</i> Y. Marín, Cheew. & Crous	<i>Pleosporaceae</i>	<i>Eleusine indica</i>	Marin-Felix et al. (2017)

Table 2.1 (continued)

Species	Family	Host	Reference
<i>Curvularia</i> sp.	<i>Pleosporaceae</i>	<i>Oryza sativa</i>	Manamgoda et al. (2012a, 2015)
		<i>Oryza</i> sp., <i>Sorghum</i> sp.	Richardson (1990)
		<i>Sorghum</i> <i>vulgare</i> , <i>Zea</i> <i>mays</i>	Giatgong (1980)
* <i>Curvularia variabilis</i> Y. Marín, Senwanna & Crous	<i>Pleosporaceae</i>	<i>Chloris</i> <i>barbata</i>	Marin-Felix et al. (2017)
		<i>Digitaria</i> <i>ciliaris</i>	Marin-Felix et al. (2017)
		<i>Imperata</i> <i>cylindrica</i>	Marin-Felix et al. (2017)
* <i>Curvularia</i> <i>verruculosa</i> Tandon & Bilgrami ex M.B. Ellis	<i>Pleosporaceae</i>	<i>Cynodon</i> <i>dactylon</i>	Marin-Felix et al. (2017)
		<i>Eleusine indica</i>	Marin-Felix et al. (2017)
		<i>Oryza sativa</i> , <i>Zea</i> sp.	Manamgoda et al. (2015)
* <i>Cytospora sacchari</i> E.J. Butler	<i>Cytosporaceae</i>	<i>Saccharum</i> <i>officinarum</i>	Giatgong (1980)
<i>Diaporthe oryzae</i> - <i>sativae</i> Punith.	<i>Diaporthaceae</i>	<i>Oryza sativa</i>	Punithalingam & Sharma (1979) as <i>Ascochyta</i> <i>oryzae</i> , Giatgong (1980) as <i>A. oryzae</i> , Udayanga et al. 2011 as <i>A. oryzae</i>
<i>Diaporthe sorghicola</i> Punith.	<i>Diaporthaceae</i>	<i>Sorghum</i> <i>vulgare</i>	Punithalingam (1975) as <i>Phomopsis sorghicola</i>
* <i>Didymella poaeicola</i> Thambug. & K.D. Hyde	<i>Didymellaceae</i>	Poaceae	Thambugala et al. (2017)
<i>Diplocarpon</i> sp.	<i>Drepanopezizaceae</i>	<i>Saccharum</i> <i>officinarum</i>	Giatgong (1980) as <i>Gloeosporium</i> sp.
<i>Diplodia</i> sp.	<i>Botryosphaeriaceae</i>	<i>Zea mays</i>	Giatgong (1980), Richardson (1990) as <i>Botryodiplodia</i> sp.
* <i>Epicoccum poaeicola</i> Thambug. & K.D. Hyde	<i>Didymellaceae</i>	Poaceae	Thambugala et al. (2017)
* <i>Epicoccum sorghinum</i> (Sacc.) Aveskamp, Gruyter & Verkley	<i>Leptosphaeriaceae</i>	<i>Saccharum</i> <i>officinale</i>	Giatgong (1980) as <i>Leptosphaeria sacchari</i>
* <i>Epicoccum</i> <i>thailandicum</i> Goonas., Thambug. & K.D. Hyde	<i>Didymellaceae</i>	on grass litter	Thambugala et al. (2017)
* <i>Exserohilum</i> <i>rostratum</i> (Drechsler) K.J. Leonard & Suggs	<i>Pleosporaceae</i>	<i>Zea mays</i>	Richardson (1990) as <i>Drechslera halodes</i>

Table 2.1 (continued)

Species	Family	Host	Reference
* <i>Exserohilum turcicum</i> (Pass.) K.J. Leonard & Suggs	Pleosporaceae	<i>Sorghum vulgare</i> , <i>Zea mays</i>	Giatgong (1980) as <i>Helminthosporium turcicum</i>
<i>Fumago</i> sp.	Pezizomycotina, Incertae sedis	<i>Saccharum officinarum</i>	Giatgong (1980)
* <i>Fusarium fujikuroi</i> Nirenberg	Nectriaceae	<i>Hordeum vulgare</i> <i>Oryza sativa</i> <i>Saccharum officinarum</i> <i>Zea mays</i>	Richardson (1990) as <i>Fusarium moniliforme</i> Ou (1985) as <i>Gibberella fujikuroi</i> , Giatgong (1980) as <i>G. fujikuroi</i> Giatgong (1980) as <i>F. moniliforme</i> , <i>G. fujikuroi</i> Giatgong (1980) as <i>F. moniliforme</i>
* <i>Fusarium incarnatum</i> (Desm.) Sacc.	Nectriaceae	<i>Oryza sativa</i>	Giatgong (1980) as <i>Fusarium semitectum</i>
* <i>Fusarium oxysporum</i> Schltdl.	Nectriaceae	<i>Zea mays</i>	Giatgong (1980) as <i>Fusarium aurantiacum</i>
* <i>Fusarium pallidoroseum</i> (Cooke) Sacc.	Nectriaceae	<i>Hordeum vulgare</i>	Richardson (1990)
* <i>Fusarium solani</i> (Mart.) Sacc.	Nectriaceae	<i>Hordeum vulgare</i>	Richardson (1990)
<i>Fusarium</i> sp.	Nectriaceae	<i>Oryza sativa</i> <i>Sorghum</i> sp. <i>Sorghum vulgare</i> <i>Zea mays</i>	Giatgong (1980), Richardson (1990) Richardson (1990) Giatgong (1980)
<i>Gloeocercospora</i> sp.	Xylariales, Incertae sedis	<i>Sorghum vulgare</i>	Giatgong (1980), Giatgong (1980) as <i>Gibberella</i> sp. Giatgong (1980)
* <i>Graminopassalora graminis</i> (Fuckel) U. Braun, C. Nakash., Videira & Crous	Mycosphaerellaceae	<i>Agropyron</i> sp., <i>Agrostis</i> sp., <i>Alopecurus</i> sp., <i>Ammophila</i> sp., <i>Anthoxanthum</i> sp., <i>Arctagrostis</i> sp., <i>Arrhenatherum</i> sp., <i>Arundinaria</i> sp., <i>Avena</i> sp., <i>Beckmannia</i> sp.	Phengsintham et al. (2012) as <i>Passalora graminis</i>

Table 2.1 (continued)

Species	Family	Host	Reference
		<i>Bromus</i> sp., <i>Calamagrostis</i> sp., <i>Cinna</i> sp., <i>Cynodon</i> <i>sp.</i> , <i>Cynosurus</i> sp., <i>Dactylis</i> sp., <i>Danthonia</i> sp., <i>Deschampsia</i> sp., <i>Digitaria</i> sp., <i>Elymus</i> <i>sp.</i> , <i>Elytrigia</i> sp., <i>Festuca</i> sp., <i>Glyceria</i> <i>sp.</i> , <i>Hierochloe</i> sp., <i>Hordeum</i> sp., <i>Hystrix</i> <i>sp.</i> , <i>Koeleria</i> sp., <i>Leersia</i> sp., <i>Leucopoa</i> <i>sp.</i> , <i>Lolium</i> sp., <i>Melica</i> sp., <i>Milium</i> <i>sp.</i> , <i>Miscanthus</i> sp., <i>Muhlenbergia</i> sp., <i>Oryzopsis</i> sp., <i>Panicum</i> sp., <i>Pennisetum</i> sp., <i>Phleum</i> sp., <i>Phragmites</i> sp., <i>Poa</i> <i>sp.</i> , <i>Puccinellia</i> sp., <i>Roegneria</i> sp., <i>Secale</i> <i>sp.</i> , <i>Sitanion</i> sp., <i>Stenotaphrum</i> sp., <i>Stipa</i> <i>sp.</i> , <i>Trisetum</i> sp., <i>Zea</i> <i>sp.</i>	
<i>Helminthosporium</i> sp.	Massarinaceae	<i>Setaria italic</i>	Lenne (1990)
* <i>Leptosphaerulina</i> <i>saccharicola</i> Phook., Jian K. Liu & K.D. Hyde	Didymellaceae	<i>Zea mays</i> <i>Saccharum</i> <i>officinarum</i>	Giatgong (1980) Phookamsak (2017)
* <i>Macrophomina</i> <i>phaseolina</i> (Tassi) Goid.	Botryosphaeriaceae	<i>Sorghum vulgare</i>	Giatgong (1980) as <i>Botryodiplodia</i> <i>phaseoli</i>
* <i>Microdochium</i> <i>albescens</i> (Thüm.) Hern.-Restr. & Crous	Microdochiaceae	<i>Oryza sativa</i>	Giatgong (1980) as <i>Rhynchosporium</i> <i>oryzae</i>
* <i>Multiseptospora</i> <i>thailandica</i> Phookamsak & K.D. Hyde	Parabambusicolaceae	<i>Thysanolaena</i> <i>maxima</i>	Liu et al. (2015b)

Table 2.1 (continued)

Species	Family	Host	Reference
* <i>Nakataea oryzae</i> (Catt.) J. Luo & N. Zhang	<i>Magnaporthaceae</i>	<i>Oryza sativa</i>	Giatgong (1980) as <i>Leptosphaeria salvinii</i>
* <i>Neosetophoma</i> <i>poaceicola</i> Goonas., Thambug. & K.D. Hyde	<i>Phaeosphaeriaceae</i>	on grass litter	Thambugala et al. (2017)
* <i>Neosphaerellopsis</i> <i>thailandica</i> Crous & Trakun.	<i>Phaeosphaeriaceae</i>	<i>Bothriochloa</i> <i>bladhii</i>	Trakunyingcharoen et al. (2014)
* <i>Nigrocornus scleroticus</i> (Pat.) Ryley	<i>Clavicipitaceae</i>	<i>Cymbopogon</i> <i>nardus</i>	Ryley (2006) as <i>Balansia sclerotica</i>
* <i>Nigrospora oryzae</i> (Berk. & Broome) Petch	<i>Sordariomycetes,</i> <i>incertae sedis</i>	<i>Zea mays</i>	Richardson (1990) as <i>Khuskia oryzae</i>
* <i>Ophiosphaerella</i> <i>agrostidis</i> Derm., M.P.S. Câmara, N.R. O'Neill, Berkum & M.E. Palm	<i>Phaeosphaeriaceae</i>	on grass litter, <i>Thysanolaena</i> <i>maxima</i>	Phookamsak et al. (2014b), Thambugala et al. (2017)
<i>Ophiosphaerella</i> -like	<i>Pleosporales,</i> <i>Incertae sedis</i>	<i>Imperata</i> <i>cylindrica</i>	Phookamsak (2017)
* <i>Paraconiothyrium</i> <i>thysanolaenae</i> Phookamsak, Chethana & K.D. Hyde	<i>Didymosphaeriaceae</i>	<i>Thysanolaena</i> <i>maxima</i>	Liu et al. (2015b)
* <i>Paraphaeosphaeria</i> <i>graminicola</i> Thambug. & K.D. Hyde	<i>Didymosphaeriaceae</i>	Poaceae	Thambugala et al. (2017)
<i>Passalora agrostidicola</i> Phengs. & U. Braun	<i>Mycosphaerellaceae</i>	<i>Agrostis</i> sp.	Braun et al. (2015)
<i>Passalora koepkei</i> (W. Krüger) U. Braun & Crous	<i>Mycosphaerellaceae</i>	<i>Saccharum</i> <i>officinarum</i>	Giatgong (1980) as <i>Cercospora koepkei</i>
* <i>Passalora vaginae</i> (W. Krüger) U. Braun & Crous	<i>Mycosphaerellaceae</i>	<i>Saccharum</i> <i>officinarum</i>	Giatgong (1980) as <i>Cercospora vaginae</i>
<i>Pestalotia andropogonis</i> Rostr.	<i>Sporocadaceae</i>	<i>Andropogon</i> <i>sorghum</i>	Nag Raj (1993)
<i>Pestalotia</i> sp.	<i>Sporocadaceae</i>	<i>Sorghum</i> <i>vulgare</i>	Giatgong (1980)
* <i>Periconia cortaderiae</i> Thambug. & K.D. Hyde	<i>Periconiaceae</i>	<i>Cortaderia</i> sp.	Thambugala et al. (2017)
* <i>Phaeocytostroma</i> <i>sacchari</i> (Ellis & Everh.) B. Sutton	<i>Diaporthaceae</i>	<i>Saccharum</i> <i>officinarum</i>	Giatgong (1980) as <i>Melanconium</i> <i>sacchari</i>
* <i>Phaeosphaeria</i> <i>chiangraina</i> Phookamsak & K.D. Hyde	<i>Phaeosphaeriaceae</i>	<i>Oryza sativa</i>	Phookamsak et al. (2014b)
* <i>Phaeosphaeria</i> <i>thysanolaenicola</i> Phookamsak & K.D. Hyde	<i>Phaeosphaeriaceae</i>	<i>Thysanolaena</i> <i>maxima</i>	Phookamsak et al. (2014b)

Table 2.1 (continued)

Species	Family	Host	Reference
* <i>Phoma insidiosa</i> Tassi	<i>Didymellaceae</i>	<i>Sorghum vulgare</i>	Giatgong (1980)
<i>Phoma</i> sp.	<i>Didymellaceae</i>	<i>Hordeum vulgare</i>	Richardson (1990)
* <i>Phyllachora chrysopogonicola</i> Tamakaew, Cheew. & K.D. Hyde	<i>Phyllachoraceae</i>	<i>Phyllachora chrysopogonicola</i>	Tamakaew et al. (2017)
<i>Phyllachora cynodontis</i> Niessl	<i>Phyllachoraceae</i>	<i>Cynodon</i> sp.	Lenne (1990)
<i>Phyllachora sacchari</i> Henn.	<i>Phyllachoraceae</i>	<i>Sorghum vulgare</i>	Giatgong (1980) as <i>Phyllachora sorghi</i>
<i>Phyllachora</i> sp.	<i>Phyllachoraceae</i>	<i>Cynodon dactylon</i>	Lenne (1990)
* <i>Phyllachora thysanolaenae</i> Tamakaew, Maharachch., K.D. Hyde & Cheew.	<i>Phyllachoraceae</i>	<i>Thysanolaena maxima</i>	Tamakaew et al. (2017)
* <i>Phyllosticta capitalensis</i> Henn.	<i>Phyllostictaceae</i>	<i>Saccharum officinarum</i>	Wikee et al (2013a, b)
<i>Phyllosticta</i> sp.	<i>Phyllostictaceae</i>	<i>Oryza sativa</i>	Giatgong (1980)
* <i>Poaceascoma helicoides</i> Phook. & K.D. Hyde	<i>Lentitheciaceae</i>	<i>Digitaria sanguinalis</i>	Phookamsak (2017)
<i>Pseudoacrodictys dimorphospora</i> Somrith. & E.B.G. Jones	<i>Ascomycota, incertae sedis</i>	<i>Arundinaria pusilla</i>	Somrithipol & Jones (2003)
* <i>Pseudopyricularia bothriochloae</i> (Crous & Cheew.) Y. Marin & Crous	<i>Pyriculariaceae</i>	<i>Bothriochloa bladhii</i>	Crous et al. (2013) as <i>Pyricularia bothriochloae</i>
* <i>Pseudotrichia rubriostiolata</i> (Phook. & K.D. Hyde) Q. Tian & K.D. Hyde	<i>Pleomassariaceae</i>	<i>Thysanolaena maxima</i>	Liu et al. (2015b)
* <i>Pseudotrichia thailandica</i> (Phook. & K.D. Hyde) Q. Tian & K.D. Hyde	<i>Pleomassariaceae</i>	<i>Thysanolaena maxima</i>	Liu et al. (2015b)
* <i>Psiloglonium colihuae</i> (Lorenzo & Messuti) E. Boehm	<i>Hysteriaceae</i>	on grass litter <i>Thysanolaena maxima</i>	Phookamsak (2017) Liu et al. (2015b)
* <i>Psiloglonium multiseptatum</i> Phookamsak & K.D. Hyde	<i>Hysteriaceae</i>	<i>Thysanolaena maxima</i>	Liu et al. (2015b)
<i>Pyrenophora</i> sp.	<i>Pleosporaceae</i>	<i>Ophiuros exaltatus</i> <i>Oryza sativa</i> , <i>Sorghum</i> sp.	Lenne (1990) as <i>Drechslera</i> sp. Richardson (1990) as <i>Drechslera</i> sp.

Table 2.1 (continued)

Species	Family	Host	Reference
* <i>Pyrenophora tritici-repentis</i> (Died.) Drechsler	Pleosporaceae	<i>Triticum vulgare</i>	Giatgong (1980) as <i>Helminthosporium tritici-vulgaris</i>
* <i>Pyricularia oryzae</i> Cavara	Pyriculariaceae	<i>Oryza sativa</i>	Giatgong (1980)
<i>Pyricularia</i> sp.	Pyriculariaceae	<i>Brachiaria mutica</i> , <i>Digitaria</i> sp., <i>Echinochloa colonum</i> <i>Eragrostis</i> sp. <i>Ischaemum rugosum</i> , <i>Leersia hexandra</i> <i>Panicum repens</i> <i>Triticum vulgare</i> <i>Zoysia matrella</i>	Giatgong (1980), Lenne (1990) Lenne (1990) Giatgong (1980) Giatgong (1980), Lenne (1990) Giatgong (1980)
* <i>Sarocladium oryzae</i> (Sawada) W. Gams & D. Hawksw.	Hypocreales, <i>Incertae sedis</i>	<i>Oryza sativa</i>	Giatgong (1980) Giatgong (1980) as <i>Acrocyllidrium oryzae</i> , Richardson (1990)
* <i>Sarocladium strictum</i> (W. Gams) Summerb.	Hypocreales, <i>Incertae sedis</i>	<i>Sorghum vulgare</i> , <i>Zea mays</i>	Giatgong (1980) as <i>Cephalosporium acremonium</i>
<i>Sclerotium</i> sp.	Sclerotiniaceae	<i>Saccharum officinarum</i>	Giatgong (1980)
* <i>Setophoma poaeicola</i> Goonas., Thambug. & K.D. Hyde	Phaeosphaeriaceae	on grass litter	Thambugala et al. (2017)
* <i>Setophoma sacchari</i> (Bitanc.) Gruyter, Aveskamp & Verkley	Phaeosphaeriaceae	<i>Saccharum officinarum</i>	Phookamsak et al. (2014a), Phookamsak (2017)
* <i>Spegazzinia neosundara</i> Thambug. & K.D. Hyde	Apiosporaceae	<i>Cortaderia</i> sp.	Thambugala et al. (2017)
* <i>Sphaerulina oryzina</i> Hara	Mycosphaerellaceae	<i>Oryza sativa</i>	Richardson (1990) as <i>Cercospora oryzae</i>
* <i>Stagonospora imperaticola</i> Phukhams., Thambug. & K.D. Hyde	Massarinaceae	<i>Imperata cylindrica</i>	Thambugala et al. (2017)
* <i>Stagonospora multiseptata</i> Thambug. & K.D. Hyde	Massarinaceae	leaves and stems of grasses	Thambugala et al. (2017)

Table 2.1 (continued)

Species	Family	Host	Reference
* <i>Stenocarpella maydis</i> (Berk.) B. Sutton	Diaporthaceae	<i>Zea mays</i>	Giatgong (1980) as <i>Diplodia zeae</i>
* <i>Sulcosporium thailandicum</i> Phookamsak & K.D. Hyde	Halotthiaceae	<i>Axonopus compressus</i>	Ariyawansa et al. (2015b)
* <i>Tetraploa yakushimensis</i> (Kaz. Tanaka, K. Hiray. & Hosoya) Kaz. Tanaka & K. Hiray.	Tetraplosphaeriaceae	<i>Imperata cylindrica</i>	Phookamsak (2017)
* <i>Ustilaginoidea virens</i> (Cooke) Takah.	Clavicipitaceae	<i>Oryza sativa</i>	Giatgong (1980)
BASIDIOMYCOTA			
<i>Anthracocystis andropogonis-aciculati</i> (Petch) McTaggart & R.G. Shivas	Ustilaginaceae	<i>Chrysopogon aciculatus</i>	Shivas et al. (2007) as <i>Sporisorium andropogonis-aciculati</i>
* <i>Anthracocystis anthistiriae</i> (Cobb) McTaggart & R.G. Shivas	Ustilaginaceae	<i>Themeda triandra</i>	Shivas et al. (2007) as <i>Sporisorium anthistiriae</i>
<i>Anthracocystis berndtii</i> (Vánky) McTaggart & R.G. Shivas	Ustilaginaceae	<i>Schizachyrium sanguineum</i>	Shivas et al. (2008) as <i>Sporisorium berndtii</i> , McTaggart et al. (2012)
<i>Anthracocystis holstii</i> (Henn.) McTaggart & R.G. Shivas	Ustilaginaceae	<i>Themeda triandra</i>	Shivas et al. (2007) as <i>Sporisorium holstii</i> , McTaggart et al. (2012)
<i>Anthracocystis likhitekarajae</i> (R.G. Shivas, Athip. & McTaggart) McTaggart & R.G. Shivas	Ustilaginaceae	<i>Ischaemum</i> sp.	Shivas et al. (2008) as <i>Sporisorium likhitekarajae</i> , McTaggart et al. 2012
<i>Anthracocystis paspali-thunbergii</i> (Henn.) McTaggart & R.G. Shivas	Ustilaginaceae	<i>Paspalum orbiculare</i>	Shivas et al. (2007) as <i>Sporisorium paspali-thunbergii</i>
<i>Anthracocystis shivasiorum</i> (Vánky) McTaggart & R.G. Shivas	Ustilaginaceae	<i>Eulalia trispicata</i>	Vánky (2008) as <i>Sporisorium shivasiorum</i> , McTaggart et al. (2012)
* <i>Anthracocystis trispicatae</i> (R.G. Shivas, Vánky & Athip.) McTaggart & R.G. Shivas	Ustilaginaceae	<i>Eulalia trispicata</i>	Vánky et al. (2006) as <i>Sporisorium trispicatae</i> , Shivas et al. (2007) as <i>S. trispicatae</i> , McTaggart et al. (2012), Piątek et al. (2015)

Table 2.1 (continued)

Species	Family	Host	Reference
* <i>Conidiosporomyces ayresii</i> (Berk.) Vánky & R. Bauer	Tilletiaceae	<i>Panicum maximum</i>	Shivas et al. (2007)
* <i>Eballistra oryzae</i> (Syd. & P. Syd.) R. Bauer, Begerow, A. Nagler & Oberw	Entylomataceae	<i>Oryza sativa</i>	Giatgong (1980) as <i>Entyloma oryzae</i>
* <i>Franzpetrakia microstegii</i> Thirum. & Pavgi	Ustilaginaceae	<i>Microstegium fasciculatum</i>	Shivas et al. (2007), Vánky (2013)
<i>Langdonia clandestina</i> (R.G. Shivas, Vánky & Athip.) McTaggart & R.G. Shivas	Ustilaginaceae	<i>Aristida balansae</i>	McTaggart et al. (2012)
		<i>Aristida setacea</i>	Vánky et al. (2006), Shivas et al. (2007) as <i>Sporisorium clandestinum</i> , McTaggart et al. (2012)
* <i>Langdonia inopinata</i> (Vánky) McTaggart & R.G. Shivas	Ustilaginaceae	<i>Aristida</i> sp.	Shivas et al. (2007) as <i>Sporisorium inopinatum</i> , McTaggart et al. (2012)
* <i>Macalpinomyces arundinellae-setosae</i> R.G. Shivas & Vánky	Ustilaginaceae	<i>Arundinella setosa</i>	Shivas et al. (2007)
<i>Macalpinomyces siamensis</i> R.G. Shivas, Vánky & Athip.	Ustilaginaceae	<i>Coelorachis striata</i>	Vánky et al. (2006), Shivas et al. (2007)
* <i>Moesziomyces bullatus</i> (J. Schröt.) Vánky	Ustilaginaceae	<i>Leersia hexandra</i> , <i>Polytrias amaura</i>	Shivas et al. (2007)
* <i>Ontoteliium coronatum</i> Syd. as <i>Uromyces coronatus</i>	Pucciniaceae	<i>Zizania latifolia</i>	Lorsuwan et al. (1984) as <i>Uromyces coronatus</i>
<i>Puccinia andropogonicola</i> Har. & Pat.	Pucciniaceae	<i>Hyparrhenia bracteata</i>	Gjaerum (1988)
* <i>Puccinia cynodontis</i> Lacroix ex Desm.	Pucciniaceae	<i>Cynodon dactylon</i>	Lenne (1990)
* <i>Puccinia duthiei</i> Ellis & Tracy	Pucciniaceae	<i>Cynodon</i> sp., <i>Dichanthium annulatum</i>	Lorsuwan et al. (1984) Lorsuwan et al. (1984) as <i>Puccinia duthiae</i>
* <i>Puccinia graminis</i> Pers	Pucciniaceae	<i>Hordeum vulgare</i>	Giatgong (1980) as <i>Puccinia graminis</i> subsp. <i>graminis</i>

Table 2.1 (continued)

Species	Family	Host	Reference
* <i>Puccinia graminis</i> f.sp. <i>avenae</i> Erikss. & Henning	<i>Pucciniaceae</i>	<i>Avena sativa</i>	Giatgong (1980)
* <i>Puccinia melanocephala</i> Syd. & P. Syd.	<i>Pucciniaceae</i>	<i>Saccharum officinarum</i>	Giatgong (1980)
* <i>Puccinia nakanishikii</i> Dietel	<i>Pucciniaceae</i>	<i>Cymbopogon citratus</i>	Lorsuwan et al. (1984)
<i>Puccinia neyraudiae</i> Syd. & P. Syd.	<i>Pucciniaceae</i>	<i>Neyraudia reynaudiana</i>	Lohsomboon et al. (1986)
* <i>Puccinia operta</i> Mundk. & Thirum.	<i>Pucciniaceae</i>	<i>Coix lacryma-jobi</i>	Lohsomboon et al. (1986)
* <i>Puccinia polysora</i> Underw.	<i>Pucciniaceae</i>	<i>Brachiaria distachya</i> <i>Zea mays</i>	Unartngam et al. (2011) Giatgong (1980), Unartngam et al. (2011)
* <i>Puccinia purpurea</i> Cooke	<i>Pucciniaceae</i>	<i>Sorghum halepense</i> <i>Sorghum vulgare</i>	Giatgong (1980)
* <i>Puccinia recondita</i> Roberge ex Desm.	<i>Pucciniaceae</i>	<i>Triticum aestivum</i>	Giatgong (1980), Lorsuwan et al. (1984)
* <i>Puccinia rubigo-vera</i> f.sp. <i>tritici</i> Carleton	<i>Pucciniaceae</i>	<i>Triticum vulgare</i>	Lorsuwan et al. (1984)
* <i>Puccinia rufipes</i> Dietel	<i>Pucciniaceae</i>	<i>Imperata cylindrica</i>	Giatgong (1980), Lorsuwan et al. (1984)
* <i>Puccinia sorghi</i> Schwein.	<i>Pucciniaceae</i>	<i>Zea mays</i>	Giatgong (1980), Lorsuwan et al. (1984)
<i>Puccinia</i> sp.	<i>Pucciniaceae</i>	<i>Sorghum</i> sp.	Giatgong (1980)
* <i>Puccinia striiformis</i> Westend	<i>Pucciniaceae</i>	<i>Triticum vulgare</i>	Giatgong (1980) as <i>Puccinia glumarum</i>
* <i>Rhizoctonia solani</i> J.G. Kühn	<i>Ceratobasidiaceae</i>	<i>Oryza sativa</i>	Giatgong (1980) as <i>Thanatephorus cucumeris</i>
* <i>Schizophyllum commune</i> Fr.	<i>Schizophyllaceae</i>	<i>Saccharum officinarum</i>	Giatgong (1980)
<i>Sporisorium amaurae</i> Vánky & C. Vánky	<i>Ustilaginaceae</i>	<i>Polytrias amaura</i>	Shivas et al. (2007)
* <i>Sporisorium arthraxonis</i> (Pat.) L. Guo	<i>Ustilaginaceae</i>	<i>Arthraxon hispidus</i>	Shivas et al. (2007)
* <i>Sporisorium cruentum</i> (J.G. Kühn) Vánky	<i>Ustilaginaceae</i>	<i>Sorghum propinquum</i>	Shivas et al. (2007)
<i>Sporisorium dichanthiicola</i> (Mundk. & Thirum.) Vánky	<i>Ustilaginaceae</i>	<i>Dichanthium caricosum</i>	Shivas et al. (2007) as <i>Sporisorium dichanthicola</i>
* <i>Sporisorium doidgeae</i> (Zundel) Langdon & Full.	<i>Ustilaginaceae</i>	<i>Bothriochloa bladhii</i>	Shivas et al. (2007)

Table 2.1 (continued)

Species	Family	Host	Reference
* <i>Sporisorium exsertum</i> (McAlpine) L. Guo	Ustilaginaceae	<i>Themeda triandra</i>	Shivas et al. (2008)
<i>Sporisorium ischaemicola</i> (L. Ling) Vánky	Ustilaginaceae	<i>Ischaemum indicum</i>	Shivas et al. (2007)
* <i>Sporisorium manilense</i> (Syd. & P. Syd.) Vánky	Ustilaginaceae	<i>Sacciolepis indica</i>	Shivas et al. (2007)
* <i>Sporisorium ophiuri</i> (Henn.) Vánky	Ustilaginaceae	<i>Rottboellia cochinchinensis</i>	Shivas et al. (2007)
<i>Sporisorium pseudosorghum</i> Vánky, R.G. Shivas & Athip.	Ustilaginaceae	<i>Pseudosorghum fasciculare</i>	Vánky et al. (2006), Shivas et al. (2007)
<i>Sporisorium sacchari</i> (Rabenh.) Vánky	Ustilaginaceae	<i>Saccharum arundinaceum</i>	Shivas et al. (2007)
* <i>Sporisorium scitamineum</i> (Syd.) M. Piepenbr., M. Stoll & Oberw.	Ustilaginaceae	<i>Saccharum officinarum</i>	Shivas et al. (2007) Giatgong (1980) as <i>Ustilago scitaminea</i> , Shivas et al. (2007) as <i>U. scitaminea</i>
<i>Sporisorium</i> sp.	Ustilaginaceae	<i>Saccharum</i> sp.	Raboin et al. (2007) as <i>U. scitaminea</i>
* <i>Sporisorium tenue</i> (Syd. & P. Syd.) Vánky	Ustilaginaceae	<i>Ischaemum indicum</i>	Shivas et al. (2007)
* <i>Stollia bothriochloae</i> (L. Ling) McTaggart & R.G. Shivas	Ustilaginaceae	<i>Bothriochloa bladhii</i>	Shivas et al. (2008)
	Ustilaginaceae	<i>Bothriochloa bladhii</i>	Shivas et al. (2007) as <i>Macalpinomyces bothriochloae</i> , McTaggart et al. (2012)
	Ustilaginaceae	<i>Bothriochloa pertusa</i>	Shivas et al. (2007) as <i>M. bothriochloae</i>
* <i>Stollia bursa</i> (Berk.) McTaggart & R.G. Shivas	Ustilaginaceae	<i>Themeda triandra</i>	McTaggart et al. (2012)
	Ustilaginaceae	<i>Themeda villosa</i>	Shivas et al. (2008) as <i>Macalpinomyces bursus</i> , McTaggart et al. (2012)
* <i>Stollia ewartii</i> (McAlpine) McTaggart & R.G. Shivas	Ustilaginaceae	<i>Sorghum nitidum</i>	Shivas et al. (2008) as <i>Macalpinomyces ewartii</i>
<i>Thecaphora</i> sp.	Glomosporiaceae	<i>Digitaria</i> sp.	Giatgong (1980), Lenne (1990) as <i>Sorosporium</i> sp.

Table 2.1 (continued)

Species	Family	Host	Reference
* <i>Tilletia barclayana</i> (Bref.) Sacc. & P. Syd.	Tilletiaceae	<i>Oryza sativa</i>	Zundel (1953) as <i>Neovossia horrida</i> , Duran & Fischer (1961), Chandrasrikul (1962) as <i>Tilletia horrida</i> , Giatgong (1980) as <i>T. horrida</i> , Shivas et al. (2007) as <i>T. horrida</i>
<i>Tilletia Chiangmaiensis</i> R.G. Shivas, Vánky & Athip.	Tilletiaceae	<i>Arundinella bengalensis</i>	Vánky et al. (2006), Shivas et al. (2007)
<i>Tilletia filisora</i> R.G. Shivas, Vánky & Athip.	Tilletiaceae	<i>Pennisetum setosum</i>	Vánky et al. (2006), Shivas et al. (2007)
<i>Tilletia isachnicola</i> R.G. Shivas, Athip. & McTaggart	Tilletiaceae	<i>Isachne globosa</i>	Shivas et al. (2008)
<i>Tilletia ischaemi</i> Vánky & N.D. Sharma	Tilletiaceae	<i>Ischaemum rugosum</i>	Shivas et al. (2007)
<i>Tilletia lageniformis</i> Vánky, C. Vánky, R.G. Shivas & Athip.	Tilletiaceae	<i>Hyparrhenia rufa</i>	Vánky et al. (2006), Shivas et al. (2007)
* <i>Tilletia setariae-parviflorae</i> Vánky & R.G. Shivas	Tilletiaceae	<i>Setaria parviflora</i>	Vánky (2007), Shivas et al. (2007)
<i>Tilletia</i> sp.	Tilletiaceae	<i>Sacciolepis indica</i>	Shivas et al. (2008)
<i>Tilletia thailandica</i> Vánky & R.G. Shivas	Tilletiaceae	<i>Eragrostis amabilis</i>	Vánky (2007), Shivas et al. (2007)
* <i>Tilletia vittata</i> (Berk.) Mundk.	Tilletiaceae	<i>Oplismenus compositus</i>	Shivas et al. (2007)
* <i>Uromyces setariae-italicae</i> Yoshino	Pucciniaceae	<i>Panicum purpurascens</i>	Giatgong (1980) as <i>Uromyces leptodermus</i> , Lenne (1990)
* <i>Ustilago coicis</i> Bref.	Ustilaginaceae	<i>Panicum</i> sp. <i>Coix lacryma-jobi</i>	Lorsuwan et al. (1984) Titatarn et al. (1983), Richardson (1990), Shivas (2007)
* <i>Ustilago cynodontis</i> (Pass.) Henn.	Ustilaginaceae	<i>Cynodon dactylon</i>	Shivas et al. (2007)
<i>Ustilago egenula</i> Syd., P. Syd. & E.J. Butler	Ustilaginaceae	<i>Eragrostis japonica</i>	Shivas et al. (2007)
* <i>Ustilago esculenta</i> Henn.	Ustilaginaceae	<i>Zizania aquatica</i> <i>Zizania latifolia</i>	Zundel (1953) Shivas et al. (2008)
<i>Ustilago maydis</i> (DC.) Corda	Ustilaginaceae	<i>Zea mays</i>	Giatgong (1980), Shivas et al. (2007)

Table 2.1 (continued)

Species	Family	Host	Reference
* <i>Ustilago neyraudiae</i> Mundk.	<i>Ustilaginaceae</i>	<i>Neyraudia reynaudiana</i>	Shivas et al. (2007)
* <i>Ustilago nuda</i> (C.N. Jensen) Kellerm. & Swingle	<i>Ustilaginaceae</i>	<i>Triticum aestivum</i>	Shivas et al. (2007) as <i>Ustilago tritici</i>
		<i>Triticum vulgare</i>	Giatgong (1980) as <i>U. tritici</i>
* <i>Ustilago planetella</i> Vánky & R.G. Shivas	<i>Ustilaginaceae</i>	<i>Eragrostis japonica</i>	Vánky (2007)
<i>Ustilago sclerachnes</i> Wakef. ex Vánky	<i>Ustilaginaceae</i>	<i>Chionachne punctata</i>	Shivas et al. (2007)
<i>Ustilago</i> sp.	<i>Ustilaginaceae</i>	<i>Brachiaria mutica</i>	Giatgong (1980)
		<i>Panicum repens</i>	Lenne (1990), Giatgong (1980)
		<i>Zoysia japonica</i>	Giatgong (1980)
* <i>Ustilago trichophora</i> (Link) Kunze	<i>Ustilaginaceae</i>	<i>Echinochloa colonum</i>	Shivas et al. (2007)
* <i>Waitea circinata</i> Warcup & P.H.B. Talbot	<i>Corticaceae</i>	<i>Oryza sativa</i>	Giatgong (1980) as <i>Rhizoctonia oryzae</i>
CHYTRIDIOMYCOTA			
* <i>Physoderma maydis</i> (Miyabe) Miyabe	<i>Physodermataceae</i>	<i>Sorghum vulgare</i>	Giatgong (1980)
		<i>Zea mays</i>	Giatgong (1980) as <i>Physoderma zae-maydis</i>

Note *molecular data available in GenBank. In bold – Type/ species first recorded from this grass host in Thailand

2.9 Discussion

This checklist of microfungi on grasses (excluding bambusicolous fungi) found in Thailand, consists of 172 taxa distributed in 24 orders, 46 families and 89 genera. Another 35 taxa remain unidentified (to species level). The species *Stagonospora sacchari* T.T. Lo & L. Ling (*Phaeosphaeriaceae*) recorded on *Saccharum officinarum* (Castellani & Germano 1977, Giatgong 1980) and *Ustilago phragmitis* Hirschh. (*Ustilaginaceae*) on *Phragmites karka* (Shivas et al. 2007) are currently listed as invalid names in Index Fungorum and therefore are not included in this checklist. The most

commonly recorded genera are *Puccinia*, *Sporisorium*, *Tilletia* and *Ustilago* (commonly known as the rusts and smuts), followed by the genera *Bipolaris/Cochliobolus*, *Curvularia*, *Cercospora*, *Colletotrichum* and *Fusarium*.



CHAPTER 3

TAXONOMIC NOVELITIES OF SAPROBIC MICROFUNGI OCCURRING ON *Axonopus compressus*, *Imperata cylindrica*, *Thysanolaena latifolia* AND *Zea mays* IN THAILAND

3.1 Introduction

3.1.1 *Axonopus compressus* and *Imperata cylindrica*

The grasses *Axonopus compressus* (broad-leaved carpet grass) and *Imperata cylindrica* (Cogon grass) are two common stout grasses of Poaceae commonly found in northern Thailand. They are mainly useful as undergrowth in the forest type ecosystem, while the broad-leaved carpet grass is popular for landscaping purposes. In some continents, Cogon grass is listed as an invasive weed (plants.ifas.ufl.edu, Lucardi et al., 2014). The USDA (Farr & Rossman, 2022) lists over 260 records of microfungi on the host genus *Axonopus* while, with 210 records for *Imperata cylindrica* worldwide. However, there is very less current research about the fungi on these hosts in Thailand. Table 3.1 lists the microfungi recorded from Thailand on both hosts.

3.1.2 *Thysanolaena latifolia*

Commonly known as “tiger grass” or “broom grass”, *Thysanolaena latifolia* Kuntze is a robust and perennial grass with a bamboo-like solid stem, growing up to 3-4 m in height and it is found abundantly in the tropical regions of the world (<http://www.fao.org>). This grass is important as cattle feed and for making brooms (with the inflorescence) or as an ornamental plant. The cultivation of the plant is highly productive and requires less fertilization. The plant is considered as a great source for producing high yields of monomeric sugar (Komolwanich et al., 2016).

Most fungi recorded on *T. latifolia* are from the Asian region (China, Hong Kong, India, Malaysia, Myanmar, Papua New Guinea, Philippines, and Thailand) while a few are from Mauritius of East Africa (Farr & Rossman, 2016). A study by Bhilabutra et al. (2010) of the fungal diversity occurring on leaves and stems of *Thysanolaena latifolia* (syn = *T. maxima*) in two field sites in Northern Thailand showed that the most common genera that occur on this host are *Leptosphaeria*, *Niptera*, *Periconia*, *Septoria*, *Stachybotrys*, *Tetraploa*, and *Verticillium*. The species diversity of *T. latifolia* could be related to the tissue type of the host with more species preferring the stems than the leaves (Bhilabutra et al., 2010). However, these fungi can differ in their abundance at certain sites due to various environmental factors (Wong & Hyde, 2001; Bhilabutra et al., 2010). The study by Bhilabutra et al. (2010) noted that species *Pycnothyriopsis* sp., *Dendrographium thysanolaenae* and *Drumopama monoseta* have been known only to occur on *T. latifolia* but whether they are host specific or not remains inconclusive.

The earliest record from this host is *Meliola panici* from the Philippines by Teodoro (1937). Studies prior to 2015 have mainly focused on recording the species diversity on *Thysanolaena* sp. Wong and Hyde (2001) recorded 20 taxa from Hong Kong, Bhilabutra et al. (2010) reported 67 taxa from Chiang Mai, Thailand, while the USDA database (Farr & Rossman, 2016) currently lists about 80 species recorded worldwide. Since 2015, close to ten new species have been introduced from Thailand with complete morphological and phylogenetic data. All species currently recorded are listed in Table 3.2.

Table 3.1 Microfungi recorded on *Axonopus compressus* and *Imperata cylindrica* from Thailand

Host species	Fungal species (genus)	Study
<i>Axonopus compressus</i>	<i>Cladosporium tenuissimum</i> Cooke	This study
	<i>Pestalotiopsis compressa</i> Goonas. & K.D. Hyde	This study
	<i>Phaeosphaeria musae</i> Sawada	This study
	<i>Sulcosporium thailandicum</i> Phook. & K.D. Hyde	Ariyawansa et al. (2015)
	<i>Striaticonidium synnematum</i> L. Lombard & Crous	This study
<i>Imperata cylindrica</i>	<i>Alfaria</i> sp.	This study
	<i>Fusarium</i> sp.	
	<i>Cochliobolus setariae</i> (S. Ito & Kurib.) Drechsler ex Dastur as ' <i>Bipolaris setariae</i> '	Marin-Felix et al. (2017)
	<i>Cladosporium tenuissimum</i> Cooke	This study
	<i>Curvularia variabilis</i> Y. Marín, Senwanna & Crous	Marin-Felix et al. (2017)
	<i>Ectophoma pomi</i> (A.S. Horne) Valenz.-Lopez, Cano, Crous, Guarro & Stchigel	This study
	<i>Puccinia rufipes</i> Dietel	Lorsuwan et al. (1984), Giatgong (1980)
	<i>Stagonospora imperaticola</i> Phukhams., Thambug. & K.D. Hyde	Thambugala et al. (2017)

Table 3.2 List of microfungi recorded on *Thysanolaena latifolia* globally

Species	Recorded from	References
<i>Annellophragmia coonoorensis</i> (Subram.) Subram. 1963	China	Zhuang (2001)
	India	Kapoor and Munjal (1967), Ellis (1971)
	Myanmar	Thaung (2006), Thaung (2008)
<i>Apiospora montagnei</i> Sacc. 1875	Myanmar	Thaung (2008)
<i>Apiospora</i> sp.	Thailand	This study
<i>Arthrinium arundinis</i> (Corda) Dyko & B. Sutton	Myanmar	Thaung (2008)
<i>Arthrinium</i> sp.	Thailand	This study
<i>Arthrobotryum coonoorens</i> Subram. 1955	India	Subramanian (1955), Salam and Rao (1957)
<i>Astrosphaeriella stellata</i> (Pat.) Sacc. 1928	Hong Kong	Lu et al. (2000), Wong and Hyde (2001)
<i>Bipolaris coicis</i> (Y. Nisik.) Shoemaker	Papua New Guinea	Sivanesan (1987)
<i>Botryosphaeria</i> sp.	Thailand	This study
<i>Cacumisporium</i> sp.	Malaysia	Johnston (1960)
<i>Cercospora</i> sp.	Mauritius	Wiehe (1948), Orieux and Felix (1968)
<i>Cochliobolus nisikadoi</i> (Tsuda, Ueyama & Nishih.) Alcorn 1983	Papua New Guinea	Sivanesan (1987)
<i>Diaporthe</i> sp.	Hong Kong	Lu et al. (2000), Wong and Hyde (2001)

Table 3.2 (continued)

Species	Recorded from	References
<i>Diaporthe yunnanensis</i> Y.H. Gao & L. Cai	Thailand	This study
<i>Dictyochaeta simplex</i>	Hong Kong	Lu et al. (2000), Wong and Hyde (2001), Zhuang (2001)
<i>Dinemasporium maximum</i>	India	Shahezaad et al (2011)
<i>Glonium</i> sp.	Hong Kong	Lu et al. (2000), Wong & Hyde (2001), Zhuang (2001)
<i>Helminthosporium sativum</i> Pammel, C.M. King & Bakke 1910	India	Misra and Prakash (1972)
<i>Janetia synnematos</i> Sivan. & W.H. Hsieh 1990	Hong Kong	Zhou et al. (1999), Lu et al. (2000), Wong and Hyde (2001), Zhuang (2001)
<i>Lasiodiplodia pseudotheobromae</i> A.J.L. Phillips, A. Alves & Crous	Thailand	This study
<i>Leptostromella thysanolaenae</i> Syd. & P. Syd. 1917	Phillippines	Teodoro (1937)
<i>Massariothea thysanolaenae</i> Thambugala & K.D. Hyde 2018	Thailand	Thambugala et al. (2018)
<i>Meliola andropogonis</i> F. Stevens & A. Roldán 1935	China	Zhuang (2001)

Table 3.2 (continued)

Species	Recorded from	References
<i>Meliola arundinis</i> Pat. 1897	China	Zhuang (2001)
	Myanmar	Thaung (2006)
<i>Meliola panici</i> Earle 1901	Phillippines	Teodoro (1937)
<i>Meliola panici</i> var. <i>panici</i> Earle 1901	China	Zhuang (2001)
<i>Microthyrium</i> sp.	Hong Kong	Lu et al. (2000), Wong and Hyde (2001)
<i>Multiseptospora thailandica</i> Phookamsak & K.D. Hyde 2015	Thailand	Liu et al. (2015)
<i>Neopestalotiopsis asiatica</i> (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous	Thailand	This study
<i>Niptera excelsior</i> (P. Karst.) Dennis 1972	Hong Kong	Lu et al. (2000), Wong and Hyde (2001)
<i>Ommatomyces</i> sp.	Hong Kong	Lu et al. (2000)
<i>Ommatomyces terrestris</i> You Z. Wang, M.K.M. Wong & K.D. Hyde 2000	Hong Kong	Wong and Hyde (2001)
<i>Paraconiothyrium thysanolaenae</i> Phookamsak, Chethana & K.D. Hyde 2015	Thailand	Liu et al. (2015)
<i>Paraphaeosphaeria thysanolaenae</i> Goonas. & K.D. Hyde	Thailand	This study

Table 3.2 (continued)

Species	Recorded from	References
<i>Petrakia</i> sp.	Hong Kong	Lu et al. (2000), Wong and Hyde (2001), Zhuang (2001)
<i>Phaeosphaeria thysanolaenicola</i>	Thailand	Phookamsak et al. (2014)
<i>Phyllachora thysanolaenae</i> Tamakaew, Maharachch., K.D. Hyde & Cheew. 2017	Thailand	Tamakaew et al. (2017)
<i>Pseudotrichia rubriostiolata</i> Phookamsak & K.D. Hyde 2015	Thailand	Liu et al. (2015)
<i>Pseudotrichia thailandica</i> Phookamsak & K.D. Hyde 2015	Thailand	Liu et al. (2015)
<i>Psiloglonium colihuae</i> (Lorenzo & Messuti) E. Boehm 2009	Thailand	Liu et al. (2015)
<i>Psiloglonium multiseptatum</i> Phookamsak & K.D. Hyde 2015	Thailand	Liu et al. (2015)
<i>Puccinia miscanthi</i> Miura 1928	China	Zhuang (2001), Wang and Zhuang (1998)
<i>Rhinocladiella</i> sp.	Hong Kong	Lu et al. (2000), Wong and Hyde (2001), Zhuang (2001)
<i>Rosellinia sublimbata</i> (Durieu & Mont.) Pass. 1879	Myanmar	Thaung (2007)
<i>Sporidesmium</i> sp.	Hong Kong	Lu et al. (2000), Wong and Hyde (2001), Zhuang (2001)

Table 3.2 (continued)

Species	Recorded from	References
<i>Stachybotrys kampalensis</i> Hansf. 1943	Hong Kong	Lu et al. (2000), Wong and Hyde (2001), Zhuang (2001)
<i>Terriera</i> sp.	Hong Kong	Lu et al. (2000), Wong and Hyde (2001)
<i>Tetraploa aristata</i> Berk. & Broome 1850	Hong Kong	Lu et al. (2000), Wong and Hyde (2001), Zhuang (2001)
	Myanmar	Thaung (2006)
<i>Tetraposporium ravenelii</i> (Farl. ex Cooke) S. Hughes 1951	Hong Kong	Lu et al. (2000), Wong and Hyde (2001), Thaung (2006)
<i>Uredo</i> sp.	Papua New Guinea	Shaw (1984)
<i>Verticillium</i> sp.	Hong Kong	Lu et al. (2000), Wong and Hyde (2001)
<i>Xenosporium</i> sp.	Myanmar	Thaung (2006)

3.1.3 *Zea Mays*

Zea mays (Maize) is an annual grass crop in Poaceae widely cultivated for food and energy and is known to be one of the top three cereals in the world, the other two being rice and wheat (<http://eol.org>). It's commonly grown in Thailand and is susceptible to many smut and seedling blight/ root rot diseases (Giatgong, 1980; Shivas et al., 2007). A few recent studies prevail on what kind of saprobic fungi occur on this host.

3.2 Materials and Methods

3.2.1 Sample Collection, Isolation and Morphological Study

Fresh samples were collected from different sites in northern Thailand (Chiang Mai, Chiang Rai, Nan, Phayao provinces). Specimens were brought to the laboratory in clean paper bags or zip lock bags and examined with a Motic SMZ 168 stereomicroscope. For ascomycetes and coelomycetes, free hand sections of the fruiting body structures were mounted in water on slides for microscopic studies and photomicrography. For hyphomycetes, fungal structures were picked and added to water on slide using a needle. Indian ink staining was used to reveal the presence of a gelatinous sheath around the ascospores. Cotton blue staining was used for observing septation and Congo red for observing internal structures. The species were examined with a Nikon ECLIPSE 80i compound light microscope and photographed with a Canon 750D digital camera fitted to the compound microscope. Measurements will be made with the Tarosoft (R) Image Frame Work program and images used for figures were processed with Adobe Photoshop CS6 Extended version 13.1.2 x32 (Adobe Systems, USA).

Ascospores or conidia were isolated when they could be found. Fruiting bodies were cut through horizontally and the contents transferred to a drop of sterile water on a flamed microscopic slide. The spore suspension was spread over a few square centimeters of a petri plate containing water agar (WA) or potato dextrose agar (PDA) and incubated at room temperature overnight. The next day, individual germinating spores were transferred to fresh PDA following the methods as detailed in Senanayake et al. (2020). Germinating spores were located by examining the PDA plate with a stereomicroscope and aseptically transferred to fresh PDA plates. After incubating at room temperature, colonies were observed for their growth. Hyphal tips were cut and transferred to agar slants in 1.5 ml microcentrifuge tubes and sterile media plates, for depositing in culture collections. After 6–12 weeks, cultures on media were examined for asexual structures. Facesoffungi numbers and Index Fungorum (2022) numbers for novel species were obtained as detailed in Jayasiri et al. (2015) and Kirk et al. (2008).

The holotypes were deposited at the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and culture isolates at Mae Fah Luang University Culture Collection (MFLUCC). Duplicate cultures would be deposited at other local and international culture collections.

3.2.2 DNA Extraction, PCR Amplification and Sequencing

Total fungal DNA was extracted from fresh fungal mycelium grown for 4–6 weeks on PDA at room temperature using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux, P.R. China) following the instructions of the manufacturer. Polymerase chain reaction (PCR) was carried out using four to seven partial gene portions mentioned below. The amplifications were performed in 25 μ L of PCR mixtures containing 9.5 μ L ddH₂O, 12.5 μ L PCR Master Mix (TIANGEN Co., China), 1 μ L of DNA template, 1 μ L of each primer (10 μ M).

The primers pairs for each gene region and their amplification conditions given in Table 3.3 were used with some modifications for certain species. The PCR amplification was confirmed on 1 % agarose electrophoresis gels stained with 4S Green Stain. The amplified PCR fragments were sent to several sequencing companies in China and Korea.

3.2.3 Phylogenetic analyses

Sequences generated from this study were analyzed with similar sequences obtained from GenBank based on blast searches and recently published data. Single gene datasets and multi-gene datasets were aligned with MAFFT (Katoh & Standley, 2013, <http://mafft.cbrc.jp/alignment/server/>;2016) and manually improved with BioEdit v.7.2.5 (Hall, 2004). Maximum-parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) analyses of the combined aligned dataset was used.

Parsimony analysis was carried with the heuristic search option in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford, 2002), with the following parameter settings: characters unordered with equal weight, random taxon addition, branch swapping with tree bisection-reconnection (TBR) algorithm, branches collapsing if the maximum branch length will be zero. Alignment gaps were treated as missing characters in the analysis of the combine data set, where they appeared in relatively conserved regions. Parsimony bootstrap analyses was

performed using the full heuristic search option, random stepwise addition, and 1000 replicates, with maxtrees set at 1000. Descriptive tree statistics for parsimony (Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI] calculated for trees generated under different optimality criteria. The Kishino-Hasegawa tests (Kishino & Hasegawa, 1989) was performed to determine whether the trees inferred under different optimality criteria are meaningfully different.

Evolutionary models for phylogenetic analyses were selected independently for each locus using MrModeltest v. 3.7 (Posada & Crandall, 1998) under the Akaike Information Criterion (AIC) implemented in both PAUP v. 4.0b10 and MrBayes v. 3. Phylogenetic reconstructions of combined gene trees will be performed using both Bayesian Inference (BI) and Maximum Likelihood (ML) criteria.

Maximum-likelihood (ML) analysis was performed in RAxML (Stamatakis, 2014) implemented in raxmlGUI v.0.9b2 (Silvestro & Michalak, 2010), employing GTR + I + G models of evolution settings of the program and Bootstrap support obtained by running 1000 to 5000 pseudo replicates.

Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronqvist, 2001) to evaluate Posterior probabilities (BYPP) (Rannala & Yang, 1996; Zhaxybayeva & Gogarten, 2002) by Markov Chain Monte Carlo sampling (MCMC). Two parallel runs were conducted, using the default settings, but with the following adjustments: Six simultaneous Markov chains were run for 1,000,000 generations and trees sampled every 100th generation and 10,000 trees were obtained. The first 20% of trees, representing the burn-in phase of the analyses was discarded. The remaining 80% trees were used to calculate PP in the majority rule consensus tree.

Phylograms were visualized with FigTree v1.4.0 program (Rambaut, 2012) and reorganized in Microsoft power point (2007) and Adobe Illustrator® CS5 (Version 15.0.0, Adobe®, San Jose, CA). Sequences derived in this study were deposited in GenBank, and the alignments in TreeBASE (www.treebase.org).

Table 3.3 Partial gene regions, primers and PCR amplification conditions used in this study

Gene	Primer		PCR protocol					Reference
	FW	RV	Initial Denaturation	Denaturation	Annealing	Extension	Final extension	
Internal transcribed spacer (ITS)	ITS5	ITS4	94°C, 3 min 1 cycle	95°C, 30 sec	56°C, 50 sec 37 cycles	72°C, 1 min	72°C, 10 min 1 cycle	White et al. (1990)
Large subunit (LSU)	LROR	LR5	94°C, 3 min 1 cycle	95°C, 30 sec	56°C, 50 sec 37 cycles	72°C, 1 min	72°C, 10 min 1 cycle	Rehner and Samuels (1994), Vilgalys and Hester (1990)
Small subunit (SSU)	NS1	NS4	94°C, 3 min 1 cycle	94°C, 30 sec	53°C, 50 sec 37 cycles	72°C, 1 min	72°C, 10 min 1 cycle	White et al. (1990)
Elongation factor-1 alpha (EF 1- α)	EF1-728F or 983F	EF1- 986R or 2218R	95°C, 10 min 1 cycle	94°C, 30 sec	55°C, 30 sec 37 cycles	72°C, 1 min	72°C, 10 min 1 cycle	Carbone and Kohn (1999)
Second largest subunit of RNA Polymerase II (RBP2)	fRPB2-5F	fRPB2-7cR	95°C, 5 min 1 cycle	95°C, 1 min	55°C, 2 min 37 cycles	72°C, 2 min	72°C, 10 min 1 cycle	Liu et al. (1999)
Beta (β) tubulin (TUB)	Bt2a	Bt2b	95°C, 10 min 1 cycle	94°C, 30 sec	55°C, 50 sec 35 cycles	72°C, 1 min	72°C, 10 min 1 cycle	Glass and Donaldson (1995)
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	gpd1	gpd2	96°C, 2 min 1 cycle	96°C, 1 min	52°C, 1 min 35 cycles	72°C, 45 sec	72°C, 10 min 1 cycle	Berbee et al. (1999), Manamgoda et al. (2012)
Actin (ACT)	ACT-512F	ACT-783R	95°C, 8 min 1 cycle	95°C, 15 sec	53°C, 30 sec 35 cycles	72°C, 1 min	72°C, 5 min 1 cycle	Carbone and Kohn (1999)

3.3 Results

3.3.1 Phylogeny and taxonomy

The classification of fungi follows the Outline of fungi and fungus-like taxa by Wijayawardene et al. (2020).

Ascomycota

Dothideomycetes sensu O.E. Erikss & Winka

Botryosphaeriales C.L. Schoch, Crous & Shoemaker

Botryosphaeriaceae Theiss. & Syd.

Lasiodiplodia Ellis & Everh.

Lasiodiplodia pseudotheobromae A.J.L. Phillips, A. Alves & Crous, Fungal Diversity 28: 8 (2008)

Index Fungorum number: IF510941; Facesoffungi number: FoF00166, Figure 3.2

Saprobic on culms of *Thysanolaena latifolia* appearing as dark brown to black lesions, surrounded by blackened areas on the inner side of the culm. **Sexual morph:** not observed. **Asexual morph:** *Conidiomata* 150–350 µm diam., 150–200 µm high (n = 20), semi-immersed or immersed in the host surface, becoming erumpent when mature, solitary, gregarious or confluent, globose to subglobose, conical, short neck, black. *Peridium* up to 25–40 µm wide, consisting of brown, cells of *textura angularis*, with thinner, layers of pale brown to hyaline cells lining the inner walls. *Ostiole* 30–55 µm diam., mostly centrally located, papillate. *Paraphyses* 2–5 µm wide, unbranched, aseptate, hyaline, cylindrical. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 7–10 µm long, 2–2.5 µm wide, hyaline, subglobose or cylindrical. *Conidia* 20–35 × 13–16 µm ($\bar{x}$ = 25 × 14 µm, n = 30), subglobose to oval, rounded at the apex and base, hyaline, aseptate, thick-walled, guttulate, becoming 1-septate, reddish-brown with the appearance of longitudinal striations when mature conidia.

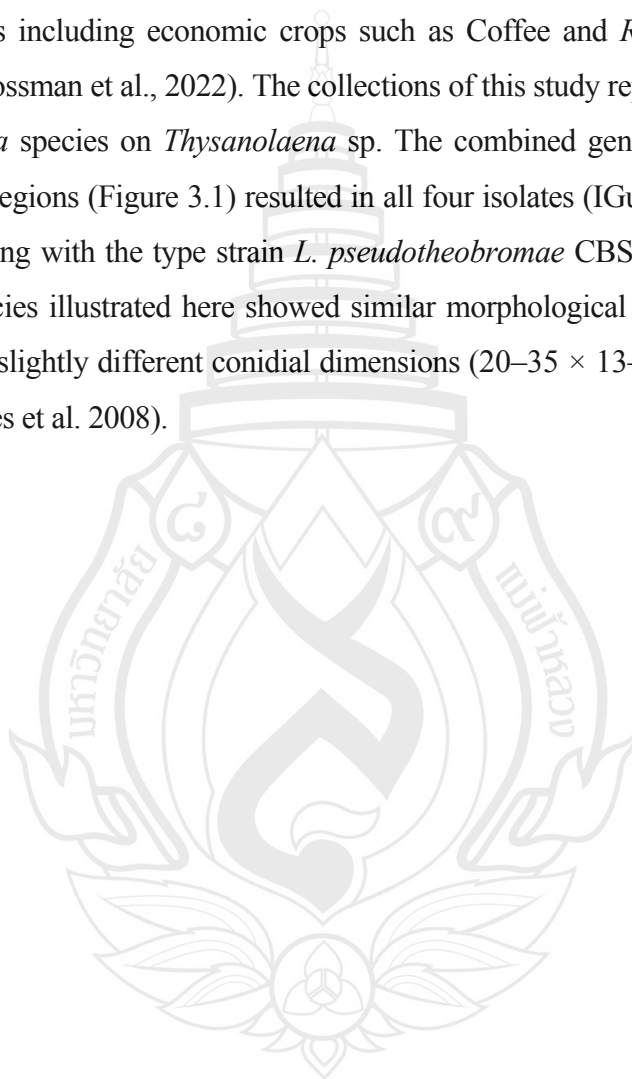
Culture characteristics: Colonies reaching 3.5 cm diam. after 7 days at room temperature, circular, with entire margin, sparse, initially white becoming greenish grey and black after 1 week.

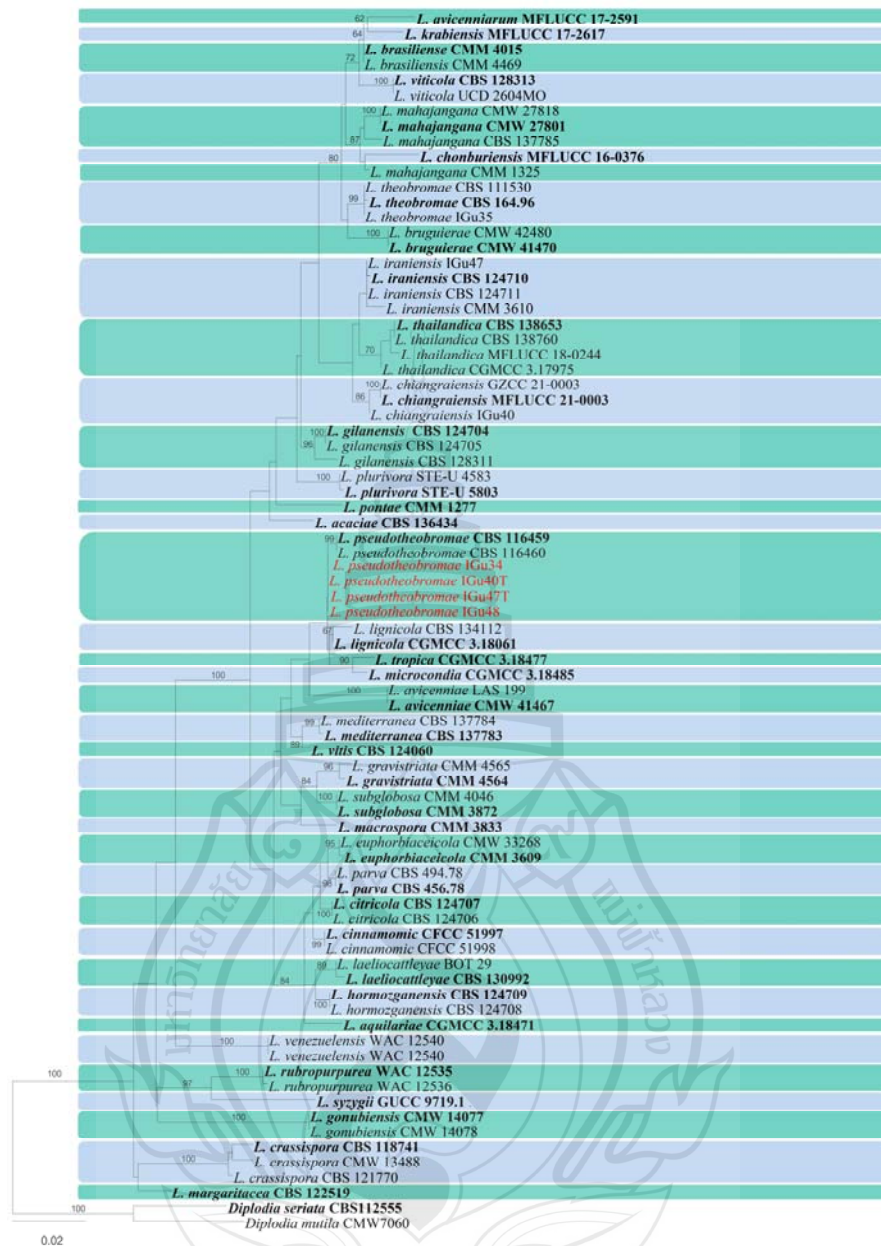
Material examined: Thailand, Chiang Rai province, on culms of *Thysanolaena latifolia*, 20 February 2019, Ishani D. Goonasekara, IGu48T; living culture, IGu48T,

ibid., on culms of *T. latifolia*, 2 February 2017, IGu34; living culture, IGu34, *ibid.*, Phayao province, on culms of *T. latifolia*, 17 July 2017, IGu40T; living culture, IGu40T, *ibid.*, on culms of *T. latifolia*, 13 February 2020, IGu47T; living culture IGu34.

GenBank Accession numbers: IGu34: ITS: ON988078; IGu40T: ITS: ON988082; IGu47T: ITS: ON988083 IGu48: ITS: ON988084.

Notes: *Lasiodiplodia pseudotheobromae* has been recorded from various flowering plants including economic crops such as Coffee and *Rosa* sp. (Alves et al., 2008; Farr & Rossman et al., 2022). The collections of this study represent the first record of *Lasiodiplodia* species on *Thysanolaena* sp. The combined gene analysis of the ITS, TEF and TUB regions (Figure 3.1) resulted in all four isolates (IGu34, IGu40T, IGu47T, IGu48T) grouping with the type strain *L. pseudotheobromae* CBS 116459 (Alves et al., 2008). The species illustrated here showed similar morphological characters to the type specimen, with slightly different conidial dimensions ($20\text{--}35 \times 13\text{--}16 \mu\text{m}$ vs. $23.5\text{--}32 \times 14\text{--}18 \mu\text{m}$, Alves et al. 2008).





Note Sequences of 80 taxa representing *Lasiodiplodia* in *Botryosphaeriaceae*. The tree was rooted to *Diplodia mutila* CMW 7060 and *D. seriata* CBS 112555. The scale bar indicates 0.02 nucleotide changes per site.

Figure 3.1 Phylogram generated from RAxML analysis based on combined ITS, TEF and TUB sequence data of *Lasiodiplodia* isolates



Note a Host in nature. b Appearance of conidiomata on host surface. c Section of conidioma. d Part of peridium. e Conidia arising from conidiogenous cells. f-h Conidia. Scale bars: c, e-h = 50 μm , d = 25 μm .

Figure 3.2 *Lasiodiplodia pseudotheobromae* (IGu48T, new host record)

Capnodiales Woron.

Cladosporiaceae Chalm. & R.G. Archibald

Cladosporium Link

Cladosporium tenuissimum Cooke, Grevillea 6: 140 (1878)

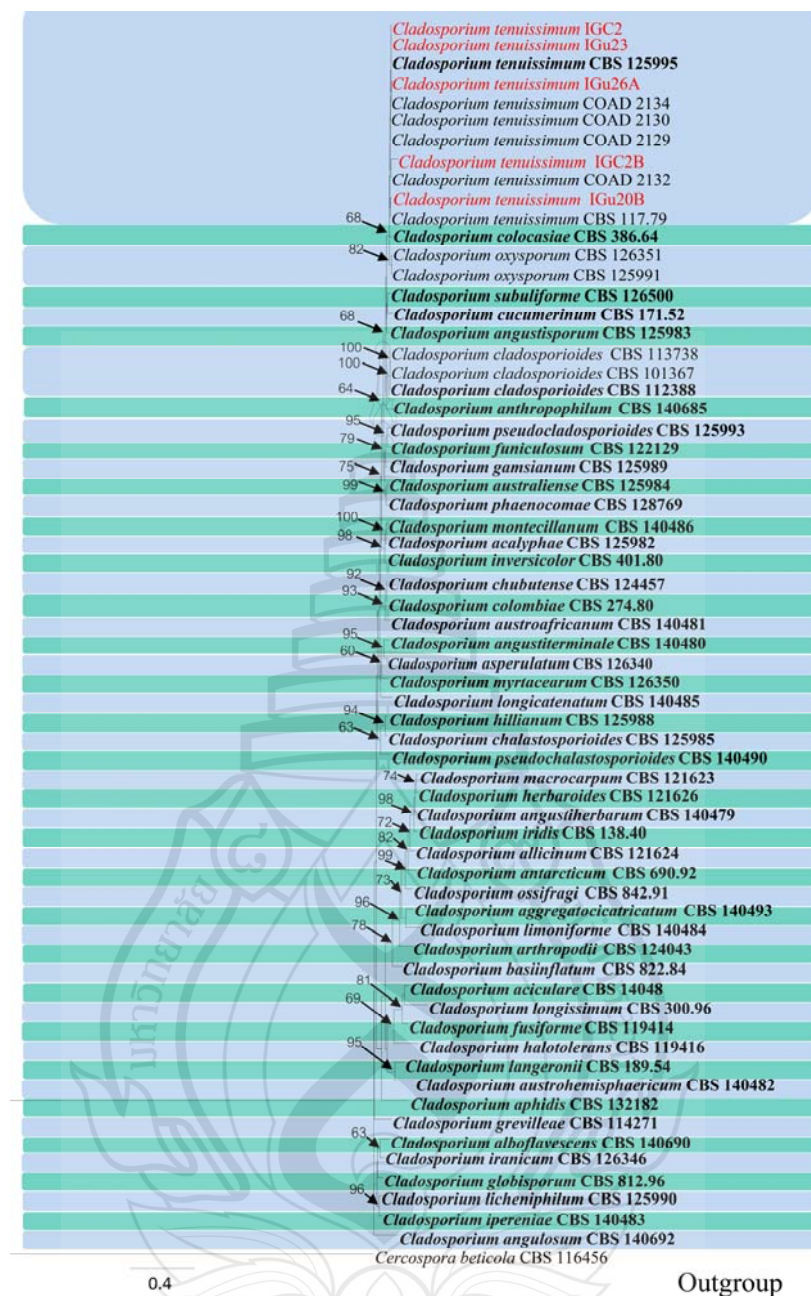
Index Fungorum number: IF145672; Facesoffungi number: FoF09313, Figure 3.4

Saprobic on dead culms and leaves of *Axonopus compressus*. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous, *Colonies* greyish to dark brown, confluent, conidiophores erect, solitary. *Mycelium* superficial, unbranched, 1–5 μm wide, septate, light brown to brown, with thick walls. *Conidiophores* solitary, erect, straight or slightly curved, unbranched, $45\text{--}85 \times 3\text{--}4 \mu\text{m}$ ($\bar{x} = 50 \times 3.5 \mu\text{m}$, $n = 40$), wider and darker at the base, gradually attenuated towards the apex, becoming lighter, septate, non-constricted at the septa, brown to dark brown, smooth. *Conidiogenous cells* $10\text{--}15 \times 3\text{--}4 \mu\text{m}$ ($\bar{x} = 12 \times 3.5 \mu\text{m}$, $n = 15$), integrated, terminal, subcylindrical, sometimes subglobose, light brown to hyaline, smooth. *Ramoconidia* not observed. *Conidia* $2\text{--}7.5 \times 2\text{--}3.5 \mu\text{m}$ ($\bar{x} = 4.5 \times 3 \mu\text{m}$, $n = 40$), light brown to olivaceous, aseptate, globose, subglobose, ellipsoid-ovoid, smooth-walled.

Culture characteristics: *Colonies* on PDA, 2.5 cm diam. after three weeks, colonies from above: dense, circular, raised or convex, surface smooth with crenate edge, growing as tufts, dark to brown in the center, becoming olivaceous towards the margin, reverse, dark brown to olivaceous green.

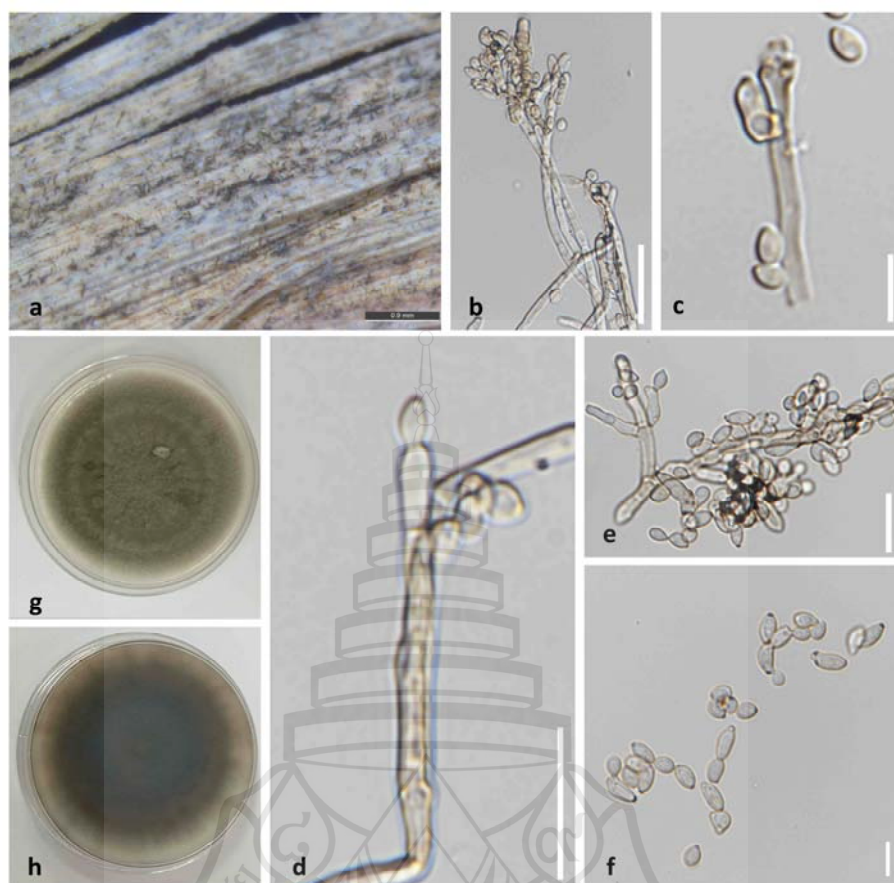
Material examined: Thailand, Chiang Rai province, on dead culms and leaves of *Axonopus compressus*, 17 July 2017, Ishani D. Goonasekara, IGu20B; living culture, IGu20B, *ibid.*, on dead leaves of *A. compressus*, 22 July 2017, IGu23; living culture, IGu23, *ibid.*, on dead culms of *Imperata cylindrica*, 7 Feb 2020, IGu26A; living culture, IGu26A, *ibid.*, on dead leaves of *Zea mays*, 13 February 2017, IGC2B; living culture, IGC2B, *ibid.*, Chiang Mai, on dead leaves and culms of *Zea mays*, 7 July 2020, IGC2B; living culture, IGC2B.

GenBank Accession numbers: IGu20B: ITS: ON997064, LSU: ON997069; IGu23: ITS: ON997065; IGu26A: ITS: ON997066; IGC2: ITS: ON997067; IGC2B: ITS: ON997068, LSU: ON997070.



Note Sequences of 65 taxa, representing *Cladosporium* in *Cladosporiaceae*. The tree was rooted to *Cercospora beticola* (CBS 116456). The scale bar indicates 0.4 nucleotide changes per site.

Figure 3.3 Phylogram generated from RAxML analysis based on combined ITS, TEF and ACT sequence data of *Cladosporium* isolates



Note a Appearance on host. b-e Conidiophores and conidia produced from conidiogenous cells. f Conidia. g Colony from above. h Colony in reverse. Scale bars: b = 100 μ m, c, d = 50 μ m, e, f = 5 μ m.

Figure 3.4 *Cladosporium tenuissimum* (IGu20B, new host record)

Notes: *Cladosporium* was introduced by Link (1816) and its members are distributed worldwide (Heuchert et al., 2005; Bensch et al., 2018; Tibpromma et al., 2019). The genus consists of three major species complexes: *C. herbarum*, *C. sphaerospermum* and *C. cladosporioides* (Marin-Felix et al., 2017). The phylogenetic analysis conducted using ITS, TEF and ACT (Figure 3.3) regions resulted in the collections from this study (IGu20B, IGu23, IGu26A, IGC2 and IGC2B), clustering within the group of the type strain of *C. tenuissimum* CBS 125995. The compared morphological characters were consistent with the description of *C. tenuissimum*.

Therefore, the collections IGu 20B and IGC2 are reported as new host records on *A. compressus* and *Z. mays* respectively.

Pleosporales Luttrell ex M.E. Barr

Didymosphaeriaceae Munk

Paraphaeosphaeria O.E. Erikss.

Paraphaeosphaeria thysanolaenae Goonas. & K.D. Hyde, *sp. nov.*, Figure 3.6

Etymology: The specific epithet “*thysanolaenae*” is in reference to the host from which the fungus was isolated.

Holotype: IGu41

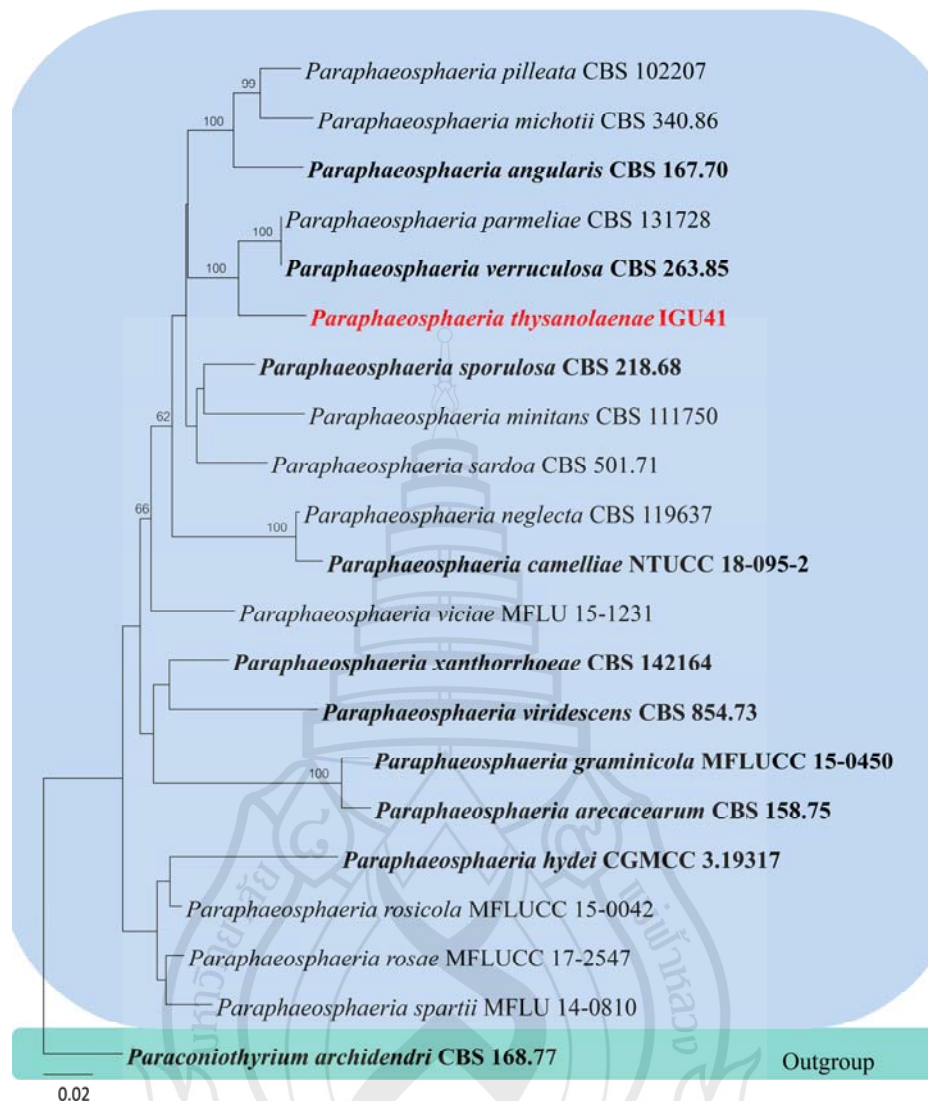
Saprobic on grasses. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* up to 1-1.3 mm wide on host, pycnidial, mostly solitary, gregarious, or in groups, scattered, semi-immersed to erumpent, uni-loculate, globose to subglobose. Locules (50–)40–180 × 50–200 µm ($\bar{x}$ = 190 × 150 µm, n = 10), globose to subglobose, ostiolate. *Conidiomatal wall* 10–15 µm wide, comprising several layers of lightly pigmented to brown, thick-walled, cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 2–2.5(–5) × 3.5–5 µm ($\bar{x}$ = 2.3 × 3.8 µm, n = 15), hyaline, smooth, ampulliform to lageniform, lining the inside layer of conidiomatal cavity. *Conidia* 4.5–5.5 × 3.5–4 µm ($\bar{x}$ = 5 × 3.2 µm, n = 40), ellipsoidal to cylindrical, initially hyaline, becoming darker brown, thin-walled, smooth, aseptate, with 1–2 guttules.

Culture characteristics: Colonies growing on PDA, reaching a diameter of 30 mm after 7 d at room temperature, smooth surfaced, flat, with entire edge, white at the center becoming greyish towards margins, moderately dense, circular; reverse grey.

Material examined: Thailand, Chiang Rai Province, near Mae Fah Luang premises, on dead culms of *Thysanolaena latifolia*., 02 February 2017, Ishani D. Goonasekara IGu41 (**holotype**); ex-type living culture IGu41.

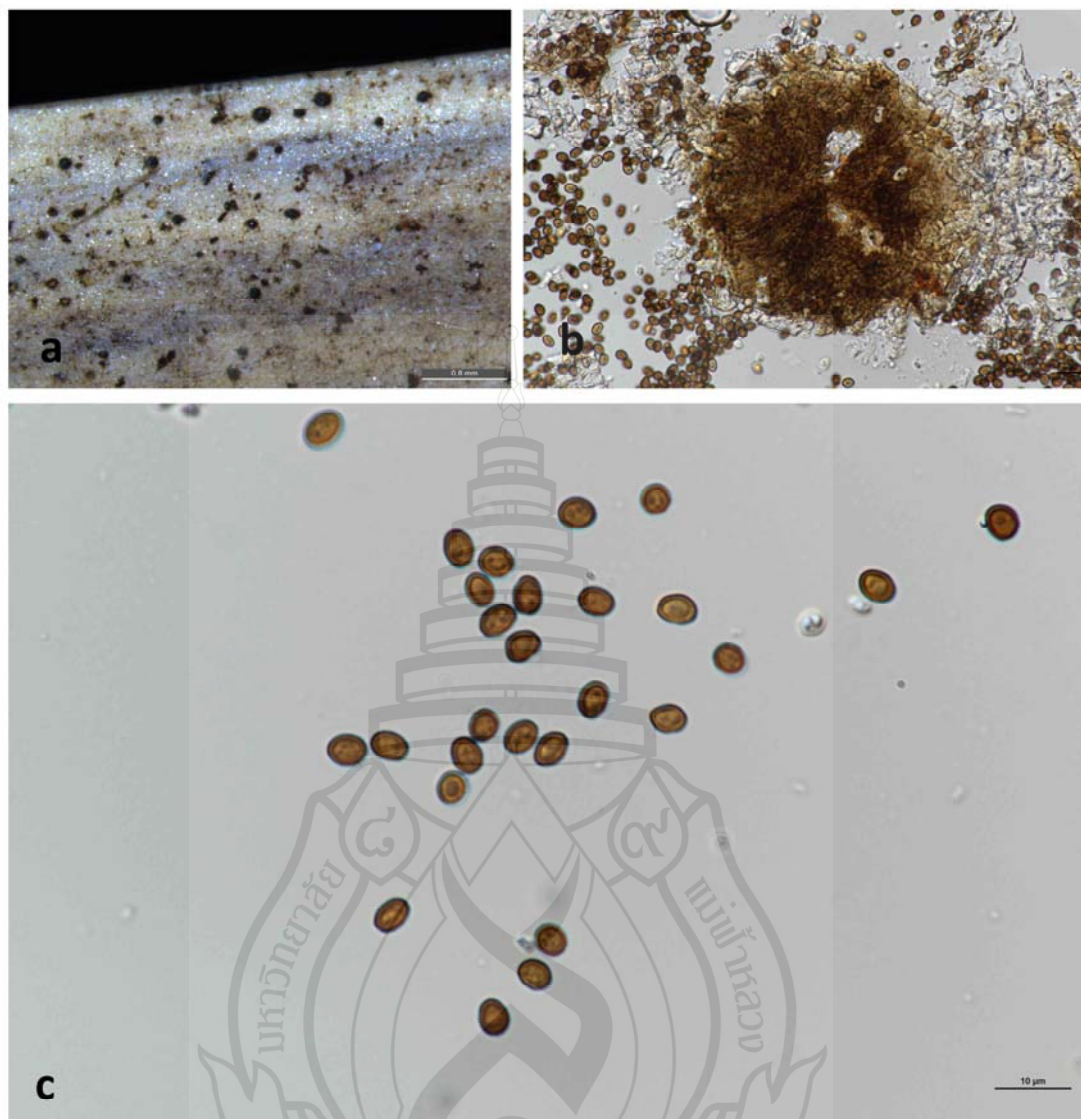
GenBank Accession numbers: ITS: ON987218; LSU: ON987218.

Notes: The *Paraphaeosphaeria* isolate generated in this study formed a clade together with *P. verruculosa* and *P. parmeliae* with strong bootstrap support (100% ML, Figure 3.5). This species closely resembles *P. graminicola* the first record of this genus on Poaceae and which was also collected in Thailand (Thambugala et al., 2017). It is distinguished by its brown conidia, conidiomata and conidial dimensions.



Note Sequences from twenty-one taxa, representing *Paraphaeosphaeriopsis* in *Didymellaceae*. The tree was rooted to *Paraconiothyrium archidendri* (CBS 168.77). The scale bar indicates 0.02 nucleotide changes per site.

Figure 3.5 Phylogram generated from RAxML analysis based on combined ITS and LSU sequence data of *Paraphaeosphaeriopsis* isolates



Note a Colonies on host. b Squash mount of conidioma. c Conidia. Scale bars: b, c = 10 μm .

Figure 3.6 *Paraphaeosphaeria thysanolaenae* sp. nov. (IGu41, holotype)

Phaeosphaeriaceae M.E. Barr

Phaeosphaeria musae Sawada, Special Publication College of Agriculture, National Taiwan University 8: 66 (1959)

Index Fungorum number: IF336200; Facesoffungi number: FoF00262, Figure 3.8

Saprobic on *Axonopus compressus*. **Sexual morph:** *Ascomata* 100–150 μm high, 110–140 μm diam., solitary, scattered, immersed, visible as slightly raised,

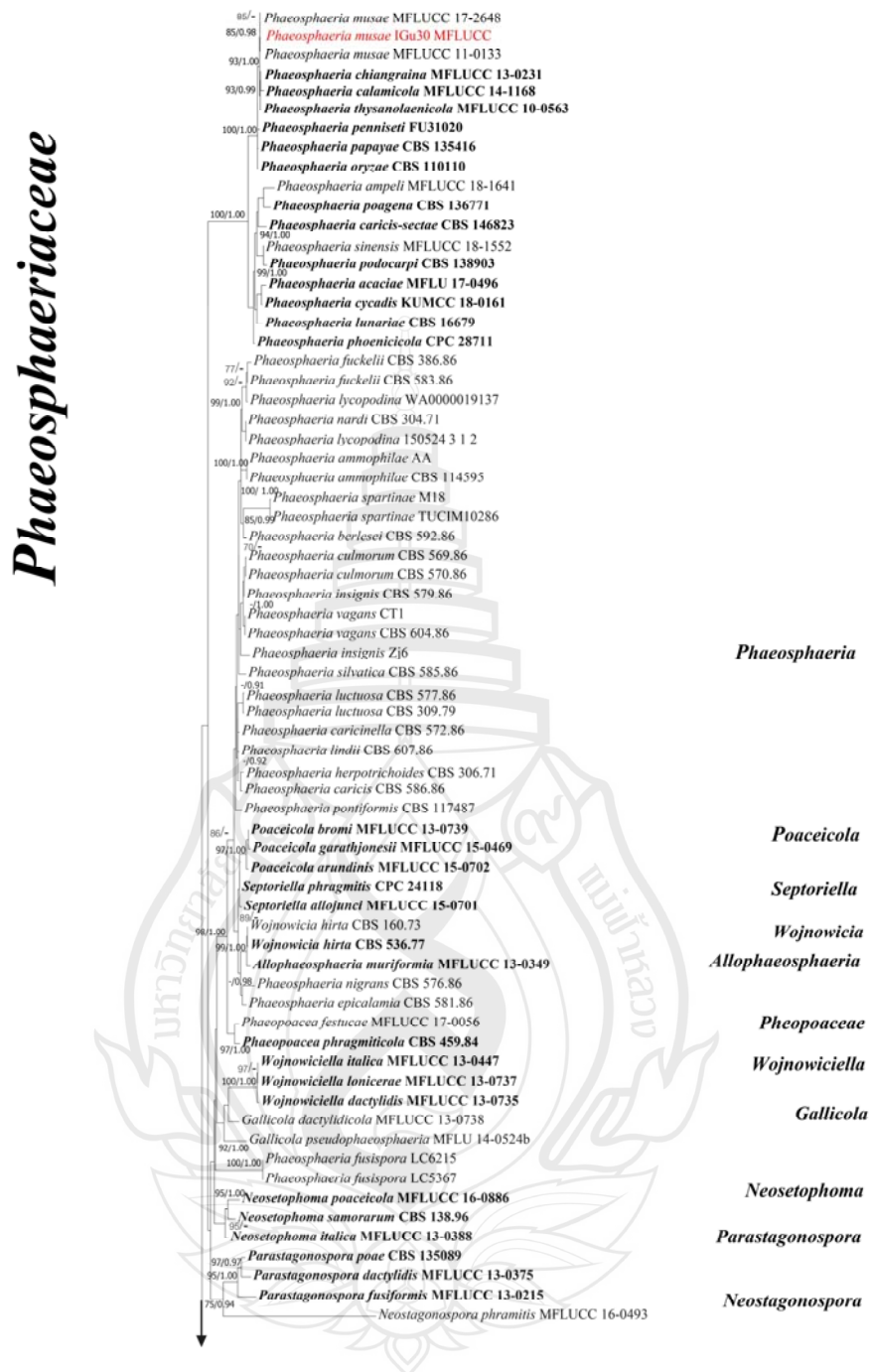
small black dots on host surface, uniloculate, dark brown, globose to subglobose, central ostiole with minute papilla. *Peridium* 8–15 μm wide, thin-walled, slightly thick at the apex, composed of 1–3 layers of brown to dark brown, multi-layered pseudoparenchymatous cells and elongated, cells of *textura angularis* towards the opening. *Hamathecium* composed of numerous, 2.5–3 μm wide, filiform, broad cellular pseudoparaphyses, with distinct septa. *Asci* (20–)60–80(–90) $\times$ (8–)10–15 μm ($\bar{x}$ = 65 $\times$ 13.5 μm , n = 20), 8-spored, bitunicate, fissitunicate, broadly cylindric-clavate to clavate, with acute ends, apically rounded. *Ascospores* (15–)25–35(–38) $\times$ 4–7.5 μm ($\bar{x}$ = 29.5 $\times$ 6.5 μm , n =40), overlapping 2–3-seriate, phragmosporous, fusiform, yellowish to dark brown, slightly constricted at the central septa, enlarged at the second cell, slightly curved, rarely straight, rough-walled, surrounded by narrow mucilaginous sheath. **Asexual morph:** Unknown.

Culture characteristics: Colonies growing on PDA, reaching a diameter of 2.5–3 cm after 7 d at room temperature, surface smooth, flat, with entire edge, white at the center becoming greyish towards margins, circular; reverse dark grey.

Material examined: Thailand, Chiang Rai Province, on dead culms of *Axonopus compressus*, 20 February 2017, Ishani D. Goonasekara IGu30; living culture IGu30.

GenBank Accession numbers: ITS: ON987234

Notes: The *Phaeosphaeria* are commonly known pathogens on Poaceae especially on cereal crops such as wheat (Phookamsak et al., 2014) The collection of *Phaeosphaeria musae* in this study is the first record of this species on *Axonopus compressus*. The isolate IGu30 grouped together with two other strains of *P. musae* MFLUCC 17-2648 and MFLUCC 11-0133 (Figure 3.7).



Note Sequences of 127 representative taxa in *Phaeosphaeriaceae* are included in the analyses. The tree was rooted to *Didymella exigua* (CBS 183.55). The scale bar indicates 0.08 nucleotide changes per site.

Figure 3.7 Phylogram generated from RAXML analysis based on combined ITS, LSU, SSU and RPB2 sequence data in *Phaeosphaeriaceae*



Figure 3.7 (continued)

Sordariomycetes O.E. Erikss. & Winka

Amphisphaeriales D. Hawksw. & O.E. Erikss.

Sporocadaceae Corda

Neopestalotiopsis Maharachch., K.D. Hyde & Crous

Neopestalotiopsis asiatica (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, in Maharachchikumbura, Hyde, Groenewald, Xu & Crous, Stud. Mycol. 79: 136 (2014).

Index Fungorum number: IF 809761, Figure 3.9

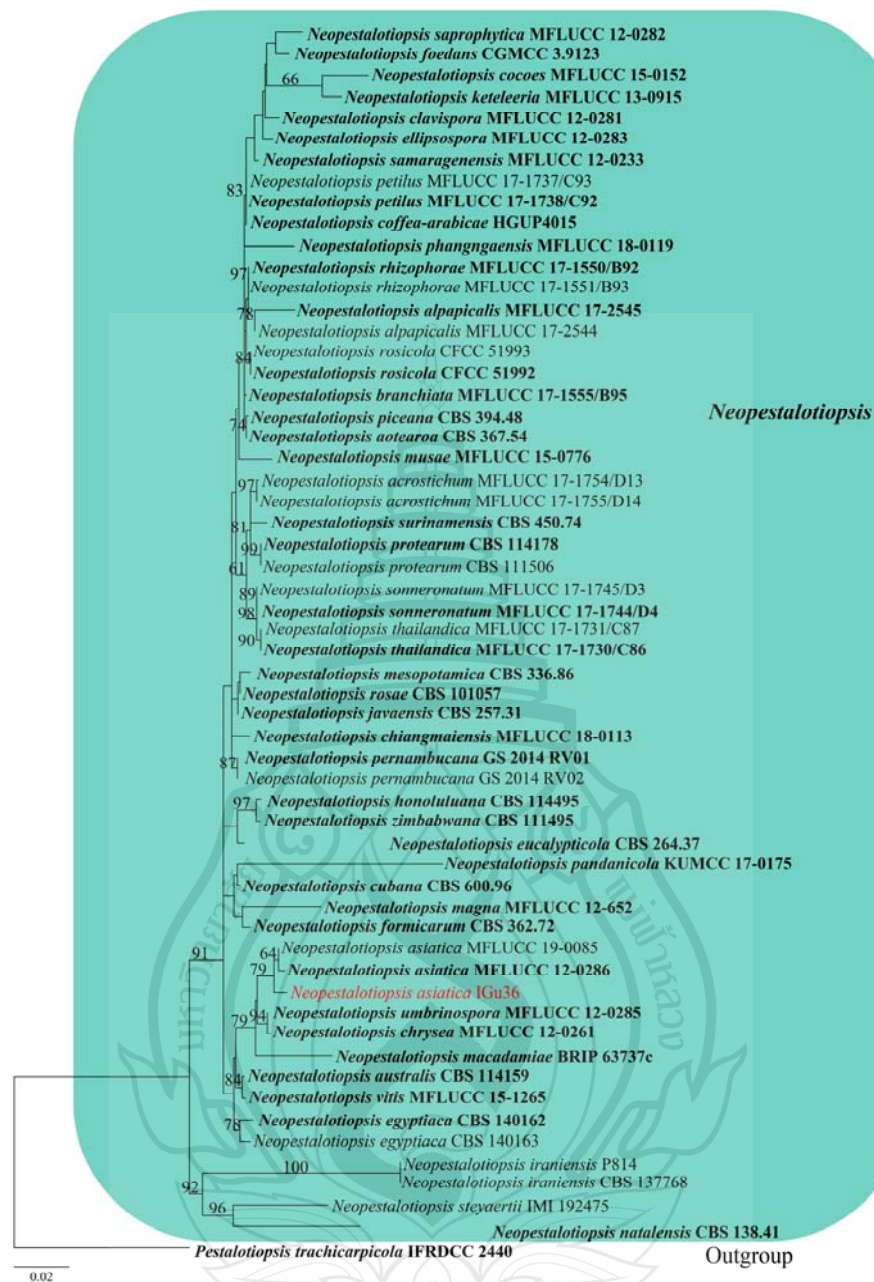
Saprobic on the dead culms of *Thysanolaena latifolia*. **Asexual morph:** *Conidiophores* indistinct. *Conidiogenous cells* hyaline, mostly reduced. *Conidia* 20–25 × 4.5–6.5 µm ($\bar{x}$ = 23 × 5.5 µm), fusiform, straight to slightly curved, 4-septate; basal cell conical, hyaline, thin and verruculose, 3–4.5 µm long ($\bar{x}$ = 3.8 µm); three median cells 13–15 µm long ($\bar{x}$ = 14 µm), dark brown, verruculose, septa and periclinal walls darker than the rest of the cell, versicoloured, second cell from base pale brown, 4–5 µm ($\bar{x}$ = 4.5 µm); third cell darker brown, 4–5 µm ($\bar{x}$ = 4.8 µm); fourth cell darker, 4–5 µm ($\bar{x}$ = 4.7 µm); apical cell 3.5–5 µm long ($\bar{x}$ = 3.35 µm), hyaline, conical to cylindrical, comprising mostly 3 apical appendages, 20–30 µm long ($\bar{x}$ = 25.6 µm), tubular, arising from the apex of the apical cell; basal appendage, 4–8 µm long ($\bar{x}$ = 5.65 µm), filiform.

Culture characteristics: Colonies on PDA reaching 6–8 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined: Thailand, Chiang Rai Province, on dead culms of *Thysanolaena latifolia*, 22 February 2017, Ishani D. Goonasekara IG36; living culture IG36.

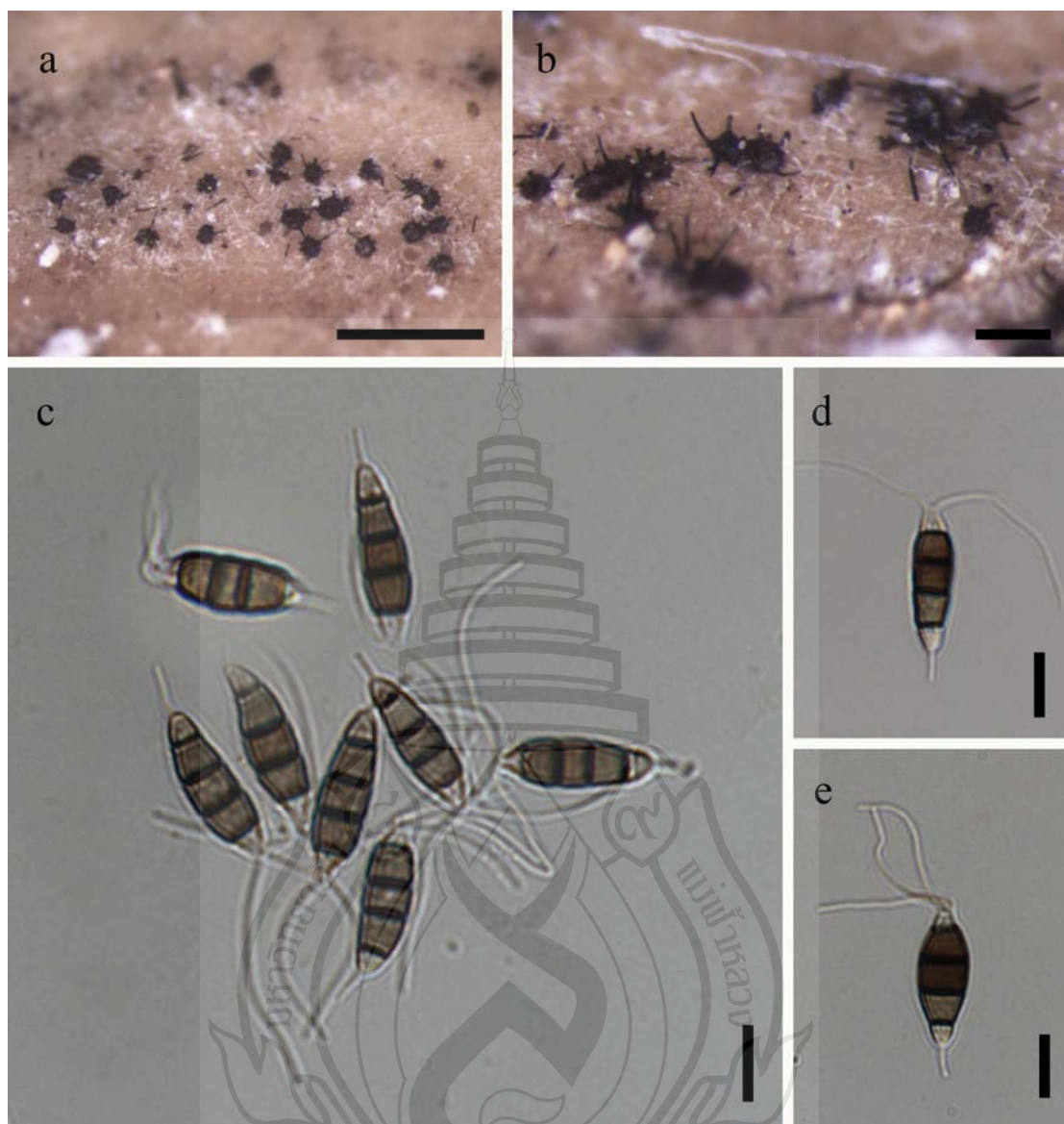
GenBank Accession numbers: ITS: ON986397

Notes: *Neopestalotiopsis asiatica* was introduced from unidentified living leaves in China (Maharachchikumbura et al., 2012). The collection described here is the first host record of this species on *Thysanolaena latifolia*. In our phylogenetic analysis, it groups together with the type strain of *N. asiatica* MFLUCC12-0285 and MFLUCC 19-0085 with 78% ML bootstrap support (Figure 3.8).



Note Sequences of 58 taxa representing *Neopestalotiopsis* are included in the analyses. The tree was rooted to *Pestalotiopsis trachicarpicola* (IFRDCC 2440). The scale bar indicates 0.02 nucleotide changes per site.

Figure 3.8 Phylogram generated from RAxML analysis based on combined ITS, TUB, TUB sequence data in *Pestalotiopsis*



Note a,b Appearance on host. c-e Conidia. Scale bars: a = 200 μm , b = 100 μm , c-e = 10 μm .

Figure 3.9 *Neopestalotiopsis asiatica* (IGu36, new host record)

***Pestalotiopsis* Steyaert**

Pestalotiopsis compressa Goonas. & K.D. Hyde, **sp. nov.**, Figure 3.11

Etymology: refers to the host from which the fungus was isolated, *Axonopus compressus*

Holotype: IGu26

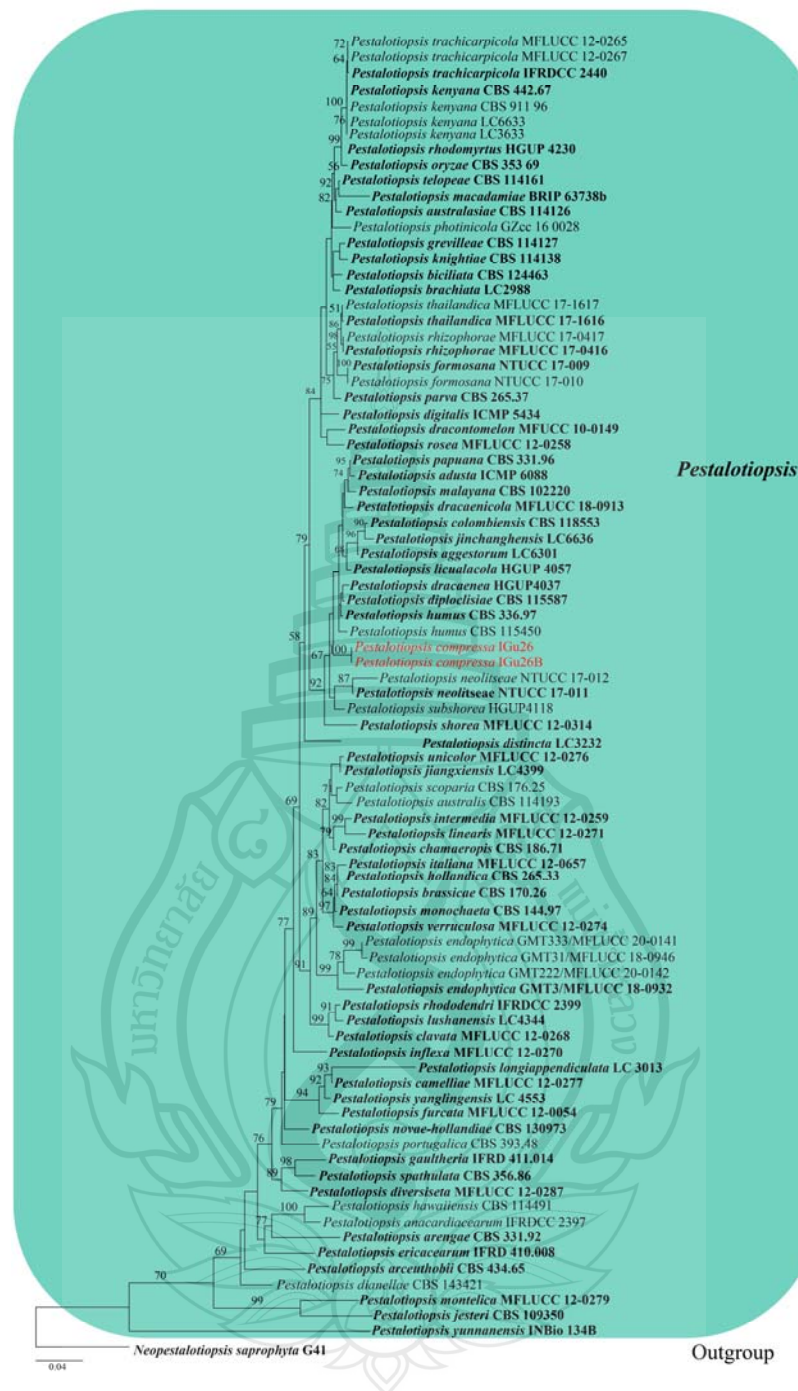
Saprobic of decaying culms of *Axonopus compressus*. **Asexual morph:** *Conidiomata* pycnidial, globose, black, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin-walled, collarette not distinct. *Conidia* 17.5–28(–30) × (4.5–)5.5–6.5(–7.1) µm ($\bar{x}$ = 25 × 6 µm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes light brown, thin and smooth-walled, 3–6 µm long ($\bar{x}$ = 4.3 µm); three median cells 15–18.5 µm long ($\bar{x}$ = 16.5 µm), light brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, 5–8 µm long ($\bar{x}$ = 7.5 µm); third cell brown, 5–6.5(–7) µm long ($\bar{x}$ = 5.6 µm); fourth cell brown, 5–6.5 µm long ($\bar{x}$ = 5.5 µm); apical cell 3.5–5 µm long ($\bar{x}$ = 4.5 µm), hyaline, conic to acute; with mostly 3 (occasionally 2) tubular appendages on apical cell, unbranched, flexuous, 15–40 µm long ($\bar{x}$ = 36 µm); single basal appendage, tubular, unbranched, centric, (1–)3.5–7.5(–12) µm long ($\bar{x}$ = 5).

Culture characteristics: Colonies on PDA reaching 5 cm diam after 7 d at room temperature, colonies circular, medium dense to highly dense in the center, aerial mycelium flat or raised, with entire edge, white from above and reverse; sporulating fruiting bodies, black.

Material examined: Thailand, Chiang Rai Province, on dead culms of *Axonopus compressus*, 20 February 2017, Ishani D. Goonasekara IGu26 (**holotype**); living culture IGu26; Thailand, MFU premises, on dead culms of *Axonopus compressus*, 2 December 2020, Ishani D. Goonasekara IGu26B; living culture IGu26B.

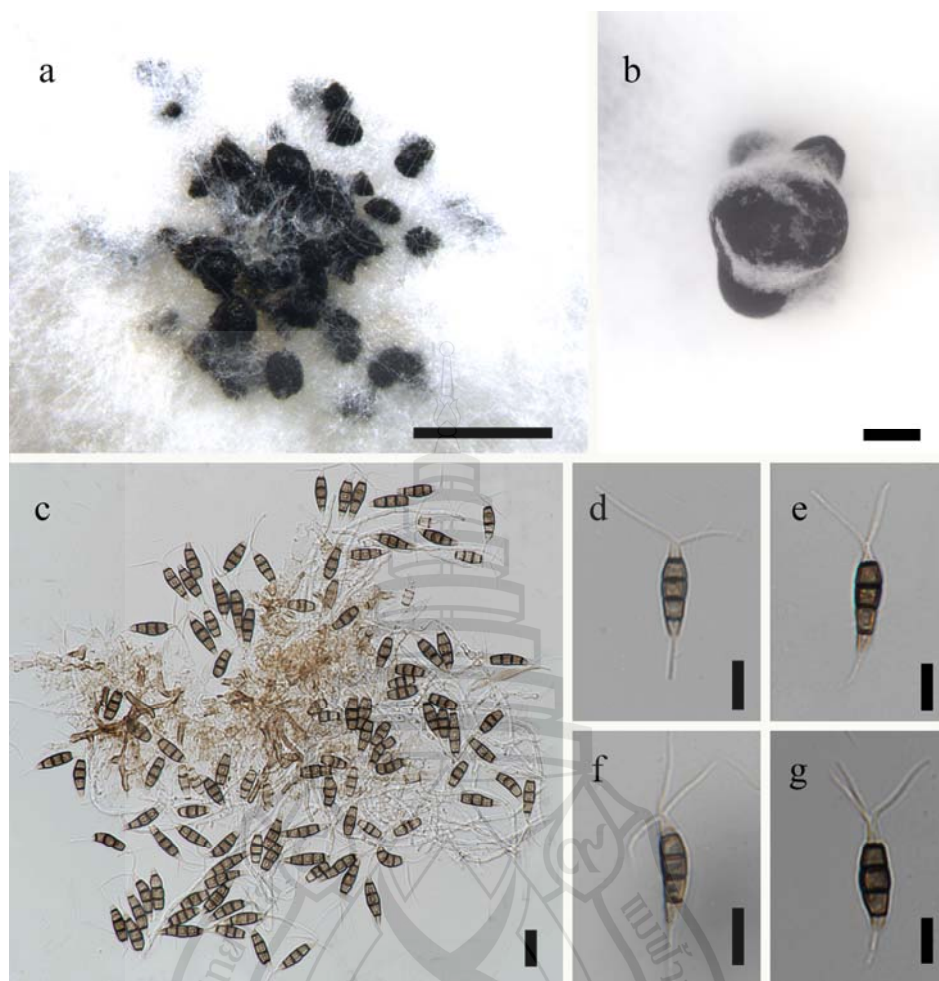
GenBank Accession numbers: IGu26: ITS: ON986417, IGu26B: ITS: ON986418.

Notes: *Pestalotiopsis compressa* is a phylogenetically distinct species in *Pestalotiopsis* (Figure 3.10). It can be distinguished from its phylogenetically related species *P. humus* from its relatively longer conidial dimensions ($\bar{x}$ = 25 × 6 µm vs. 20 × 6 µm, Maharachchimbura et al., 2012)



Note Sequences of 83 taxa representing *Pestalotiopsis* are included in the analyses. The tree was rooted to *Neopestalotiopsis saprophyta* (G41). The scale bar indicates 0.04 nucleotide changes per site.

Figure 3.10 Phylogram generated from RAxML analysis based on combined ITS, TUB, TUB sequence data in *Pestalotiopsis*



Note a, b Appearance on host. c-e Conidia. Scale bars: a = 200 μm , b = 100 μm , c-e = 10 μm .

Figure 3.11 *Pestalotiopsis compressa* sp. nov. (IGu26, holotype)

Diaporthales Nannf.

Diaporthaceae Höhn. ex Wehm.

Diaporthe Nitschke

Diaporthe yunnanensis Y.H. Gao & L. Cai, in Gao, Liu, Duan, Crous & Cai, IMA Fungus 8(1): 180 (2017)

Index Fungorum number: IF820686, Figure 3.13

Saprobic on *Thysanolaena latifolia*. **Sexual morph:** undetermined. **Asexual morph:** *Conidiomata* pycnidial, 250–600 μm in diam, globose to subglobose, semi-

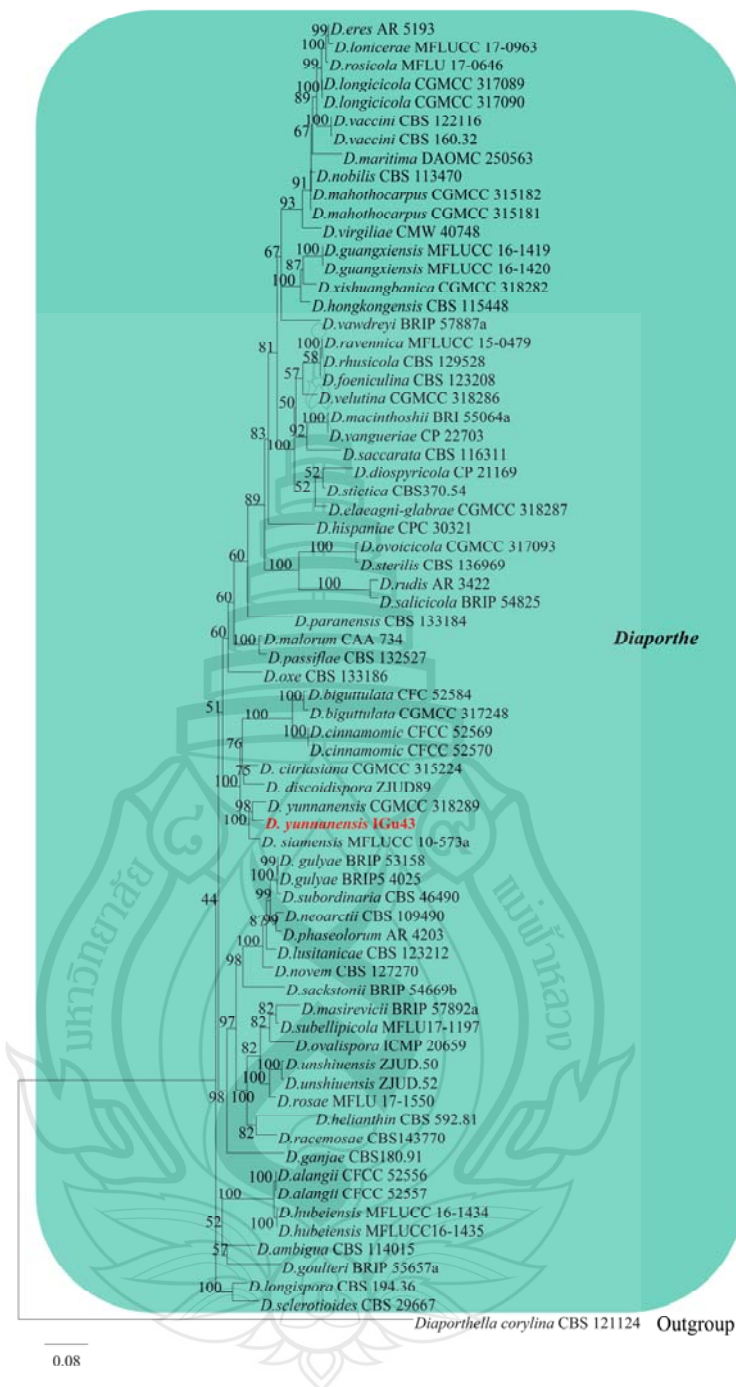
immersed to erumpent, solitary or aggregated together, dark brown to black. *Conidia* exuding from the pycnidia in white to cream drops. *Conidiophores* doliiform, straight or slightly curved. *Alpha conidia* $3\text{--}5.5 \times 1\text{--}2 \mu\text{m}$ ($\bar{x} = 4.5 \times 1.8$, $n = 40$), hyaline, fusiform, biguttulate, one end obtuse and the other, acute. *Beta conidia* $15\text{--}30 \times 1\text{--}2 \mu\text{m}$ ($\bar{x} = 25.5 \times 1.5$, $n = 40$), hyaline, aseptate, slightly curved, with truncate base.

Culture characteristics: Colonies growing on PDA, reaching a diameter of 3-5 cm after 7 d at room temperature, surface yellowish white, flat, sparse, with undulated edge, reverse beige to off white with dark concentric rings occuing in the colony.

Material examined: Thailand, Chiang Rai Province, on dead culms of *Thysanolaena latifolia*, 20 February 2017, Ishani D. Goonasekara IGu43; living culture IGu43.

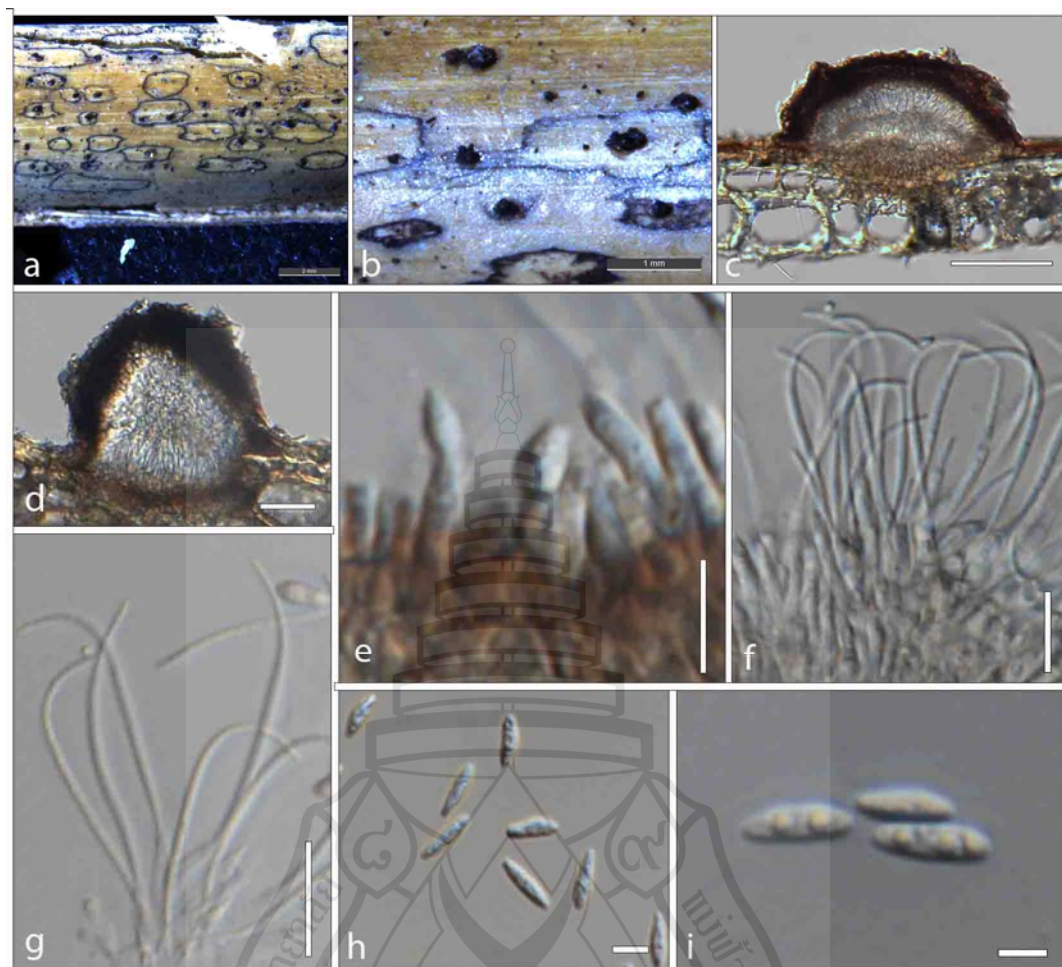
GenBank Accession numbers: ITS: ON986213

Notes: *Diaporthe yunnanensis* was introduced from healthy coffee leaves in Yunnan province, China (Gao et al., 2017). The collection described here is the first host record of *Diaporthe* on *Thysanolaena latifolia*. In our phylogenetic analysis, it groups with *D. yunnanensis* CGMCC 318289 with 98% ML bootstrap support (Figure 3.12).



Note Sequences of 71 taxa representing *Diaporthe* are included in the analyses. The tree was rooted to *Diaporthella corylina* (CBS 121124). The scale bar indicates 0.08 nucleotide changes per site.

Figure 3.12 Phylogram generated from RAxML analysis based on combined ITS, TUB, TEF, CAL and HIS sequence data in *Diaporthe*



Note a,b Appearance on host. b, d Section of conidioma. e Conidia arising from conidiogenous cells. f,g Beta conidia. h,i Alpha conidia. Scale bars: c, d = 50 μm , e-g = 5 μm , h,i = 2 μm .

Figure 3.13 *Diaporthe yunnanensis* (IGu43, new host record)

***Hypocreales* Lindau**

***Bionectriaceae* Samuels & Rossman**

***Clonostachys chiangmaiensis* Goonas. & K. D. Hyde, sp. nov., Figure 3.15**

Etymology: Named after the province from where it was collected – Chiang Mai

Holotype: IGC3

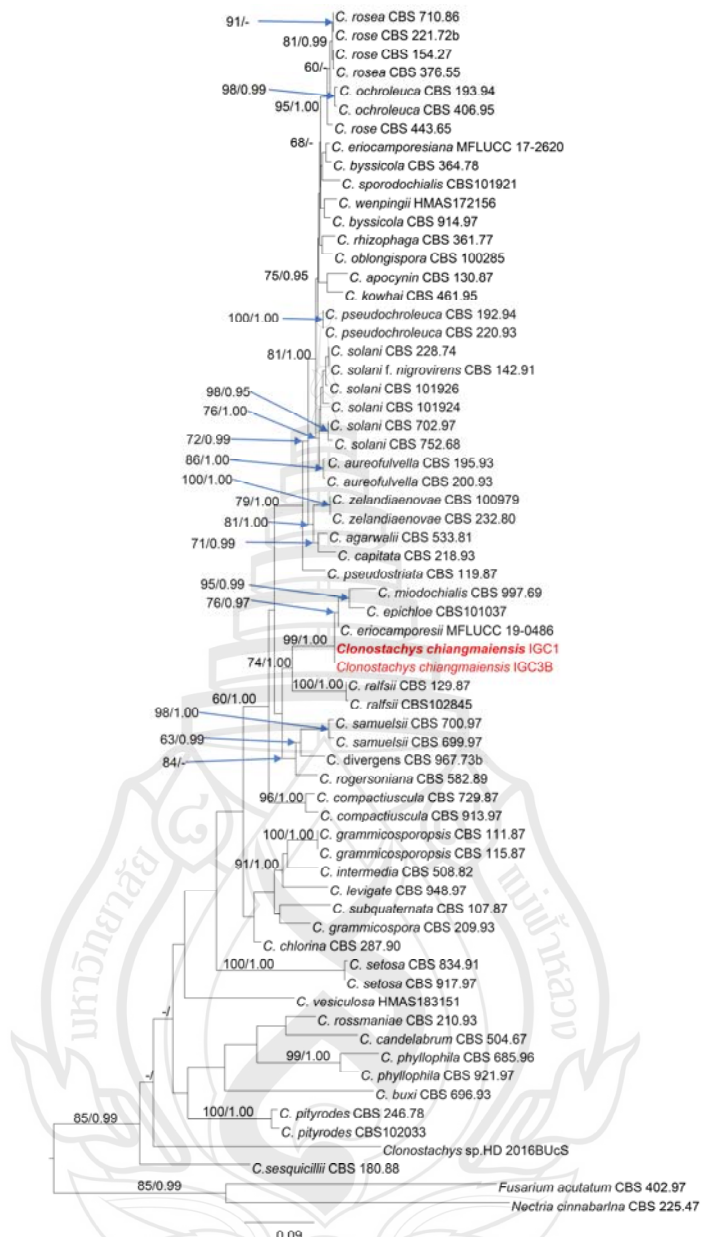
Saprobic on decaying leaves on *Zea mays*. **Sexual morph:** Undetermined. **Asexual morph:** Coelomycetous. *Hyphae* 2–8 µm wide, septate, hyaline, smooth, thin-walled, branched. *Conidiophores* wide at the base, macronematous, mononematous, erect, mostly straight, smooth or verrucose, thin-walled, septate, hyaline, cylindrical, tapering with base cell wall slightly verrucose, simple with 1 phialide or verticillate in the upper part. *Conidia* 3–5 × 1–1.5 µm, oblong, fusoid, doliiform, hyaline, thin-walled, smooth.

Culture characteristics: Colonies growing on PDA, reaching a diameter of 5 cm after 7 d at room temperature, surface yellowish white, flat, sparse, with entire edge, reverse white from above and reverse.

Material examined: Thailand, Chiang Mai Province, on decaying leaves of *Zea mays*, 7 July 2020, Ishani D. Goonasekara IGC3B (**holotype**); living culture IGC3B, *ibid.* Thailand, Chiang Mai Province, on decaying leaves of *Zea mays*, 7 July 2020, Ishani D. Goonasekara IGC3; living culture IGC3.

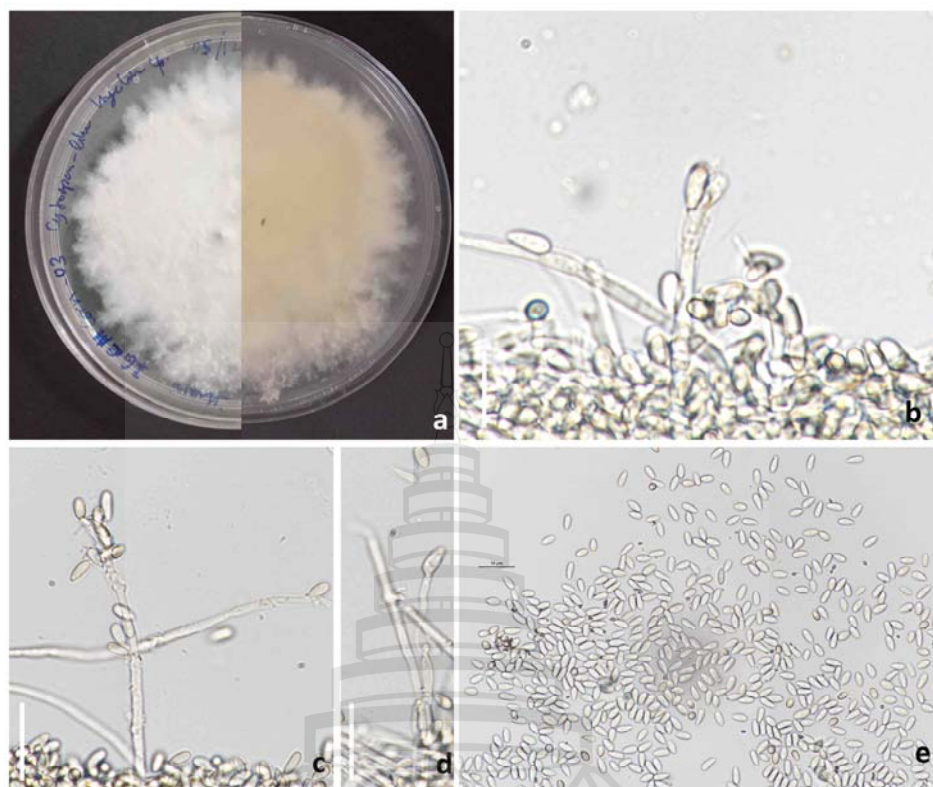
GenBank Accession numbers: IGC3: ITS: ON986632, IGC3B: ITS: ON986633; LSU: ON986841.

Notes: *Clonostachys* was introduced by Corda (1839) based on *C. araucaria* Corda. *Clonostachys* species are characterised by penicillate, sporodochial or dimorphic conidiophores and phialidic conidiogenous cells, producing hyaline conidia (Schroers, 2001). The sexual morphic genus *Bionectria*, the sexual morph genus was linked with *Clonostachys* and synonymised under *Clonostachys* by Rossman et al. (2013). Our collection of *Clonostachys* is the first report of the species on *Zea mays* and is phylogenetically distinct within the genus, forming a distinct lineage sister to *C. eriocamporesii* MFLUCC 19-0486 (Figure 3.14).



Note Sequences of 65 taxa representing *Clonostachys* are included in the analyses. The tree was rooted to *Fusarium acutatum* (CBS 402.97) and *Nectria cinnabarina* (CBS 225.47). The scale bar indicates 0.08 nucleotide changes per site.

Figure 3.14 Phylogram generated from RAxML analysis based on combined ITS and TUB sequence data in *Clonostachys*



Note a Colony growing on PDA. b Mycelium and formation of conidia. e Conidia. Scale bars: b = 20 μm , c, d = 15 μm , e = 10 μm .

Figure 3.15 *Clonostachys chiangmaiensis* sp. nov. (IGC3B, holotype)

Stachybotryaceae L. Lombard & Crous

Striaticonidium L. Lombard & Crous

Striaticonidium synnematum L. Lombard & Crous, in Lombard, Houbraken, Decock, Samson, Meijer, Réblová, Groenewald & Crous, *Persoonia* 36: 230 (2016), Figure 3.17

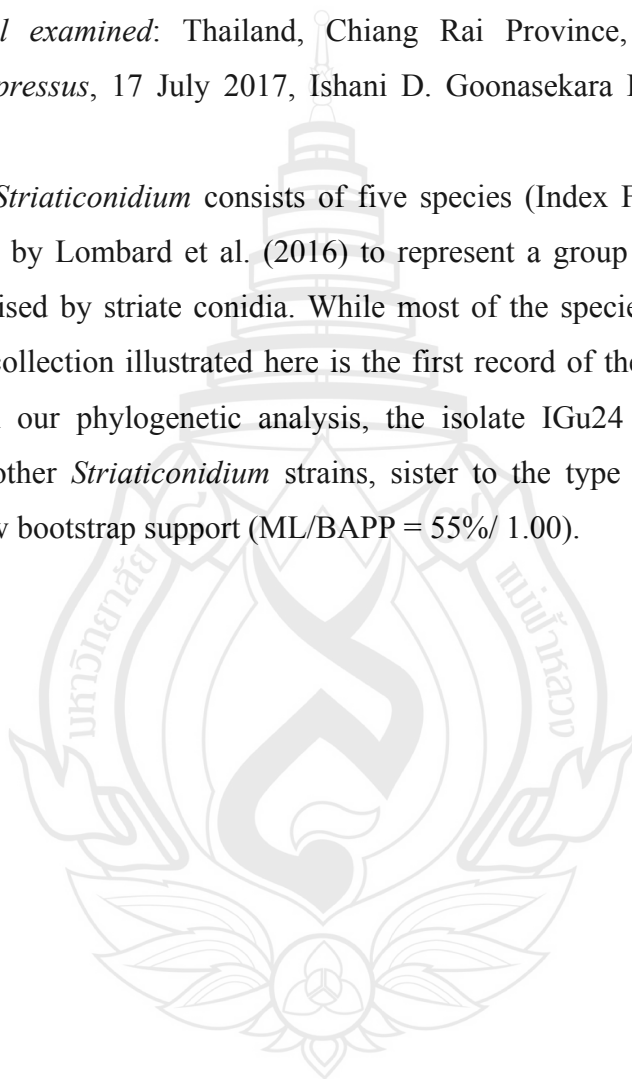
Conidiomata synnematos, solitary, 60–90 μm high, 40–70 μm wide at the base, 45–85 μm at the apex, unbranched, cylindrical, broadening towards the apex, consisting of bundles of parallel, longitudinal, tightly compacted hyphae, terminating in whorls of 1–3 conidiogenous cells, surrounded by marginal hyphae arising from the stroma and covered with olivaceous green slimy mass of conidia. *Marginal hyphae* 2–3.5 μm wide, sinuous, mostly unbranched, hyaline to olivaceous green, verrucose, with a blunt apex. *Stroma* well developed, consisting of hyaline or light brown cells of

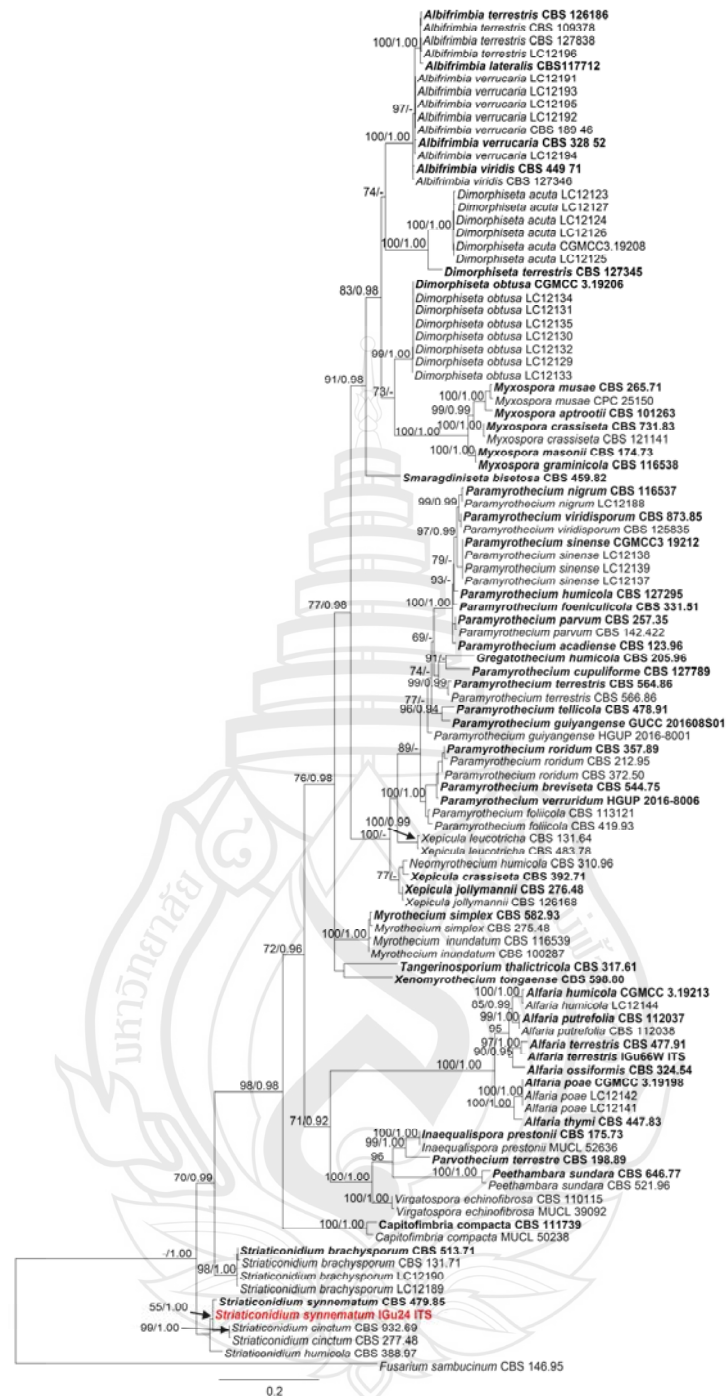
of *textura globulosa* to *textura angularis*. *Conidiogenous cells* phialidic, cylindrical to subcylindrical, hyaline, smooth, $10\text{--}40 \times 1\text{--}2 \mu\text{m}$. *Conidia* aseptate, longitudinally striate, hyaline to olivaceous green, fusiform to ellipsoidal, $6\text{--}8 \times 1.5\text{--}3.5 \mu\text{m}$ ($\bar{x} = 7 \times 2.5 \mu\text{m}$), with a distinct apical hilum.

Culture characteristics: Colonies growing on PDA up to 3cm in 7 days, dense mycelium, white to greyish white and reverse olivaceous green.

Material examined: Thailand, Chiang Rai Province, on dead culms of *Axonopus compressus*, 17 July 2017, Ishani D. Goonasekara IGu24; living culture IGu24.

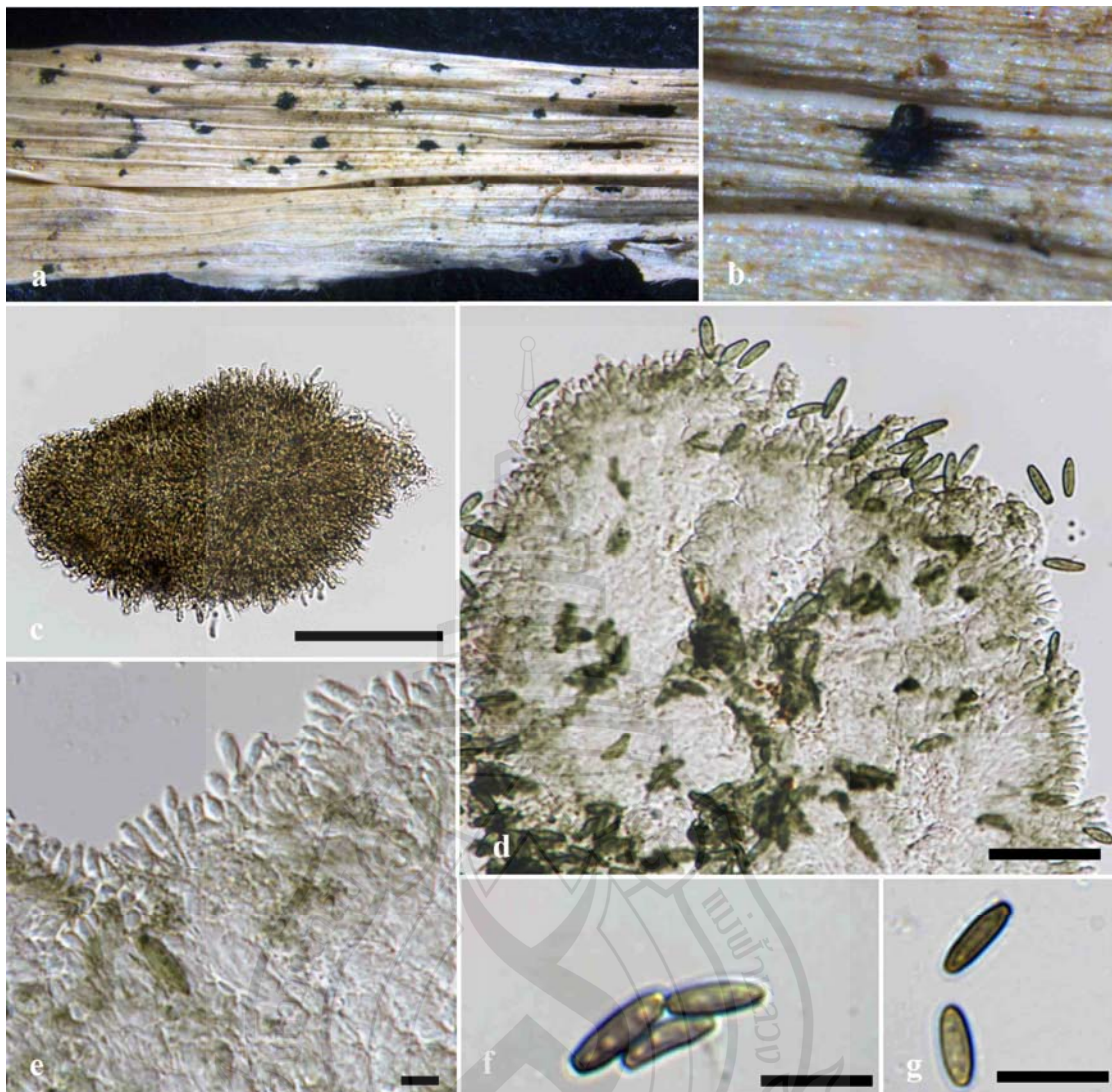
Notes: *Striaticonidium* consists of five species (Index Fungorum, 2022) and was introduced by Lombard et al. (2016) to represent a group of myrothecium-like fungi characterised by striate conidia. While most of the species have been isolated from soil, the collection illustrated here is the first record of the genus on *Axonopus compressus*. In our phylogenetic analysis, the isolate IGu24 from this collection grouped with other *Striaticonidium* strains, sister to the type *S. synnematum* CBS 479.85 with low bootstrap support (ML/BAPP = 55%/ 1.00).





Note Sequences of 106 taxa representing *Striaticonidium* and related genera are included in the analyses. The tree is rooted to *Fusarium sambucinum* (CBS 146.95). The scale bar indicates 0.2 nucleotide changes per site.

Figure 3.16 Phylogram generated from RAxML analysis based on combined ITS and TUB sequence data in *Stachybotryaceae*



Note a Appearance on host surface. b Fruiting body exuding spores. c, d Squash mount of fungal structures e Conidiogenous cells with attached to conidia. f, g Conidia. Scale bars: c, f, g = 50 μm , d = 10 μm , e = 100 μm .

Figure 3.17 *Striaticonidium synnematum* (IGu24, new host record)

3.4 Discussion

Numerous fungal species occur on a wide range of hosts in *Poaceae*. In this study, we show the diversity and taxonomy of several grass inhabiting fungal species with molecular sequence data and descriptions linked to morphological illustrations.

The majority of the species are asexual morphs are mostly prevalent in culms of grasses. Out of over a total of 350 collections, taxa were recorded on 176 samples. Out of these collections, three new species are introduced based on morphology and phylogenetic evidence. They are *Paraphaeosphaeria thysanolaenae* isolated from *Thysanolaena latifolia*, *Pestalotiopsis compressa* on *Axonopus compressus*, and *Clonostachys chiangmaiensis* on *Zea mays*. *Cladosporium* was the most commonly encountered genus during the collections although they were almost always the species commonly inhabiting grass litter and other grass hosts. *Cladosporium tenuissimum* was identified from *A. compressus*, *I. cylindrica* and *Z. mays*.

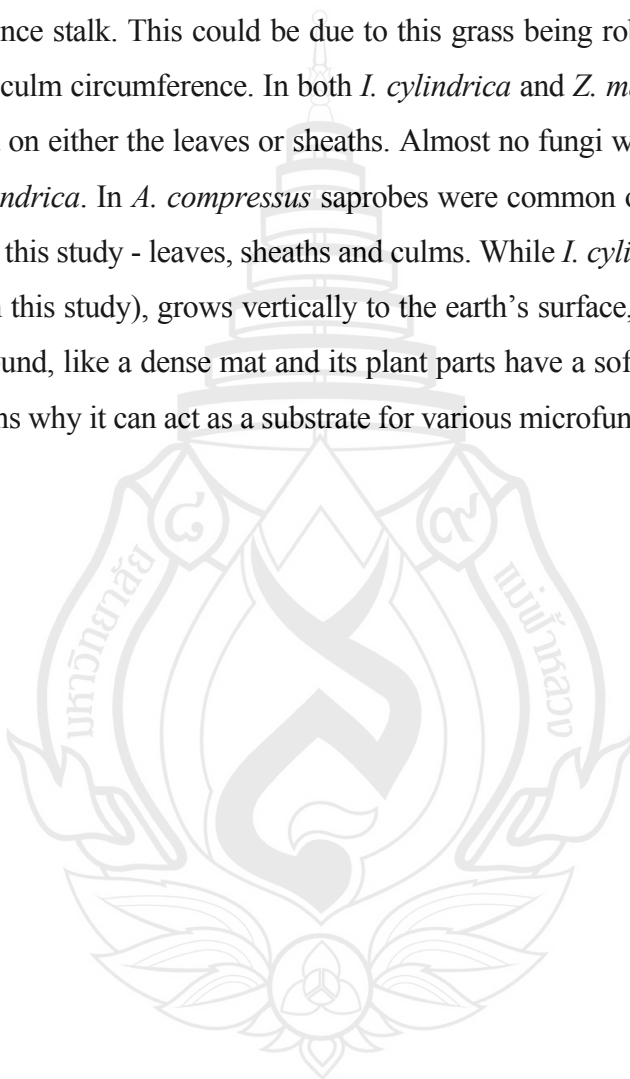
The most re-occurring genera for *A. compressus* were *Cladosporium*, *Curvularia* and *Fusarium*. Other than these unidentified species of *Arthrimum*, *Bipolaris*, *Discosia*, *Epicoccum*, *Pseudopithomyces*, were found inhabiting this grass. Prior to this study, only one species *Sulcosporium thailandicum* was recorded. Furthermore, a *Puccinia* sp. was collected for the first time on *A. compressus* as is a new record for this host. *Phaeosphaeria musae* and *Striaticonidium synnematum* have been identified based on their morphology and molecular data and are new host records. Other than *Cladosporium*, *Alternaria* sp. and *Epicoccum* sp. were commonly recorded on *I. cylindrica*. Other genera recorded once or a few times are *Alfaria*, *Curvularia*, *Ectophoma*, *Lophiostoma*, *Nigrospora*, *Pithomyces* and *Tetraploa*.

Prior to this study there have been eight records of fungi on *T. latifolia*. During this study *Cladosporium*, *Spegazzinia*, *Phaeosphaeria* were commonly found in our collections. Apart from these, species of *Arthrimum*, *Lasiodiplodia*, *Simplicillium*, *Paraconiothyrium*, *Leptosphaeria*, *Ophiosphaerella* and *Tetraploa* were encountered associated with this grass. *Diaporthe yunnanensis*, *Neopestalotiopsis asiatica*, *Lasiodiplodia theobromae* are new records on *T. latifolia* illustrated in this chapter.

Despite the various taxa recorded in literature (Farr & Rossman, 2022), our collections of *Zea mays* did not include a high variety of microfungi as expected. As with the other hosts, *Cladosporium* was very evident, followed several records of *Alternaria*, *Bipolaris*, *Curvularia*, *Epicoccum*, *Passalora* and *Xylaria*. The less variety in saprobic microfungi could be due to the random collections from limited areas in northern Thailand. Also, most decaying plant samples found seem to be treated with pesticides or

subjected to burning. It could also be that the Maize varieties grown in this region are less susceptible to invasion by fungi.

Asexual morphs were quite common among all the collections of the four grass hosts. Between the two tall grasses *T. latifolia* and *Zea mays* the former was host to many different kinds of asexual and sexual morphs. Microfungi were more likely found on the culms of *T. latifolia* while some hyphomycetous fungi like *Cladosporium* were observed in the inflorescence stalk. This could be due to this grass being robust, bamboo like and having a higher culm circumference. In both *I. cylindrica* and *Z. mays* microfungi almost always occurred on either the leaves or sheaths. Almost no fungi were observed in culms of *Imperata cylindrica*. In *A. compressus* saprobes were common on all three main parts observed during this study - leaves, sheaths and culms. While *I. cylindrica* (the other short grass selected in this study), grows vertically to the earth's surface, *A. compressus* grows closer to the ground, like a dense mat and its plant parts have a softer texture. This could one of the reasons why it can act as a substrate for various microfungi.



CHAPTER 4

MICROFUNGI ON GRASS LITTER IN THAILAND

4.1 Introduction

Fungi are heterotrophs and are an essential part of the ecosystem. They play dynamic roles in all ecosystems as epiphytes, fungicolous fungi, biotrophs, hemi-biotrophs and saprobes (Hawksworth, 2001; Hyde et al., 2013; De Silva et al., 2016; Hernandez-Restrepo et al., 2017). Thus, they are morphologically, phylogenetically and ecologically highly diverse (Hawksworth, 2004; Blackwell, 2011; Blackwell & Vega, 2018). The global fungal diversity estimation has always been contentious and has changed over the years (Hawksworth, 1991, 2001; Rossmann, 1994; Cannon, 1997; May, 2000; O'Brien et al., 2005; Blackwell, 2011; Hawksworth & Lücking, 2017; Baldrian et al., 2021), however, while 1.08 million species are currently published, many species are still undescribed (Hyde et al., 2020b; Baldrian et al., 2021). Numerous researchers have pointed out the reason for the missing fungal diversity is due to fungi being poorly studied in many countries, and on many hosts and substrates (Hyde et al., 2018, 2020). It was revealed that 96% of fungal species in northern Thailand may be new to science (Hyde et al., 2018). Moreover, the favorable climatic conditions in Thailand make it a most desired place for fungal collections. However, most of the geographical regions and host species in Thailand are still under studied. For instance, grasses are agriculturally, economically and ecologically dominant plant species, but their fungal diversity in Thailand has not been fully explored or has been studied only with regard to a few important hosts (i.e., bamboo, *Carex* sp., *Saccharum* sp., *Triticum* sp.) or pathogenic groups such as rusts and smuts (Hyde et al., 2001; Somrithipol & Jones, 2003; Bhilabutra et al., 2006; Dai et al., 2017; Thambugala et al., 2017; Goonasekara et al., 2018). Hence, as an effort to clarify their taxonomy

based on morphology coupled with molecular phylogeny, the continuous investigation of the fungal diversity of on grasses in Thailand is being carried out. During our survey, several interesting fungal species belonging to *Chaetosphaeriaceae*, *Coniothyriaceae*, *Didymellaceae*, *Phaeosphaeriaceae* and *Sporocadaceae* were collected from decaying grasses/ grass litter in northern Thailand. Morphological and multi-gene phylogenetic analyses were performed to establish their taxonomic placements.

4.2 Materials and Methods

4.2.1 Sample Collection, Morphological Studies and Isolation

Samples were collected from Mushroom Research Center (M.R.C.), Chiang Mai, and brought back to the laboratory in paper bags. Morphological features of the fungi were examined using a Motic SMZ 168 dissecting microscope. Free-hand sections of the fungal fruiting structures were obtained and mounted in water on a slide to observe their microscopic features. Micro-morphologies were examined using a Nikon ECLIPSE 80i compound microscope and photographed using a Canon EOS 750D digital camera fitted to the microscope. The images were processed using Adobe Photoshop CS5 Extended version 12.0 software (Adobe Systems, USA). The Tarosoft (R) Image Frame Work program v. 0.9.7 was used for taking measurements. Isolates were obtained by single spore isolation as detailed in Chomnunti et al. (2014) in a petri dish containing 2% water agar and incubated overnight at room temperature. Germinating spores were then transferred aseptically onto potato dextrose agar (PDA) and further incubated at room temperature to obtain pure colonies. Herbarium material is deposited in the herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU) and living cultures are deposited at Mae Fah Luang University Culture Collection (MFLUCC). Facesoffungi and Index Fungorum numbers were obtained as described in Jayasiri et al. (2015) and Index Fungorum (2021).

4.2.2 DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was extracted from fresh mycelia grown on PDA, following the manufacturer's standard protocol described in the DNA extraction kit (Biospin

Fungus Genomic DNA Extraction Kit). The internal transcribed spacers (ITS), large subunit rDNA (LSU), small subunit rDNA (SSU), RNA polymerase II second largest subunit (RPB2) and β -tubulin (TUB) gene regions were amplified using ITS5 and ITS4 (White et al., 1990), LROR and LR5 (Vilgalys & Hester, 1990; Rehner & Samuels, 1994), NS1 and NS4 (White et al., 1990), 5F and 7CR (Liu et al., 1999) and Bt2a and Bt2b (Glass & Donaldson, 1995), respectively following the conditions mentioned in Goonasekara et al. (2022). PCR products were verified using 1% agarose gel electrophoresis, stained with 4S Green Stain. Purification and sequencing of PCR products were carried out at Shanghai Sangon Biological Engineering Technology and Services Co., China. Sequences derived from this study are deposited in GenBank.

4.2.3 Phylogenetic Analyses

Sequences generated from this study were subjected to BLAST (NCBI) searches to obtain the closest matches in GenBank and from recent publications (Tables 4.1–4.5). The multiple alignments were made with MAFFT v. 7 at the webserver (<http://mafft.cbrc.jp/alignment/server>), using default settings (Katoh & Standley, 2013). The alignment was refined manually with BioEdit v. 7.0.5.2 (Hall, 1999) where necessary. Evolutionary models for phylogenetic analyses were independently selected for each gene region following the Akaike Information Criterion (AIC) of the MrModeltest v. 3.7 (Nylander, 2004), implemented using both PAUP v. 4.0b10 and MrBayes v. 3. Phylogenetic reconstructions of combined gene trees were performed using maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) criteria.

Maximum likelihood analysis was performed using RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al., 2008; Stamatakis, 2014) in the CIPRES Science Gateway platform (Miller et al., 2010) with GTR + I + G as the model of evolution and bootstrap support obtained by running 1000 pseudoreplicates. The MP analysis was performed using PAUP v. 4.0b10 (Phylogenetic Analysis Using Parsimony) (Swofford, 2002). Ambiguously aligned regions were excluded and gaps were treated as missing data. The trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Maxtrees were set up to 1000,

branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony tree length [TL], consistency index [CI], retention index [RI], relative consistency index [RC] and homoplasy index [HI] were calculated for the maximum parsimonious tree. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications resulting from MP analysis, each with ten replicates of random stepwise addition of taxa (Felsenstein, 1985). The Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa, 1989) were performed to determine whether the trees were significantly different. The BI analysis was conducted using MrBayes v. 3.1.2 (Huelsenbeck & Ronquist, 2001) to evaluate posterior probabilities (BYPP) (Rannala & Yang, 1996; Zhaxybayeva & Gogarten, 2002) by Markov chain Monte Carlo sampling (BMCMC). Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generation. The distribution of log-likelihood scores was examined to determine the stationary phase for each search and to decide if extra data runs were required to achieve convergence, using the Tracer v. 1.6 program (Rambaut et al., 2014). The first 10% of generated trees representing the burn-in phase were discarded and the remaining trees were used to calculate posterior probabilities of the majority rule consensus tree (critical value for the topological convergence diagnostic was set to 0.01). Phylograms were visualized using FigTree v1.4.0 program (Rambaut, 2012) and re-edited and formatted using Microsoft PowerPoint (2013) and Adobe Photoshop CS6 extended version 13.1.2 software. The final tree alignments were submitted to TreeBASE (<http://www.treebase.org/>).

Table 4.1 GenBank accession numbers of taxa included in the phylogenetic study of *Chaetosphaeriaceae*.

Species	Strain/ Voucher no.	GenBank accession no.	
		LSU	ITS
<i>Dinemasporium americana</i>	CBS 127127	JQ889290	JQ889274
<i>D. bambusicola</i>	MAFF 244322	-	AB900860
<i>D. bambusicola</i>	MAFF 244323	-	AB900861

Table 4.1 (continued)

Species	Strain/ Voucher no.	GenBank accession no.	
		LSU	ITS
<i>D. cruciferum</i>	MAFF 244325	-	AB900893
<i>D. cruciferum</i>	MAFF 244327	-	AB900895
<i>D. decipiens</i>	CBS 592.73	JQ889291	JQ889275
<i>D. ipomoeae</i>	CBS 138898	KP004474	KP004446
<i>D. iriomotense</i>	MAFF 244329	-	AB900891
<i>D. iriomotense</i>	MAFF 244330	-	AB900892
<i>D. longicapillatum</i>	MAFF 239569	-	AB900867
<i>D. longicapillatum</i>	MAFF 244346	-	AB900866
<i>D. longicapillatum</i>	MAFF 244347	-	AB900868
<i>D. morbidum</i>	MFLUCC 13-0401	MT183469	MT185507
<i>D. morbidum</i>	CBS 129.66	JQ889296	JQ889280
<i>D. morbidum</i>	CBS 995.97	JQ889297	JQ889281
<i>D. nelloi</i>	MFLUCC 13-0482	KP711363	KP711358
<i>D. parastrigosum</i>	MFLUCC 20-0162	MW114411	MW114342
<i>D. parastrigosum</i>	MAFF 244348	-	AB900888
<i>D. parastrigosum</i>	MAFF 244349	-	AB900889
<i>D. polygonum</i>	CBS 516.95	JQ889292	JQ889276
<i>D. pseudodecipiens</i>	MAFF 244351	-	AB900886
<i>D. pseudodecipiens</i>	MAFF 244352	-	AB900887
<i>D. pseudodecipiens</i>	MFLU 15-3635	MT183470	MT185508
<i>D. pseudodecipiens</i>	MFLU 19-2865	MT183471	MT185509
<i>D. pseudoindicum</i>	CBS 127402	MH876021	JQ889277
<i>D. pseudostrigosum</i>	CBS 717.85	JQ889294	JQ889278
<i>D. pseudostrigosum</i>	CBS 825.91	JQ889295	JQ889279
<i>D. pseudostrigosum</i>	MFLU 15-0588	MT183472	MT185510
<i>D. pseudostrigosum</i>	MFLUCC 16-0894*	OL824838	OL824834
<i>D. pseudostrigosum</i>	MFLUCC 16-0896*	OL824839	OL824837
<i>D. rishiriense</i>	MAFF 244353	-	AB900863
<i>D. sasae</i>	MAFF 244354	-	AB900883

Table 4.1 (continued)

Species	Strain/ Voucher no.	GenBank accession no.	
		LSU	ITS
<i>D. spinificis</i>	BCRC 34948	-	KT380642
<i>D. spinificis</i>	BCRC 34997	-	KT380643
<i>D. strigosum</i>	CBS 520.78	JQ889298	JQ889282
<i>D. strigosum</i>	CBS 828.84	JQ889299	JQ889283
<i>D. trichophoricola</i>	CBS 136772	KJ869173	KJ869115
<i>Menispora tortuosa</i>	AFTOL-ID 278	AY544682	KT225527
<i>M. tortuosa</i>	CBS 214.56	MH869135	MH857588

Note *The newly generated isolates

Table 4.2 GenBank accession numbers of species included in the phylogenetic study for *Coniothyriaceae*.

Species	Strain/ Voucher no.	GenBank accession no.			
		LSU	SSU	ITS	RPB2
<i>Coniothyrium carteri</i>	CBS 105.91	KF251712	GQ387533	KF251209	KF252214
<i>C. cereale</i>	CBS 157.78	JX681080	-	MH861123	-
<i>C. Chiangmaiense</i>	MFLUCC 16-0891*	KY550384	KY550385	KY568987	KY607015
<i>C. dolichi</i>	CBS 124143	GQ387610	GQ387549	JF740182	-
<i>C. glycines</i>	CBS 124141	GQ387598	GQ387537	KF251211	KF252216
<i>C. palmarum</i>	CBS 400.71	JX681084	EU754054	MH860184	KT389592
<i>C. multiporum</i>	CBS 501.91	GU238109	GU238225	JF740186	-
<i>C. telephii</i>	CBS 188.71	MH871840	GQ387538	MH860059	KT389593
<i>C. telephii</i>	CBS 101636	GQ387601	GQ387540	JF740190	-
<i>C. sidae</i>	CBS 135108	MH878127	-	KF251149	KF252158
<i>Phaeosphaeria oryzae</i>	CBS 110110	MH874442	NG_061080	MH862850	KF252193

Note *The newly generated isolate

Table 4.3 GenBank accession numbers of species included in the phylogenetic study for *Didymellaceae*.

Species	Strain/ Voucher no.	GenBank accession no.			
		LSU	ITS	RPB2	TUB
<i>Didymella exigua</i>	CBS 183.55	MH868977	MH857436	XM_033597068	XM_033591772
<i>Epicoccum brasiliense</i>	CBS 120105	GU238049	GU237760	KT389627	GU237588
<i>E. draconis</i>	CBS 186.83	GU238070	GU237795	KT389628	GU237607
<i>E. henningsii</i>	CBS 104.80	GU238081	GU237731	KT389629	GU237612
<i>E. huancayense</i>	CBS 105.80	NG_069776	MH861244	KT389630	GU237615
<i>E. mackenziei</i>	MFLUCC 16-0335	KX698028	KX698039	KX698035	KX698032
<i>E. nigrum</i>	CBS 125.82	GU237974	FJ426995	KT389631	FJ427106
<i>E. nigrum</i>	MFLUCC 17-1162	MG818850	MG807029	MG859837	MG859832
<i>E. nigrum</i>	CBS 173.73	MH872357	MH860655	KT389632	FJ427107
<i>E. pimprinum</i>	PD 77/1028	GU237977	FJ427050	KT389633	FJ427160
<i>E. pimprinum</i>	CBS 246.60	MH869525	MH857973	MT018100	FJ427159
<i>E. plurivorum</i>	CBS 558.81	GU238132	MH861377	KT389634	GU237647
<i>E. poaceicola</i>	MFLUCC 15-0448	KX954396	KX965727	KX898365	KY197980
<i>E. sorghinum</i>	CBS 627.68	GU237979	FJ427072	KT389636	FJ427178
<i>E. sorghinum</i>	CBS 179.80	GU237978	FJ427067	KT389635	FJ427173
<i>E. thailandicum</i>	MFLUCC 16-0892*, KUMCC 17-0026	KY703620	KY703619	-	-
<i>E. tritici</i>	MFLUCC 16-0277	KX954393	KX965724	-	-
<i>E. tritici</i>	MFLUCC 16-0598	KX954392	KX965723	KX891172	-
<i>E. tritici</i>	MFLUCC 16-0276, MFLU 16- 2569	KX954391	KX926426	-	KY197979

Note *The newly generated isolate

Table 4.4 GenBank accession numbers of species included in the phylogenetic study for *Phaeosphaeriaceae*.

Species	Strain/ Voucher no.	GenBank accession no.			
		LSU	SSU	ITS	RPB2
<i>Allophaeosphaeria muriformia</i>	MFLUCC 13-0349	KP765681	KP765682	KP765680	-
<i>Ampleomyces quisqualis</i>	CBS 129.79	JX681064	EU754029	HQ108038	-
<i>Didymella exigua</i>	CBS 183.55	EU754155	EU754056	GU237794	GU357800
<i>Equiseticola fusispora</i>	MFLUCC 14-0522	KU987669	KU987670	KU987668	-
<i>Gallicola dactylidicola</i>	MFLUCC 13-0738	KP684147	KP684148	KP744469	-
<i>G. pseudophaeosphaeria</i>	MFLU 14-0524b	KT326693	-	KT326692	-
<i>Leptospora galii</i>	KUMCC 15-0521	KX599548	NG_068364	NR_168158	-
<i>L. rubella</i>	CPC 11006	DQ195792	DQ195803	DQ195780	-
<i>L. thailandica</i>	MFLUCC 16-0385	KX655549	KX655554	KX655559	-
<i>Neosetophoma clematidis</i>	MFLUCC 13-0734	KP684153	KP684154	KP744450	-
<i>N. italica</i>	MFLUCC 13-0388	KP711361	KP711366	KP711356	-
<i>N. poaeicola</i>	MFLUCC 16-0886*	KY550382	KY550383	KY568986	-
<i>N. samorarum</i>	CBS 138.96	KF251664	GQ387517	KF251160	KF252168
<i>Neosulcatispora agaves</i>	CPC 26407	KT950867	-	KT950853	-
<i>Neostagonospora caricis</i>	CBS 135092	KF251667	-	KF251163	KF252171
<i>Ne. phramitis</i>	MFLUCC 16-0493	KX910090	KX950401	KX926416	KX880498
<i>Nodulosphaeria digitalis</i>	MFLUCC 15-2716	KU058720	-	KU058710	-
<i>No. italica</i>	MFLU 16-1359	KX672158	-	KX672153	-
<i>Ophiosimulans tanacetii</i>	MFLUCC 14-0525	KU738891	KU738892	KU738890	-
<i>Ophiosphaerella agrostidis</i>	MFLUCC 11-0152	KM434281	KM434290	KM434271	-

Table 4.4 (continued)

Species	Strain/ Voucher no.	GenBank accession no.			
		LSU	SSU	ITS	RPB2
<i>Op. agrostidis</i>	MFLUCC 16-0895*	MF197563	MF351604	MF351996	MF351995
<i>Op. agrostidis</i>	MFLUCC 12-0007	KM434282	KM434291	KM434272	KM434308
<i>Paraphoma dioscoreae</i>	CPC 11361	KF251673	-	KF251169	KF252177
<i>P. radicina</i>	CBS 111.79	KF251676	EU754092	KF251172	KF252180
<i>Parastagonospora dactylidis</i>	MFLUCC 13-0375	KU058722	-	KU058712	-
<i>Pa. fusiformis</i>	MFLUCC 13-0215	KX910088	KX950403	KX926418	KX863711
<i>Pa. nodorum</i>	CBS 110109	KF251681	EU754076	KF251177	KF252185
<i>Pa. poaceicola</i>	MFLUCC 15-0471	KX910092	KX950404	KX926419	KX880499
<i>Pa. poae</i>	CBS 135089	KF251682	-	KF251178	KF252186
<i>Phaeopoacea festucae</i>	MFLUCC 17-0056	KY824767	KY824769	KY824766	KY824768
<i>Phaeopoacea phragmiticola</i>	CBS 459.84	KF251691	KY090700	KY090640	KF252195
<i>Phaeosphaeria chiangraina</i>	MFLUCC 13-0231	KM434280	KM434289	KM434270	KM434307
<i>Ph. musae</i>	MFLUCC 11-0133	KM434277	KM434287	KM434267	KM434304
<i>Ph. oryzae</i>	CBS 110110	KF251689	GQ387530	KF251186	KF252193
<i>Ph. papayae</i>	CBS 135416	MH877574	no	MH866082	no
<i>Phaeosphaeria</i> sp.	CPC 12131	KF251700	-	KF251197	530746961
<i>Ph. thysanolaenicola</i>	MFLUCC 10-0563	KM434276	KM434286	KM434266	KM434303
<i>Phaeosphaeriopsis glaucopunctata</i>	MFLUCC 13-0220	KJ522477	KJ522481	KJ522473	-
<i>Pha. triseptata</i>	MFLUCC 13-0271	KJ522479	KJ522484	KJ522475	KJ522485
<i>Poaceicola arundinis</i>	MFLUCC 15-0702	KU058726	MG520988	KU058716	-
<i>Po. bromi</i>	MFLUCC 13-0739	KU058727	-	KU058717	-

Table 4.4 (continued)

Species	Strain/ Voucher no.	GenBank accession no.			
		LSU	SSU	ITS	RPB2
<i>Po. garathjonesii</i>	MFLUCC 15-0469	KX954390	KY205717	KX926425	KX898363
<i>Populocrescentia forlicesenensis</i>	MFLUCC 14-0651	KT306952	KT306955	KT306948	-
<i>Pseudophaeosphaeria rubi</i>	MFLUCC 14-0259	NG_067543	KX765300	NR_154351	-
<i>Septoriella allojunci</i>	MFLUCC 15-0701	KU058728	MG520997	KU058718	-
<i>S.a phragmitis</i>	CPC 24118	KR873279	-	KR873251	-
<i>S. subcylindrospora</i>	MFLUCC 13-0380	KT314183	KT314185	KT314184	-
<i>Setophoma chromolaena</i>	CBS 135105	KF251747	-	KF251244	KF252249
<i>Se. poaeicola</i>	MFLUCC 16-0880*	KY550386	KY550387	KY568988	KY609967
<i>Se. sacchari</i>	CBS 333.39	KF251748	GQ387525	KF251245	KF252250
<i>Se. terrestris</i>	CBS 335.87	GQ387589	GQ387528	KF251247	KF252252
<i>Se. vernoniae</i>	CPC 23123	KJ869198	-	KJ869141	-
<i>Vagicola vagans</i>	CBS 604.86	KF251696	-	KF251193	KF252200
<i>Vrystaatia aloecicola</i>	CBS 135107	KF251781	-	KF251278	KF252283
<i>Wojnowicia hirta</i>	CBS 536.77	KR873278	-	KR873249	KR873324
<i>Wojnowicia hirta</i>	CBS 160.73	EU754222	EU754123	KR873248	KR873323
<i>Wojnowiciella dactylidis</i>	MFLUCC 13-0735	KP684149	KP684150	KP744470	-
<i>Wo. italica</i>	MFLUCC 13-0447	-	NG_063613	KX342923	KX430004
<i>Wo. loniceriae</i>	MFLUCC 13-0737	KP684151	KP684152	NR_154502	-
<i>Xenoseptoria neosaccardoi</i>	CBS 128665	KF251784	-	KF251281	KF252286

Note *The newly generated isolates

Table 4.5 GenBank accession numbers of species included in the phylogenetic study for *Sporocadaceae*.

Species	Strain/ Voucher no.	GenBank accession no.	
		LSU	ITS
<i>Adisciso tricellulare</i>	NBRC 32705	AB593728	AB594796
<i>A. yakushimense</i>	HHUF 29671	AB593721	AB594789
<i>Bartalinia robillardoides</i>	CBS 122705	KJ710438	KJ710460
<i>Broomella rosae</i>	MFLU 16-0244	MG828990	MG828874
<i>B. vitalbae</i>	CBS 140412	-	KT949895
<i>Ciliochorella</i> sp.	IMI 052255	AF382379	-
<i>C. castaneae</i>	HHUF 28799	AB433277	-
<i>Discostroma tostum</i>	NBRC 32626	AB593727	AB594795
<i>Hymenoplella hippophaeicola</i>	CBS 140410	MH878678	KT949901
<i>H. lakefuiensis</i>	HKUCC 7303	MZ420203	MZ420208
<i>Immersidiscosia eucalypti</i>	MAFF 242781	AB593725	AB594793
<i>Monochaetia kansensis</i>	PSHI2004Endo1030	DQ534037	DQ534046
<i>Morinia acaciae</i>	CBS 137994	MH554221	MH554002
<i>M. pestalozzioides</i>	F 090354	-	AY929325
<i>Neopestalotiopsis protearum</i>	CBS 114178	JN712564	JN712498
<i>N. rosae</i>	CBS 101057	KM116245	KM199359
<i>Pestalotiopsis knightiae</i>	CBS 114138	KM116227	KM199310
<i>P. malayana</i>	CBS 102220	KM116238	KM199306
<i>Phlogicylindrium eucalyptorum</i>	CBS 111689	KF251708	KF251205
<i>P. uniforme</i>	CBS 131312	JQ044445	JQ044426
<i>Pseudopestalotiopsis cocos</i>	CBS 272.29	MH866532	MH855069
<i>P. theae</i>	MFLUCC 12-0055	KM116282	JQ683727
<i>Robillarda africana</i>	CBS 122.75	KR873281	KR873253
<i>R. africana</i>	MFLUCC 16-0888*	OL824828	OL824826

Table 4.5 (continued)

Species	Strain/ Voucher no.	GenBank accession no.	
		LSU	ITS
<i>R. australiana</i>	CBS 143882	MH554301	MH554091
<i>R. mangiferae</i>	KUMCC 18-0180	MK353086	MK353084
<i>R. roystoneae</i>	CBS 115445	MH874545	KR873254
<i>R. roystoneae</i>	CPC 25020	KR873287	KR873259
<i>R. sessilis</i>	CBS 114312	KR873284	KR873256
<i>R. terra</i>	CBS 587.71	KJ710459	KJ710484
<i>Seimatosporium rosicola</i>	MFLU 16-0239	MG829069	MG828958
<i>S. rosicola</i>	MFLUCC 15-0564	MG829070	MG828959
<i>Seiridium marginatum</i>	CBS 140403	MH878679	KT949914
<i>S. marginatum</i>	CBS 140404	MH878681	KT949916
<i>S. papillatum</i>	CBS 340.97	DQ414531	MH862649
<i>Strickeria kochii</i>	C149	-	KT949920
<i>S. kochii</i>	CBS 140411	MH878680	KT949918
<i>Truncatella</i> sp.	UWR 156	-	MN654348
<i>T. hartigii</i>	CBS 118148	DQ278928	DQ278913
<i>T. restionacearum</i>	CMW 18755	DQ278929	DQ278915

Note *The newly generated isolate

4.3 Results

4.3.1 Phylogenetic Analyses

4.3.1.1 Chaetosphaeriaceae

Combined analysis of the LSU and ITS sequence data contained 39 strains of *Chaetosphaeriaceae* (Table 4.1, Figure 4.1). This combination of sequence data was performed after confirming that the topologies of the trees obtained from each gene were overall congruent. Bootstrap values obtained for ML, MP and posterior probabilities values resulted in BI (BYPP) analysis are given at each node (Figure

4.1). The Bayesian analysis resulted in 10,001 trees after 1,000,000 generations. The first 1000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 9001 trees were used for calculating posterior probabilities in the majority rule consensus tree presented in Figure 4.1. The RAxML analysis of the combined dataset yielded a best scoring tree with a ML optimization likelihood value of - 4038.813875. The matrix had 270 distinct alignment patterns, with 29.29% of undetermined characters or gaps. Parameters for the GTR + I + G model of the combined LSU and ITS were as follows: estimated base frequencies; A = 0.223009, C = 0.273209, G = 0.297238, T = 0.206543; substitution rates AC = 0.851505, AG = 1.760246, AT = 2.121245, CG = 1.397494, CT = 5.708727, GT = 1.000000; proportion of invariable sites I = 0.627261; gamma distribution shape parameter α = 0.665235. The maximum parsimony dataset consisted of 1405 characters, of which 1179 characters were constant, 148 were parsimony-informative and 78 were parsimony-uninformative. The most parsimonious tree showed values as follows: CI = 0.749, RI = 0.833, RC = 0.624, HI = 0.251.

4.3.1.2 *Coniothyriaceae*

A combined dataset of the LSU, SSU, ITS and RPB2 sequence data of 11 strains of *Coniothyrium* was analyzed (Table 4.2, Figure 4.2). This combination of sequence data was performed after confirming that the topologies of the trees obtained from each gene were overall congruent. The RAxML analysis of the combined dataset yielded a best scoring tree with a ML optimization likelihood value of - 9626.467674 is presented here (Figure 4.2) The matrix had 507 distinct alignment patterns, with 24.61% of undetermined characters or gaps. Parameters for the GTR model of the combined LSU, SSU, ITS and RPB2 dataset were as follows: estimated base frequencies; A = 0.253518, C = 0.219239, G = 0.270692, T = 0.256551; substitution rates AC = 1.622208, AG = 2.613923, AT = 1.760614, CG = 0.905032, CT = 4.865486, GT = 1.000000; proportion of invariable sites I = 0.475366; gamma distribution shape parameter α = 0.020000. Bootstrap values obtained for ML are given at each node (Figure 4.2).

4.3.1.3 *Didymellaceae*

A combined dataset of the LSU, ITS, RPB2 and TUB sequence data included 18 strains of *Epicoccum* and *Didymella exigua* CBS 183.55 as the outgroup

taxon (Table 4.3, Figure 4.3). The topology of each gene tree was confirmed to be similar before combining. Bootstrap values obtained for ML are given at each node (Figure 4.3). The RAxML analysis of the combined dataset yielded a best scoring tree with a ML optimization likelihood value of - 8040.023131. The matrix had 461 distinct alignment patterns, with 15.09% of undetermined characters or gaps. Parameters for the GTR model of the combined LSU, ITS, RPB2 and TUB were as follows: estimated base frequencies; A = 0.244593, C = 0.239408, G = 0.275316, T = 0.240683; substitution rates AC = 1.649436, AG = 5.078462, AT = 1.842071, CG = 1.431841, CT = 13.224979, GT = 1.000000; proportion of invariable sites I = 0.731251; gamma distribution shape parameter α = 0.738417.

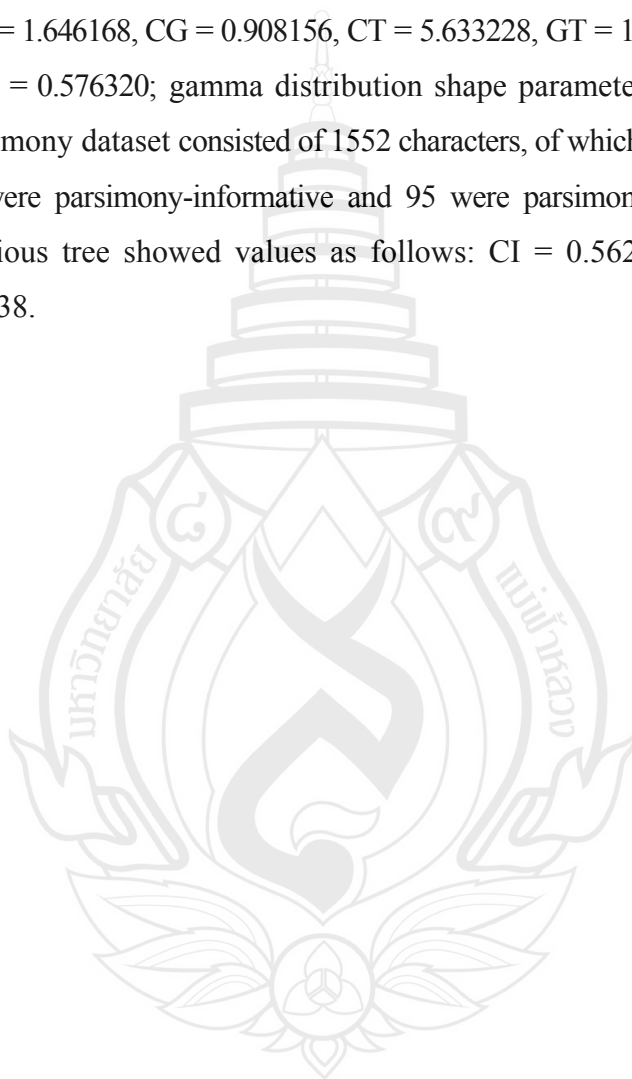
4.3.1.4 *Phaeosphaeriaceae*

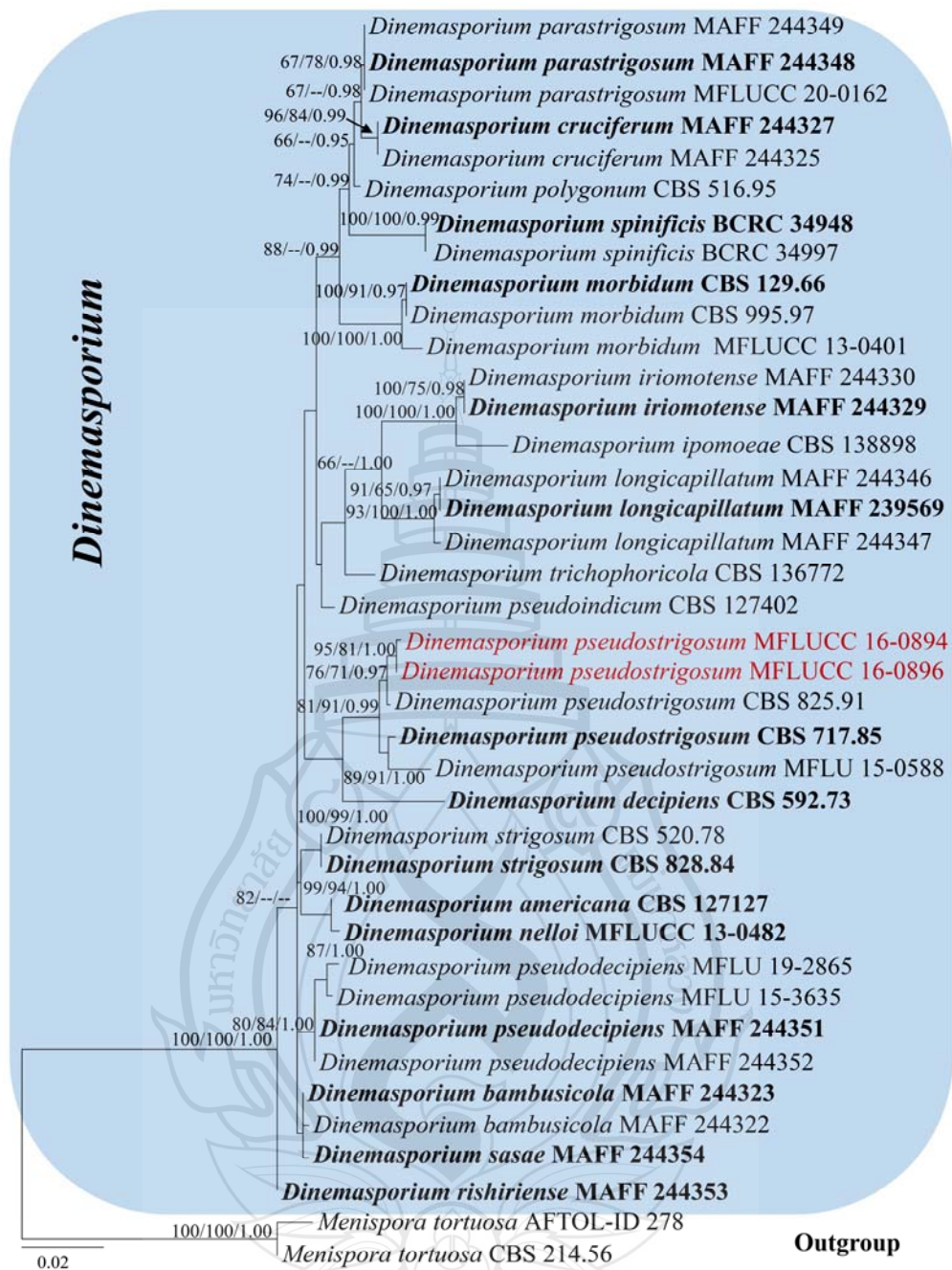
A combined dataset of LSU, SSU, ITS and RPB2 sequence data of 60 strains in *Phaeosphaeriaceae* was analyzed (Table 4.4, Figure 4.4). The combination was done after comparing the topologies of each gene tree to confirm their similarity. Bootstrap values obtained for ML are given at each node (Figure 4.4). The RAxML analysis of the combined dataset yielded a best scoring tree with a ML optimization likelihood value of - 22780.075160 and is presented in Figure 4.4. The matrix had 1343 distinct alignment patterns, with 33.91% of undetermined characters or gaps. Parameters for the GTR + I + G model of the combined LSU, SSU, ITS and RPB2 were as follows: estimated base frequencies; A = 0.253926, C = 0.218490, G = 0.266520, T = 0.261065; substitution rates AC = 1.740088, AG = 3.548734, AT = 2.179207, CG = 1.019239, CT = 6.546030, GT = 1.000000; proportion of invariable sites I = 0.564449; gamma distribution shape parameter α = 0.547066.

4.3.1.5 *Sporocadaceae*

A combined dataset of the LSU and ITS sequence data of 40 strains of *Sporocadaceae* was analyzed (Table 4.5, Figure 4.5). This combination of sequence data was performed after confirming that the topologies of the trees obtained from each gene were overall congruent. Bootstrap values obtained for ML, MP and posterior probabilities values resulted in BI analysis (BYPP) are given at each node (Figure 4.5). The Bayesian analysis resulted in 10,001 trees after 1,000,000 generations. The first 1000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 9001 trees were used for calculating posterior

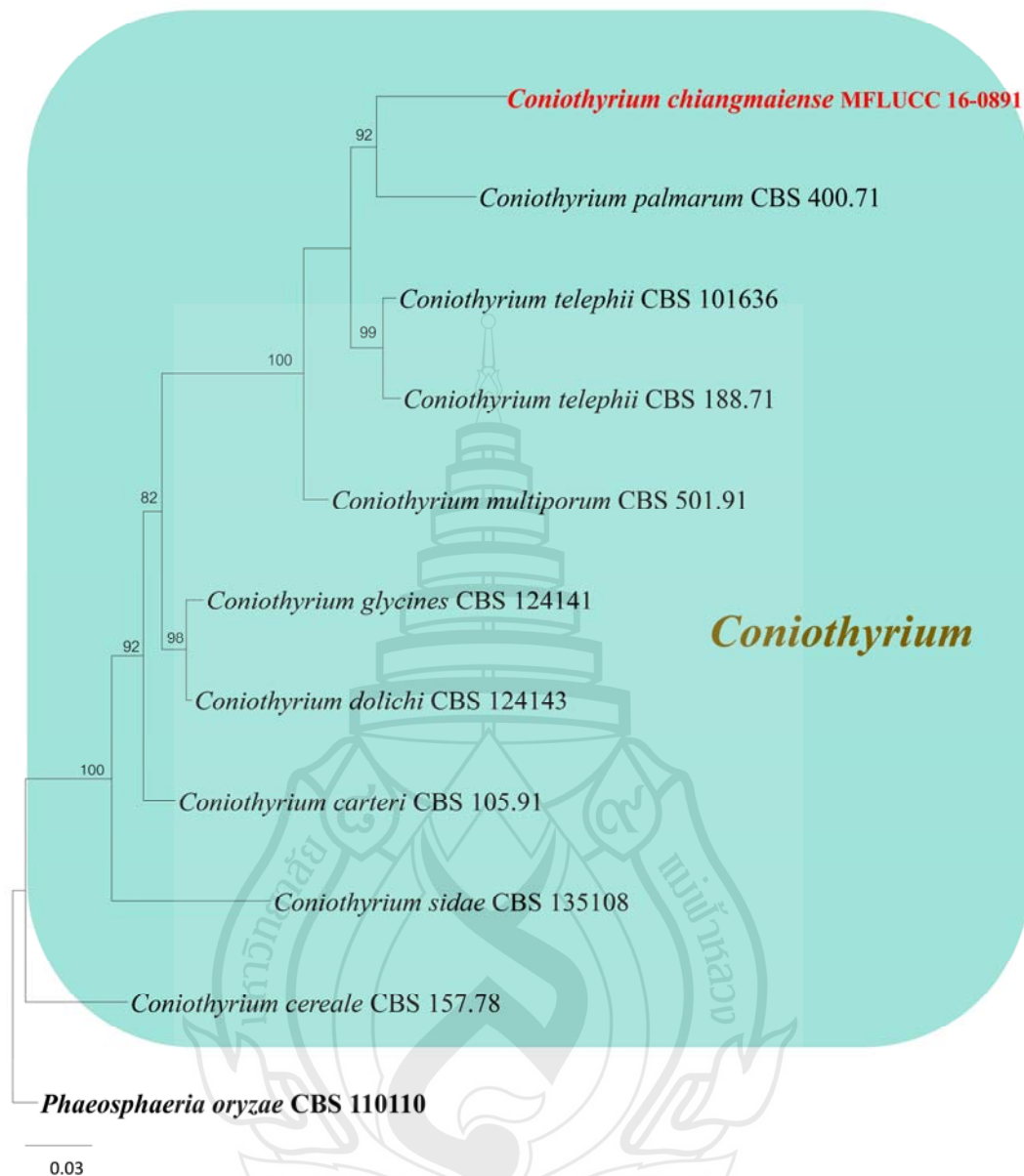
probabilities in the majority rule consensus tree presented in Figure 4.5. The RAxML analysis of the combined dataset yielded a best scoring tree with a ML optimization likelihood value of - 6399.182313. The matrix had 512 distinct alignment patterns, with 15.20% of undetermined characters or gaps. Parameters for the GTR + I + G model of the combined LSU and ITS were as follows: estimated base frequencies; A = 0.261195, C = 0.210681, G = 0.260975, T = 0.267149; substitution rates AC = 0.862479, AG = 3.134306, AT = 1.646168, CG = 0.908156, CT = 5.633228, GT = 1.000000; proportion of invariable sites I = 0.576320; gamma distribution shape parameter α = 0.617880. The maximum parsimony dataset consisted of 1552 characters, of which 1196 characters were constant, 261 were parsimony-informative and 95 were parsimony-uninformative. The most parsimonious tree showed values as follows: CI = 0.562, RI = 0.774, RC = 0.435, HI = 0.438.





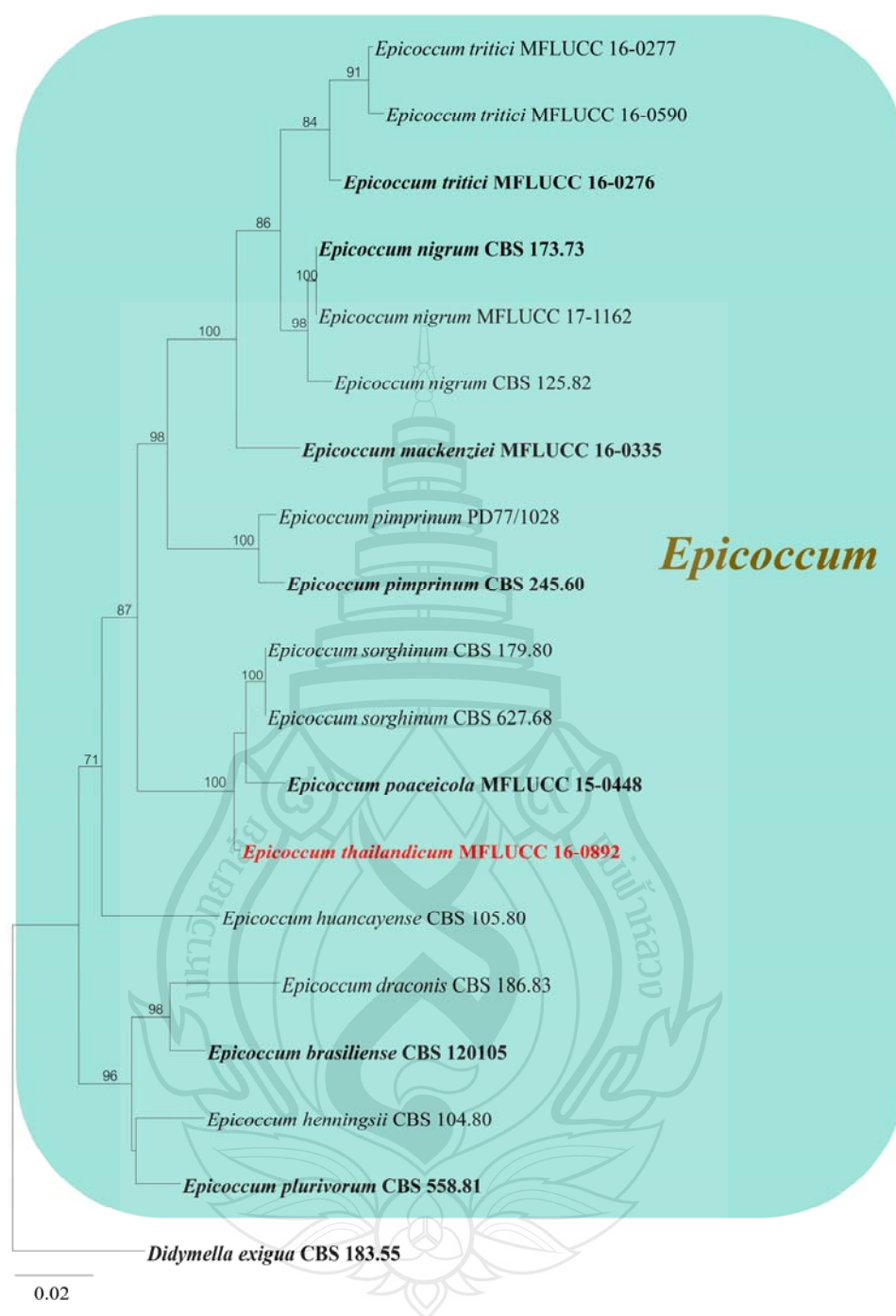
Note Bootstrap support values for ML and MP higher than 65% and Bayesian posterior probabilities (BYPP) greater than 0.90 are given above each branch, respectively. The new isolates are in red. Ex-type strains are in bold. The tree is rooted to *Menispora tortuosa* (AFTOL-ID 278 and CBS 214.56).

Figure 4.1 RAxML tree based on a combined dataset of LSU and ITS sequences representing *Dinemasporium* in *Chaetosphaeriaceae*



Note Bootstrap support values equal or greater than 80 % are given at the nodes. The ex-type strains are in bold and the new isolate in red. The tree is rooted to *Phaeosphaeria oryzae*.

Figure 4.2 Phylogram resulting from ML analysis of a combined LSU, SSU, ITS and RPB2 dataset of *Coniothyrium*, *Coniothyriaceae*



Note Maximum likelihood bootstrap support values equal to or greater than 70 % are given at the nodes. The ex-type strains are in bold and the new isolate in red. The tree is rooted to *Didymella exigua* CBS 183.55.

Figure 4.3 Phylogram resulting from ML analysis of LSU, ITS, RPB2 and TUB sequences representing *Epicoccum* in *Didymellaceae*

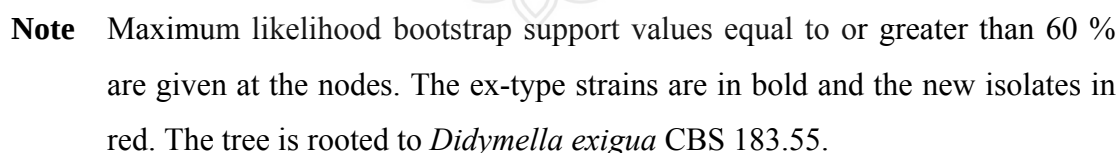
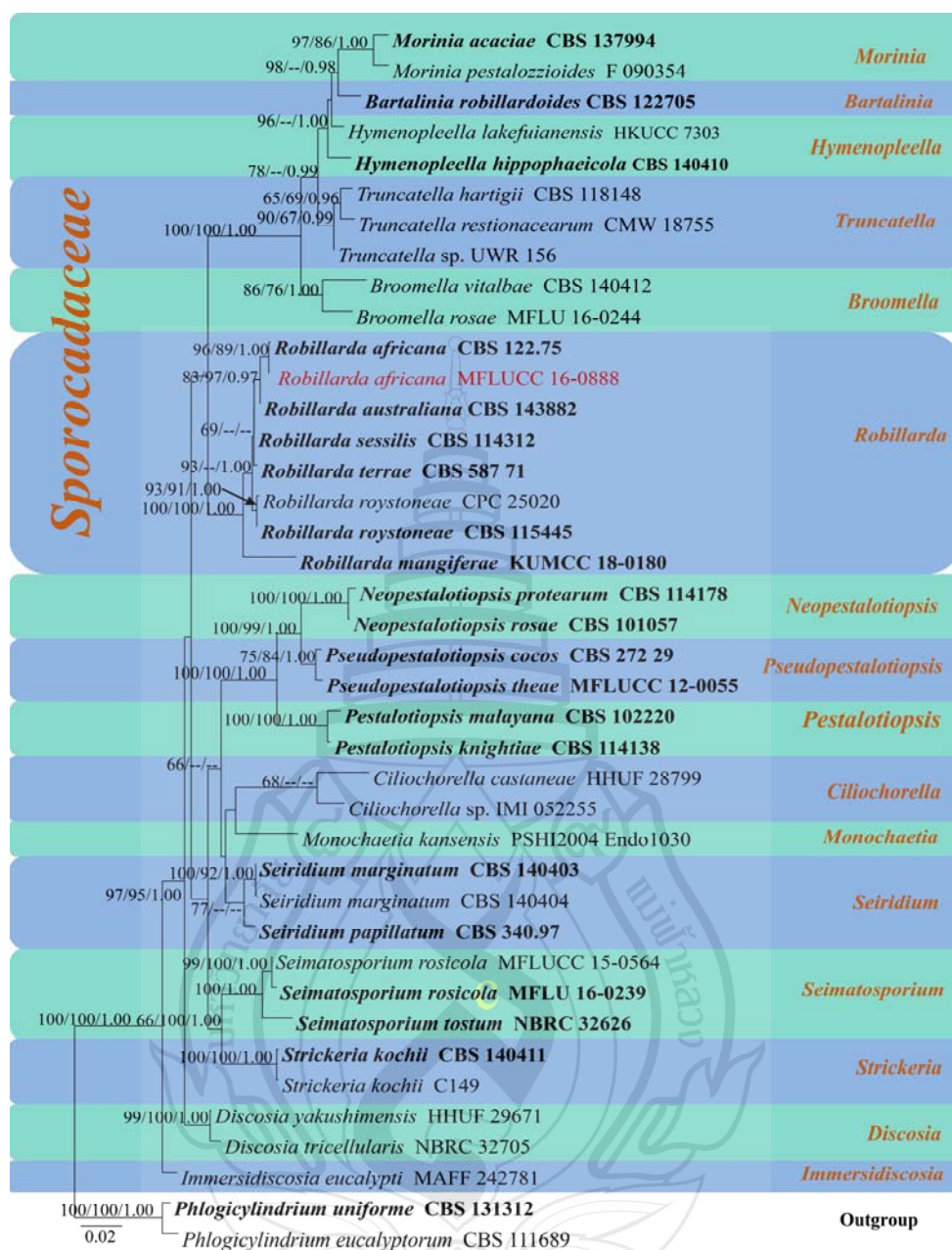


Figure 4.4 Phylogram resulting from ML analysis of LSU, SSU, ITS and RPB2 sequences of *Phaeosphaeriaceae*



Note Bootstrap support values for ML and MP higher than 65% and Bayesian posterior probabilities (BYPP) greater than 0.90 are given above each branch, respectively. The new isolates are in red. Ex-type strains are in bold. The tree is rooted to *Phlogicylindrium eucalyptorum* (CBS 111689) and *P. uniforme* (CBS 131312).

Figure 4.5 RAxML tree based on a combined dataset of LSU and ITS sequence data of *Sporocadaceae*

4.3.2 Taxonomy

Chaetosphaeriaceae Réblová, M.E. Barr & Samuels

Notes: This family, introduced by Locquin (1984), was not considered as validly published due to not having a proper description. Therefore, Réblová et al. (1999) re-established this family to accommodate *Chaetosphaeria* as the type genus and six genera (*Ascocodinaea*, *Melanochaeta*, *Melanopsammella*, *Porosphaerella*, *Porosphaerellopsis* and *Striatosphaeria*). *Chaetosphaeriaceae* species are widely distributed as saprobes (Réblová et al., 1999; Maharachchikumbura et al., 2016; Lin et al., 2019; Hyde et al., 2020a). Forty-three genera are accepted in Chaetosphaeriaceae (Hyde et al. 2020a). The latest treatments by Lin et al. (2019) and Hyde et al. (2020a) for *Chaetosphaeriaceae* have been followed.

Dinemasporium Lév.

Notes: Lévillé (1846) introduced *Dinemasporium* with *D. graminum* (Lib.) Lév. as the type species. *Dinemasporium* members have superficial, setose, cupulate conidiomata, discrete or integrated conidiogenous cells and fusiform, naviculate or allantoid, aseptate conidia, with one setula at each end, with or without lateral appendages (Crous et al., 2012; Maharachchikumbura et al., 2016; Hyde et al., 2020a). This genus is broadly distributed on various woody plants and some species have been recorded from soil and human sputum (Hussaini et al., 2000; Crous et al., 2012; Deshmukh & Verekar, 2012; Hashimoto et al., 2015; Liu et al., 2015). There are 43 *Dinemasporium* species listed in Index Fungorum (2021).

Dinemasporium pseudostrigosum Crous, Persoonia 28: 134 (2012)

Index Fungorum number: IF800164; Facesoffungi number: FoF 07316 (Figure 4.6)

Saprobic on culms of dead grass. **Sexual morph:** Undetermined. **Asexual morph:** Coelomycetous. *Conidiomata* 60–100 × 120–150 µm ($\bar{x}$ = 80 × 135 µm, n = 15), stromatic, brown to black, superficial, solitary to gregarious, occasionally confluent, pulvinate, oval to rounded in outline, cupulate, with incurved margins, unilocular, setose. *Conidiomatal setae* 200–250 µm long, 3–5 µm wide, arising from the basal stroma, straight or curved, septate, brown but pale at the apex, thick-walled, acute, unbranched. *Conidiomatal wall* 8–10 µm wide, of *textura angularis* with cells brown to pale brown, thick-walled. *Conidiophores* lining the basal stroma, rectangular to cylindrical, smooth, pale brown to hyaline, unbranched. *Conidiogenous*

cells $6\text{--}11 \times 2\text{--}3 \mu\text{m}$ ($\bar{x} = 9 \times 2.3 \mu\text{m}$, $n = 20$), phialidic, subcylindrical to lageniform, hyaline, smooth. *Conidia* $8\text{--}10 \times 2\text{--}3.5 \mu\text{m}$ ($\bar{x} = 9 \times 3 \mu\text{m}$, $n = 40$), hyaline, naviculate to fusiform or ellipsoid, obtuse or slightly acute at the apex, slightly truncate at the base, unicellular, eguttulate or guttulate, smooth, bearing $3\text{--}6 \mu\text{m}$ long, unbranched, single tubular appendage at each end.

Culture characteristics: Colonies on PDA reaching 4–5 cm diam. after 2 weeks at 28–30 °C, medium sparse, irregular, slightly raised, surface smooth with undulate edge, fairly fluffy and strongly irregular, wrinkled, folded margins, colony from above white, reverse pale yellow becoming greyish brown just surrounding the center as the colony matures, grey to pale yellow at the margins, no pigmentation observed.

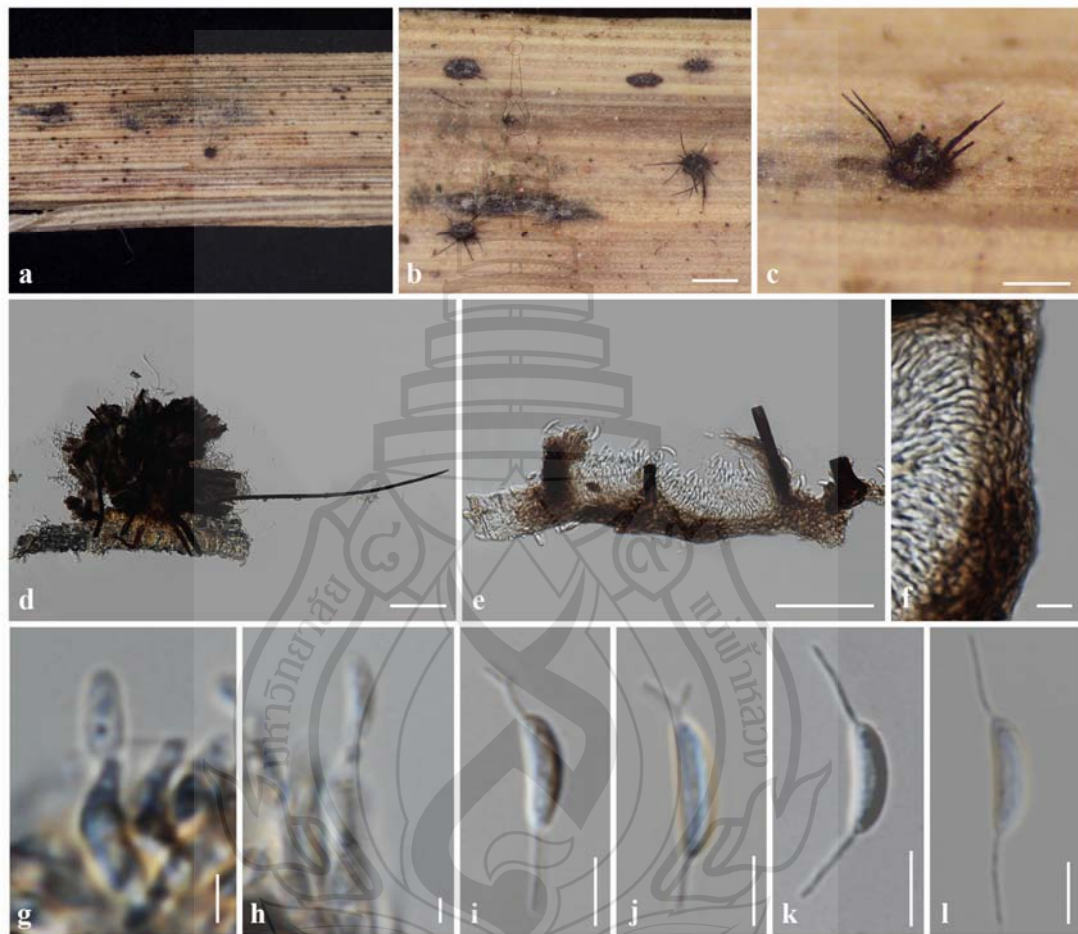
Known hosts: Bamboo species, dead grass (Poaceae), *Elymus farctus* (Poaceae), *Stigmaphyllon sagraeanum* (Malpighiaceae) (Crous et al., 2012; Hashimoto et al., 2015; Li et al., 2020; this study).

Known distribution: Germany, Italy, Japan, Thailand (Crous et al., 2012; Hashimoto et al., 2015; Li et al., 2020; this study).

Material examined: Thailand, Chiang Mai Province: Mae Teang District, Mushroom Research Center (M.R.C.), on dead culms of unidentified grass, 24 March 2016, Ishani D. Goonasekara, IGM34 (MFLU 22-0006), living culture MFLUCC 16-0894; *ibid.*, on dead culms of unidentified grass, 28 March 2016, Ishani D. Goonasekara, IGM38 (MFLU 22-0007), living culture, MFLUCC 16-0896.

Notes: The new isolates from this study (MFLUCC 16-0894 and MFLUCC 16-0896) cluster with *D. pseudostrigosum* isolates (CBS 717.85, CBS 825.91 and MFLU 15-0588) in a strongly supported clade (81% ML, 91%, 0.99 BYPP, Figure 4.1). In particular, they have a close relationship with the strain CBS 825.91 showing high statistical support (76% ML, 71% MP, 0.97 BYPP). Based on phylogenetic results and compared morphology, they are identified as *Dinemasporium pseudostrigosum*. This taxon was originally described in Crous et al. (2012) from *Triticum aestivum* in Germany. Morphologically, our collections resemble the collections of *D. pseudostrigosum* (CBS 717.85, CBS 825.91) in having stromatic, brown to black, superficial, unilocular, setose conidiomata, subcylindrical to lageniform conidiogenous cells and naviculate to fusiform or ellipsoid, aseptate,

hyaline conidia with tubular appendages at each end (Crous et al., 2012). However, the collections from Thailand (this study) have relatively smaller conidia ($8\text{--}10 \times 2\text{--}3.5 \mu\text{m}$) than of the type species ($12\text{--}13 \times 3 \mu\text{m}$) described by Crous et al. (2012). This is the first record of *D. pseudostrigosum* for Thailand.



Note (a, b) Appearance of conidiomata on host. (c) Close-up of conidioma. (d) Squash mount of conidioma with seta. (e) Section of conidioma. (f) Conidiomatal wall. (g, h) Conidiogenous cells and developing conidia. (i–l) Conidia. Scale bars: b, c = 500 μm , d, e = 50 μm , g, h = 2 μm , f, i–l = 5 μm .

Figure 4.6 *Dinemasporium pseudostrigosum* (MFLU 22-0006, new geographical record)

***Coniothyriaceae* W.B. Cooke**

Notes: *Coniothyriaceae* was introduced by Cooke (1983b) and was considered as a synonym of *Leptosphaeriaceae* (Kirk et al., 2008; de Gruyter et al., 2009). It is currently accepted as a distinct family in *Pleosporales* (Hyde et al., 2013; Quaedvlieg et al., 2013; Wijayawardene et al., 2016; Wijayawardene et al., 2020) and consists of the five genera *Coniothyrium*, *Foliophoma*, *Neoconiothyrium*, *Ochrocladosporium* and *Staurosphaeria*.

***Coniothyrium* Corda**

Notes: *Coniothyrium* and coniothyrium-like species are considered to be polyphyletic, grouping in several clades within the order *Pleosporales* (Hyde et al., 2013; Verkley et al., 2014; Chen et al., 2015). However, *Coniothyriaceae*, *Pleosporales* is represented by the strains of *Coniothyrium* sensu stricto (Verkley et al., 2014; Chen et al., 2015; Wijayawardene et al., 2016). Several phoma-like species have been placed in *Coniothyrium* based on morphological similarities (De Gruyter et al., 2013). A sexual morph of *Coniothyrium* was collected in this study and phylogenetic analysis confirmed its placement in *Coniothyriaceae*.

Coniothyrium chiangmaiense* Goonas., Thambugala & K.D. Hyde, *sp. nov.

Index Fungorum number: IF552972; Facesoffungi number: FoF 03192; Figure 4.7

Etymology: Referring to the province Chiang Mai, where the species was first encountered.

Holotype: MFLU 16-2854

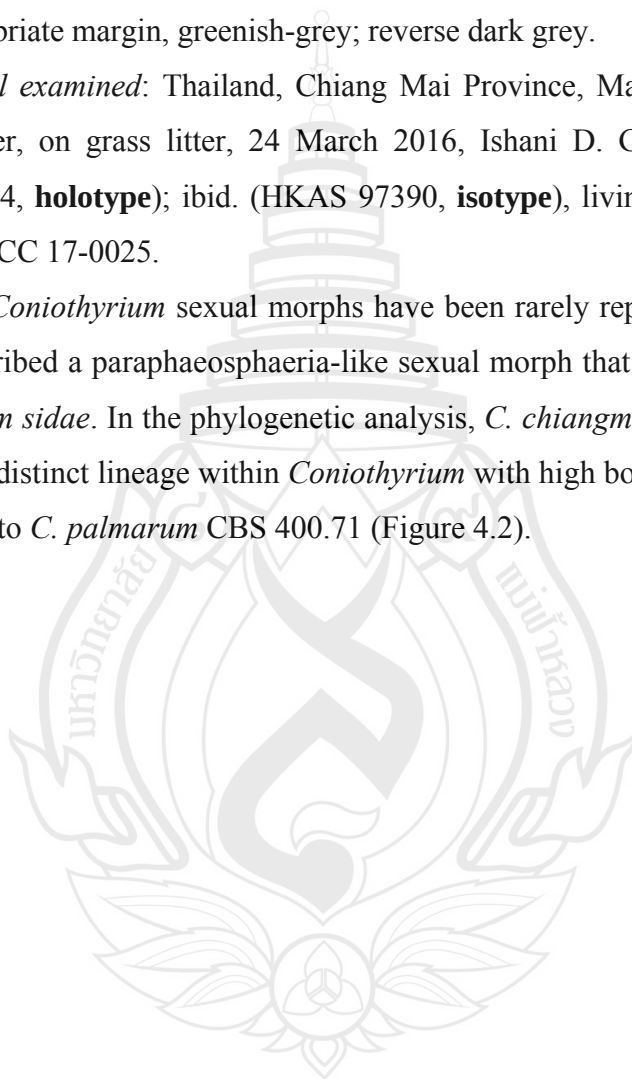
Saprobic on dead stem of grass litter, forming black, elongated, raised structures, ascostromata opening through slits along the length. **Sexual morph:** *Ascostromata* 0.13–0.25 mm long, 0.1–0.18 mm wide, 0.1–0.2 mm high, solitary, immersed, fusiform. *Ascomata* perithecial, 120–270 µm high, 200–380 µm diameter, 3–4 perithecia immersed within the ascostromata, subglobose or globose with a flattened base, dark brown, with centrally located short ostiole. *Peridium* 6.8–9 µm wide, thick to thin-walled, of unequal thickness, poorly developed at the base and merging with host tissue, composed of several layers of outer brown to inner hyaline, pseudoparenchymatous cells of *textura prismatica*. *Hamathecium* of dense, 1–2.2 µm wide, filamentous, indistinct, septate, cellular pseudoparaphyses, not constricted at the septum. *Asci* 60–75 × 5.5–8 µm ($\bar{x}$ = 67 × 7 µm, n = 15), 8-spored, bitunicate,

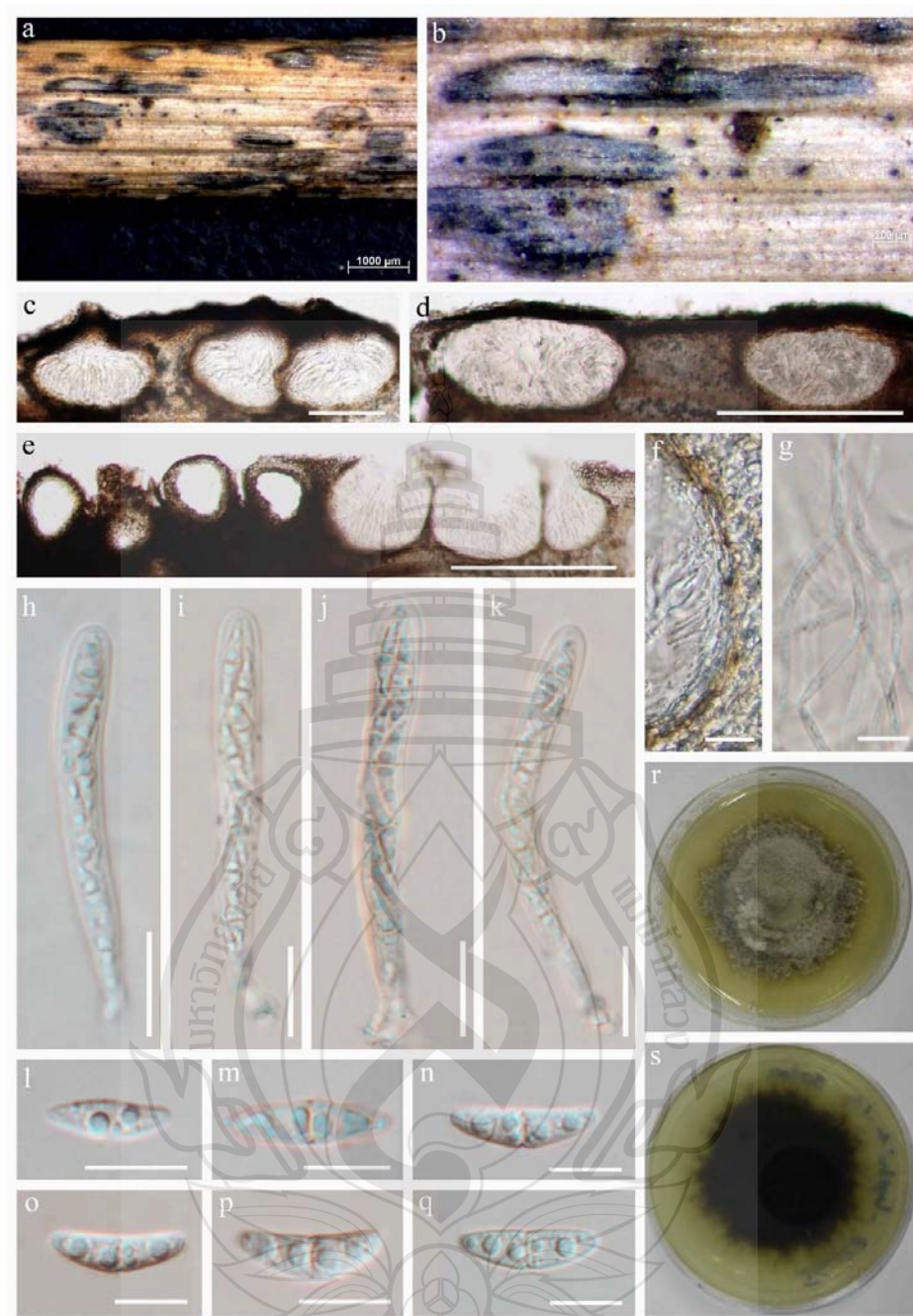
fissitunicate, cylindric-clavate, with broad flat pedicel, apex rounded. *Ascospores* 13–17 × 3.5–5 µm ($\bar{x}$ = 15 × 4.2 µm, n = 20), overlapping uni-seriate, hyaline, fusiform, with rounded to acute ends, 1-septate, slightly constricted at the septum, cells broader near the septum, smooth-walled, guttulate. **Asexual morph:** Undetermined.

Culture characteristics: Conidia germinating on PDA within 12 h reaching a diameter of 25 mm after 7 d at 25 °C, irregular, dense, floccose and velvety, slightly raised with fimbriate margin, greenish-grey; reverse dark grey.

Material examined: Thailand, Chiang Mai Province, Mae Taeng, Mushroom Research Center, on grass litter, 24 March 2016, Ishani D. Goonasekara IGM 28 (MFLU 16-2854, **holotype**); *ibid.* (HKAS 97390, **isotype**), living culture, MFLUCC 16-0891, KUMCC 17-0025.

Notes: *Coniothyrium* sexual morphs have been rarely reported. Quaedvlieg et al. (2013) described a paraphaeosphaeria-like sexual morph that developed in culture of *Coniothyrium sidae*. In the phylogenetic analysis, *C. Chiangmaiense* MFLUCC 16-0891 formed a distinct lineage within *Coniothyrium* with high bootstrap support (92% ML) and sister to *C. palmarum* CBS 400.71 (Figure 4.2).





Note (a) Appearance of ascostromata on host surface. (b) Close up of ascostromata (c–e) Vertical sections through ascostromata showing locules. (f) Peridium of locule. (g) Pseudoparaphyses. (h–k) Asci. (l–q) Ascospores. (r, s) Colony on PDA (from above and reverse). Scale bars: c–e = 250 μm , f = 20 μm , g, l–q = 10 μm , h–k = 15 μm .

Figure 4.7 *Coniothyrium chiangmaiense* (MFLU 16-2854, holotype)

Didymellaceae Gruyter, Aveskamp & Verkley

Notes: Species of *Didymellaceae* include plant pathogens, saprobes and endophytes associated with a wide range of hosts and substrates worldwide (Hyde et al., 2013; Chen et al., 2015; Liu et al., 2015). A revision of this family based on morphology and multi-gene (ITS, LSU, RPB2 and TUB) phylogenetic analysis was provided by Chen et al. (2015).

Epicoccum Link

Notes: Initially, *Epicoccum* was known as the hyphomycetous asexual morph in *Didymellaceae* (Hyde et al., 2013). However, as a result of combined molecular data analysis, five *Phoma* species were recombined into *Epicoccum* by Chen et al. (2015), emending the genus to include coelomycetous synasexual morphs.

Epicoccum thailandicum Goonas., Thambugala & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF552996; Facesoffungi number: FoF 03215; Figure 4.8

Etymology: The specific epithet “thailandicum” refers to the country where the taxon was first collected.

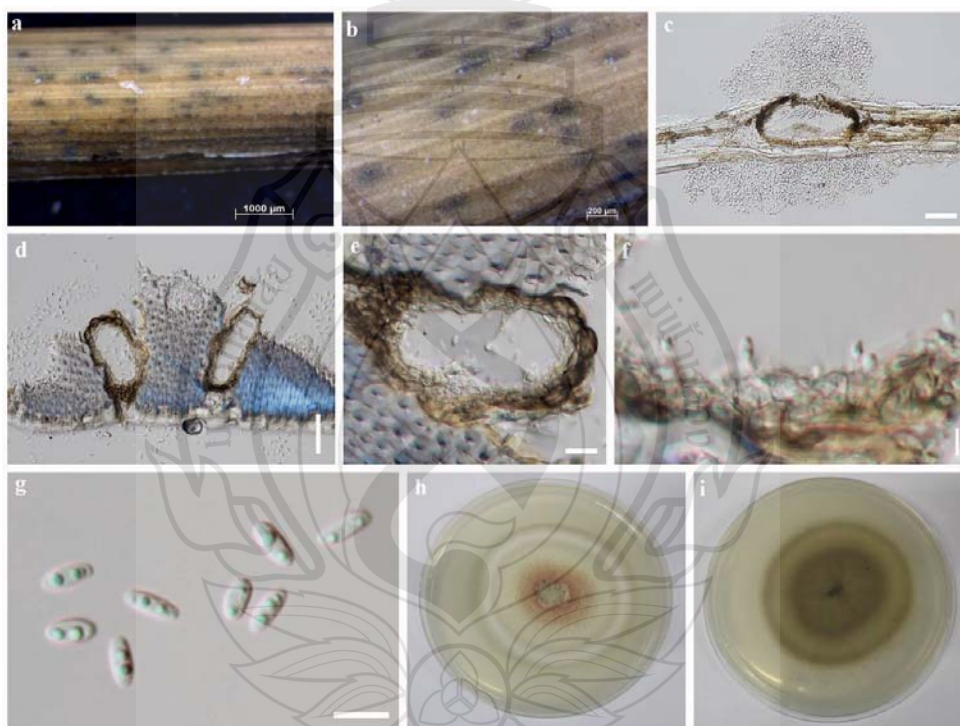
Holotype: MFLU 16-2855

Saprobic on grass litter. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* 100–150 µm diameter, 50–70 µm high ($\bar{x} = 127 \times 60$ µm, $n = 10$), pycnidial, solitary, in linear rows, immersed, visible as black elongated patches on host surface, subglobose with a flattened base. *Conidiomata* wall 8.5–16 µm wide, comprising outer thick-walled, dark brown to light brown cells of *textura angularis* to *textura prismatica* and inner hyaline cells of *textura angularis*, with outer cells at base gradually fusing with host tissues and indistinguishable. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 3–4.5 × 2–3 µm ($\bar{x} = 3.5 \times 2.3$ µm, $n = 10$), holoblastic, hyaline, doliform or obpyriform, smooth. *Conidia* 7.5–10 × 3–4.5 µm ($\bar{x} = 8.5 \times 3.5$ µm, $n = 30$), oblong, with rounded ends, hyaline, aseptate, guttulate, smooth-walled.

Culture characteristics: Conidia germinating on PDA within 12 h reaching a diameter of 51 mm after 7 d at 25 °C, circular, flat, surface smooth, with entire edge, pale orange at the center and greyish towards the edge, with white margin; reverse yellowish-grey.

Material examined: Thailand, Chiang Mai Province, Mae Taeng, Mushroom Research Center, on grass litter, 24 March 2016, Ishani D. Goonasekara IGm 30 (MFLU 16-2855, **holotype**) ibid. (HKAS 97391, **isotype**), living culture, MFLUCC 16-0892, KUMCC 17-0026.

Notes: *Epicoccum thailandicum* is morphologically similar to *E. sorghinum* (Sacc.) Aveskamp et al., but distinct in conidial dimensions (*E. thailandicum* $7.5\text{--}10 \times 3\text{--}4.5 \mu\text{m}$ and *E. sorghinum* $5 \times 2 \mu\text{m}$) (Saccardo, 1878). In the phylogenetic analysis (Figure 4.3) carried out in this study, *E. thailandicum* forms a strongly supported clade (100% ML) within the genus and therefore is introduced as a new species.



Note (a, b) Appearance of conidiomata on host (c, d) Section through conidiomata (e) Vertical section through conidioma showing conidioma wall. (f) Conidia arising from conidiogenous cells (g) Conidia (h) Colony from above (i) Colony from below. Scale bars: c, d = $50 \mu\text{m}$, e = $100 \mu\text{m}$, f = $5 \mu\text{m}$, g = $10 \mu\text{m}$.

Figure 4.8 *Epicoccum thailandicum* (MFLU 16-2855, holotype)

***Phaeosphaeriaceae* M.E. Barr**

Notes: Members of the family *Phaeosphaeriaceae* are commonly found on many Poaceae host species (Quaedvlieg et al., 2013; Phookamsak et al., 2014). In the present study, two new species and the first record of an asexual morph of *Ophiosphaerella agrostidis* is described.

***Neosetophoma* Gruyter, Aveskamp & Verkley**

Notes: Gruyter et al. (2010) erected the genus to accommodate *Phoma samarorum* Desm. Subsequently, two asexual species *N. clematidis* and *N. italica* were added (Liu et al., 2015).

Neosetophoma poaceicola* Goonas., Thambugala & K.D. Hyde, *sp. nov.

Index Fungorum number: IF552974; Facesoffungi number: FoF 03194; Figure 4.9

Etymology: in reference to holotype occurring on grasses (*Poaceae*).

Holotype: MFLU 16-2852

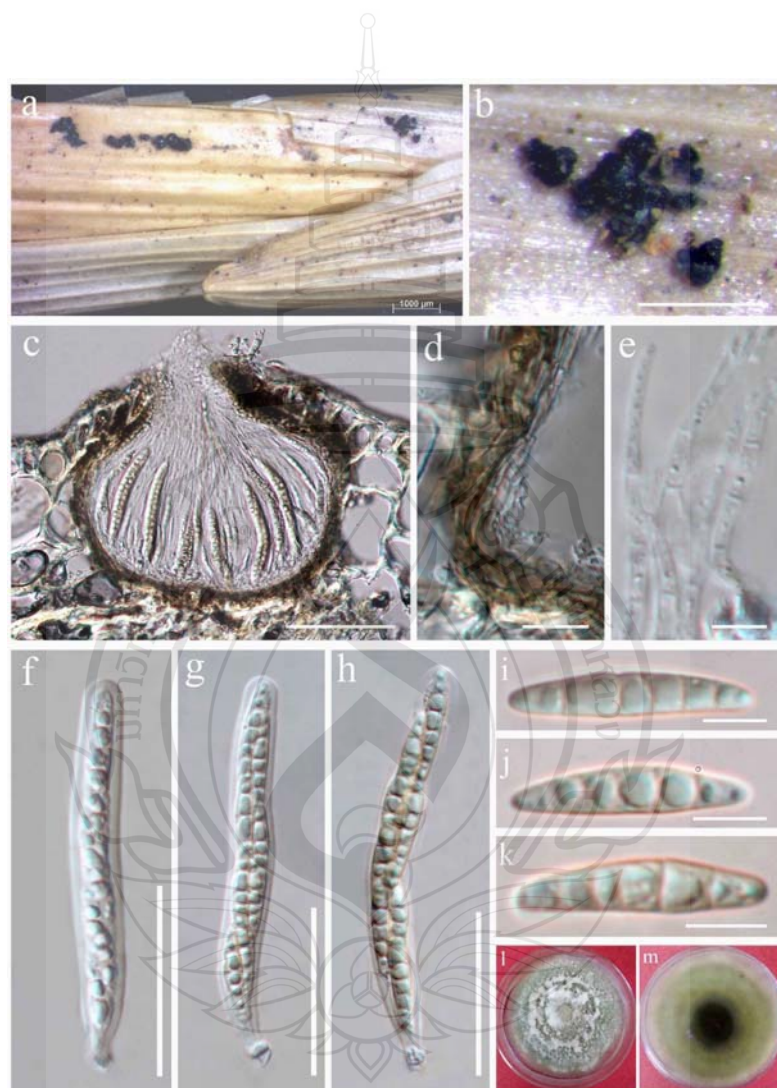
Saprobic on dead grass, as raised, black, shiny globular structures on host surface. **Sexual morph:** *Ascomata* 130–145 µm high, 135–160 µm diameter, solitary, gregarious, immersed under epidermis, becoming erumpent through host surface, globose to subglobose glabrous short ostiolate. *Peridium* 8.5–14.5 µm wide, thick-walled, of equal thickness, composed of several layers of dark brown to brown, pseudoparenchymatous cells of *textura angularis*. *Hamathecium* of dense 0.9–1.8 µm wide, filamentous, indistinct, septate, cellular pseudoparaphyses, slightly constricted at the septa, embedded in a hyaline gelatinous matrix. *Asci* 55–82 × 7–9 µm ($\bar{x}$ = 70.5 × 8.4 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindric-clavate with short rounded pedicel, apically rounded. *Ascospores* 18.5–22.5 × 3.5–5 µm ($\bar{x}$ = 20.5 × 4.5 µm, n = 20), overlapping uni-seriate to bi-seriate, hyaline, fusiform, with acute ends, occasionally 1-septate, cell near the septum slightly larger, smooth-walled, guttulate. **Asexual morph:** Undetermined.

Culture characteristics: Conidia germinating on PDA within 12 h reaching a diameter of 20 mm after 7 d at 25 °C, circular, floccose, flat with even margin, initially pale yellow becoming grey, circular; reverse yellowish-grey.

Material examined: Thailand, Chiang Mai Province, Mae Taeng, Mushroom Research Center, on grass litter, 18 March 2016, Ishani D. Goonasekara IGM 22 (MFLU 16-2852,

holotype); ibid. (HKAS 97388, **isotype**), living culture, MFLUCC 16-0886, KUMCC 17-0023.

Notes: *Neosetophoma* was introduced as an asexual genus and the new species, *N. poaceicola* represents the first record of a sexual morph of this genus. In the phylogenetic analysis (Figure 4.4) the new isolate MFLUCC 16-0886 groups with other *Neosetophoma* isolates with high bootstrap support (84% ML).



Note (a) Appearance of ascomata on host. (b) Close up of ascoma (c) Vertical section through ascoma. (d) Peridium. (e) Pseudoparaphyses. (f–h) Asci. (i–k) Ascospores. (l, m) Culture characteristics (from above and reverse). Scale bars: b = 500 μm , c = 100 μm , d = 20 μm , e, i–k = 10 μm , f–h = 50 μm .

Figure 4.9 *Neosetophoma poaceicola* (MFLU 16-2852, holotype)

***Ophiosphaerella* Speg.**

Notes: *Ophiosphaerella* was introduced by Spegazzini (1909) and is typified by *Op. graminicola*. Most species of this genus are pathogens or saprobes occurring worldwide on Poaceae and Cyperaceae (Phookamsak et al., 2014a).

Ophiosphaerella agrostidis Dem., M.P.S. Câmara, N.R. O'Neill, Berkum & M.E. Palm

Index Fungorum number: IF 464614; Facesoffungi number: FoF 00258; Figure 4.10

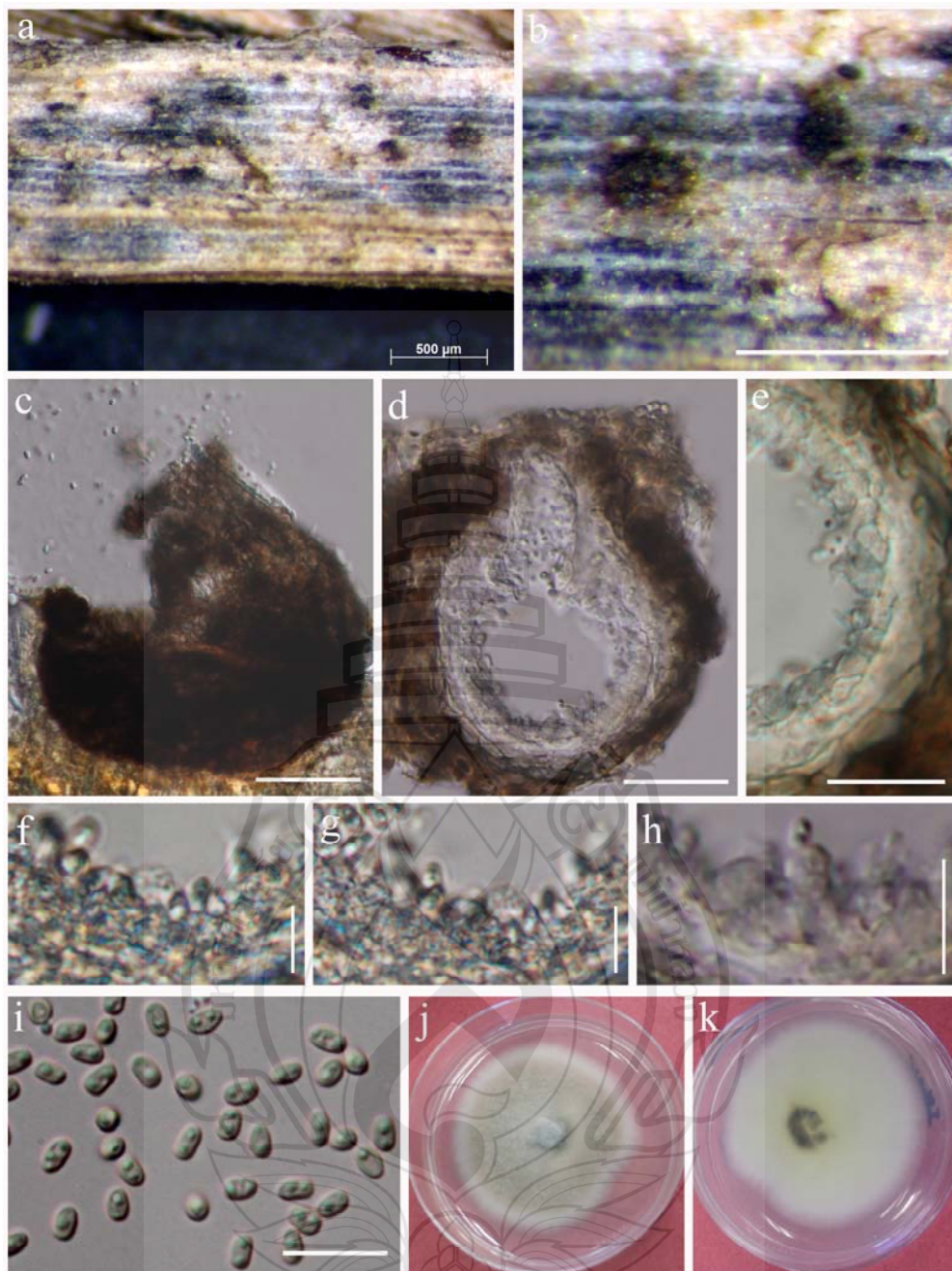
Saprobic on stems of grasses. **Sexual morph:** See Phookamsak et al. (2014a).

Asexual morph: *Conidiomata* 50–60 µm diameter 65–80 µm high ($\bar{x} = 58.5 \times 75.5$ µm, $n = 6$), solitary or scattered, immersed to semi-immersed, appearing as black, slightly raised lesions on host surface, globose to subglobose, ostiolate, papillate. Ostioles 30–33 µm high, 14–18 µm diameter ($\bar{x} = 31 \times 16$ µm, $n = 6$). *Conidiomatal walls* 6.5–11 µm wide, comprising several layers of outer, flattened, dark brown cells of *textura prismatica* and inner, hyaline cells of *textura angularis* to *textura globulosa*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 2–3.5 × 1.5–3 µm ($\bar{x} = 3 \times 2.5$ µm, $n = 8$), lining the conidiomatal cavity, holoblastic, hyaline, doliiform to ampulliform. *Conidia* 2.5–3.5 × 1.2–2 µm ($\bar{x} = 3 \times 1.5$ µm, $n = 45$), pale brown, oblong, aseptate, guttulate, smooth-walled.

Culture characteristics: Conidia germinating on PDA within 12 hours reaching a diameter of 18 mm after 7 days at 25 °C, circular, flat, surface smooth to velvety, with edge entire, grey with a lighter margin; reverse pale yellow.

Material examined: Thailand, Chiang Mai Province, Mae Taeng, Mushroom Research Center, on decaying grass (Poaceae), 24 March 2016, Ishani D. Goonasekara IGM 35 (MFLU 16-2856), living culture MFLUCC 16-0895, KUMCC 17-0027.

Notes: Our isolate MFLUCC 16-0895 which is a paraphaeosphaeria-like asexual morph grouped with two strains of *Ophiosphaerella agrostidis* (MFLUCC 11-0152 and MFLUCC 12-0007, Figure 4.4). Based on this phylogenetic evidence we assigned this specimen as an asexual morph record of *Op. agrostidis*. No asexual morph has been reported before for the species. *Scolecosporiella* has been recorded as the asexual morph of *Op. herpotricha*. However, there is no phylogenetic evidence to confirm this (Phookamsak et al., 2014a).



Note (a) Appearance of conidiomata on host surface (b) Close up of conidiomata (c) Squash mount of conidiomata (d) Section through conidiomata (e) Conidiomata wall (f-h) Conidiogenous cells (i) Conidia (j, k) Colony characteristics (from above and reverse) Scale bars: b = 200 µm, c-e = 25 µm, f-h = 5 µm, i = 10 µm.

Figure 4.10 *Ophiosphaerella agrostidis* (MFLU 16-2856)

Setophoma Gruyter, Aveskamp & Verkley

Notes: *Setophoma* was introduced by de Gruyter et al. (2010) to accommodate *Phoma terrestris* H.N. Hansen and *Pyrenochaeta sacchari* Bitancourt in the family *Phaeosphaeriaceae*. Four species epithets are listed in Index Fungorum (2017).

Setophoma poaceicola Goonas., Thambugala & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF552993; Facesoffungi number: FoF 03212; Figure 4.11

Etymology: in reference to holotype occurring on grasses (Poaceae).

Holotype: MFLU 16-2850

Saprobic on dead culm of grass, appearing as black, flat to slightly raised lesions, sometimes coalescing creating larger lesions. **Sexual morph:** *Ascomata* perithecial, solitary, scattered to gregarious, glabrous, 90–110 µm high, 70–100 µm diameter, globose, dark brown to black, with centrally located short ostiole. *Peridium* 10–15 µm wide, composed of several layers of outer thick-walled, cells of *textura prismatica* and inner, hyaline cells of *textura prismatica* to *textura angularis*. *Hamathecium* comprising many, filamentous, 0.5–0.9 µm wide, indistinctly septate, anastomosing pseudoparaphyses. *Asci* 30–70 × 6.5–8 µm ($\bar{x}$ = 48.5 × 7 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical, with short double lobed pedicel, apex rounded, ocular chamber present. *Ascospores* 14–16 × 3–4 µm ($\bar{x}$ = 15 × 3.5 µm, n = 20), overlapping uni-seriate to bi-seriate, light brown, fusiform, with acute ends, 3-septate, smooth-walled, guttulate. **Asexual morph:** Undetermined.

Culture characteristics: Conidia germinating on PDA within 12 h, growing into an irregular colony, reaching a diameter of 15 mm after 7 d at 25 °C, flat with a filamentous margin, dull and wrinkled surface, opaque, dark brown, orange-brown at the margin; reverse same.

Material examined: Thailand, Chiang Mai Province, Mae Taeng, Mushroom Research Center, on grass litter, 9 March 2016, Ishani D. Goonasekara IGM 05 (MFLU 16-2850, **holotype**); *ibid.* (HKAS 97386, **isotype**), living culture, MFLUCC 16-0880, KUMCC 17-0021.

Notes: This is the first record of a *Setophoma* species from Thailand. In the phylogenetic analysis, the ex-type strain of *S. poaceicola* forms a strongly supported clade (100% ML) within the genus (Figure 4.4).



Note (a) Appearance of ascomata on host surface (b) Close up of ascomata (c, d) Vertical sections through ascomata (e) Peridium (f) Pseudoparaphyses (g–k) Asci (l–q) Ascospores (r) Colony - from above (s) Colony – reverse. Scale bars: a = 1000 μm , b = 200 μm , c = 100 μm , d, g = 50 μm , e, f, h–k = 20 μm , l–q = 10 μm .

Figure 4.11 *Setophoma poaceicola* (MFLU 16-2850, holotype)

***Sporocadaceae* Corda**

Notes: *Sporocadaceae*, also known as pestalotioid fungi, was introduced by Corda (1842) to accommodate *Sporocadus* as the type genus. Species of *Sporocadaceae* are cosmopolitan and have diverse lifestyles as endophytes, plant pathogens, saprobes, and associated with a wide range of host plants (Bonthond et al., 2018; Liu et al., 2019; Hyde et al. 2020a). Typically, appendage bearing coelomycetes genera are included in this family and have been subjected to several taxonomic treatments (Nag Raj, 1993; Jaklitsch et al., 2016; Maharachchikumbura et al., 2016; Liu et al., 2019). Thirty genera are accepted in *Sporocadaceae* (Liu et al., 2019; Hyde et al., 2020a). In this study, we follow Hyde et al. (2020a) as the most recent treatment for *Sporocadaceae*.

***Robillarda* Sacc.**

Notes: *Robillarda* was introduced by Saccardo (1880) and typified by *R. sessilis* Sacc. *Robillarda* is characterized in having flexuous, narrow tubular, aseptate appendages and holoblastic conidiogenous cells, proliferating sympodially or percurrently near the apex (Crous et al., 2015; Jaklitsch et al., 2016; Hyde et al., 2020a). *Robillarda* species are distributed worldwide, on various host species (Crous et al., 2015; Liu et al., 2019; Hyde et al., 2020a). There are 17 *Robillarda* species listed in Index Fungorum (2021).

***Robillarda africana* Crous & Giraldo López, IMA Fungus 6(1): 184 (2015)**

Index Fungorum number: IF812797; Facesoffungi number: FoF 10700; Figure 4.12

Saprobic on grass litter. **Sexual morph:** Undetermined. **Asexual morph:** Coelomycetous. *Conidiomata* 100–140 × 170–230 µm ($\bar{x}$ = 125 × 190 µm, n = 20), pycnidial, scattered, globose to subglobose, semi-immersed, unilocular, dark brown to black, ostiolate. *Conidiomatal wall* 30–40 µm, brown to dark brown, arranged in a *textura angularis*, cells towards the inside hyaline, at the outside darker, fusing and indistinguishable from the host tissues. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 4–5 × 2–3 µm ($\bar{x}$ = 4.5 × 2.5 µm, n = 20), phialidic, ampulliform to subcylindrical, hyaline, smooth, guttulate, deliquescing at maturity. *Conidia* 10–12 × 1.5–3 µm ($\bar{x}$ = 11 × 2.2 µm, n = 30), cylindrical to fusiform, straight or slightly curved, 1-septate, slightly constricted at median septum, hyaline to

pale brown, rounded at both ends, smooth-walled, 3 extracellular appendages, 11–16 μm long, arising from the conidial apex.

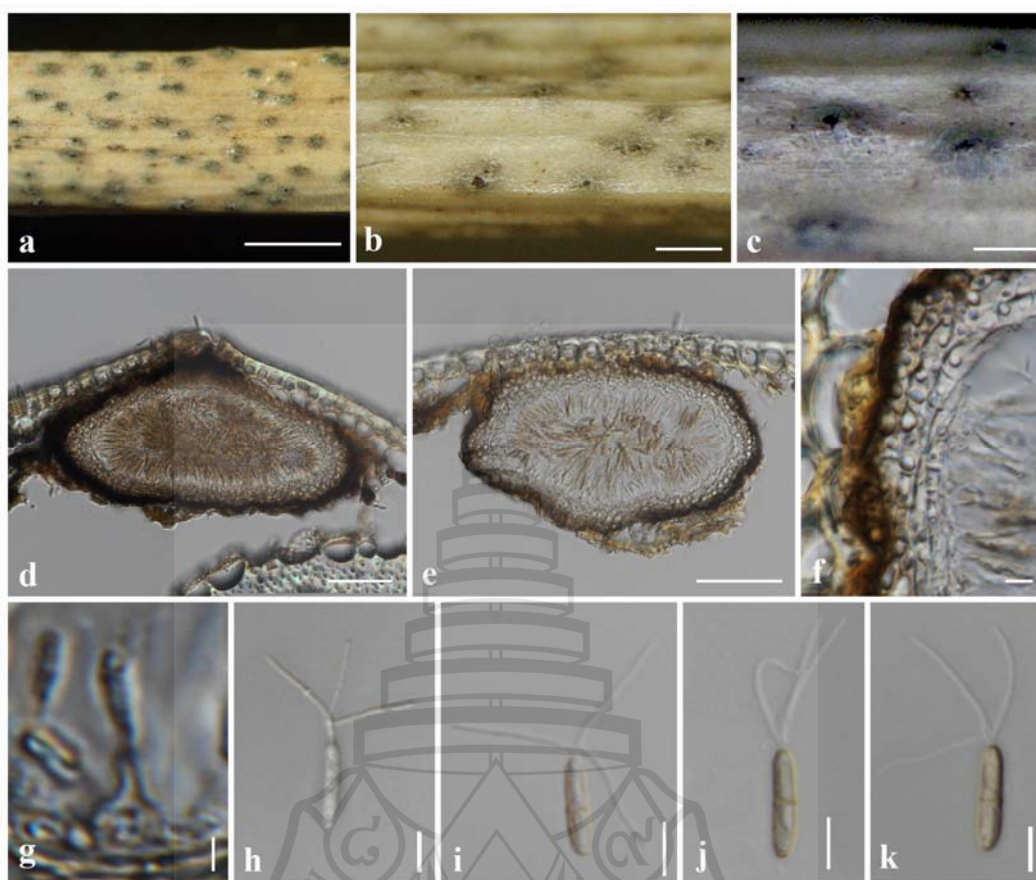
Culture characteristics: Colonies on PDA reaching 35 mm diam. after 15 days at 28–30 °C, medium to medium sparse, circular, flat, dull, entire, thinly hairy, slightly irregular margins, smooth, colony from above olive to dark grey at the center, with pale grey to white margins, reverse darker grey, punctiform-like at the center, with greyish and pale yellowish middle area and white margins, no pigmentation observed.

Material examined: Thailand, Chiang Mai Province: Mae Teang District, Mushroom Research Center (M.R.C.), on dead culms of unidentified grass (Poaceae), 18 March 2016, Ishani D. Goonasekara, IGM 25 (MFLU 22-0005), living culture, MFLUCC 16-0888.

Known hosts: dead grass (Poaceae) (this study).

Known distribution: South Africa, Thailand (Crous et al., 2015; this study).

Notes: As morphological characters examined largely overlap with those of *Robillarda africana* (CBS 122.75), we report our collection (MFLUCC 16-0888) as a new host and geographical record of *R. africana* from dead grass in Thailand. *Robillarda africana* (CBS 122.75) was initially introduced by Crous et al. (2015) from South Africa (exact location and substrate unknown). Both isolates (MFLUCC 16-0888 and CBS 122.75) have pycnidial, globose to subglobose, semi-immersed, unilocular conidiomata, ampulliform to subcylindrical, hyaline conidiogenous cells ($4\text{--}5 \times 2\text{--}3 \mu\text{m}$ vs $3\text{--}10 \times 2\text{--}4 \mu\text{m}$) and cylindrical to fusiform, straight or slightly curved, 1-septate, hyaline to pale brown conidia ($10\text{--}12 \times 1.5\text{--}3 \mu\text{m}$ vs $11\text{--}12 \times 2.5\text{--}3 \mu\text{m}$) with extracellular appendages (Crous et al. 2015). Phylogeny also shows that our collection clusters with *R. africana* (CBS 122.75) with strong support (96% ML, 89% MP, 1.00 BYPP, Figure 4.5).



Note (a, b) Appearance of conidiomata on host. (c) Close-up of conidiomata. (d, e) Sections of conidiomata. (f) Conidiomatal wall. (g) Conidiogenous cells and developing conidia. (h–k) Conidia. Scale bars: a = 1 mm b = 500 μ m, c = 200 μ m, d, e = 50 μ m, f = 10 μ m, g = 2 μ m, h–k = 5 μ m.

Figure 4.12 *Robillarda africana* (MFLU 22-0005, new host and geographical record)

4.4 Discussion

The presence of appendages in coelomycetous fungi is quite fascinating as they play a pivotal role in both aquatic and terrestrial environments, assisting in the dispersal and attachment of conidia to substrata (Jones, 2006; Crous et al., 2012). Researchers have made considerable efforts to identify such fungi and discuss their roles in nature (Sutton & Sellar, 1966; Nag Raj, 1993; Jones, 1994, 2006; Crous et al., 2012). For instance, Sutton and Sellar (1966) treated appendage-bearing

coelomycetes according to the structure (mucilaginous or cellular) and position of appendages on the conidium. Nag Raj (1993) provided a broader revision on coelomycetous genera with diagnostic morphological characters of the appendages, such as shape (filiform, attenuated or podiform), position (apical, basal, lateral, or in combination), patterns of distribution on the conidium and some structural changes. The character of having appendage-bearing conidia, has often provided a basis for the separation of taxa in coelomycetous genera in *Sodariomycetes* i.e., *Coniella*, *Dinemasporium*, *Phyllosticta*, *Neopestalotiopsis*, *Pestalotiopsis*, *Pseudopestalotiopsis*, *Robillarda*, *Seimatosporium*, *Seiridium*, *Truncatella* (Wulandari et al., 2009; Barber et al., 2011; Crous et al., 2014; Senanayake et al., 2015; Alvarez et al., 2016; Jaklitsch et al., 2016).

Despite having 43 species listed in *Dinemasporium* (Index Fungorum, 2021), molecular data is available for only 20 species (Hyde et al., 2020a). The type species *D. graminum* lacks molecular data. There is a lack of knowledge regarding the sexual morph of *Dinemasporium* species (Wijayawardene et al., 2017). Therefore, there is a need to recollect representatives of *Dinemasporium* and further obtain molecular data to clarify their phylogenetic relationships, especially for type species. In this study, we provide taxonomic details for *D. pseudostrigosum* which was collected from grass litter for the first time in Thailand.

Among the seventeen *Robillarda* species listed in Index Fungorum (2021), only six (*R. africana*, *R. australiana* F. Liu, L. Cai & Crous, *R. mangiferae* Thiyag., Wanas., Phookamsak & K.D. Hyde, *R. roystoneae* Crous & Giraldo López, *R. sessilis* (Sacc.) Sacc., *R. terrae* Crous & Giraldo López) have molecular data. In this study, we provide the morphology and phylogeny of the first collection of *R. africana* on dead grass from Thailand. Since most *Robillarda* species lack molecular data, further collections are required for further taxonomic evaluation.

CHAPTER 5

TAXONOMIC NOVELTIES IN *Phaeosphaeriaceae*, AN OVERVIEW WITH NEW RECORDS OF *Pyrenophora trichostoma* (*Pleosporaceae*) ON GRASSES FROM ITALY AND RUSSIA

5.1 Introduction

Among the pathogenic microfungi occurring on grasses, the asexual morph genera in *Phaeosphaeriaceae* are commonly reported and are of great importance (Lamprecht et al., 2011; Phookamsak et al., 2014, 2017; Bakhshi et al., 2015). *Parastagonospora* is one such genus. *Parastagonospora avenae* (A.B. Frank) Quaedvl., Verkley & Crous and *P. nodorum* (Berk.) Quaedvl., Verkley & Crous (the generic type), are both pathogens that cause leaf and glume blotch on cereal crops such as barley, wheat and rye, leading to heavy losses of yield (Cunfer, 2000; Stukenbrock et al., 2006; Vergnes et al., 2006; Quaedvlieg et al., 2013; Phookamsak et al., 2014).

In this study two novel *Parastagonospora* species, one on *E. repens* from Russia and the other on *D. glomerata* from Italy are introduced based on phylogenetic analyses of ITS, LSU and RPB2 sequence data complete with descriptions and illustrations. *Elymus repens* (L.) Gould, commonly known as couch grass, is important for grazing animals, used in traditional Austrian medicine and is considered an invasive weed in some parts of the world (Werner & Rioux, 1977; Klein, 2011; Vogl et al., 2013). *Dactylis glomerata* L. (cocksfoot) is a commonly found grass distributed in temperate regions throughout Asia, Europe and North Africa (Sánchez Márquez et al., 2007). It is widely used as fodder or for pastures (Hackney & Dear, 2007). Five fungal species have been recorded from *Elymus* spp. in Russia (Farr & Rossman, 2019) while more than ten species have been recorded on *D. glomerata* from Italy (Farr & Rossman 2019).

Septoriella belonging to *Phaeosphaeriaceae* is typified by *Septoriella phragmitis* Oudem. (Crous et al., 2015). Species of this genus have been reported worldwide, from a range of hosts families including Juncaceae, Poaceae and Rosaceae, while *Septoriella hirta* is noted as an important secondary pathogen on grasses (Sprague, 1950; Marin-Felix et al., 2019; Farr & Rossman, 2019). A novel *Septoriella* species occurring on *Anthoxanthum odoratum* from Italy is described in this study.

Pyrenophora consists of endophytes, pathogens and saprobes that are widely distributed on various cereal crops and grass hosts. From our collections in Russia, *P. trichostoma* was found for the first time, occurring on two grasses *Bothriochloa ischaemum* and *Bromopsis inermis*. This study presents the history of the species by providing details of its morphology, distribution, phylogeny, economic impact as a pathogen and current status in research.

5.2 Materials and Methods

5.2.1 Literature survey

Published articles in journals, books, web-based resources such as reports on host plants or disease management, USDA database (Farr & Rossman, 2020) and research theses were referred for the literature review on *Pyrenophora trichostoma*. Host names given in the original citation were checked according to The Plant List (<http://www.theplantlist.org>).

5.2.2 Sample Collection, Morphological Studies and Isolation

Samples were collected in Italy and Russia, into paper bags and brought to the laboratory. A Motic SMZ 168 dissecting microscope was used to observe the fungal morphological features as described in Goonasekara et al. (2019). Photographs were obtained through a Canon EOS 600D digital camera fixed to a Nikon Ni compound microscope. Adobe Photoshop CS5 Extended version 12.0 software (Adobe Systems, USA) was used to process the photos. Measurements were taken with The Tarosoft (R) Image Frame Work program v. 0.9.7 software. The fungus was isolated using single spore isolation, in a petri-dish containing 2% water agar (Chomnunti et al., 2014) and incubated at 16–18°C, overnight. Germinating spores were transferred aseptically onto

fresh potato dextrose agar (PDA), and further incubated at 16–18°C. Pure colonies were subcultured into new PDA plates. Herbarium material is deposited in the herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU) and living cultures are deposited at in Kunming Culture Collection (KUMCC) and duplicated at Mae Fah Luang University Culture Collection (MFLUCC). The Facesoffungi and Index Fungorum numbers were obtained according to Jayasiri et al. (2015) and Index Fungorum (2020).

5.2.3 DNA Extraction, PCR Amplification and DNA Sequencing

Genomic DNA was extracted from fresh mycelia grown on PDA, following the manufacturer's standard protocol described in the DNA extraction kit (Biospin Fungus Genomic DNA Extraction Kit). Polymerase chain reactions (PCR) were processed using the primer pairs ITS5 and ITS4, to amplify the internal transcribed spacers (ITS) (White et al., 1990), LROR and LR5 for large subunit rDNA (LSU) (Vilgalys & Hester, 1990) and fRPB2-5F and fRPB2-7CR to amplify the partial RNA polymerase II second largest subunit (RPB2) (Liu et al., 1999). The amplification reaction was completed in a 25 µl total volume containing 1µl DNA, 9.5 µl Taq polymerase and PCR buffer mix, 12.5 µl double distilled water and 1 µl of each primer. The PCR thermal cycling program for LSU and ITS gene regions were as follows: an initial denaturing step of 94 °C for 3 mins, followed by 35 amplification cycles of 94°C for 30 s, annealation at 57°C for 45 s, elongation at 72°C for 60 s and a final extension step of 72°C for 10 min. For the RPB2 gene region an initial step of 95 °C for 5 min, followed by 40 cycles of amplification at 95 °C for 1 min, annealation at 52 °C for 2 min, elongation at 72 °C for 90 s, and final extension at 72 °C for 10 min was followed. PCR products were verified by running 1% agarose gel electrophoresis, stained with 4S Green Stain. Purification and sequencing of PCR products were carried out at Shanghai Sangon Biological Engineering Technology and Services Co., China. Sequences generated in this study are deposited in GenBank. Small subunit rDNA (SSU) sequence data for both *Parastagonospora elymi* and *P. macrouniseptata*, and TEF for *P. elymi* were obtained but not used in the phylogenetic tree and are available on request.

5.2.4 Phylogenetic Analysis

Sequence results were subjected to BLASTn (NCBI) searches to obtain matching sequences from GenBank related to *Parastagonospora* (Table 5.1), *Pyrenophora* (Table

5.2) and *Septoriella*, and from recent publications (Li et al., 2015; Tennakoon et al., 2016; Karunarathna et al., 2017; Thambugala et al., 2017; Marin-Felix et al., 2019). Single gene sequences were assembled in BioEdit v. 7.0.9.0 (Hall, 1999). Multiple sequence alignments were generated with MAFFT v. 7 (<http://mafft.cbrc.jp/alignment/server/index.html>) and manually improved using MEGA v.6 for maximum alignment and minimum gaps. Evolutionary models for phylogenetic analyses were independently selected for each gene region following the Akaike Information Criterion (AIC) of the MrModeltest v. 3.7 (Nylander, 2004). Phylogenetic reconstructions of combined gene trees were performed using maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) criteria.

The ML analysis was performed using RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al., 2008; Stamatakis, 2014) in the CIPRES Science Gateway platform (Miller et al., 2010) with GTR+I+G as the model of evolution and bootstrap support obtained by running 1000 pseudo replicates. The MP analysis was performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford, 2002). Ambiguously aligned regions were excluded and gaps were treated as missing data. The trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Maxtrees were setup to 1000, branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony tree length [TL], consistency index [CI], retention index [RI], relative consistency index [RC] and homoplasy index [HI] were calculated. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications resulting from maximum parsimony analysis, each with ten replicates of random stepwise addition of taxa (Felsenstein, 1985). The Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa, 1989) were performed to determine whether the trees were significantly different. The BI analysis was conducted using MrBayes v. 3.1.2 (Huelsenbeck & Ronquist, 2001) to evaluate posterior probabilities (BYPP) (Rannala & Yang, 1996, Zhaxybayeva & Gogarten, 2002) by Markov chain Monte Carlo sampling (BMCMC). Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generation. First The burn-in phase of 20% was set and critical value for the topological convergence diagnostic set to 0.01.

Phylograms were visualized using FigTree v1.4.0 program (Rambaut, 2012) and re-edited and formatted using Microsoft Power Point (2013) and Adobe Photoshop CS6 extended version 13.1.2 software. The final tree alignment was submitted to TreeBASE.

Table 5.1 GenBank accession numbers of the strains used for phylogenetic analysis of *Parastagonospora*

Taxon	Strain numbers	GenBank accession numbers		
		ITS	LSU	RPB2
<i>Camarosporioides phragmitis</i>	MFLUCC 13-0365 ^T	KX572340	KX572345	-
<i>Didymocyrtis cladonicola</i>	CBS 128025	KP170641	MH877975	-
<i>D. consimilis</i>	CBS 129140	MH865190	MH876627	-
<i>D. foliaceiphila</i>	CBS 129141	KP170648	MH876628	-
<i>Juncaceicola alpina</i>	CBS 456.84	KF251181	KF251684	KF252188
<i>J. luzulae</i>	MFLUCC 16-0780 ^T	KX449529	KX449530	-
<i>Melnikia anthoxanthii</i>	MFLUCC 14-1011 ^T	-	KU848204	-
<i>Neosetophoma samarorum</i>	CBS 138.96 ^T	KF251160	KF251664	KF252168
<i>Neostagonospora caricis</i>	CBS 135092 ^T	KF251163	KF251667	KF252171
<i>N. phragmitis</i>	MFLUCC 16-0493	KX926416	KX910090	KX880498
<i>Ophiosphaerella agrostidis</i>	MFLUCC 16-0895	MF351996	MF197563	MF351995
<i>O. agrostidis</i>	MFLUCC 12-0007	KM434272	KM434282	KM434308
<i>Paraphoma radicina</i>	CBS 111.79 ^T	KF251172	KF251676	KF252180
<i>Parastagonospora allouniseptata</i>	MFLUCC 13-0386 ^T	KU058711	KU058721	-
<i>Pa. avenae</i>	CBS 289.69	KF251174	KF251678	KF252182
<i>Pa. avenae</i>	CBS 290.69	KF251175	KF251679	KF252183
<i>Pa. caricis</i>	CBS 135671/S615 ^T	KF251176	KF251680	KF252184
<i>Pa. cumpignensis</i>	MFLU 15-1480 ^T	KU842388	KU842389	-
<i>Pa. dactylidis</i>	MFLUCC 13-0375 ^T	KU058712	KU058722	-
<i>Pa. elymi</i>	*KUMCC 16-0125 ^T	MN002867	MN002870	-
<i>Pa. forlicesenica</i>	MFLUCC 13-0557 ^T , MFLUCC 15-0461	KY769660	KY769661	-

Table 5.1 (continued)

Taxon	Strain numbers	GenBank accession numbers		
		ITS	LSU	RPB2
<i>Pa. fusiformis</i>	MFLUCC 13-0215 ^T	KX926418	KX910088	KX863711
<i>Pa. italica</i>	MFLUCC 13-0377 ^T	KU058714	KU058724	-
<i>Pa. macrouniseptata</i>	*KUMCC 16-0111 ^T	MN002869	MN002868	MN019669
<i>Pa. minima</i>	MFLUCC 13-0376 ^T	KU058713	KU058723	-
<i>Pa. nigrans</i>	CBS 307.79	KF251184	KF251687	KF252191
<i>Pa. nodorum</i>	CBS 110109	KF251177	KF251681	KF252185
<i>Pa. nodorum</i>	CBS 287.52	-	JX681113	-
<i>Pa. poaeicola</i>	MFLUCC 15-0471 ^T , ICMP 21415	-	KX910092	KX880499
<i>Pa. poae</i>	CBS 135089	KF251178	KF251682	KF252186
<i>Pa. poagensa</i>	CBS 136776 ^T	KJ869116	KJ869174	-
<i>Pa. uniseptata</i>	MFLUCC 13-0387 ^T	KU058715	KU058725	-
<i>Phaeosphaeria chiangraina</i>	MFLUCC 13-0231 ^T	KM434270	KM434280	KM434307
<i>Ph. thysanolaenicola</i>	MFLUCC 10-0563 ^T	KM434266	KM434276	KM434303
<i>Sclerostagonospora lathyri</i>	MFLUCC 14-0958	MG828955	MG829066	-
<i>S. opuntiae</i>	CBS 118224	DQ286768	DQ286772	-
<i>Sclerostagonospora sp.</i>	CBS 118152	JX517283	JX517292	-
<i>Scolicosporium minkeviciusii</i>	MFLUCC 12-0089 ^T	-	KF366382	-
<i>Wojnowicia dactylidis</i>	MFLUCC 13-0735 ^T	KP744470	KP684149	-
<i>Wojnowicia spartii</i>	MFLUCC 13-0402	KU058719	KU058729	-
<i>Wojnowiciella eucalypti</i>	CPC 25024 ^T	KR476741	KR476774	-
<i>Wojnowiciella viburni</i>	MFLUCC 12-0733	KC594286	KC594287	-
<i>Xenoseptoria neosaccardoi</i>	CBS 128665 ^T	KF251281	KF251784	KF252286
<i>Yunnanensis phragmitis</i>	MFLUCC 17-0315	MF684862	MF684863	-
<i>Yunnanensis phragmitis</i>	MFLUCC 17-1361 ^T	MF684869	MF684865	-

Note *Isolates generated in this study. T = Ex-type strains.

Table 5.2 GenBank accession numbers of the strains used for phylogenetic analysis of *Pyrenophora*

Taxon	Culture code	GenBank accession numbers	
		ITS	LSU
<i>Bipolaris yamadae</i>	CBS 202.29 ^{ET}	MH855044	MH866508
<i>B. panici-miliacei</i>	CBS 199.29 ^{LT}	MH855043	MH866507
<i>Pyrenophora avenicola</i>	CBS 307.84 ^T	MK539972	MK540042
<i>P. bisepitata</i>	CBS 319.69	MK539974	MK540044
	CBS 108963	MK539975	JN712532
<i>P. bromi</i>	CBS 311.68	MK539976	MH870851
	DAOMC 127414	JN943666	JN940074
<i>P. chaetomioides</i>	CBS 195.31	MK539978	MH866633
<i>P. cynosuri</i>	CBS 127918 ^T	MK539980	MK540047
<i>P. dactylidis</i>	DAOMC 92161	JN943667	JN940087
<i>P. dictyoides</i>	DAOMC 63666	JN943653	JN940080
	CBS 258.80	MK539981	MK540048
<i>P. erythrospila</i>	CBS 312.69	MK539983	MK540051
	CBS 108941	MK539984	MK540052
<i>P. fugax</i>	CBS 509.77	MK539985	MK540053
<i>P. grahamii</i>	CBS 315.69	MK539986	MK540054
	CBS 128043	MK539987	MH876230
<i>P. japonica</i>	CBS 281.31 ^{RS}	MK540004	MK540067
<i>P. leucospermi</i>	CBS 111083 ^T	JN712467	JN712533
	CBS 111505	MK539989	JN712542
	CBS 114493	MK539990	JN712545
<i>P. lolii</i>	CBS 240.48	MK539991	MK540055
<i>P. nisikadoi</i>	CBS 190.29 ^{ET}	KM257054	KM243296
	CBS 119213	EU552124	MK540056
<i>P. nobleae</i>	CBS 259.80	MK539994	MK540058
	CBS 127936	MK539996	MK540060
<i>P. novozelandica</i>	CBS 127934 ^T	MK539997	MK540061
<i>P. phaeocomes</i>	DAOMC 222769	JN943649	JN940093
<i>P. poae</i>	CBS 319.68 ^{RS}	MK539998	MK540062
	CBS 128045	MK539999	MH876232

Table 5.2 (continued)

Taxon	Culture code	GenBank accession numbers	
		ITS	LSU
<i>P. pseudoerythrospila</i>	CBS 127931 ^T	MK540000	MK540063
<i>P. semeniperda</i>	DAOMC 213153	JN943665	JN940088
<i>P. sieglingiae</i>	CBS 127930	MK540002	MK540065
<i>P. teres</i> f. <i>maculata</i>	CBS 228.76 ^T	MK540003	MK540066
<i>P. teres</i>	CBS 336.29	MK540007	MH877692
<i>P. tetrarrhenae</i>	DAOMC 171966	JN943663	JN940090
	CBS 127924	MK540011	MH877965
<i>P. trichostoma</i>	CBS 328.53	MK540012	MK540072
	CBS 391.54	MK540013	MK540073
	CBS 392.54	MK540014	MK540074
	KUMCC 16-0120*	MN736436	MN733192
	KUMCC 16-0123*	MW041570	MW041254
<i>P. triseptata</i>	CBS 128047	MK540015	MH877983
<i>P. tritici-repentis</i>	CBS 259.59	MK540017	MK540075
<i>P. tritici-repentis</i>	CBS 191.29	MK540018	MK540076
<i>P. tritici-repentis</i>	CBS 127922	MK540019	MK540077
<i>P. variabilis</i>	CBS 127920 ^T	MK540020	MK540078
<i>P. wirreganensis</i>	CBS 109896	MK540021	MK540079

Note *Isolates generated in this study. ET = epitype, LT = lectotype, RS = reference strain, T = type

5.3 Results

5.3.1 An Overview of the Species *Pyrenophora trichostoma*

Pyrenophora is a distinct genus in *Pleosporales* as it lacks pseudoparaphyses in the hamathecium and have asci with large apical rings and visible ocular chambers (Sivanesan, 1984; Ariyawansa et al., 2014). The genus was introduced by Rebentisch (1804) based on the type *Pyrenophora phaeocomes* (Rebent.) Fr. *Drechslera* has been confirmed as the asexual morph (Zhang & Berbee, 2001; Ariyawansa et al., 2014; Marin-Felix et al., 2019; Hyde et al., 2020). *Pyrenophora* species are widespread and a few are

well-known plant pathogens (Tekauz, 1983; Lamari & Bernier, 1989; Kingsland, 1991; Gupta & Loughman, 2001; Ariyawansa et al., 2014). Marin-Felix et al. (2019) accepted 27 species in *Pyrenophora* based on a multi-gene analysis consisting of ITS, LSU, GAPDH and RPB2 sequence data.

Pyrenophora trichostoma was originally described as *Sphaeria trichostoma*, a species introduced by Fries (1823) in *Hypoxylaceae* and later synonymized to this name (Fries 1870). However, no holotype is designated in the original description. Wehmeyer (1961) re-introduced *Pyrenophora trichostoma* as the sexual morph of *Helminthosporium* species that were reported on several grasses such as *Bromus*, *Hordeum*, *Poa*, *Secale* and *Triticum*. This name was then widely used to identify the tan spot or yellow leaf spot pathogen on wheat (Wiese, 1977; Hosford, 1982). Later, the species was synonymized under *P. tritici-repentis* for having similar morphological characters, such as producing ascospores with mucilaginous sheaths (Ciuffetti & Tuori, 1999; Sivanesan, 1984). Based on phylogenetic analysis, Kodsueb et al. (2006) considered them as two distinct species, citing differences in the base pairs of *P. tritici-repentis* and *P. trichostoma* sequences. In the study by Marin-Felix et al. (2019) *P. trichostoma* was accepted as a phylogenetically distinct species.

5.3.1.1 Characters of the sexual/asexual morph

During a revision of *Drechslera*, *Pyrenophora tritici-repentis* was proposed as the sexual morph of the hyphomycete *Drechslera tritici-repentis* (Died.) Shoem. by Shoemaker (1959, 1962). Subsequent studies have confirmed the link between the sexual and asexual morphs of these genera based on phylogeny (Zhang & Berbee, 2001; Ariyawansa et al., 2014). The *Pyrenophora* sexual morph has immersed to semi-immersed ascomata with necks surrounded by brown to reddish brown setae. The hamathecium comprises no pseudoparaphyses, clavate to saccate shaped asci with large apical rings, and muriform, terete ascospores (Ariyawansa et al., 2014; Marin-Felix et al., 2019; Hyde et al., 2020). *Pyrenophora trichostoma* has 330 µm long and 40 µm wide, 8-spored asci, with broad-oblong, asymmetric, muriform, yellow, 52 × 20 µm sized ascospores that are 4–6 septate, with constrictions at the mid septum (Fuckel, 1870; translated from Latin). There is no asexual morph reported yet.

In pure culture this species usually forms flat or umbonate colonies, greenish, grey to white, or olivaceous black, which later develop an orangish, brownish or pinkish

tint. For sporulation, several media have been proposed as suitable: sterilized rice or wheat straw on PDA, PNA, OA and MEA for asexual morphs, and Sach's agar for the sexual morphs (Marin-Felix et al., 2019).

5.3.1.2 Diversity of the species

Pyrenophora trichostoma is widely distributed with most occurrences being from Europe and USA (Far & Rossman, 2020). A compilation of all records of the species from various hosts and regions is listed in Table 5.3.

Table 5.3 Reports of *Pyrenophora trichostoma* on various hosts worldwide

Host	State/ Country	References
<i>Agropyron repens</i>	Canada	Conners (1967)
<i>A. smithii</i>	USA	Krupinsky (1982)
<i>Avena</i> sp.	Italy	Greuter et al. (1991)
<i>Bothriochloa ischaemum</i> *	Russia	This study
<i>Bromopsis inermis</i> *	Russia	This study
<i>Bromus</i> sp.	England	Dennis (1978)
<i>Calamagrostis arundinacea</i>	Poland	Chlebicki (1993)
<i>Dactylis</i> sp.	Portugal	Checa (2004), Unamuno (1941)
	Spain	Checa (2004)
<i>Elymus junceus</i>	USA	Krupinsky (1982), Krupinsky and Berdahl (1984), Checa (2004)
<i>Festuca nigricans</i>	Portugal	Checa (2004)
	Spain	Checa (2004)
<i>Hierochloe alpina</i> ,	Alaska	Cash (1953)
<i>Hordeum vulgare</i>	China	Tai (1979)
<i>H. vulgare</i> var. <i>nudum</i>	China	Tai (1979)
<i>Juncus balticus</i> var. <i>montanus</i>	USA	Cooke (1955)
<i>Luzula sylvatica</i>	Austria	Scheuer (1988)
<i>Poa pratensis</i>	Alaska	Cash (1953)
<i>Puccinellia borealis</i>	Alaska	Cash (1953)
<i>P. distans</i>	Poland	Chlebicki (1993)
<i>Secale cereal</i>	Portugal	Checa (2004)
	Spain	Checa (2004)
	USA	Index of pl dis US 1960, Hanlin (1963)
<i>Triticum aestivum</i>	Bolivia	Farr and Stevenson (1963)
	Brazil	Mendez et al. (1998)
	USA	Index of Pl dis US (1960), Hanlin (1963)

Note *molecular data available in GenBank

5.3.1.3 Molecular data

The LSU and ITS genes are used as DNA barcodes for *Pyrenophora* for classification at genus level, while ITS, GAPDH and TEF1 are effective in species identification (Marin-Felix et al., 2019). There is no molecular data available in GenBank for the reports mentioning *P. trichostoma* as a pathogen. Yet, the pathogenicity of *Pyrenophora* species is widespread and established, and as a result, researchers have looked into developing molecular genetic markers for rapid identification of the species (Moreno et al., 2012).

5.3.1.4 Economic importance and pathogenicity

A form of *Pyrenophora trichostoma* that severely infected wheat of North Dakota was reported by Hosford (1971) in 1968 and 1969. The disease symptoms showed light-brown lesions with yellow halos on leaves, and many ascostromata present on wheat stubble. The pathogenicity of the fungus varied, as well as the resistance of different wheat cultivars towards the pathogen. This depended on how long the leaves were exposed to free moisture (Hosford, 1971). The species was also found pathogenic causing leaf spots on *Elymus arenarius* and *Elymus cinereus* from North Dakota (Krupinsky & Berdahl, 1984). As a result of these diseases, cereal crops have faced a high reduction in grain yield and quality leading to the decrease in crop productivity (Porta-Puglia et al., 1986; Wiese, 1987; Sivanesan, 1987).

5.3.2 Phylogenetic Analysis

5.3.2.1 *Parastagonospora*

A combined analysis of the ITS, LSU and RPB2 sequence data of 45 strains consisting of *Parastagonospora* and other representative genera in *Phaeosphaeriaceae* were used to confirm the phylogenetic placement of our strains, with *Neosetophoma samarorum* (CBS 138.96) as the outgroup taxon. This combination of sequence data was performed after confirming that the topologies of the trees obtained from each gene were overall congruent. Bootstrap values obtained for MP and ML analyses and PP values resulted in BI analysis are given at each node (Figure 5.1). The Bayesian analysis resulted in 10,001 trees after 1,000,000 generations. The first 1000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 9001 trees were used for calculating posterior probabilities in the majority rule consensus tree presented in Figure

5.1. The average standard deviation of split frequencies was 0.010067. The RAxML analysis of the combined dataset yielded a best scoring tree with a ML optimization likelihood value of -11145.503449. The matrix had 796 distinct alignment patterns, with 37.7% of undetermined characters or gaps. Parameters for the GTR + I + G model of the combined ITS, LSU and RPB2 were as follows: estimated base frequencies; A = 0.249573, C = 0.222647, G = 0.273922, T = 0.253857; substitution rates AC = 1.771863, AG = 3.436302, AT = 1.824852, CG = 0.770295, CT = 7.693174, GT = 1.000000; proportion of invariable sites I = 0.001000; gamma distribution shape parameter α = 0.146118. The maximum parsimony dataset consisted of 2584 characters, of which 1906 characters were constant, 505 were parsimony-informative and 173 were parsimony-uninformative. The most parsimonious tree showed values as follows: CI = 0.620, RI = 0.707, RC = 0.438, HI = 0.380. Both KUMCC 16-0125 and KUMCC 16-0111 strains clustered within *Parastagonospora* with KUMCC 16-0111 forming a sister clade to *P. allouniseptata* with strong statistical support (96% ML/ 88% MP/ 1.00 PP). KUMCC 16-0125 grouped closely with *P. avenae*, *P. caricis*, *P. forlicesenica* and *P. italica* with moderate support (61% ML/ 49% MP/ 0.93 PP).

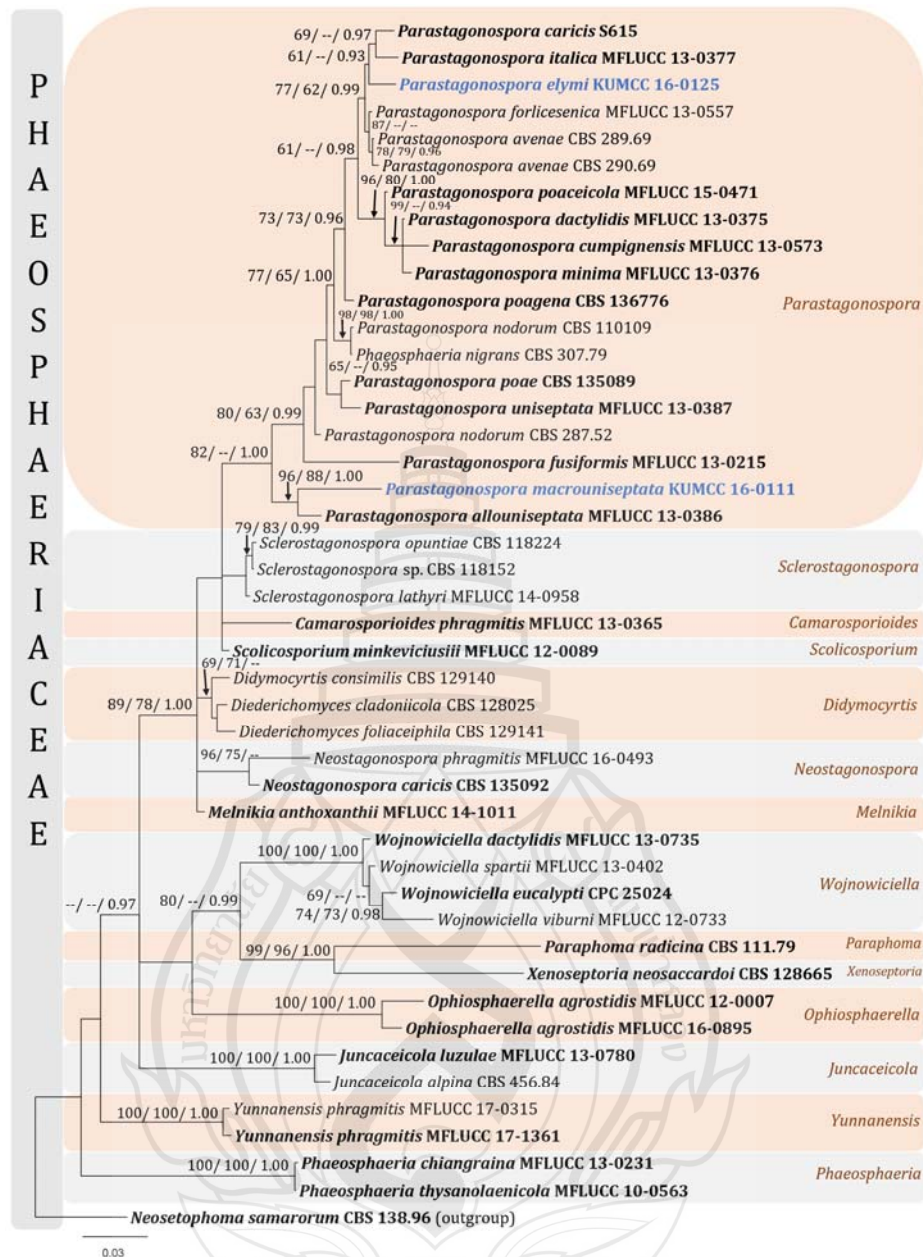
5.3.2.2 *Pyrenophora*

A combined dataset of the LSU and ITS sequences of 46 *Pyrenophora* strains were subjected to the phylogenetic analyses, with *Bipolaris yamadae* CBS 202.29 and *B. panici-miliacei* CBS 199.29 as the outgroup taxa. Bootstrap values equal to or greater than 60% obtained for MP and ML analyses and BYPP values equal to or greater than 0.90 resulting from the BI analysis are given at each node (Figure 5.2). The MP dataset consisted of 1780 characters, of which 1465 characters were constant, 266 were parsimony-informative and 49 were parsimony-uninformative. The most parsimonious tree with a tree length of 900, had the following values: CI = 0.563, RI = 0.777, RC = 0.438, HI = 0.437 and is presented in Figure 5.2. The tree topology of the ML and BI analysis did not differ significantly from that of the MP analysis. The RAxML analysis of the combined dataset yielded a best scoring tree with a ML optimization likelihood value of -7078.475836. The matrix had 461 distinct alignment patterns, with 13.71% of undetermined characters or gaps. Parameters for the GTR model of the combined ITS and LSU were as follows: estimated base frequencies; A = 0.245334, C = 0.229315, G = 0.279163, T = 0.246188; substitution rates AC = 1.521880, AG = 1.485545, AT =

1.366202, CG = 0.879594, CT = 3.736353, GT = 1.000000; gamma distribution shape parameter α = 0.104747. The BI analysis resulted in 40,002 trees after 1,000,000 generations. The first 2000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 9001 trees were used for calculating posterior probabilities in the majority rule consensus. The average standard deviation of split frequencies was 0.010067. The two isolates obtained in this study, KUMCC 16-0120 and KUMCC 16-0123, together with the other *Pyrenophora trichostoma* isolates CBS 328.53, CBS 391.54, CBS 392.54 and KUMCC 16-0120 formed a clade with strong bootstrap support (100% MP/100% ML/1 BYPP), sister to *P. bromi* (Figure 5.2).

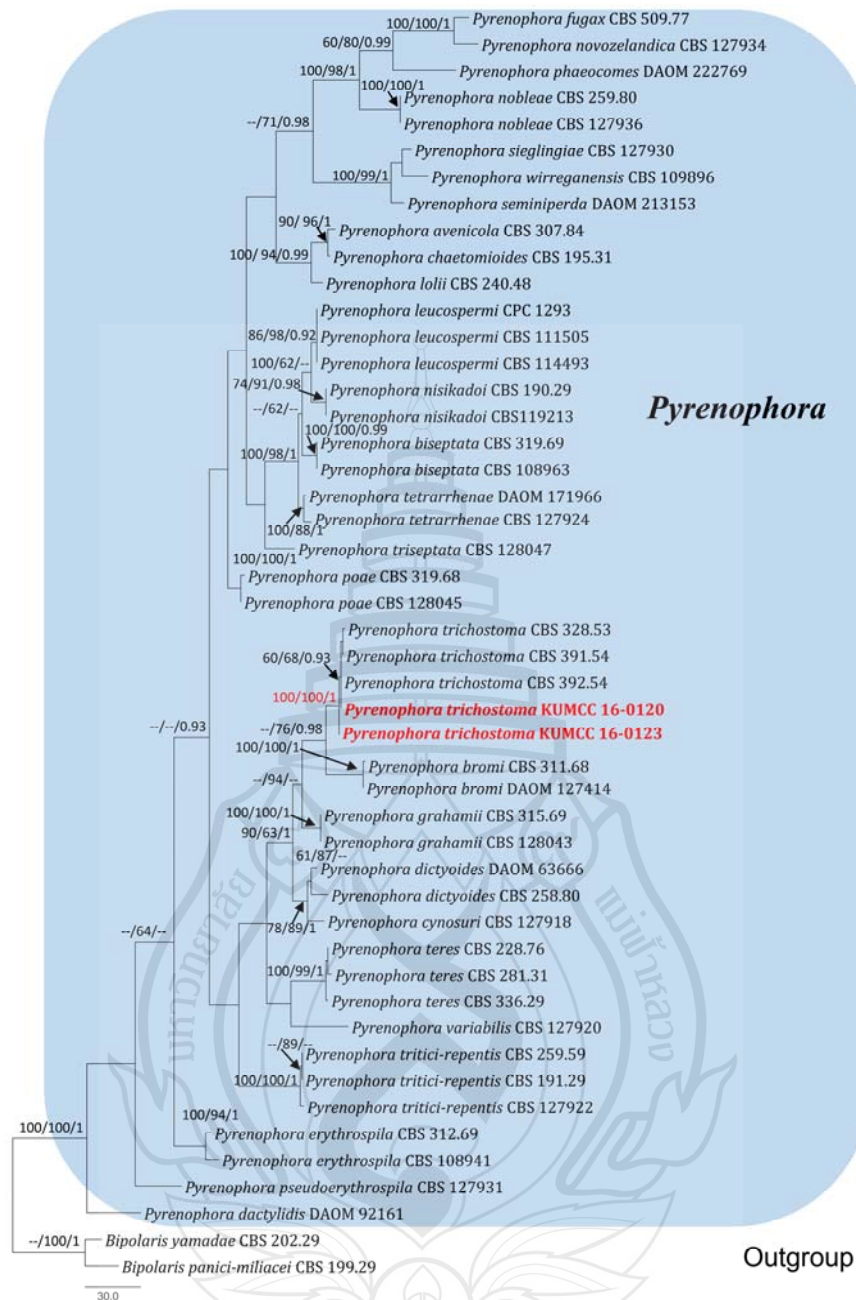
5.3.2.3 *Septoriella*

A combined analysis of the ITS sequence data of 22 strains representing *Septoriella* and other related species in *Phaeosphaeriaceae* were used to confirm the phylogenetic placement of KUMCC 16-0113 with *Neostagonospora caricis* (CBS 135092) as the outgroup taxon (Figure 5.3). Bootstrap values resulting from ML analyses are given at each node (Figure 5.2). The RAxML analysis of the combined dataset yielded a best scoring tree with a ML optimization likelihood value of -1722.479324. The matrix had 114 distinct alignment patterns, with 6.54% of undetermined characters or gaps. Parameters for the GTR model were as follows: estimated base frequencies; A = 0.254364, C = 0.208971, G = 0.301756, T = 0.234909; substitution rates AC = 2.920787, AG = 10.005708, AT = 6.429214, CG = 0.833835, CT = 16.759049, GT = 1.000000; proportion of invariable sites I = 0.001000; gamma distribution shape parameter α = 0.155805. The maximum parsimony dataset consisted of 900 total characters, of which 824 characters were constant, 10 were parsimony-informative and 66 were parsimony-uninformative. The most parsimonious tree showed values as follows: CI = 0.975, RI = 0.900, RC = 0.878, HI = 0.025.



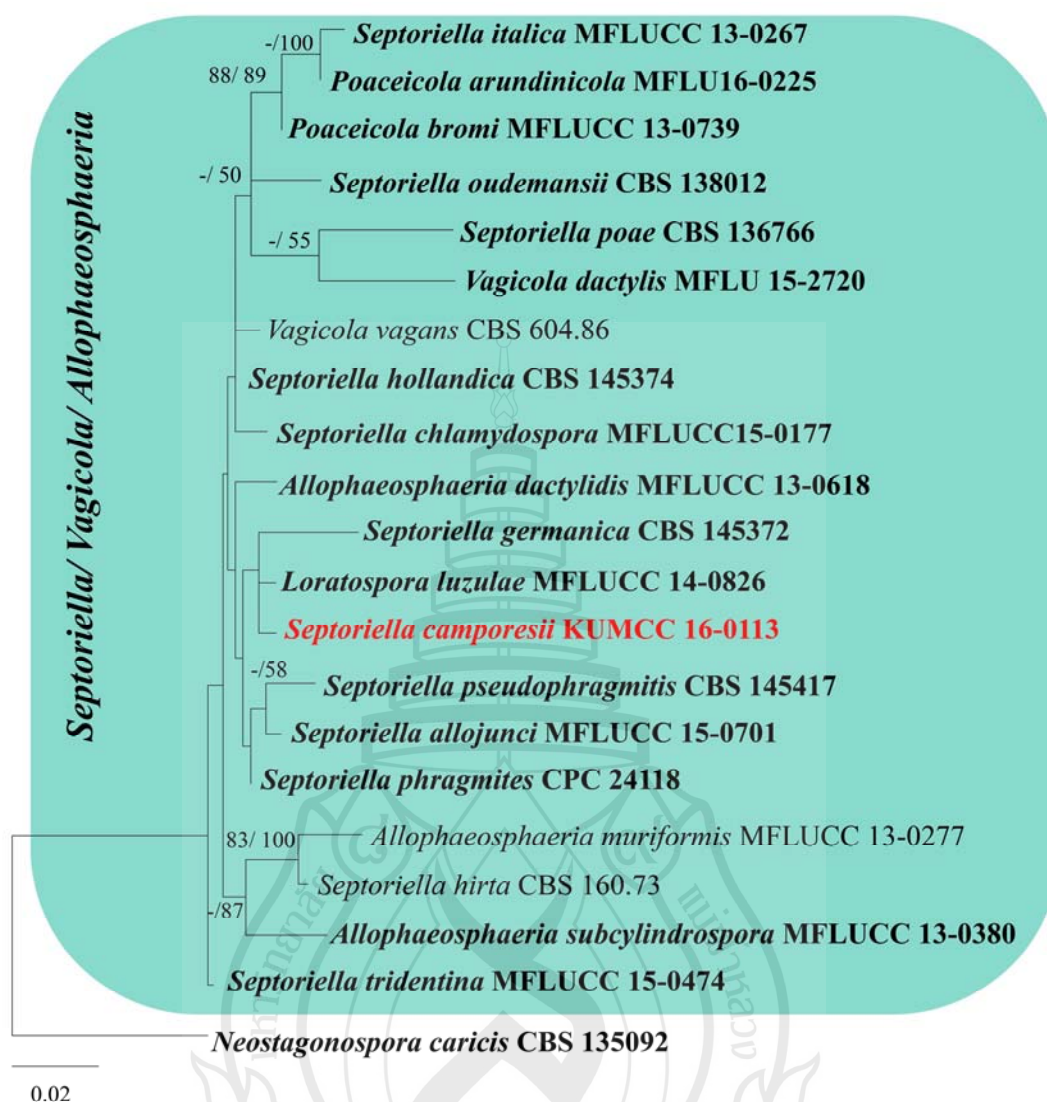
Note Maximum likelihood bootstrap values (ML) and maximum parsimony values (MP) equal or greater than 60% are given at the nodes. Bayesian posterior probabilities (PP) of more than 0.90 are given at the nodes. Ex-type strains are in bold; the new isolates are in blue. The tree is rooted to *Neosetophoma samarorum* CBS 138.96.

Figure 5.1 Bayesian majority consensus tree based on ITS, LSU and RPB2 sequences of *Parastagonospora* and related genera in *Phaeosphaeriaceae*



Note Bootstrap values for MP and ML analyses equal to or greater than 60 % and Bayesian posterior probabilities (BYPP) greater than 0.9 are given at the nodes, respectively. The tree is rooted to *Bipolaris yamadae* CBS 202.29 and *B. panici-miliacei* CBS 199.29. Ex-type strains are in bold. The newly generated isolates for this study are indicated in red.

Figure 5.2 Phylogram generated from MP analysis based on combined LSU and ITS sequence data for *Pyrenophora* isolates



Note Bootstrap values for MP and ML analyses equal to or greater than 50 % are given at the nodes, respectively. The tree is rooted to *Neostagonospora caricis* CBS135092. Ex-type strains are in bold. The newly generated isolate in this study is in red.

Figure 5.3 Phylogram generated from ML analysis based ITS sequence data for isolates of *Septoriella* and related genera

5.3.3 Taxonomy

Phaeosphaeriaceae M.E. Barr

Notes: *Phaeosphaeriaceae* M.E. Barr is an important family of the order *Pleosporales* and was introduced by Barr (1979) with *Phaeospharia* I. Miyake as the

generic type (Zhang et al., 2009, 2012; Hyde et al., 2013; Phookamsak et al., 2014, 2017). It consists of species that are mostly phytopathogenic on economically important crops, saprobic or endophytic on plants, and some associated with lichens or human infections (Shoemaker & Babcock, 1989; Kirk et al., 2008; Zhang et al., 2009; Lawrey et al., 2012; Phookamsak et al., 2014; Wijayawardene et al., 2014; Jayasiri et al., 2015b; Li et al., 2015; Tennakoon et al., 2016; Ahmed et al., 2017; Senanayake et al., 2018). Phookamsak et al. (2014) comprehensively reviewed the family *Phaeosphaeriaceae*, accepting 30 genera (including 17 asexual morph genera) based on morphological data, with the addition of multi-gene (ITS, LSU, SSU, RPB2 and TEF1) analyses. Several more genera have been added to *Phaeosphaeriaceae* following recent studies (Liu et al., 2015; Ariyawansa et al., 2015; Crous et al., 2015; Li et al., 2015; Phukhamsakda et al., 2015; Tennakoon et al., 2016; Hyde et al., 2017; Thambugala et al., 2017; Karunarathna et al., 2017; Phookamsak et al., 2017, 2019; Wanasinghe et al., 2018; Bakhshi et al., 2019; Yang et al., 2019; Maharachchikumbura et al., 2019). The majority of asexual morphs in *Phaeosphaeriaceae* are coelomycetous (Li et al., 2015). The family comprises more than 400 species from over 60 genera (Phookamsak et al., 2017, 2019; Wijayawardene et al., 2018; Bakhshi et al., 2019).

***Parastagonospora* Quaedvl., Verkley & Crous**

Notes: *Parastagonospora* was introduced by Quaedvlieg et al. (2013) to accommodate several species previously classified as *Leptosphaeria*, *Phaeosphaeria*, *Septoria* and *Stagonospora*. This genus is characterized by a sexual morph having immersed ascomata with slightly papillate ostiole, bitunicate, shortly stipitate asci, fusoid, subhyaline to pale brown, septate ascospores and a coelomycetous asexual morph with hyaline, cylindrical, granular to multi-guttulate, transversely euseptate conidia (Quaedvlieg et al., 2013; Li et al., 2015; Thambugala et al., 2017). *Parastagonospora* species are widespread occurring mostly on wheat, grasses such as *Dactylis* sp. and *Poa* sp. as well as other cereal crops (Quaedvlieg et al., 2013; Li et al., 2015; Ghaderi et al., 2017).

Parastagonospora elymi* Goonas., Bulgakov & K. D. Hyde, *sp. nov.

Index Fungorum number: IF556608; Facesoffungi number: FoF06260; Figure 5.4

Etymology: Named after the host genus *Elymus* from which it was collected.

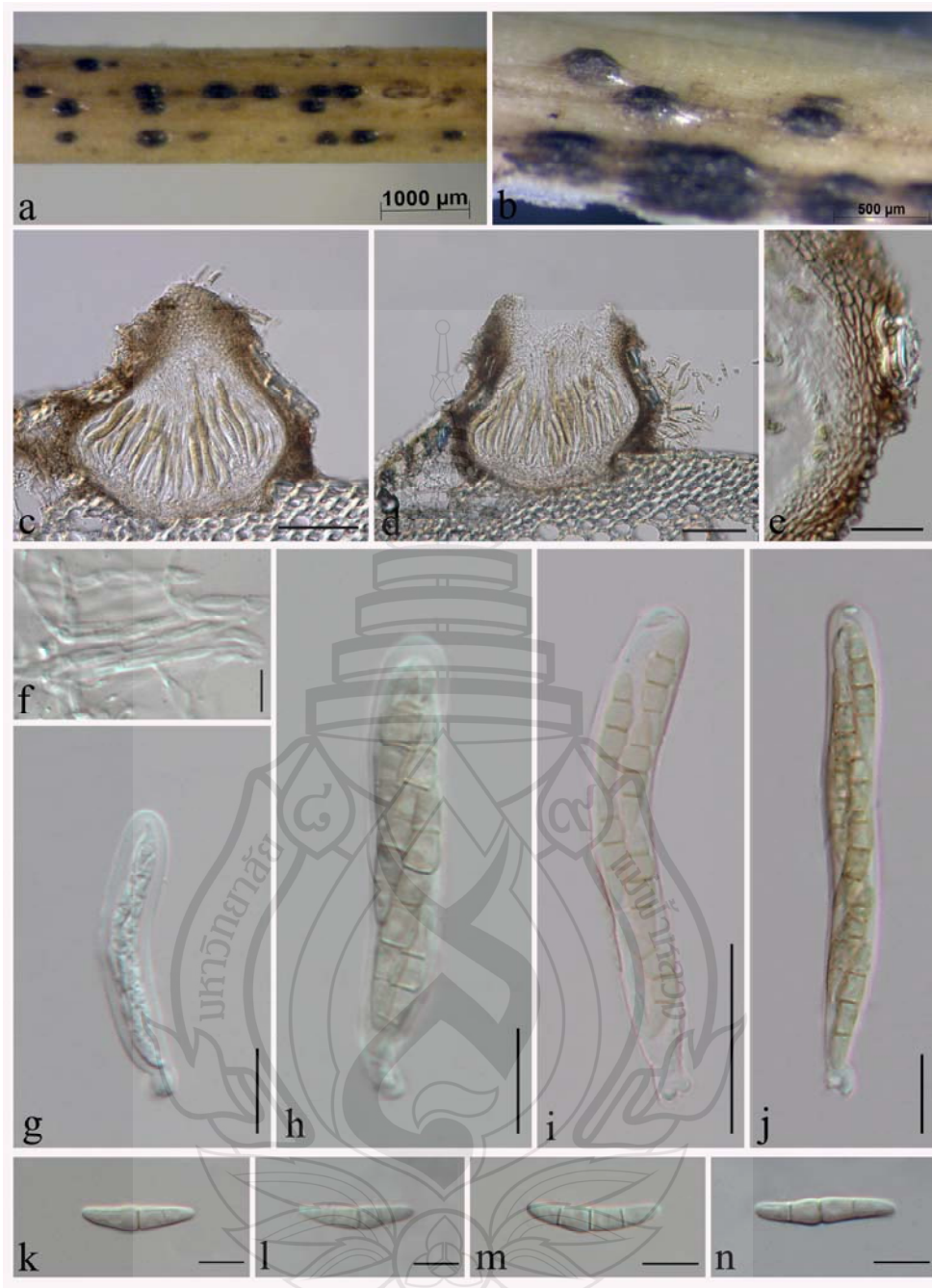
Holotype: MFLU 15-2243

Saprobic on dead stems of *Elymus repens*, appearing as black, raised, shiny, oval lesions on host surface. **Sexual morph:** *Ascomata* perithecial, solitary, scattered to gregarious, glabrous, semi-immersed to superficial, 100–150 µm high, 145–200 µm diam. (n = 10), subglobose to conical, dark brown, with central ostiole. *Peridium* 15–20 µm wide, composed of several layers, outer layers; brown, thick-walled cells of *textura prismatica* to *textura angularis* and inner layers, hyaline to lightly pigmented, thin-walled cells of *textura angularis*. *Hamathecium* composed of many filamentous, septate, 1.5–2 µm wide, cellular pseudoparaphyses, paraphyses present. *Asci* 55–95 × 7–13 µm ($\bar{x}$ = 80 × 10.5 µm, n = 20), cylindrical, 6–8 spored, bitunicate, fissitunicate, rounded at the apex, with bi-lobed pedicel. *Ascospores* 21.5–25.5 × 3.5–5 µm ($\bar{x}$ = 22.5 × 4.5 µm, n = 20), uniseriate to overlapping bi-seriate, hyaline, fusiform, with acute ends, straight or slightly curved, 3-septate at maturity, cells near the mid septum, especially second cell from apex slightly swollen, constricted at the middle septum, smooth-walled. **Asexual morph:** undetermined.

Culture characteristics: Colonies reaching 15–20 mm diam. in 4 weeks at 16–18 °C on PDA, woolly and raised, margins irregular, from above grey, reverse grey to white.

Material examined: Russia, Rostov region, Krasnosulinsky district, Donskoye forestry, artificial forest (47.85472° N, 40.2318907° E, on dead stems of *Elymus repens* (L.) Gould [=*Elytrigia repens* (L.) Nevski] (Poaceae), 28 June 2015, Timur S. Bulgakov T-539 (MFLU 15-2243, **holotype**); living culture, KUMCC 16-0125, MFLUCC 16-0887.

Notes: In the phylogenetic analyses, *Parastagonospora elymi* groups closely with the two strains of *P. avenae* (CBS 289.69 and CBS 290.69). Morphologically, *P. elymi* is similar to the sexual morph of *P. avenae* (= *Phaeosphaeria avenaria*) in the characteristics of their asci and ascospores (cylindrical asci, with 8, overlapping biseriate, fusiform ascospores), however their dimensions of the ascomata vary. *Parastagonospora elymi* ascomata are subglobose or conical, 145–200 µm wide and 100–150 µm high, while *P. avenae* has smaller, globose ascomata that are 120–150 µm wide, 120–150 µm high (Shoemaker & Babcock, 1989, this study). The ascospores of *P. avenae* are guttulate and surrounded by a sheath, while those of *P. elymi* have neither guttules nor a sheath (Shoemaker & Babcock, 1989, this study).



Note (a) Appearance of ascomata on host (b) Close up of ascomata (c,d) Vertical section of ascoma (e) Peridium (f) Pseudoparaphyses (g–j) Asci (k–n) Ascospores. Scale bars: c, d = 100 µm, e = 50 µm, f = 10 µm, g–j = 20 µm, k–n = 15 µm.

Figure 5.4 *Parastagonospora elymi* (MFLU 15-2243, holotype)

Parastagonospora macrouniseptata Goonas., Camporesi & K. D. Hyde, *sp. nov.*

Index Fungorum number: IF55660, Facesoffungi number: FoF 06261; Figure 5.5

Etymology: “uniseptata” referring to its similarity to *P. uniseptata* and “macro” to its larger conidiomata.

Holotype: MFLU 15-0774

Saprobic on dead stem of *Dactylis glomerata* L. (Poaceae) appearing as black dots on the host surface. **Sexual morph:** undetermined. **Asexual morph:** Coelomycetous. *Conidiomata* 150–180(–190) μm wide, (120–)130–160 μm high ($\bar{x}$ = 167 $\times$ 145 μm , n = 10), dark brown to black, pycnidial, solitary to gregarious, immersed, globose to subglobose, unilocular, glabrous, ostiolate. *Ostiole* central, short papilla. *Conidiomatal wall* 8–18 μm wide ($\bar{x}$ = 13.7 μm), composed of thin, dark brown, pseudoparenchymatous cells, gradually merging with hyaline cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 4.2 $\times$ 3 μm , hyaline, phialidic, ampulliform to lageniform, discrete, determinate, aseptate, smooth, arising from the inner layers of conidioma. *Conidia* (14–)15–18(–20) $\times$ (1–)2–2.5 μm ($\bar{x}$ = 17.3 $\times$ 2 μm , n = 30), hyaline, cylindrical to subcylindrical, rounded at apex, slightly truncate at base, 1-septate, slightly constricted at the septum, smooth-walled, guttulate.

Culture Characteristics: Colonies reaching 15–20 mm diam. in 4 weeks at 18–20 °C on PDA, dense, margins undulate to irregular, from above white, slightly raised and umbonate in the center, becoming grey at the margins, reverse grey to white.

Material examined: Italy, Province of Forlì-Cesena, near Predappio Alta, on dead aerial stem of *Dactylis glomerata* L. (Poaceae), 15 January 2012, Erio Camporesi IT 109 (MFLU 15-0774, **holotype**; HKAS 96288, **isotype**); living culture, KUMCC 16-0111, MFLUCC 16-0885.

Notes: *Parastagonospora macrouniseptata* is morphologically similar to *P. allouniseptata* in having globose to subglobose, pycnidia and hyaline, cylindrical, 1-septate conidia. Both species are described from the same host. However, *P. macrouniseptata* has larger conidiomata ($\bar{x}$ = 167 $\times$ 145 μm) than *P. allouniseptata* (60–90 μm high, 70–90 μm diam.) (Li et al., 2015). Phylogenetically, *P. macrouniseptata* forms a sister clade with *P. allouniseptata*, within the *Parastagonospora* clade with high bootstrap support. The differences in base pairs between the two species were 14 for the ITS region, six for LSU and 20 for the TEF gene region.



Note (a) Specimen from above. (b) Conidiomata on host. (c) Squash mount of conidioma. (d) Section through conidioma. (e) Wall of conidioma. (f, g) Conidiogenous cells. (h–k) Conidia. (l) Colony on PDA. Scale bars: c, d=50 µm, e–k=20 µm.

Figure 5.5 *Parastagonospora macrouniseptata* (MFLU 15-0774, holotype)

***Septoriella* Oudem.**

Notes: *Septoriella* is characterized by having pycnidial, unilocular conidiomata, that produce subcylindrical to fusiform, euseptate, pale brown conidia with a mucoid appendage at each end (Crous et al., 2015; Li et al., 2015; Marin-Felix et al., 2019). Based on ITS and LSU combined data Marin-Felix et al. (2019) synonymized the genera *Allophaeosphaeria*, *Poaceicola* and *Vagicola* with *Septoriella*, however, in the outline by Wijayawardene et al. (2020) they are listed as separate genera.

Septoriella camporesii* Goonas. & Hyde, *sp. nov.

Index Fungorum number: IF556918; Facesoffungi number: FoF 06804, Figure 5.6

Etymology: Named in honour of the collector Erio Camporesi

Holotype: MFLU 15-1259

Saprobic on dead leaf of *Anthoxanthum odoratum* L. (Poaceae), appearing as black ellipsoidal, raised spots on the host surface. Sexual morph: undetermined. **Asexual morph:** Coelomycetous. *Conidiomata* 95–120 µm wide, 80–95 µm high, dark brown to black, pycnidial, solitary to gregarious or scattered, semi-immersed to erumpent, globose, unilocular, glabrous. *Wall of conidiomata* 10–20 µm wide, in the upper and lateral parts, composed of outer dark brown and inner light brown to hyaline cells of *textura prismatica*, at the base, pale brown to hyaline cells of *textura prismatica* gradually merging with host tissue. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 2 × 3 µm, hyaline, phialidic, doliform, discrete, determinate, smooth, arising from the inner layers of conidima. *Conidia* 15.3–19.2 × 2.7–3.5 µm ($\bar{x}$ = 17 × 3.2 µm, n = 30), hyaline when immature, becoming light brown to pale greenish-brown, oblong to subcylindrical, rounded at apex, slightly truncate at base, 1–3-septate at maturity, septa less conspicuous in immature spores, smooth-walled, greenish specks-like globules present in the cell matrix.

Culture Characteristics: Colonies showing moderate growth, circular, reaching 40 mm diam. in 4 weeks at 18–20 °C on PDA, medium dense and woolly, dull surface, convex, margins entire, from above white at the center, becoming grey at the margins, reverse grey.

Material examined: Italy, near Camposonardo - Santa Sofia (province of Forlì-Cesena [FC]), on dead leaf of *Anthoxanthum odoratum* L. (Poaceae), 17 September 2012, Erio Camporesi, IT 724 (MFLU 15-1259, **holotype** = HKAS 96289, **isotype**), living culture, KUMCC 16-0113.

GenBank numbers: SSU: MN648202; LSU: MN648201; ITS: MN648204.

Notes: *Septoriella camporesii* shares similar characteristics with other *Septoriella* species in having fusiform to subcylindrical pale-brown, euseptate conidia with rounded ends. However, the conidia in *S. camporesii* lack distinct mucoid appendages at both ends, which distinguishes it from other species. Characteristically it is mostly similar to *Septoriella subcylindrospora* (W.J. Li) Y. Marín & Crous in having smooth-walled, subcylindrical conidia with a truncate base, but the conidiomata of *S. camporesii* are semi-immersed to erumpent while in *S. subcylindrospora* they are immersed to semi-

immersed (Marin-Felix et al., 2019). In the phylogenetic analysis it groups within the clade represented by the type of the genus *Septoriella phragmitis* (ex-epitype strain CPC 24118) but with low bootstrap support.



Note (a) Appearance of conidiomata on the host surface. (b) Close up of conidiomata. (c, d) Section through conidiomata. (e) Conidioma wall. (f) Conidia arising from conidiogenous cells. (g–n) Conidia. (o, p) Colony characteristics from above (o), below (p). Scale bars: c, f = 50 μ m, d, e, g–o = 20 μ m, a, b = not to scale

Figure 5.6 *Septoriella camporesii* (MFLU 15-1259, holotype)

***Pleosporaceae* Nitschke**

Notes: Introduced by Nitschke (1869) in the order Pleosporales, this family comprises 23 genera (Wijayawardene et al., 2020). *Alternaria*, *Bipolaris*, *Curvularia* and *Pyrenophora* are some notable plant pathogenic genera in Pleosporaceae.

***Pyrenophora* Fr.**

Notes: *Pyrenophora* typified by *Pyrenophora phaeocomes* was introduced by Rebentisch (1804). The absence of pseudoparaphyses and smaller ascospore dimensions distinguishes *Pyrenophora* from other genera of Pleosporaceae (Sivanesan, 1984; Ariyawansa et al., 2014). *Pyrenophora* species are characterized by immersed to semi-immersed ascomata with necks surrounded by brown to reddish brown setae, absence of pseudoparaphyses, clavate to saccate asci with large apical rings, and muriform, terete ascospores (Ariyawansa et al., 2014; Marin-Felix et al., 2019).

Pyrenophora trichostoma (Fr.) Fr., Jb. nassau. Ver. Naturk. 23-24: 215 (1870) [1869-70]

Index Fungorum number: IF196497; Facesoffungi number: FoF 06984, Figure 5.7

Saprobic on dead stems of *Bromopsis inermis* (Leyss.) Holub **Sexual morph:** *Ascomata* 320–420 µm high 300–345 µm diam. ($\bar{x}$ = 400 × 320 µm, n = 10), black, immersed to semi-immersed or becoming erumpent, solitary or scattered, uniloculate, globose to subglobose, or conical, visible as shiny black dome shaped structures on the host surface, conspicuous, surrounded by setae, glabrous, non-papillate, black. *Ostiole* short, immersed, round, with a pore-like opening. *Setae* dark brown, darker towards the base, septate, tapering towards the apex. *Peridium* 16–26 µm wide, thin, multiple-layered cells of outer dark brown *textura angularis*, and inner cells of pale brown to hyaline flattened *textura angularis* to *textura prismatica*. *Hamathecium* consisting of irregular shaped cellular matter, pseudoparaphyses absent. *Asci* 250–350 × 45–65 µm, ($\bar{x}$ = 310 × 50 µm, n = 20), 8-spored, bitunicate, fissitunicate, slightly curved, broadly cylindric-clavate, smooth-walled, with a bi-lobed, short pedicel, apex rounded, with a distinct ocular chamber. *Ascospores* 35–70 × 15–35 µm, ($\bar{x}$ = 55 × 20 µm, n = 40), overlapping biserial, ellipsoidal, asymmetrical, muriform, with 3 transverse septa, 1–2 longitudinal septa, constricted at the septa, thick-walled, cells with longitudinal septa larger or swollen, hyaline when immature, pale to golden brown when mature, end cells slightly lighter, conical and broadly to narrowly rounded at the ends, surrounded by a thick mucilaginous sheath, guttulate. **Asexual morph:** Undetermined.

Culture characteristics: Colonies on PDA reaching approximately 2.5 cm diam after 7 days at 16–18°C, pinkish white, circular, flat, with dense mycelium, smooth margins, becoming orangish red and reverse brown.

Known distribution (based on molecular data): Kansas State, USA (Vu et al., 2019), Russia (this study)

Known hosts (based on molecular data): *Bothriochloa ischaemum*, *Bromopsis inermis* (this study).

Material examined: Russia, Rostov region, Shakhty city district, 20th anniversary of Red Army microdistrict, Solyonaya Balka (Salty gully), at margin of artificial forest, on dead stems of *Bromopsis inermis* (Leyss.) Holub (Poaceae), 21 May 2015, Timur S. Bulgakov, T-431 (MFLU 15-2135), living culture KUMCC 16-0123; Russia, Rostov region, Shakhty city, 20th anniversary of Red Army microdistrict, Solyonaya Balka (Salty gully), on dying stems and leaves of *Bothriochloa ischaemum* (L.) Keng (Poaceae), 21 May 2015, Timur S. Bulgakov, T-336 (MFLU 15-2040), living culture, KUMCC 16-0120.

Notes: Our species illustrated here has $310 \times 50 \mu\text{m}$ sized, broadly cylindric-clavate and slightly curved asci containing broad, asymmetric, muriform, $55 \times 20 \mu\text{m}$ sized ascospores. These morphological characters resemble *P. trichostoma* as described by Fuckel (1870). Phylogenetically, the two isolates from this study KUMCC 16-0123 (on *Bromopsis inermis* and described here) and KUMCC 16-0120 (on *Bothriochloa ischaemum*) clusters with three strains CBS. 328.53, CBS 391.54 and CBS 392.54 identified as *P. trichostoma* (Marin-Felix et al., 2019). There were no base pair differences present in the LSU or ITS gene regions among all these strains. Therefore, we identify our species as *P. trichostoma*. Both are new host records, while the collection from *B. ischaemum* is designated as the first record of *Pyrenophora trichostoma* from Russia.



Note (a) Appearance of ascomata on host. (b) Close up of ascoma. (c) Section of ascoma. (d) Setae. (e) Peridium. (f) Contents of the hamathecium. (g) Ocular chambers of asci. (h–k) Immature to mature asci. (l–o) Ascospores. (p) Ascospore surrounded in a sheath (stained with Indian Ink). Scale bars: c, h–k = 100 µm, d, f, g = 20 µm, e, l–p = 10 µm.

Figure 5.7 *Pyrenophora trichostoma* (MFLU 15-2135, new host record)

5.4 Discussion

As most sexual morphs in *Phaeosphaeriaceae* are morphologically similar, it is their asexual morph characteristics, coupled with molecular data, that are essential in classification (Phookamsak et al., 2017). In most cases, the addition of protein coding genes are necessary to clearly distinguish species in *Phaeosphaeriaceae* (Phookamsak et al., 2014). Based on the phylogenetic results of this study, the combined use of ITS, LSU and RPB2 sequence data satisfactorily segregated the species of *Parastagonospora*, although this may not be sufficient for all genera in the family. There is a need for broader collections, and more importantly, the re-collection of type species, in order to firmly establish the phylogenetic placements of certain genera, especially those with poor species representation (Phookamsak et al., 2014, 2017; Tennakoon et al., 2016). It is doubtful whether most species of this family are host specific (Phookamsak et al., 2014) as initially thought, hence more fresh collections are necessary.

Pyrenophora trichostoma is a known severe pathogen on cereal crops, especially wheat. While there are numerous records of the species being pathogenic, there is a lack of proper taxonomic studies for identification and classification. Therefore, further recollections and establishing a suitable type herbarium for future studies is necessary. The addition of more molecular data will help in species identification, in clarifying its phylogenetic placement and aid in developing modern DNA techniques for plant disease identification.

CHAPTER 6

TAXONOMIC STUDIES IN *Distoseptisporaceae*, *Parabambusicolaceae*, *Pseudotruncatellaceae*, *Mycosphaerellaceae* AND *Sporocadaceae*

6.1 Introduction

This chapter is a collection of various fungi of interest that were isolated and studied from collections made in China, Italy and Thailand. These include two new species *Distoseptispora thysanolaenae* (*Distoseptisporaceae*) and *Parabambusicola thysanolaenae* (*Parabambusicolaceae*) from grasses in China. *Seimatosporium quercina* (*Sporocadaceae*) is a new species on *Quercus robur* from Germany. *Pseudotruncatella camporesii* (*Pseudotruncatellaceae*) was isolated from *Helichrysum* sp. and is the third species introduced in this genus. *Pseudocercospora tamarindi* (*Mycosphaerellaceae*), introduced from *Tamarindus indica* causing leaf spots on its host, is a collection from Thailand. Several new host and geographical records for *Pestalotiopsis verruculosa*, *Pseudopestalotiopsis kubahensis*, *Seiridium neocupressi* and *Truncatella spartii* in *Sporocadaceae* are described.

6.2 Material and Methods

6.2.1 Sample Collection, Morphological Studies and Isolation

Samples were collected during 2012–2017 from various locations in China, Germany and Italy into paper bags and brought to the laboratory. Specimens were examined using a Motic SMZ 168 dissecting microscope in order to observe morphological features. Free-hand sections of the fungal structures were obtained and mounted in water on a slide to observe their microscopic features. The slides were

examined using a Nikon Ni compound microscope and photographed using a Canon EOS 600D digital camera fitted to the microscope. The images were processed using Adobe Photoshop CS5 Extended version 12.0 software (Adobe Systems, USA). The Tarosoft (R) Image Frame Work program v. 0.9.7 was used for taking measurements. Isolates were obtained by single spore isolation as detailed in Chomnunti et al. (2014) in a Petri-dish containing 2% water agar and incubated overnight at 16–18 °C. Germinating spores were then transferred aseptically onto potato dextrose agar (PDA), and further incubated at 16–18 °C. Pure colonies were subcultured onto malt extract agar (MEA) and potato dextrose agar (PDA). Herbarium material is deposited in the herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU) and living cultures are deposited at Mae Fah Luang University Culture Collection (MFLUCC) and duplicated in Kunming Culture Collection (KUMCC). Facesoffungi and Index Fungorum numbers are processed as described in Jayasiri et al. (2015) and Index Fungorum (2019).

6.2.2 DNA Extraction, PCR Amplification and DNA Sequencing

DNA extraction, PCR amplification, DNA sequencing Genomic DNA was extracted from the fresh mycelia grown on PDA, following the manufacturer's standard protocol described in the DNA extraction kit (Biospin Fungus Genomic DNA Extraction Kit). Polymerase chain reactions (PCR) were carried out using the following primer pairs: ITS5 and ITS4 to amplify the internal transcribed spacers (ITS) (White et al., 1990), LROR and LR5 for large subunit rDNA (LSU) (Vilgalys & Hester, 1990), NS1 and NS4 for the small subunit nuclear rRNA (SSU) (White et al., 1990), Bt2a and Bt2b for the beta-tubulin (TUB) gene (Glass & Donaldson, 1995), EF1-983F and EF1-2218R (Rehner, 2001; Currie et al., 2003) or EF1-728F and EF1-986R (Carbone & Kohn, 1999) or EF2 (O'Donnell et al., 1998) for the translation elongation factor 1-alpha (TEF1) gene. The amplification reaction was performed in a 25 µl reaction volume containing 1µl DNA, 9.5 µl Taq polymerase and PCR buffer mix, 12.5 µl double distilled water and 1 µl of each primer. The thermal cycling program followed the protocols of Goonasekara et al. (2016) and related studies for each species with some modifications for some gene regions. The PCR products were verified using 1% agarose gel, stained with 4S Green stain. Purification and sequencing of PCR products were carried at

Shanghai Sangon Biological Engineering Technology & Services Co., China. Newly generated sequences are deposited in GenBank.

6.2.3 Phylogenetic Analysis

Sequence results generated were subjected to BLAST (NCBI) searches to obtain the closest matches in GenBank as well as from recently published sequences. The single gene sequences were initially aligned using MEGA version 6 (Tamura et al., 2013) or BioEdit v. 7.0.9.0 (Hall, 1999). Multiple sequence alignments were generated with MAFFT v. 7 (<http://mafft.cbrc.jp/alignment/server/index.html>) and manually improved using MEGA v.6 for maximum alignment and minimum gaps. Maximum parsimony analysis (MP) was performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford, 2002). Ambiguously aligned regions were excluded and gaps were treated as missing data. The trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Maxtrees were setup to 5000, branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI] were calculated for the Maximum Parsimonious Tree (MPT). The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications resulting from maximum parsimony analysis, each with ten replicates of random stepwise addition of taxa (Felsenstein, 1985). The Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa, 1989) were performed to determine whether the trees were significantly different. Bayesian analyses (BA) were performed using MrBayes 3.2.0 (MrBayes v.3.2.0; Ronquist et al., 2012). Suitable models were first selected using models of nucleotide substitution for each gene, as determined by MrModeltest v. 2.2 (Nylander, 2004), and included for each gene partition. The likelihood parameters for BI were based on various models as determined by the model test. Four simultaneous Markov Chain Monte Carlo (MCMC) chains were run for 1,000,000 generations and trees were sampled every 100th generation, with 10,000 trees obtained. The first 20% of the sampled trees were discarded as 'burn-in'. The resulting trees were viewed in FigTree v.1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>). Bootstrap values obtained from ML and MP analysis and Bayesian Posterior Probabilities (PP) are given at each node, accordingly.

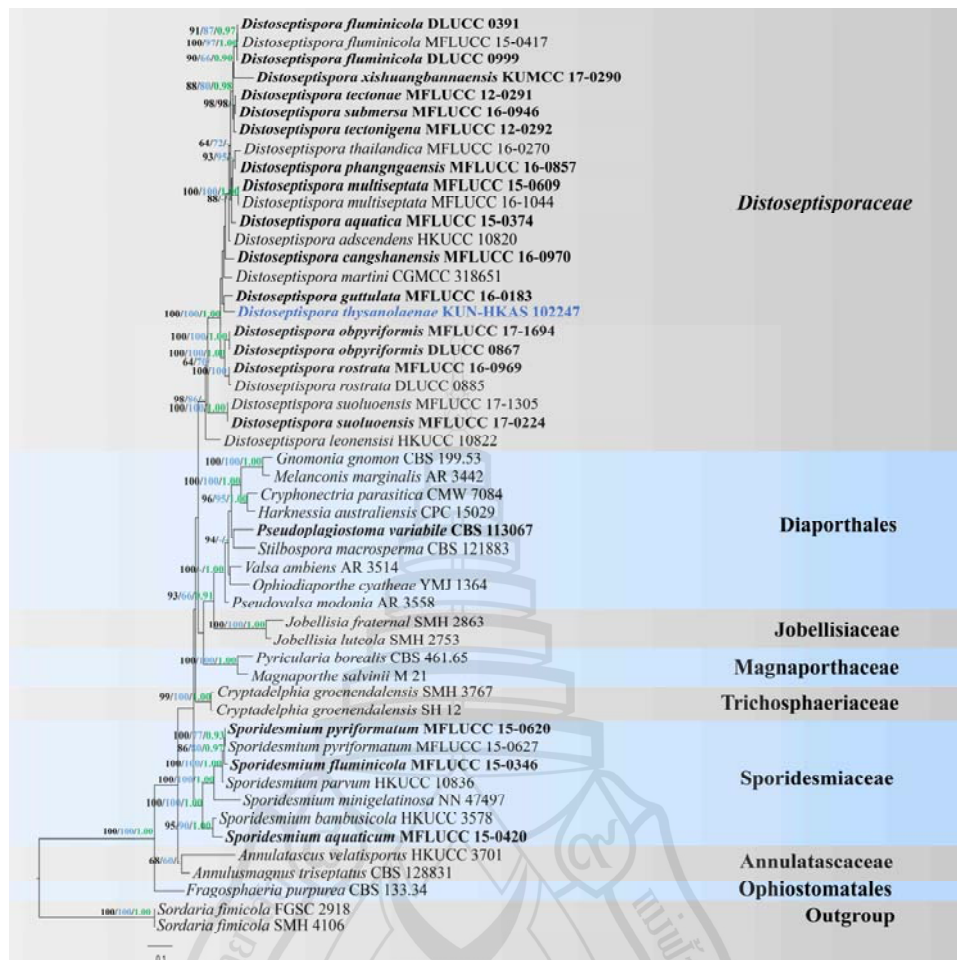
6.3 Results

6.3.1 Phylogenetic analysis

6.3.1.1 *Distoseptisporaceae*

A total of 51 strains of related sequences were obtained from GenBank and included in the analyses. The final dataset was a combination of ITS, LSU and TEF gene regions which comprised 2409 characters including gaps. Single gene analyses were carried out and compared with each species, to compare the topology of the tree and clade stability. The tree is rooted with *Sordaria fimicola* (SMH 4106, FGSC 2918). Tree topology of the ML analysis was similar to the MP and BI. The best scoring RAxML tree with a final likelihood value of -420217019.410141 is presented (Figure 6.1). The matrix had 1186 distinct alignment patterns, with 38.62% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.237054, C = 0.262859, G = 0.290202, T = 0.209884; substitution rates AC = 1.172995, AG = 2.180726, AT = 1.594744, CG = 1.095103, CT = 6.416481, 4205 GT = 1.000000; gamma distribution shape parameter α = 0.352856. The maximum parsimonious dataset consisted of 1183 constant characters, 751 parsimony-informative and 475 parsimony-uninformative characters. The parsimony analysis of the data matrix resulted in the maximum of two equally most parsimonious trees with a length of 2985 steps (CI = 0.590, RI = 0.726, RC = 0.428, HI = 0.410) in the first tree.

Phylogenetic analyses based on a combined ITS, LSU and TEF sequence dataset show that the taxon KUMCC 18-0182 clusters with other *Distoseptispora* species (Figure 6.1). The species is sister to *D. guttulata* J. Yang & K.D. Hyde (MFLUCC 16-0183). A comparison of the ITS and TEF nucleotide bases shows that *D. thysanolaenae* differs from *D. guttulata* with 59 nucleotide bases of the ITS and 64 nucleotide bases of the TEF regions. Following the guidelines of Jeewon and Hyde (2016) it is introduced as a new species.



Note RAxML (black) and maximum parsimony (blue) bootstrap support values $\geq 60\%$ are shown respectively above the nodes. Bayesian posterior probabilities ≥ 0.95 BYPP indicated in green. The scale bar indicates 0.1 changes. The ex-type strains are in bold and the new isolate is in blue.

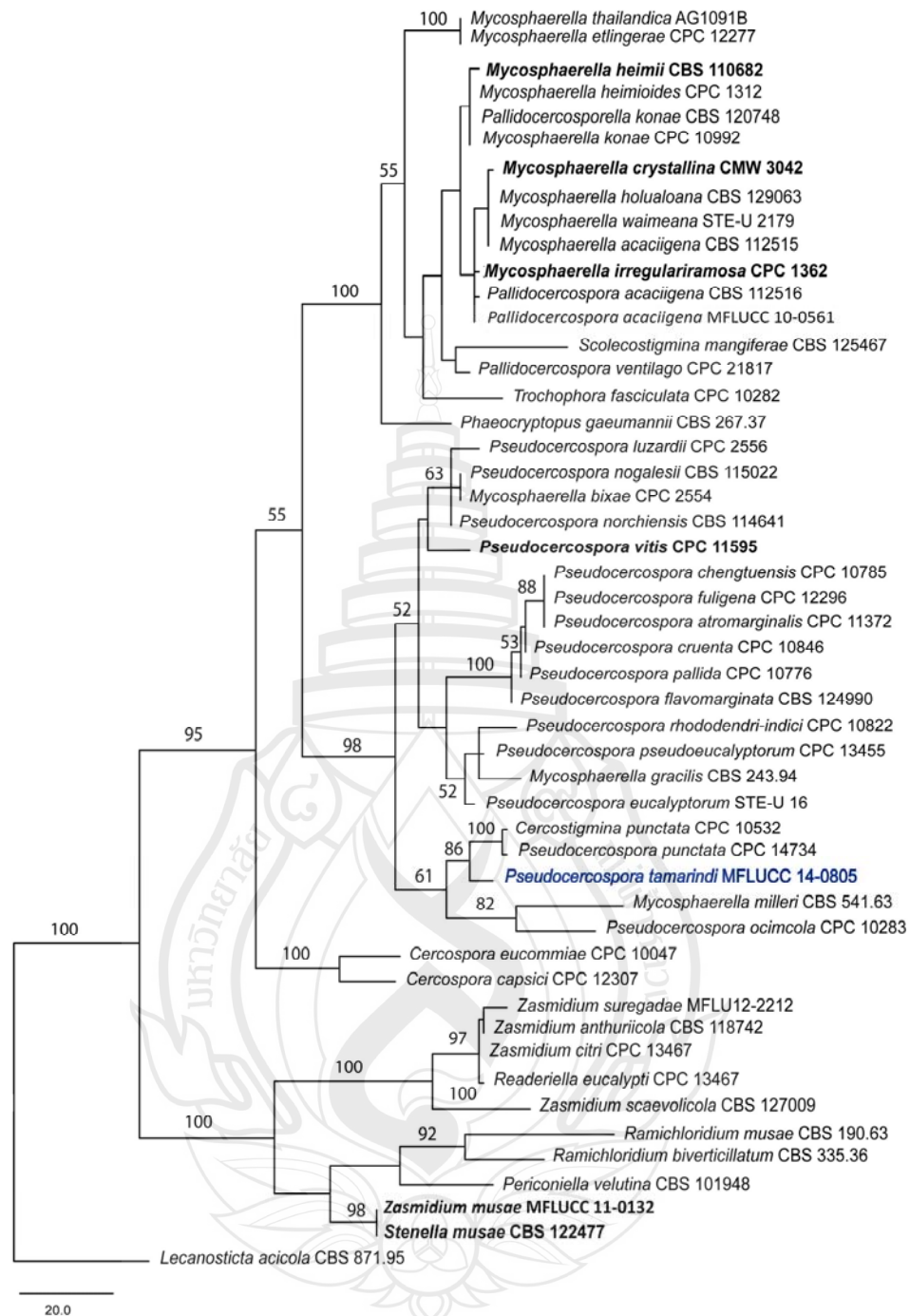
Figure 6.1 Phylogenetic tree generated by ML analysis of ITS, LSU and TEF sequence data of *Distoseptisporaceae* and relates families in *Diaporthomycetidae*

6.3.1.2 *Mycosphaerellaceae*

A combined dataset of the LSU and ITS gene regions of 46 taxa in *Mycosphaerellaceae* and related species of *Capnodiales* were analyzed. The final data matrix comprised 1358 characters including gaps. Single gene analyses were carried out and to compare the topology of the tree and clade stability. *Lecanosticta acicula*

CBS 871.95 was selected as the outgroup taxon. The best scoring RAxML tree with a final likelihood value of -5357.378316 is presented (Figure 6.2). The matrix had 315 distinct alignment patterns, with 14.44% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.254028, C = 0.214897, G = 0.256427, T = 0.274649; substitution rates AC = 1.669093, AG = 4.094948, AT = 2.100113, CG = 1.335509, CT = 6.709933, GT = 1.000000; gamma distribution shape parameter α = 0.740345. The resulting phylogram shows the taxon MFLUCC 14-0805 forming a distinct lineage sister to *Pseudocercospora punctata* CPC 14734 (Figure 6.2).



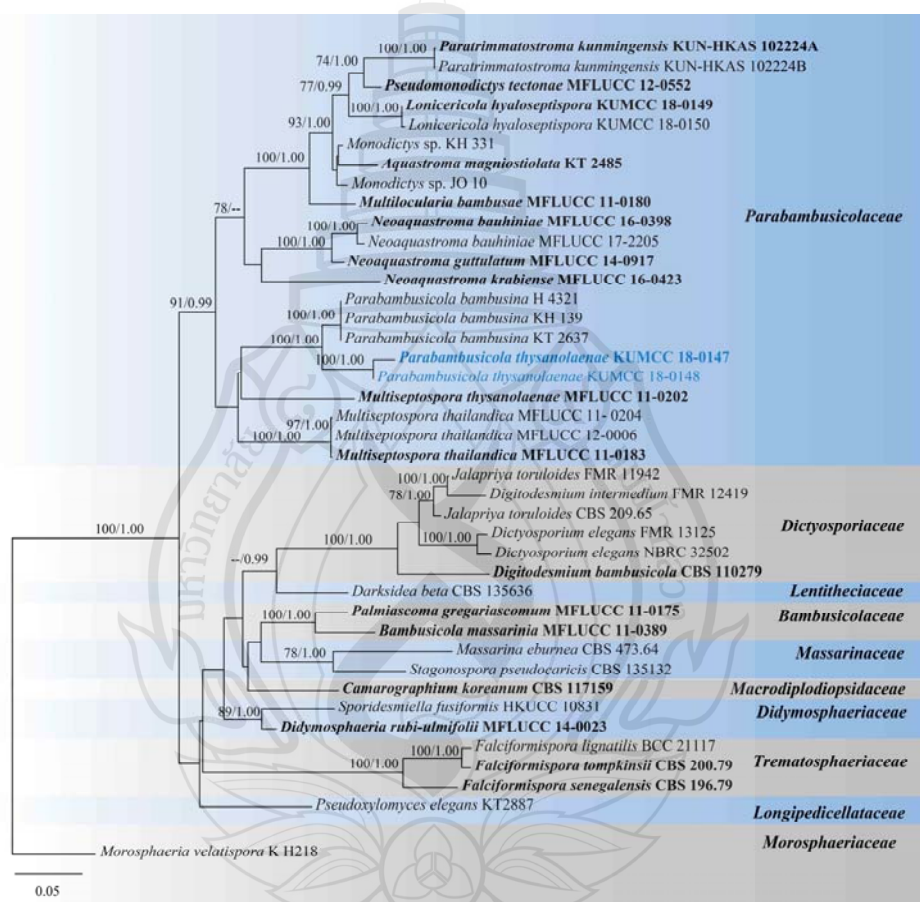


Note Maximum likelihood bootstrap support values greater than 50 % are indicated above the branches of the nodes. The ex-types (reference strains) are in bold; the new isolate is in blue. The tree is rooted with *Lecanosticta acicola* CBS 871.95.

Figure 6.2 Phylogram generated from ML analysis based on combined LSU and ITS sequence data for *Pseudocercospora* and related genera

6.3.1.3 *Parabambusicolaceae*

A combined multigene dataset of the SSU, ITS, LSU and TEF regions were analyzed which included 42 taxa of *Parabambusicolaceae* and its related families (Phukhamsakda et al., 2018). The matrix comprised 3580 characters with gaps. The best scoring RAxML tree with a final likelihood value of -14853.890740 is presented (Figure 6.3). The isolates *P. thysanolaenae* KUMCC 18-0147 and KUMCC 18-0148 clustered with *P. bambusina* forming a well-separated lineage (100% ML and 1.00 BYPP; Figure 6.3).



Note Bootstrap values above 70% and Bayesian posterior probabilities above 0.90 are given at each branch. The tree is rooted with *Morosphaeria velatispora* K H218 (*Morosphaeriaceae*). Type species are in bold and new isolates are in blue.

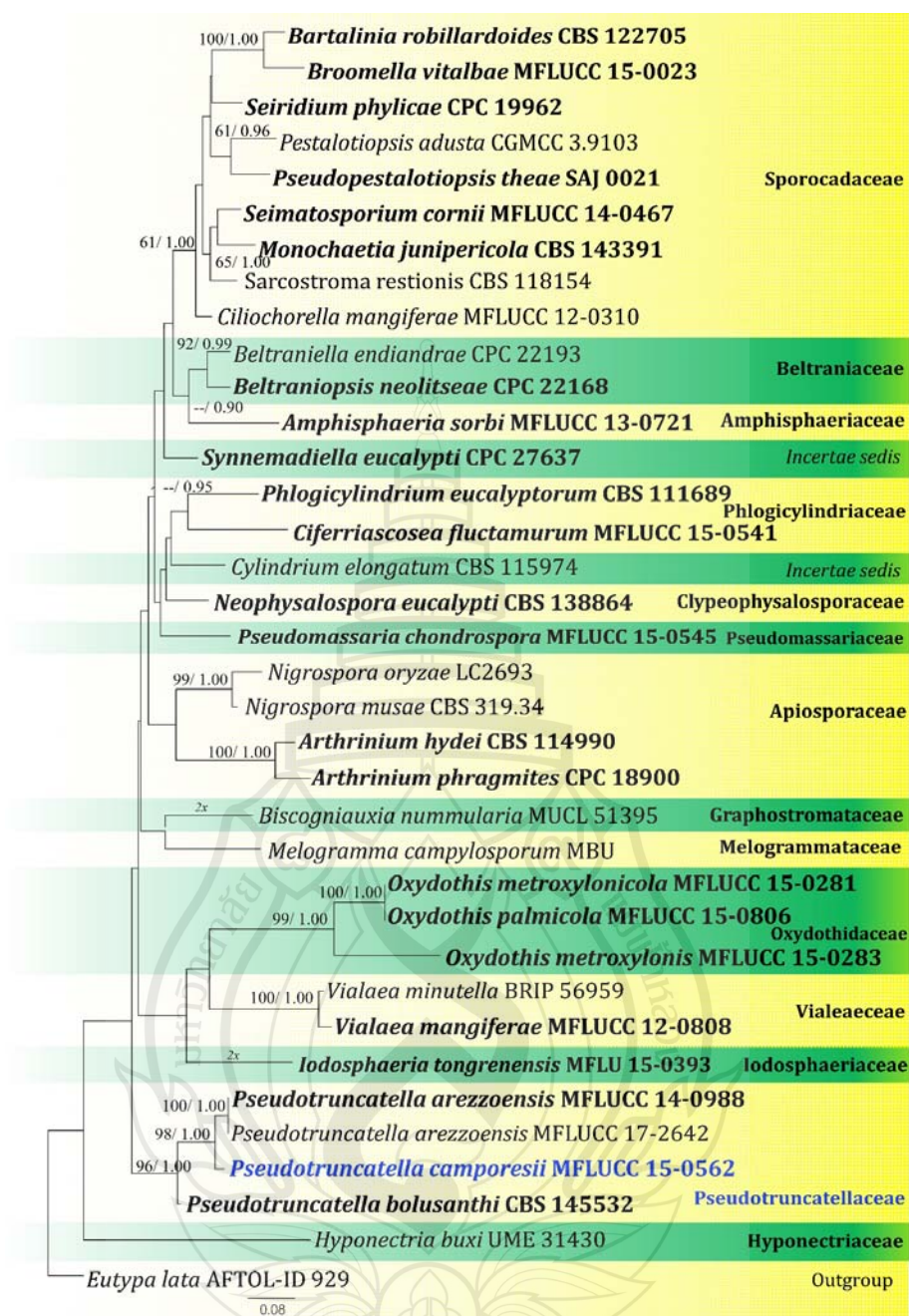
Figure 6.3 Phylogram showing the best ML tree obtained from SSU, ITS, LSU and TEF sequences in *Parabambusicolaceae* and related families

6.3.1.4 *Pseudotruncatellaceae*

Related sequences were obtained from Perera et al. (2018), Crous et al. (2019) and BLAST results. Thirty-six strains were included in the analysis which comprised of 659 characters after alignment. *Eutypa lata* (AFTOL-ID 929) in *Diatrypaceae* (*Xylariales*) was selected as the outgroup taxon. Tree topology of the maximum likelihood analysis was similar to the Bayesian analysis. The best RaxML tree with a final likelihood value of -6953.648717 is presented (Figure 6.4). The matrix had 434 distinct alignment patterns, with 20.77% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.248803, C = 0.249335, G = 0.221058, T = 0.280804; substitution rates AC = 0.899580, AG = 1.933534, AT = 1.335762, CG = 0.977859, CT = 2.816070, GT = 1.000000; gamma distribution shape parameter α = 0.372495.

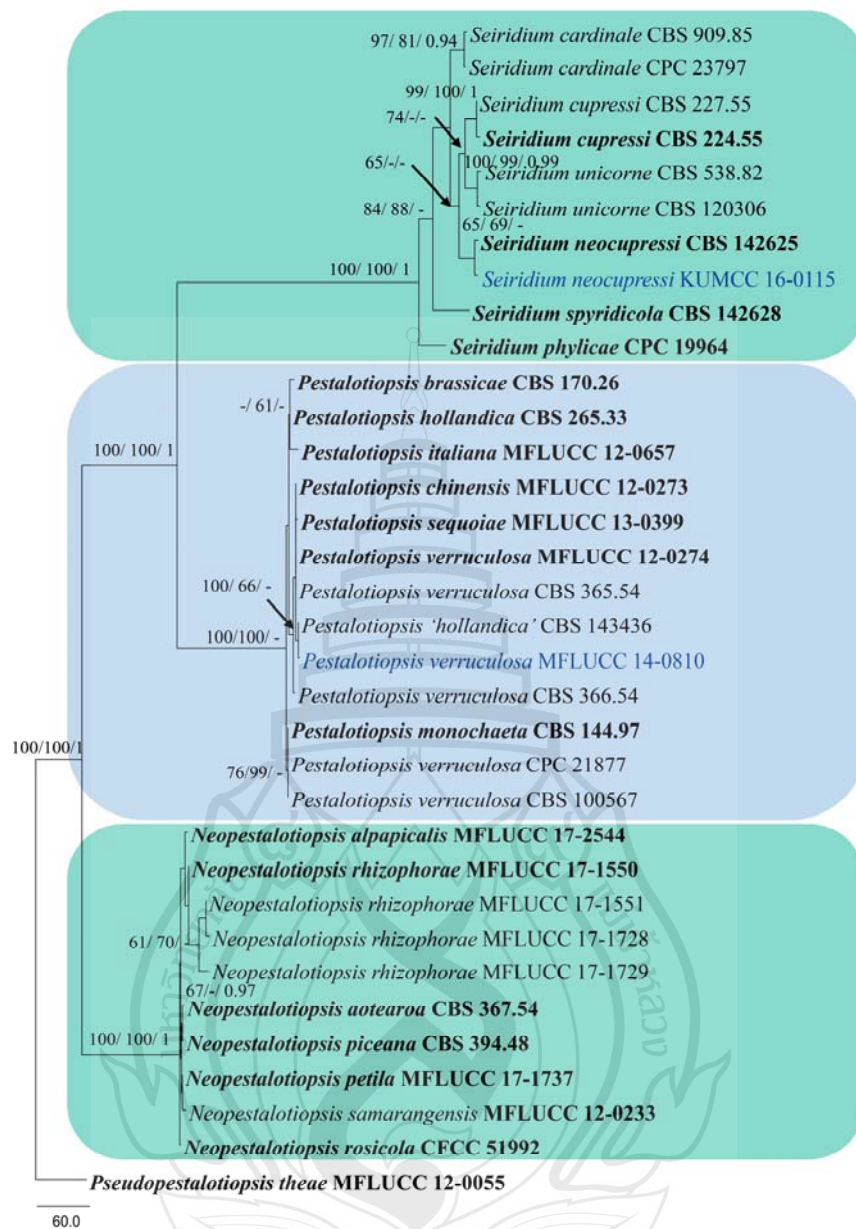
6.3.1.5 *Sporocadaceae*

Seiridium and *Pestalotiopsis*: Thirty-four strains are included in the data matrix comprising 2960 characters after alignment (902 characters for LSU, 621 characters for ITS, 880 characters for TUB, 542 characters for TEF). Bootstrap 50% majority-rule consensus tree is presented in Figure 6.5. The combined dataset contained 2222 constant, 146 parsimony uninformative and 592 parsimony informative characters. Gaps were treated as missing data. The alignment was subjected to 1000 bootstrap replicates. Statistics generated from MP analysis are as follows: TL = 1038, CI = 0.876, RI = 0.974, RC = 0.853, HI = 0.124. The tree topology of the maximum likelihood (ML) analysis and Bayesian analysis did not differ significantly from the maximum parsimony analysis. The best RAxML tree selected had a final likelihood value of -9228.684058.



Note Bootstrap values for maximum likelihood (ML) equal to or greater than 60% and clade credibility values greater than 0.90 (the rounding of values to 2 decimal proportions) from Bayesian-inference analysis are given at the nodes. The tree is rooted to *Eutypa lata* (AFTOL-ID 929). Ex-type strains are in bold and black, the new isolate is indicated in blue bold.

Figure 6.4 Phylogram generated from ML analysis based on ITS sequence data representing *Pseudotruncatellaceae* and closely related families



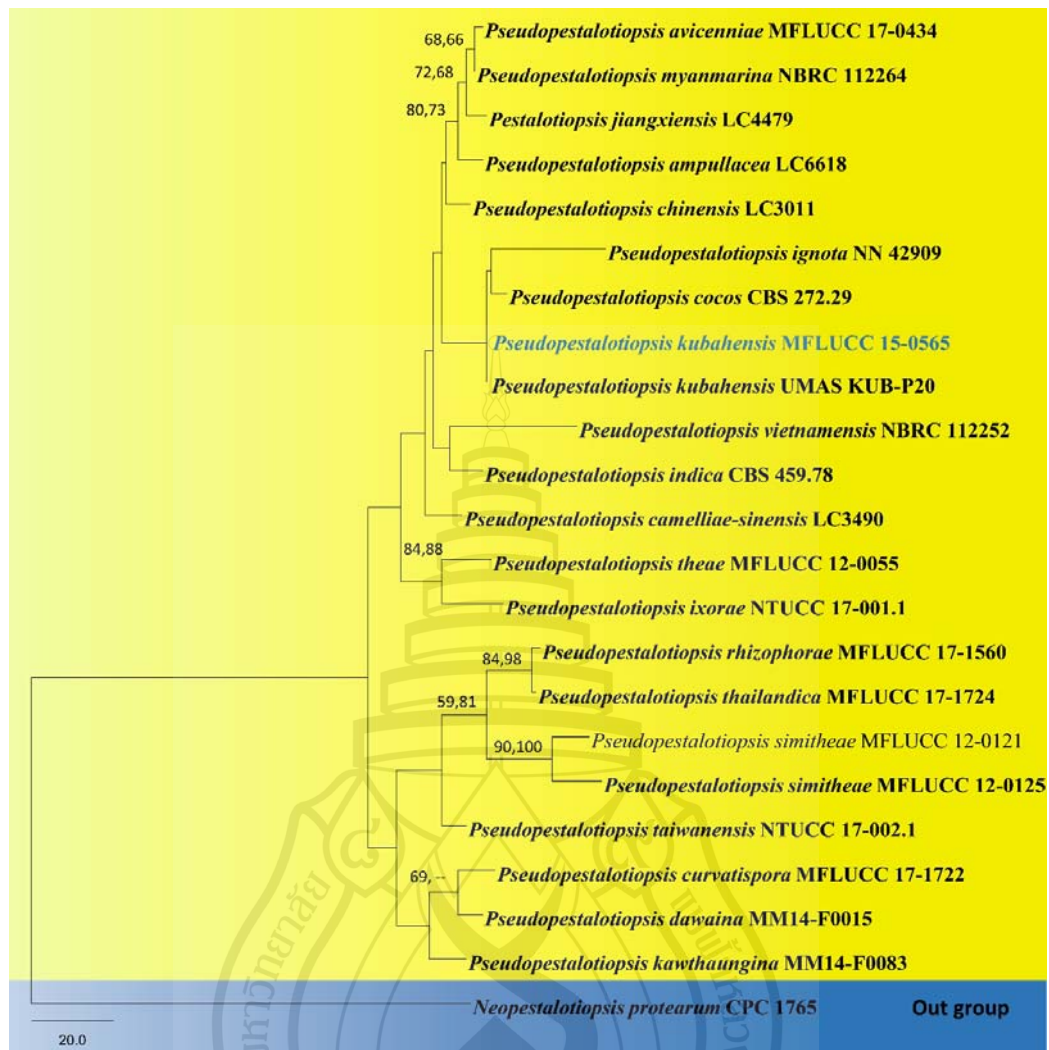
Note Bootstrap values for MP and ML analyses equal to or greater than 60% and Bayesian posterior probabilities greater than 0.9 are given near the nodes, respectively. The tree is rooted with *Pseudoestalotiopsis theae* (MFLUCC 12-0055). Ex-type strains are in bold and black. The newly generated sequences are indicated in blue.

Figure 6.5 Phylogram generated from MP analysis based on combined LSU, ITS, TUB and TEF sequence data of taxa representing *Pestalotiopsis* and *Seiridium* in *Sporocadaceae*

Pseudopestalotiopsis: Twenty-three strains were included in the combined gene analyses comprising 2323 characters after alignment (572 characters for ITS, 793 characters for TUB, 948 characters for TEF). *Neopestalotiopsis protearum* (CPC 1765) is used as the outgroup taxon. Bootstrap 50% majority-rule consensus tree is presented (Figure 6.6). The combined dataset contained 1953 constant, 270 parsimony uninformative and 100 parsimony informative characters. Gaps were treated as missing data. The alignment was subjected to 1000 bootstrap replicates. Statistics generated from MP analysis are as follows: TL = 492, CI = 0.852, RI = 0.741, RC = 0.631, HI = 0.148. The tree topology of the maximum likelihood (ML) analysis did not differ significantly from the maximum parsimony analysis. The best RaxML tree selected had a final likelihood value of -5981.645344.

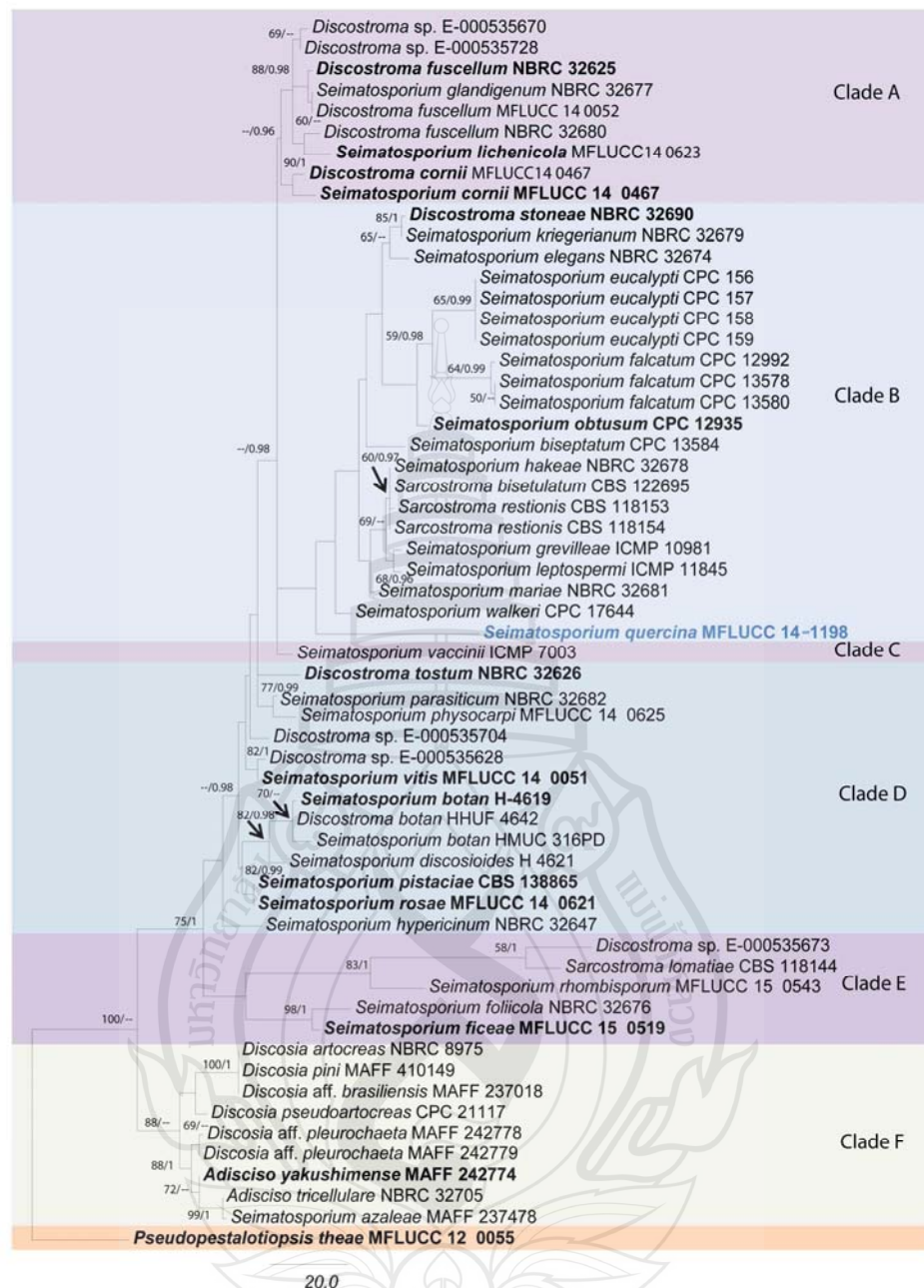
Seimatosporium: A combined analysis of the LSU and ITS sequence data of 60 strains consisting of *Adisciso*, *Discosia*, *Discostroma*, *Sarcostroma* and *Seimatosporium* were used to confirm the phylogenetic placement of our strain, with *Pseudopestalotiopsis theae* (MFLUCC 12-0055) as the outgroup taxon. The phylogenetic analysis of maximum parsimony (MP) and Bayesian analyses resulted in a similar topology. The alignment had a total of 1363 characters including gaps. Parsimony analyses indicated that 837 characters were constant, 375 variable characters were parsimony-uninformative and 175 characters were parsimony informative. The new strain, MFLUCC 14-1198, grouped with the ex-epitype *S. rosae* (MFLUCC 14-0621) forming a distinct clade with low bootstrap support (Figure 6.7).

Truncatella: Forty-two strains were included in the analysis of the ITS region which comprised of 627 characters after alignment. *Pseudopestalotiopsis theae* MFLUCC 12-0055 was selected as the outgroup taxon. Tree topology of the ML analysis was similar to the Bayesian analysis. The best RaxML tree with a final likelihood value of -2433.96530 is presented (Figure 6.8). The matrix had 241 distinct alignment patterns, with 10.86% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.263514, C = 0.235405, G = 0.207066, T = 0.294015; substitution rates AC = 0.725755, AG = 2.691745, AT = 1.060247, CG = 0.947216, CT = 2.334094, GT = 1.000000; gamma distribution shape parameter α = 0.233602.



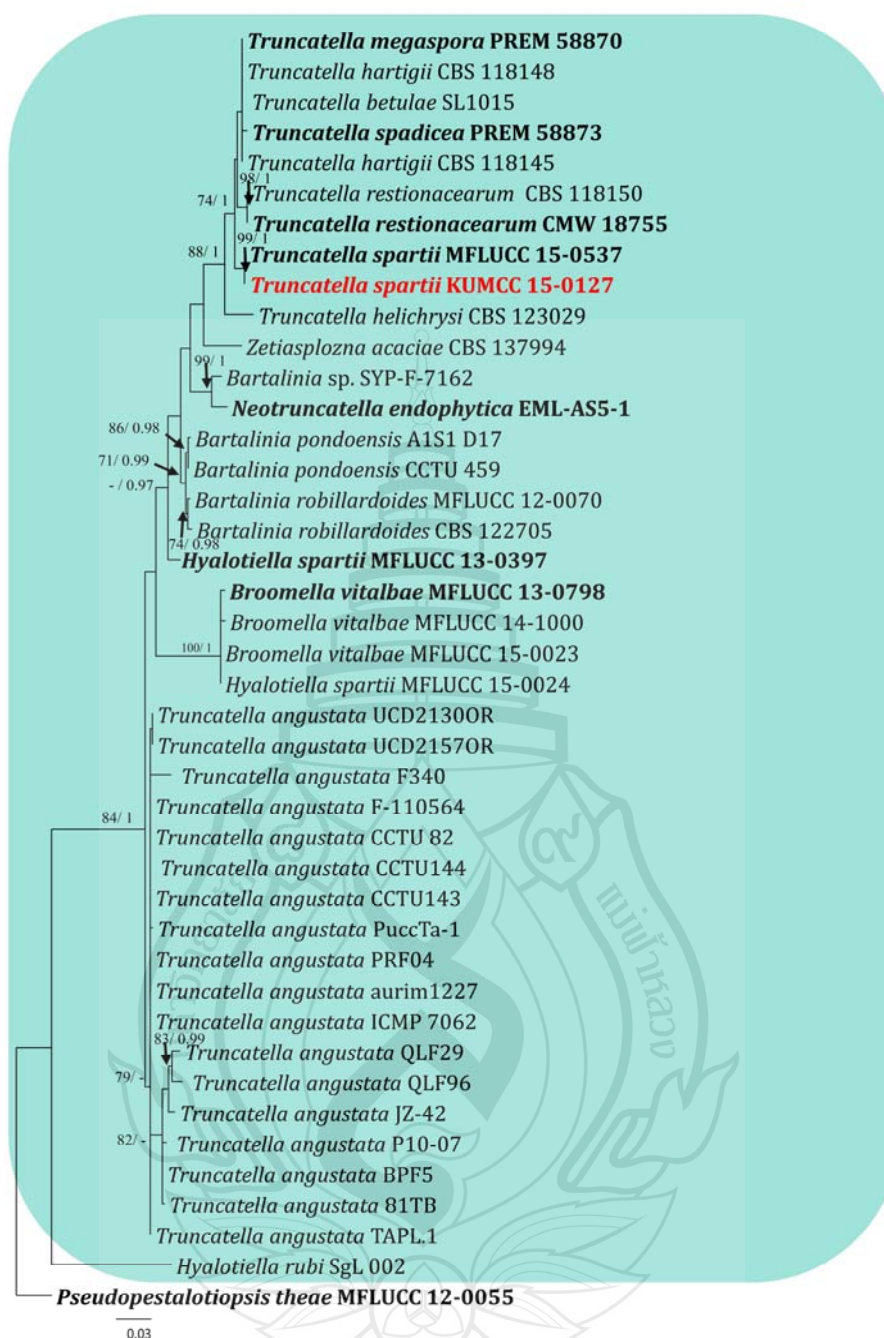
Note Bootstrap values for MP and ML analyses equal to or greater than 50 are placed above or below the branches, respectively. Ex-type strains are in bold and black. The newly generated sequence is indicated in blue.

Figure 6.6 Phylogram generated from MP analysis based on combined ITS, TUB, and TEF sequence data in *Pseudoestalotiopsis*



Note MP bootstrap support values higher than 50% and Bayesian posterior probabilities (PP) above 95% (MP/PP) are given at the nodes. The tree is rooted to *Pseudoestalotiopsis theae* (MFLUCC 12-0055). The strain numbers are given after the species names. The new strain is indicated in blue bold and ex-type strains are in black bold.

Figure 6.7 Maximum Parsimony (MP) majority rule consensus tree for the analyzed isolates of *Seimatosporium* based on LSU and ITS sequence data



Note Maximum likelihood bootstrap support values higher than 70% and Bayesian posterior probabilities greater than 0.96 are indicated above or below branches. The new isolate is shown in red and ex-type strains in black bold. The tree is rooted to *Pseudopestalotiopsis theae* MFLUCC 12-0055.

Figure 6.8 Phylogram generated from ML analysis based on ITS sequence data for *Truncatella* isolates

6.3.2 Taxonomy

Distoseptisporaceae K.D. Hyde & McKenzie

Notes: *Distoseptisporaceae* is a family introduced by Su et al. (2016), classified in *Diaportheomycetidae* families *incertae sedis* by Wijayawardene et al. (2018). It consists of one hyphomycetous genus, *Distoseptispora* which is typified by *D. fluminicola*. Thirteen *Distoseptispora* species have been introduced through previous studies, out of which eleven are from freshwater, and two from terrestrial habitats (Su et al., 2016; Hyde et al., 2016; Xia et al., 2017; Yang et al., 2018; Luo et al., 2018).

Distoseptispora thysanolaenae Goonas., Dayarathne, Phookamsak & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF555408; Facesoffungi number: FoF 05011; Figure 6. 9

Etymology: Refers to the host from which the species was isolated

Holotype: KUN-HKAS 102247

Saprobic on culms of *Thysanolaena maxima*. **Sexual morph:** Undetermined.

Asexual morph: *Conidiophores* macronematous, mononematous, light to dark brown, 2–8-septate, smooth, usually flexuous or sometimes straight, unbranched, cylindrical, 30–80 μm long ($\bar{x}$ = 52 μm , SD = 10, n = 30), 3.5–5.5 μm wide ($\bar{x}$ = 4.5 μm , SD = 0.7, n = 30), rounded at the apex. *Conidiogenous cells* monoblastic, integrated, terminal, determinate, hyaline to light brown, cylindrical. *Conidia* acrogenous, solitary, narrow and elongated obclavate, slightly curved, 8–14-distoseptate, thick-walled, light to dark brown, tapering towards a rounded, flat apex, cell at the apex light brown or hyaline, cell at base truncate with flat base, with conspicuous spore attachment loci, guttulate, 21.5–80 μm long ($\bar{x}$ = 53 μm , SD = 16, n = 30), 6.5–12.8 μm wide ($\bar{x}$ = 9.5 μm , SD = 1.8, n = 30), smooth-walled.

Culture characteristics: Colonies on PDA reaching 43–45 mm diam. after 4 weeks at 20–25°C, colonies dense, circular, flat, surface slightly rough, rugose with edge entire, 4170 floccose to velvety; colony from above, greenish grey at the margin, brown-grey at the centre, from below dark grey at the margin, black at the centre; not producing pigmentation in PDA.

Materials examined: China, Yunnan Province, Xishuangbanna, Mengla County, Xishuangbanna Tropical Botanical Garden (XTBG), on dead culms of *Thysanolaena maxima* (Roxb.) Kuntze (Poaceae), 22 April 2017, R. Phookamsak, IS004 (KUN-HKAS 102247, **holotype**), living culture, KUMCC 18-0182 (IS004A), KUMCC 18-0183 (IS004B).

GenBank numbers: ITS = MK045851; LSU = MK064091; TEF = MK086031.

Notes: Distoseptispora thysanolaenae is the sixth species of this genus to be introduced from a terrestrial habitat. The previously introduced species are *D. martini* (J.L. Crane & Dumont) J.W. Xia & X.G. Zhang, *D. tectonae* Doilom & K.D. Hyde, *D. tectonigena* Doilom & K.D. Hyde, *D. thailandica* Tibpromma & K.D. Hyde and *D. xishuangbannaensis* Tibpromma & K.D. Hyde (Hyde et al., 2016; Xia et al., 2017; Luo et al., 2018; Tibpromma et al., 2018). *Distoseptispora thysanolaenae* can be distinguished from these species by its 2–8-septate conidiophores that are $30\text{--}80 \times 3.5\text{--}5.5 \mu\text{m}$ and narrow elongated, obclavate, light to dark brown conidia that are $21.5\text{--}80 \times 6.5\text{--}12.8 \mu\text{m}$, 8–14-distoseptate and smooth-walled. *Distoseptispora martini* has longer conidiophores ($50\text{--}110 \times 3.5\text{--}4.5 \mu\text{m}$), shorter and wider conidia ($15\text{--}20 \times 11\text{--}16 \mu\text{m}$) that are ellipsoid, oblate or subglobose (Xia et al., 2017). Both *D. tectonae* and *D. tectonigena* differ from our taxon in their conidiophore dimensions (up to $40 \times 4\text{--}6 \mu\text{m}$, up to $110 \times 5\text{--}11 \mu\text{m}$, respectively) and conidial dimensions ($(90\text{--})130\text{--}140(170) \times 13\text{--}14 \mu\text{m}$, $148\text{--}225(360) \times 11\text{--}12 \mu\text{m}$, respectively) and number of septa. *Distoseptispora thysanolaenae* shares similar morphological features with *D. guttulata* but can be distinguished from the latter in having shorter conidia ($21.5\text{--}80 \mu\text{m}$ vs. $75\text{--}130 \mu\text{m}$, Yang et al., 2018).



Note On host substrate: (a) Appearance of colonies on host surface. (b, d, e) Conidiophores. (c, f) Conidiophores with attached conidia. (g–i) Variable shapes of conidia. In vitro: (j) Sporulation of conidia on PDA after 4 weeks. (k–m) Conidiophores with attached conidia. (n–s) Variable shapes of conidia. Scale bars a = 500 μm , b–i, k–r = 20 μm , s = 50 μm .

Figure 6.9 *Distoseptispora thysanolaenae* (KUN-HKAS 102247, holotype)

***Mycosphaerellaceae* Lindau**

Notes: The family *Mycosphaerellaceae* was introduced by Lindau (1896) with *Mycosphaerella* as the type genus. It was initially placed in the order *Dothideales* (Hawksworth et al., 1995). Kirk et al. (2001) introduced a separate order – *Mycosphaerellales* for the family, but later again it was placed in the order *Capnodiales* by Kirk et al. (2008). This placement of *Mycosphaerellaceae* in *Capnodiales* has been phylogenetically confirmed in studies by Crous et al. (2007, 2009a, b). The family includes 14 sexual morph-typified genera, namely *Achorodopsis*, *Brunneosphaerella*, *Cymadothea*, *Euryachora*, *Gillotia*, *Melanodopsis*, *Mycosphaerella*, *Placocrea*, *Polysporella*, *Pseudostigmidium*, *Sphaerellothecium*, *Sphaerulina*, *Stigmidium* and *Wernerella* (Lumbsch & Huhndorf, 2010). More than 30 asexual genera linked to the type genus *Mycosphaerella* are also included in the family (Hyde et al., 2013), while further genera have subsequently been added in recent studies (Crous et al., 2013; Quaedvlieg et al., 2013). These asexual genera consist of some of the most common and destructive plant pathogens that affect a wide variety of host plants, especially economically important fruit and vegetable crops, and cereals (Farr et al., 1995; Crous & Braun, 2003). Here we introduce a new species from tamarind in northern Thailand.

***Pseudocercospora* Speg.**

Pseudocercospora was introduced by Spegazzini (1910) with *Pseudocercospora vitis* as the type species. The genus comprises asexual morphs or species with *Mycosphaerella*-like sexual morphs, which includes many important plant pathogens (Hyde et al., 2013). They are characterized morphologically by their conidiophores and conidia being pigmented and by the conidiogenous loci being inconspicuous or at least unthickened and not darkened (Crous & Braun, 2003; Crous et al., 2013).

Pseudocercospora tamarindi* Goonas. & K.D. Hyde, *sp. nov.

Index Fungorum number: IF550996; Facesoffungi number: FoF00473; Figure 6.10

Etymology: in reference to the host *Tamarindus indica*.

Holotype: MFLU 14-0805

Associated with lesions on living leaves of *Tamarindus indica*. **Sexual morph:** Undetermined. **Asexual morph:** *Leaf spots* epiphyllous, indistinct, subcircular but mostly irregular, 0.5–1 cm diam., at most 2 cm diam. when confluent, pale brown to golden brown, delimited by leaf mid rib. *Caespituli* amphigenous but predominantly epiphyllous,

abundant on leaf spots, dense, dark, punctiform. *Stromata* superficial, globular, pale to dark brown 11–25 μm diam., cells rounded, oblong or irregular, 2–3 μm diam., wall thickness less than 0.3 μm . *Conidiophores* fasciculate, arising from the stromata, brown becoming pale brown towards the apex, smooth, 0–2-septate, often short or one-cells, straight to slightly curved, apex rounded to truncate, unbranched, $3\text{--}12 \times 1\text{--}2 \mu\text{m}$ ($\bar{x} = 7.37 \times 1.81 \mu\text{m}$, $n = 20$), wall thickness less than 0.25 μm . *Conidiogenous cells* $5\text{--}7 \times 0.8\text{--}2 \mu\text{m}$ ($\bar{x} = 5.66 \times 1.59$, $n = 10$), integrated, terminal or conidiophores reduced to conidiogenous cells, occasionally branched, smooth, hyaline to pale brown, integrated, proliferating percurrently, conidiogenous loci inconspicuous. *Conidia* $(13\text{--})17\text{--}25\text{--}(30) \times (1.8\text{--})2\text{--}3\text{--}(3.5) \mu\text{m}$ ($\bar{x} = 21 \times 2.38 \mu\text{m}$, $n = 30$), solitary, subhyaline to brown, cylindrical to obclavate, some showing constrictions at septa, some guttulate, subacute to obtuse apex, obconically subtruncate base, 2–14-septate, hila neither thickened or darkened, 0.7–2 μm diam.

Culture characters: Colonies on PDA show slow growth, attaining 10–12 mm diam. after 3 weeks at 25 °C, dark-grey to black at the margin, grey to pale grey in the centre; reverse dark-green to black; medium dense with dense center, irregular, convex surface, smooth lobate margin, hairy, nonpigmented.

Material examined: Thailand, Chiang Rai Province, Mueang Chiang Rai District, on living leaves of *Tamarindus indica* L. (Fabaceae), 12 February 2014, K.D. Hyde IG018 (MFLU 14-0805, **holotype**); living culture, MFLUCC 14-0805.

GenBank numbers: ITS: KP744461; LSU: KP744506; SSU: KP753965.

Notes: This new species is the first cercosporoid to be described from the host *Tamarindus indica* in Thailand. Previously, a species *Cercospora tamarindi* had been recorded on leaves of *Tamarindus indica* in Uttar Pradesh, India (Khan et al., 1988; Index Fungorum, 2015). *Pseudocercospora tamarindi* differs from this species by having conidiophores that lack distinct spore scars with much less thick wall ($>0.25 \mu\text{m}$) and darker coloured conidia. Although they both have a similar range for the number of septa (–14), the spores of *P. tamarindi* are much smaller $(13\text{--})17\text{--}25\text{--}(30) \times (1.8\text{--})2\text{--}3\text{--}(3.5) \mu\text{m}$ compared to those of *C. tamarindi* $(38.4\text{--}93 \times 3.8 \mu\text{m}$ according to Khan et al., 1988). A molecular analysis based on a combined gene analysis of ITS and LSU shows that *Pseudocercospora tamarindi* clusters within the major clade that includes the type species *Pseudocercospora vitis* with a bootstrap value of 84% but is distant from the type species. Yet it is clearly separated from other *Pseudocercospora* species. *Pseudocercospora*

tamarindi is related with *Pseudocercospora punctata* (Wakef.) B. Sutton (= *Cercostigmina punctata* Wakef.) which has verruculose conidia (which the former lacks). It is also morphologically similar, but different in conidial size and the number of septa to *Pseudocercospora ocimicola* which has 1–8 septate conidia of (15–)25–75(–85) $\times$ 2–4 μ m (Crous et al., 2013).



Note (a) Symptoms on leaves (upper surface). (b, c) Close up of leaf spot. (d, e) Stroma with attached conidiophores and conidia. (f) Stromatal cells. (g-p) Conidia of different sizes and maturity. (q) Colony on PDA (3 weeks). Scale bars: b = 500 μ m, c = 50 μ m, d-f, h-o = 10 μ m, g, p = 20 μ m.

Figure 6.10 *Pseudocercospora tamarindi* (MFLU 14-0805, holotype)

Parabambusicolaceae Kaz. Tanaka & K. Hiray.

Notes: The latest treatments by Tanaka et al. (2015), Li et al. (2016), Phukhamsakda et al. (2016, 2018), Wanasinghe et al. (2017), and Dayarathne et al. (2018) of *Parabambusicolaceae* have been followed.

Parabambusicola Kaz. Tanaka & K. Hiray.

Notes: The latest treatment for this genus has been provided by Tanaka et al. (2015). Previously, only *P. bambusina* was accommodated in this genus (Index Fungorum, 2019). Here, a second species, *P. thysanolaenae*, collected from *Thysanolaena maxima* in Yunnan, China is described.

Parabambusicola thysanolaenae Goonas., Jayasiri, Phookamsak & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF555596; Facesoffungi number: FoF 04964; Figure 6.11

Etymology: Named after the host from which the fungus was isolated.

Holotype: KUN-HKAS 102222

Saprobic on dead stems of *Thysanolaena maxima*, appearing as raised, dome-shaped, black structures on host surface. **Sexual morph:** *Ascomata* 430–600 µm wide, 130–170 µm high, ($\bar{x} = 535 \times 150$ µm, $n = 15$), mostly clustered together, sometimes solitary, semi-immersed, globose in surface view, hemispherical with a flattened base in cross section. *Ostiolar* neck short, central. *Peridium* 25–60 µm wide, lateral walls composed of numerous layers of inner, hyaline, flattened cells to outer, pale brown to brown *textura angularis* cells and pale brown to brown, globular or polygonal cells showing no conspicuous layers at the base. *Hamathecium* composed of numerous, 1.5–3 µm wide, filamentous, septate pseudoparaphyses. *Asci* (50–)80–120 µm × (10–)25–33 µm ($\bar{x} = 107 \times 28$ µm, $n = 30$), 8-spored, bitunicate, broadly cylindrical to clavate, rounded at the apex, with a short-pedicel. *Ascospores* overlapping bi-seriate to tri-seriate, 45–55 µm × 7.5–11 µm ($\bar{x} = 46.5 \times 9$ µm, $n = 35$), fusiform, mostly 5-septate, occasionally 6–7-septate, primary septum mostly median, slightly constricted at the mid-septum, hyaline, smooth, slightly curved with an entire sheath, large guttules present at immature state. **Asexual morph:** unknown.

Culture characteristic: Colonies on PDA reaching 30–32 mm diam. after 3 weeks at room temperature (20–30°C). Colony dense, circular, flattened, surface smooth, with entire edge, velvety to floccose; from above greenish grey to dark green,

paler at the edge; from below, black; produced dark brown pigmentation around colony on agar medium. Colonies on MEA reaching 28–30 diam. after 3 weeks at room temperature (20–30°C). Colony dense, circular, flattened, surface smooth, with entire edge, floccose to cottony; from above cream to pale yellowish; from below, yellowish brown, paler at the edge; not producing pigmentation on agar medium.

Material examined: CHINA, Yunnan Province, Xishuangbanna, Mengla County, Xishuangbanna Tropical Botanical Garden (XTBG), on dead stems of *Thysanolaena maxima* (Roxb.) Kuntze (Poaceae), 22 April 2017, R. Phookamsak, IS003 (KUN-HKAS 102222, **holotype**), KUMCC 18-0147 (IS003A), KUMCC 18-0148 (IS003B).

GenBank numbers: KUMCC 18-0147: ITS: MK098190; LSU: MK098199; SSU: MK098205; TEF: MK098209, KUMCC 18-0148: ITS: MK098193; LSU: MK098198; SSU: MK098202; TEF: MK098211.

Notes: *Parabambusicola thysanolaenae* shares similar peridium and asci characteristics with *P. bambusina* but could be distinguished from the latter in having wider ascomata ($535 \times 150 \mu\text{m}$ vs. $300\text{--}500 \times 150\text{--}300 \mu\text{m}$), absence of a beak-like structure and wider peridium ($25\text{--}60 \mu\text{m}$ vs. $10\text{--}20 \mu\text{m}$). Ascospores of *P. thysanolaenae* are 5-septate and shorter ($46.5 \times 9 \mu\text{m}$) compared to the 3–5-septate ascospores of *P. bambusina* ($54.7 \times 8.4 \mu\text{m}$, Tanaka & Harada, 2003). Phylogenetically it clusters with *P. bambusina* in a well-supported clade.



Note (a) Appearance of ascomata on host substrate. (b) Section through ascoma. (c) Section through peridium. (d) Ostiolate. (e) Asci immersed in hyaline, cellular pseudoparaphyses, stained with Indian ink. (f) Ascus. (g–i) Ascospores. (j, k) Ascospores stained with Indian ink. Scale bars: a = 500 µm, b = 100 µm, c, d = 50 µm, e–k = 20 µm.

Figure 6.11 *Parabambusicola thysanolaenae* (KUN-HKAS 102223, holotype)

Pseudotruncatellaceae Crous

Notes: The family *Pseudotruncatellaceae* was established by Crous et al. (2019) to accommodate the genus *Pseudotruncatella* in *Amphisphaeriales*. It currently consists

of three members, and is phylogenetically affiliated to the family *Hyponectriaceae*. Morphologically, *Pseudotruncatellaceae* is similar to *Truncatella* in *Sporocadaceae*, but is phylogenetically distinct from the latter.

Pseudotruncatella R.H. Perera, Camporesi, Maharachch. & K.D. Hyde

Notes: *Pseudotruncatella* was introduced by Perera et al. (2018) as a monotypic genus typified by *P. arezzoensis* R.H. Perera, Camporesi, Maharachch. & K.D. Hyde. This species was found on *Cytisus scoparius* and *Helichrysum italicum* from Italy. Subsequently, Crous et al. (2019) introduced a second species to this genus, *Pseudotruncatella bolusanthi* Crous, collected on *Bolusanthus speciosus* from South Africa. *Pseudotruncatella* is characterized by its truncatella-like, fusoid to clavate conidia, bearing 1 or 3 apical appendages and dark brown, thick-walled, median cells. The sexual morph is unknown undetermined.

Pseudotruncatella camporesii Goonas. & Hyde *sp. nov.*

Index Fungorum number: IF556928; Facesoffungi number: FoF 06822; Figure 6.12

Etymology: Named in honour of the collector Erio Camporesi

Holotype: MFLU 15-0796

Saprobic on dead stem of *Helichrysum* sp. **Sexual morph:** Undetermined
Asexual morph: *Conidiomata* appearing as black, shiny, raised or flattened, rounded structures on host surface. 170–250 µm high, 280–400 µm wide ($\bar{x}$ = 200 × 340 µm, n = 10) acervular to pycnidoid, scattered or gregarious, uniloculate, globose or oval with a flattened base, semi-immersed to superficial, sometimes erumpent. *Conidiomata* wall comprising several indistinct cell layers of golden brown to pale brown cells of *textura angularis* to *textura prismatica*, and towards the upper part of the fruiting body, more of *textura prismatica* and *textura globulosa*. *Conidiophores* arising from the basal and lateral cells of the conidioma cavity, cylindrical, reduced to conidiogenous cells, unbranched, smooth, hyaline. *Conidiogenous cells* 10–38 × 1.5–3 µm ($\bar{x}$ = 26 × 2 µm, n = 20), cylindrical, hyaline, smooth, hyaline, smooth, determinate, holoblastic, proliferating percurrently at apex, aseptate. *Conidia* 28–35 × 6–8.5 µm, ($\bar{x}$ = 31 × 7.3 µm, n = 40), fusoid, straight to slightly curved, 2-septate, slightly constricted at second septum from base, basal cell 5.8–12 × 2.5–3.5 µm ($\bar{x}$ = 9 × 2.8 µm), 15–30 × 1.5–2 mm, narrowly obconic, truncate at base, hyaline, smooth-walled, thin-walled, second and third cells from the base, together 18.5–26 × 5.5–9 µm ($\bar{x}$ = 22 × 6.8 µm), dark brown, smooth-

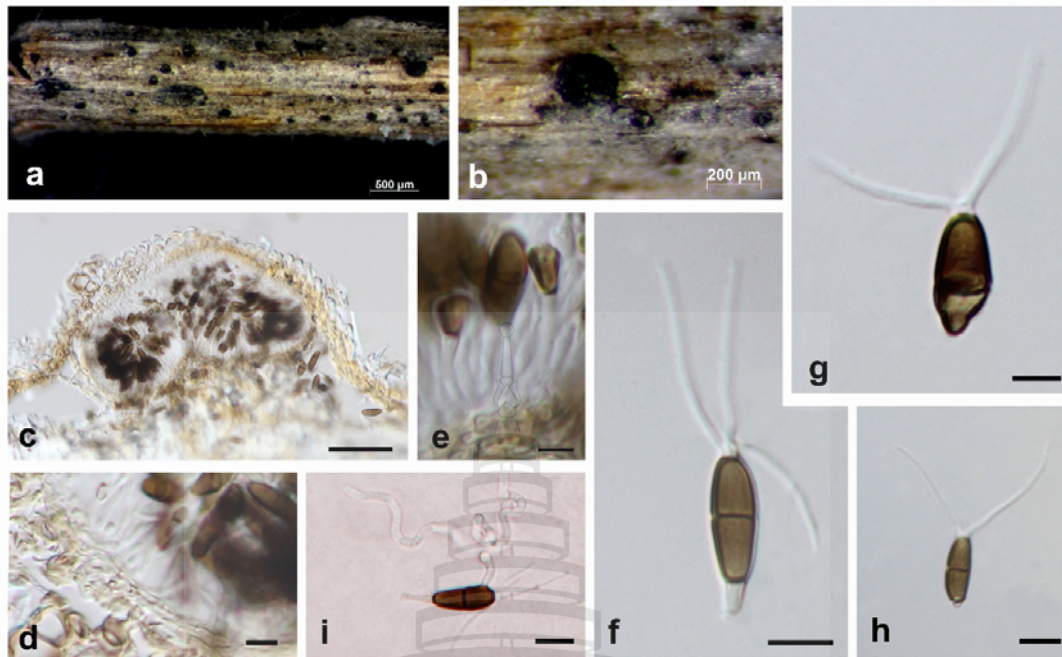
walled, thick-walled, slightly guttulate, fusoid, bearing three tubular apical appendages, 18.6–25.8 μm ($\bar{x}$ = 23.2 μm) long, smooth, tubular, unbranched.

Culture Characteristics: Colonies on PDA growing up to 20 mm in diam. at 16–18 °C after seven days. Colony irregular in shape, flat to effuse, surface smooth, with undulate margin, yellowish-white, reverse grey.

Material examined: Italy, near Passo delle Forche - Galeata (province of Forlì-Cesena [FC]), on dead stem of *Helichrysum* sp. (Asteraceae), Erio Camporesi, 16 July 2014, IT 2001 (MFLU 15-0796, **holotype**, MFLU 15-0797 = HKAS96293, **isotype**), living culture, MFLUCC 15-0562.

GenBank numbers: ITS: MN648203.

Notes: *Pseudotruncatella arezzoensis* and *P. camporesii* are reported on *Helichrysum* sp. as saprobes. The two species can be distinguished by their conidiomata dimensions (230–275 μm high, 255–280 μm wide vs. 170–250 μm high, 280–400 μm wide). *P. arezzoensis* has pycnidoid, immersed to semi-immersed conidiomata, while in *P. camporesii* they are acervular to pycnidoid, semi-immersed to superficial (Perera et al., 2018). Comparatively *P. camporesii* has longer conidiogenous cells and larger, 2-septate conidia (28–35 $\times$ 6–8.5 μm vs. 20–25 $\times$ 5.4–6.5 μm , Perera et al., 2018). Furthermore, *P. arezzoensis* produced 3-septate conidia with no constriction at the septum while the conidia in our species were 2-septate and slightly constricted at the septum. Likewise, *P. bolusanthi* have 2-septate conidia showing constriction at mid-septum (Crous et al., 2019). Yet, it is morphologically distinct in having smaller conidiomata of 150–200 μm in diam., with 0–3-septate, branched conidiophores and shorter conidia (17–20 $\times$ 6.5–7 μm), with one apical appendage (Crous et al., 2019). In the phylogenetic analysis, our new isolate clustered with *P. arezzoensis* with high bootstrap support (96% MLBS, 1.00 PP, Figure 6.4).



Note (a) Conidiomata on dead stem of *Helichrysum* sp. (b) A close-up of conidiomata (c) Cross section of conidiomata (d) Conidiomata wall (e) Developing conidia from conidiogenous cells (f-h) Conidia (i) Germinating spore. Scale bars c = 50 μm, d, e, g, h = 10 μm, f, i = 20 μm.

Figure 6.12 *Pseudotruncatella camporesii* (MFLU 15-0796, holotype)

***Sporocadaceae* Corda**

Notes: A revision of the family based on both morphology and molecular data was provided by Liu et al. (2019). The results showed 30 well-supported monophyletic clades, of which included 23 known genera and seven new. Several emendations for species and the types of *Diploceras*, *Discosia*, *Monochaetia*, *Sporocadus* and *Truncatella* have been made (Wijayawardene et al., 2020).

***Pestalotiopsis* Steyaert**

Notes: *Pestalotiopsis* is widely distributed, often occurring as pathogens, saprobes and endophytes (Maharachchikumbura et al., 2014, 2016; Wijayawardene et al., 2017). They have been reported from various terrestrial habitats, especially on crop plants, mangroves and animals (Norphanphoun et al., 2019; Farr & Rossman, 2019). The genus established by Steyaert (1949) has over 350 species epithets listed in Index Fungorum

(2020). The diversity, host distribution, morphology, and phylogenetic relationships of *Pestalotiopsis* species based on ITS, β -TUB and TEF1- α molecular data is well studied (Jeewon et al., 2002; Maharachchikumbura et al., 2011, 2012, 2013, 2014; Liu et al., 2019; Hyde et al., 2020).

Pestalotiopsis verruculosa Maharachch. & K.D. Hyde, in Maharachchikumbura
Index Fungorum number: IF800527; Facesoffungi number: FoF 06983; Figure 6.13

Saprobic on dead cone of *Picea excelsa* appearing as rounded to elongated, slightly raised black lesions erupting through irregular slits on host surface tissue.

Asexual morph: *Conidiomata* semi-immersed to erumpent or partially erumpent, exuding brown to black conidial masses, acervulus, mostly oval to elongate, 175–250 μ m in diam., solitary, black. *Conidiomata wall* indistinct, composed of light brown cells fusing with host tissue. *Conidiophores* not observed. *Conidiogenous cells* discrete or integrated, hyaline, smooth-walled, cylindrical to lageniform. *Conidia* broad fusiform, mostly straight, occasionally slightly curved, 4-septate, slightly constricted at septa, $25.5\text{--}32 \times 8.7\text{--}9.5$ μ m, ($\bar{x} = 30.5 \times 9.2$ μ m, $n = 40$), basal cell obconic with a truncate base, colourless, smooth-walled, 4.5–5.5 μ m long; three median cells 16–20 μ m long, subcylindrical or doliiform, concolourous, brown to dark brown, second cell from base sometimes lighter, septa darker than the rest of the cell (second cell from base 6–8 μ m long; third cell 6–8 μ m long; third cell 6–8 μ m); apical cell conic, hyaline, thin-walled, smooth, 3.6–4.5 μ m long; with 4 or occasionally 3, tubular, unbranched, flexuous, apical appendages arising from the apical tip, 10–25.5 μ m long, not knobbed; basal appendage single, unbranched, filiform, centric, up to 5.5–9.5 μ m long. **Sexual morph:** undetermined.

Culture characteristics: Colonies on PDA reaching approximately 7 cm in diam. after 7 days at 16–18 °C, greyish-white, round colony, with entire margins and reverse dark brown at the center becoming pale yellow towards the margins.

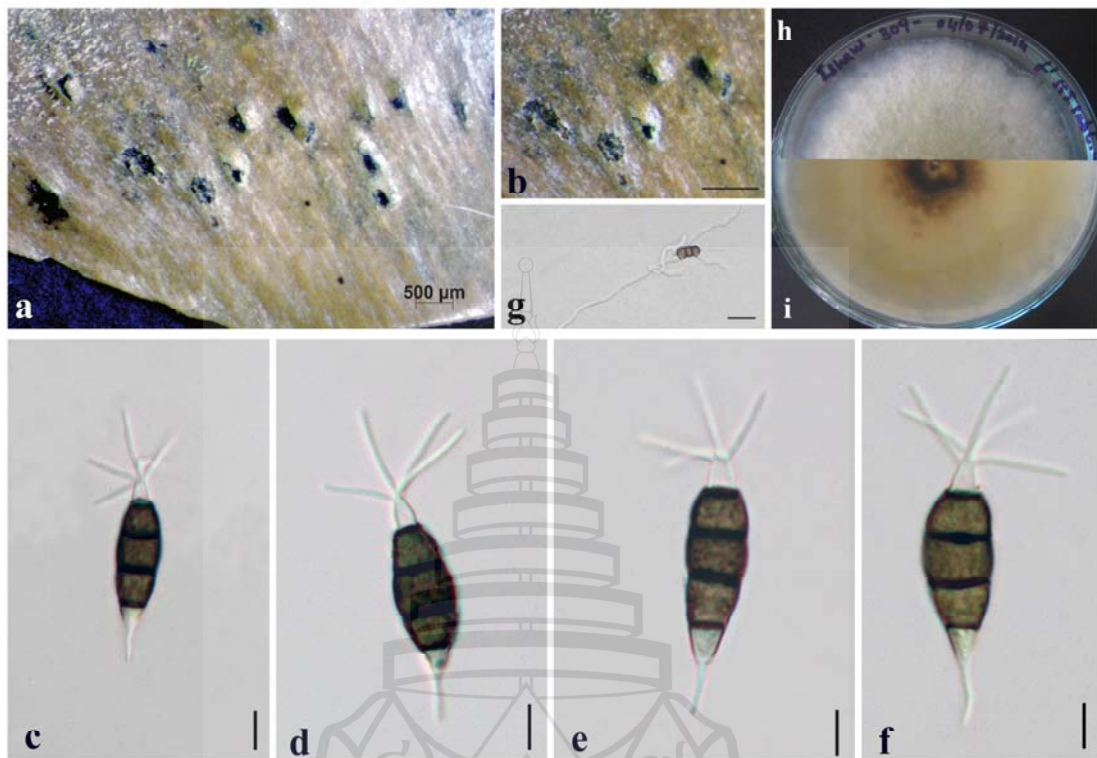
Material examined: Italy, Forlì-Cesena Province, Camposonardo - Santa Sofia, on dead cone of *Picea excelsa*, 6 May 2012, Erio Camporesi, IT 309 (MFLU 15-0777), living culture, MFLUCC 14-0810.

GenBank numbers: ITS: MN733174.

Known distribution (based on molecular data): China (Maharachchikumbura et al., 2012), Italy (this study).

Known hosts (based on molecular data): *Picea excelsa* (this study), *Rhododendron* sp. (Maharachchikumbura et al., 2012).

Notes: *Pestalotiopsis verruculosa* was introduced as an endophyte by Maharachchikumbura et al. (2012) from living leaves of *Rhododendron* species. It was distinguished from other related taxa in having larger conidia ($28\text{--}35 \times 9\text{--}11 \mu\text{m}$) and 3–4, but longer apical appendages ($25\text{--}40 \mu\text{m}$). Our strain had similar conidial dimensions ($25.5\text{--}32 \times 8.7\text{--}9.5 \mu\text{m}$), but shorter appendages ($10\text{--}25.5 \mu\text{m}$). *Pestalotiopsis verruculosa* also shares similar morphological characters with *P. hollandica* in having $25.5\text{--}33 \times 8.5\text{--}10 \mu\text{m}$ (Maharachchikumbura et al., 2014) sized conidia bearing 1–4 appendages. In their recent study of *Sporocadaceae* and phylogenetic analysis of *Pestalotiopsis*, Liu et al. (2019) suggested that *P. brassicae*, *P. hollandica*, *P. italiana*, *P. monochaeta*, *P. sequoiae* and *P. verruculosa* may be synonymous, however, additional genes are necessary to resolve this. Our phylogenetic analysis presents results similar to Liu et al. (2019). Our isolate MFLUCC 14-0810 grouped with the type strain of *P. verruculosa* (MFLUCC 12-0274). Based on the phylogenetic results and morphological comparisons we identify our strain as *P. verruculosa*.



Note (a, b) Appearance of conidiomata on the host. (c–f) Conidia. (g) Germinating spore. (h, i) Culture on PDA (h: from the above. i: reverse view). Scale bars: a, b = 500 μ m, c–f = 20 μ m, g = 50 μ m.

Figure 6.13 *Pestalotiopsis verruculosa* (MFLU 15-0777, new host and geographical record)

Pseudopestalotiopsis Maharachch., K.D. Hyde & Crous

Notes: Maharachchikumbura et al. (2014) established the genus *Pseudopestalotiopsis*. It was differentiated from its two sister genera *Pestalotiopsis* and *Neopestalotiopsis* based on conidial pigmentation and molecular phylogeny. As it is in *Pestalotiopsis*, the median cells in the conidia of *Pseudopestalotiopsis* are concolourous, but are much darker (brown to dark brown) and bear knobbed apical appendages (Maharachchikumbura et al., 2014). In *Neopestalotiopsis* the median cells are versicolorous. *Pseudopestalotiopsis* species have been reported from terrestrial to mangrove habitats, as saprobes or as pathogens associated with leaf spots (Maharachchikumbura et al., 2014, 2016; Norphanphoun et al., 2019).

Pseudopezalotiopsis kubahensis Lateef, M. Sepiah & Bolhassan

Index Fungorum number: IF812829; Facesoffungi number: FoF 01310; Figure 6.14

Endophytic or pathogenic causing irregular shaped lesions on branches of *Lonicera* sp. appearing as black, flattened patches. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* semi-immersed to erumpent, exuding brown to black conidial masses, acervulus, subglobose to globose with a flattened base, 250–300 µm in diameter, solitary, dark brown to black. *Conidiomata wall* composed of light brown, thin-walled, flattened cells of *textura prismatica*, indistinct at the base, mostly fused with host tissue. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* arising from the innermost layers of the conidiomata, discrete, hyaline, smooth-walled, filiform to lageniform. *Conidia* broad fusiform, straight to slightly curved, 4-septate, slightly constricted at septa, 22.5–26.5 × 6.5–8.5 µm, ($\bar{x}$ = 25 × 7.5 µm, n = 40) basal cell conic with a truncate base, thin and smooth-walled, 2.5–3.5 µm long; three median cells 16–19.5 µm long, doliiform, concolourous, golden brown to dark brown, septa darker than the rest of the cell (second cell from base 4–6 µm long; third cell 3.5–4.5 µm long; fourth cell 5–6.5 µm long); apical cell conic, hyaline, thin and smooth-walled, 2.5–4 µm long; with 3 or occasionally 2 tubular apical appendages arising from the apical tip, flexuous, rarely branched, 15–35(40) µm long, occasionally knobbed; basal appendage not observed.

Culture characteristics: Colonies on PDA reaching approximately 7 cm diameter after 7 days at 16–18 °C, greyish-white, irregular, with undulate margins and black, gregarious conidiomata sporulating; reverse pale yellow.

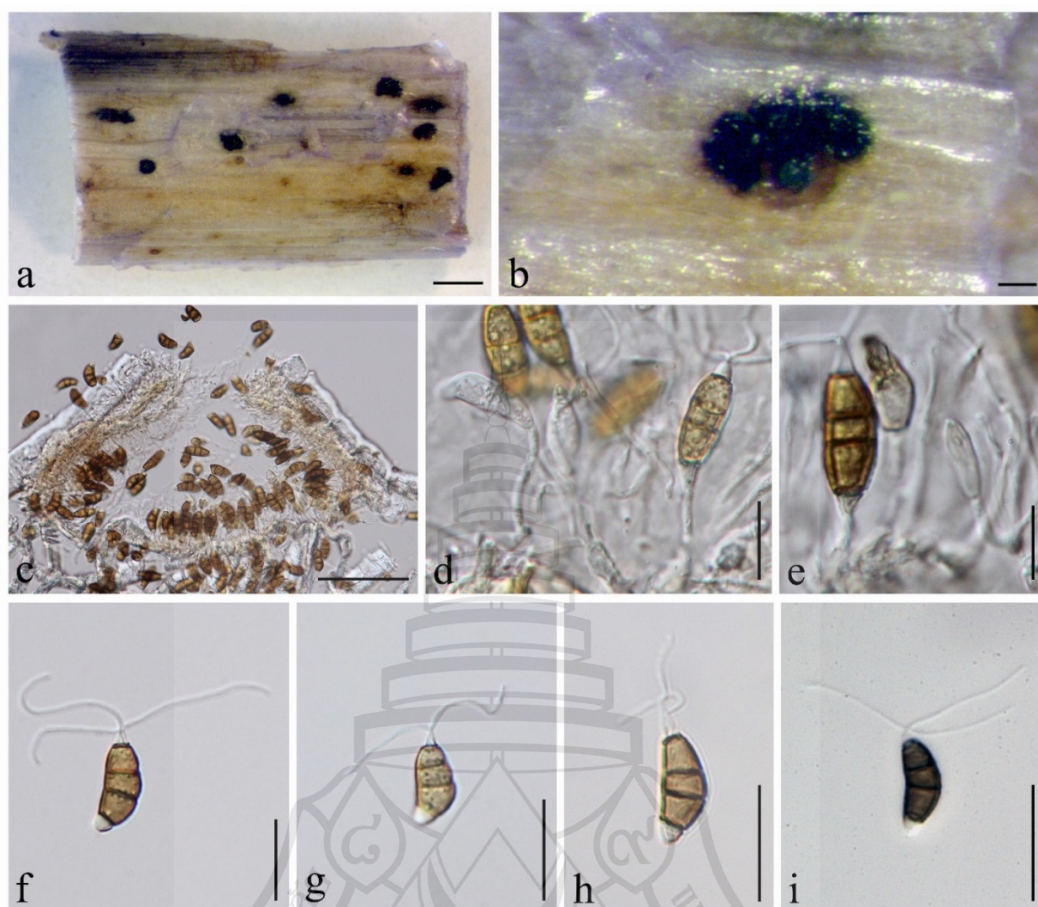
Known distribution (based on molecular data): Italy (this study), Malaysia (Lateef et al., 2015)

Known hosts (based on molecular data): *Lonicera* sp. (this study), *Macaranga* sp. (Lateef et al., 2015)

Material examined: Italy, Province of Forlì-Cesena [FC], near Converselle - Castrocaro Terme e Terra del Sole, on living branch of *Lonicera* sp. (L.) (Caprifoliaceae), 13 December 2014, E. Camporesi IT 2294 (MFLU 15-0844), living culture, MFLUCC 15-0565.

GenBank Number: ITS: MG818971.

Notes: In the phylogenetic analysis, the *Pseudopestalotiopsis* isolate (MFLUCC 15-0565) collected in the present study grouped with the type strain of *Ps. kubahensis* (UMAS KUB-P20), a species introduced from *Macaranga* sp. (Figure 6.6). in Malaysia. Both produce spores with golden brown to dark, concolourous, median cells (Lateef et al., 2015). The conidial dimensions of the species from Malaysia is $27\text{--}30 \times 5.6\text{--}7.3 \mu\text{m}$ and $22.5\text{--}26.5 \times 6.5\text{--}8.5 \mu\text{m}$ for our collection. The lengths of apical appendages for UMAS KUB-P20 and MFLUCC 15-0565 are $16\text{--}29.5 \mu\text{m}$ and $15\text{--}35 \mu\text{m}$ respectively. However, morphologically our collection differs from *Ps. kubahensis* by lacking a basal appendage. The relationship between the two strains is poorly resolved, as for both isolates only ITS data is available for sequence comparison. In a similar result obtained by Maharachchikumbura et al. (2016b) it was emphasized that the variation within the ITS region of *Pseudopestalotiopsis* species is limited, however, a combination of ITS, TUB and TEF gene data gave the best resolution as compared to any single marker for resolving *Pestalotiopsis* and related taxa. In the present study, we prefer to maintain our collection (MFLUCC 15-0565) as *Ps. kubahensis* until we have obtained more sequence data. This is the first *Pseudopestalotiopsis* sp. to be recorded on *Lonicera* sp. and is the first record from a temperate region, as all previous species introduced were from tropical regions.



Note (a) Appearance of conidiomata on host. (b) Close-up of a conidioma. (c) Cross section of a conidioma. (d, e) Conidia arising from conidiogenous cells. (f–i) Conidia. Scale bars: a = 1000 μm , b = 200 μm , c = 100 μm , d, e = 10 μm , f–i = 20 μm .

Figure 6.14 *Pseudopestalotiopsis kubahensis* (MFLU 15-0844, new host and geographical record)

Seimatosporium Corda

Notes: *Seimatosporium* was introduced by Corda (1833), based on *S. rosae* Corda as the type species. The taxonomy of *Seimatosporium* has been studied by Sutton (1963, 1980), Shoemaker and Müller (1964), von Arx (1981) and Nag Raj (1993). The genus is characterized by having conidia with pigmented median cells, with or without an apical appendage, and a single, branched or unbranched basal appendage (Sutton, 1980; Nag Raj, 1993). Nag Raj (1993) arranged *Seimatosporium* species into five groupings based on their conidial features, namely: *Seimatosporium* sensu stricto (type species: *S. botan*

Sat. Hatak. & Y. Harada), *Sporocadus* Corda (type species: *S. lichenicola* (Corda) Shoemaker & E. Müll.), *Sarcostroma* Cooke (type species: *S. foliicola* (Berk.) Shoemaker), *Diploceras* (Sacc.) Died. (type species: *S. hypericinum* (Ces.) B. Sutton) and *Vermisporium* H.J. Swart & M.A. Will. (type species: *S. leptospermi* R.G. Bagn. & Sheridan). However, Tanaka et al. (2011) showed that *Diploceras*, *Sarcostroma*, *Sporocadus* and *Vermisporium*, which lack sexual morphs, clustered in *Seimatosporium* sensu stricto based on LSU and ITS sequence data, suggesting that they should exist within *Seimatosporium*. In their phylogenetic analyses, Senanayake et al. (2015) placed *Seimatosporium* in *Discosiaceae*, along with the genera *Adisciso*, *Discosia*, *Discostroma* and *Sarcostroma*. Norphanphoun et al. (2015) designated an epitype for the type species, *Seimatosporium rosae*. Several species of *Seimatosporium* are known to have *Discostroma* sexual morphs (Nag Raj, 1993; Okane et al., 1996; Hatakeyama & Harada, 2004; Hyde et al., 2011; Wijayawardene et al., 2012), while phylogenetic analyses showed that they cluster in a monophyletic clade (Barber et al., 2011; Tanaka et al., 2011; Norphanphoun et al., 2015). Wijayawardene et al. (2016) proposed the continued use of *Discostroma* and *Seimatosporium*, as the type species of the former genus lacks DNA sequence data. Currently, there are 79 species epithets listed in Index Fungorum (2016) for *Seimatosporium*. In this study, we introduce a new species of *Seimatosporium* from *Quercus robur*, collected in Germany. A morphological comparison of species described from the same host as well as a key for identification is provided in the discussion.

Seimatosporium quercina Goonas., R.K. Schumach. & K.D. Hyde, *sp. nov.*

Index Fungorum Number: IF552011; Facesoffungi number: FoF 01819; Figure 6.15

Etymology: quercina, refers to the host genus

Holotype: MFLU 15-0760

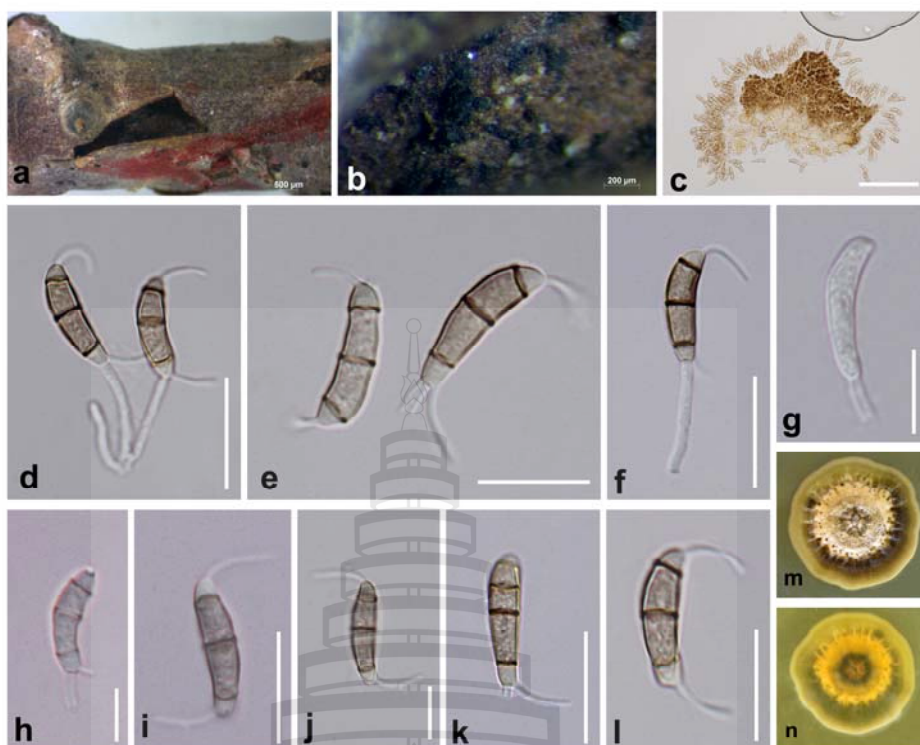
Saprobic on *Quercus robur* L. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* up to 3500 µm wide, acervular, immersed when immature, becoming erumpent at maturity, globose to subglobose, unilocular, dark brown to black, dehiscing by an irregular split of the host epidermis. *Conidiomata* wall not observed. *Conidiophores* arising from the base of the cavity, 7–9.3 µm, hyaline, filiform to cylindrical, branched at the base, smooth. *Conidiogenous cells* holoblastic, annellidic, cylindrical, hyaline, smooth. *Conidia* concolourous, falcate, 3-septate, without constrictions at the septa, 6–8.5(–9) × 1–3 µm ($\bar{x}$ = 7.7 × 1.7 µm, n=30), consists of a flattened hilum at base, smooth-

walled; basal cell obconic with a truncate base, pale brown to hyaline, 1–1.5 μm long ($\bar{x}$ = 2.3 μm), with a long, tubular, simple and unbranched, eccentric appendage, 2–5 μm long ($\bar{x}$ = 2.9 μm); middle cells cylindrical, pale brown to medium brown, second cell from base 2.1–3.3 μm long ($\bar{x}$ = 2.5 μm), third cell from base 1.8–3.1 μm long ($\bar{x}$ = 2.2 μm), together 4.5–6.5 μm long ($\bar{x}$ = 5.2 μm), transverse septa dark brown and fairly thick; apical cell conical and rounded at apex, hyaline to pale brown, 0.5–1.6 μm long ($\bar{x}$ = 1.1 μm), with a long, tubular, unbranched, central appendage, 2–4 μm long ($\bar{x}$ = 3.5 μm).

Culture characteristics: Colonies on MEA show moderate growth, reaching 2 cm diam. after 7 days at 16–18 °C, colonies composed of dense mycelium, circular, smooth, margins undulate, pale grey and umbonate at the very centre, becoming pale to orangish-brown and effuse in the center region, darker brown to pale brown towards the margin on the upper side Brown with a darker brown margin at the center, becoming orangish yellow and pale yellow towards the margin of the colony on the lower side.

Material examined: Germany, on hornbeam wood (*Carpinus* sp.), elev. = 40 m, on *Quercus robur* (Fagaceae) twig, attached on a freshly fallen branch, 23 February 2014, René K. Schumacher. 23.02.2014-028 (MFLU 15-0760! **holotype**, HKAS 91483! **isotype**), ex-type living culture, MFLUCC 14-1198, KUMCC 16-0005.

Notes: Three species of *Seimatosporium* have been reported on *Quercus* spp. (Farr & Rossman, 2016), but are morphologically distinct from *S. quercina*, which has 3-septate conidia measuring 6–8.5(–9) $\times$ 1–3 μm , with a single appendage at each end. *Seimatosporium glandigenum* (Bubák & Gonz. Frag.) B. Sutton reported on *Q. ballota* (from Spain) and *S. lichenicola* (Corda) Shoemaker & E. Müll. reported on *Q. ilex* (from Italy) have 3-septate, nonappendaged, larger conidia measuring 15–18 $\times$ 5–6.5 μm and 13–15 $\times$ 5.5–6.5 μm , respectively (Sutton, 1980; Greuter et al., 1991; Nag Raj, 1993). *Seimatosporium caninum* (Brunaud) B. Sutton reported on *Q. incana* in France has 2-septate conidia measuring 9.5–12 $\times$ 4.5–5.5 μm (Sutton 1980) and lacks appendages. Our species is further compared to morphologically similar taxa reported in Sutton (1980) and Nag Raj (1993) in the discussion, with a taxonomic key provided, to distinguish species.



Note (a) Symptoms on twig of *Quercus robur*. (b) Close up of acervuli. (c) Conidia with conidiophores attached to the base of conidioma. (d) Conidia attached to conidiophores. (e–l) Conidia at different stages of maturity. (m, n) Colony on MEA (m-above, n-below). Scale bars: c = 50 µm; d–l = 10 µm.

Figure 6.15 *Seimatosporium quercina* (MFLU 15-0760, holotype)

***Seiridium* Nees**

Seiridium, introduced by Nees (1817) is a widespread genus occurring on various terrestrial hosts as saprobes or pathogens (Tsopelas et al., 2007). The coelomycetous asexual morph produces 6-celled conidia (Jeewon, 2003; Maharachchikumbura et al., 2014), while the sexual morphs have been identified as *Blogiascospora* and *Lepteutypa* (Nag Raj & Kendrick, 1985; Senanayake et al., 2015). *Blogiascospora marginata* was confirmed to be the sexual morph of the type species *Seiridium marginatum* based on molecular data (Jaklitsch et al., 2016).

***Seiridium neocupressi* G. Bonthond**

Index Fungorum number: IF823299; Facesoffungi number: FoF 09147; Figure 6.16

Saprobic on twigs, branches of dicotyledons. **Asexual morph:** *Conidiomata* pycnidial to sporodochial, mostly solitary, immersed, unilocular, conic or subglobose with flattened base, dark brown to black. *Conidiomata wall* comprising thin-walled cells of *textura angularis*, sometimes fusing at the base into the host tissue. *Conidiophores* septate, cylindrical, branched, hyaline or pale brown, thin-walled. *Conidiogenous cells* discrete, hyaline, cylindrical, smooth- and thin-walled, proliferating percurrently, with visible collarettes and minute periclinal thickenings. *Conidia* $22\text{--}28 \times 7\text{--}9 \mu\text{m}$ ($\bar{x} = 26 \times 8 \mu\text{m}$), lunate to falcate, curved, 5-septate, rarely 4- or 6-septate, not striate, occasionally a short apical appendage present, euseptate with no visible pores, basal cell obconic with a truncate base, hyaline, walls smooth, $3.5\text{--}4 \mu\text{m}$; four median cells, smooth, cylindrical to doliiform, brown to dark brown, and septa darker than the rest of the cells, second cell from base $5\text{--}6 \mu\text{m}$ long, third cell $5\text{--}6 \mu\text{m}$ long, fourth cell $5\text{--}6 \mu\text{m}$ long, fifth cell $4.5\text{--}5.5 \mu\text{m}$ long, apical cell conical, hyaline, smooth, $3.5\text{--}4 \mu\text{m}$ long. **Sexual morph:** Unknown.

Culture characteristics: Colonies on PDA reaching approximately 5 cm in diam. after 7 days at $16\text{--}18^\circ\text{C}$, circular, raised, with a filamentous margin, greyish-white at the center, then dark grey, white at the edge, reverse, greyish-white.

Material examined: ITALY, Forli-Cesena Province, near Camposonardo - Santa Sofia, on dead and land cone of *Picea excelsa*, 15 April 2015, Erio Camporesi IT2444 (MFLU 15-0813), living culture, KUMCC 16-0115.

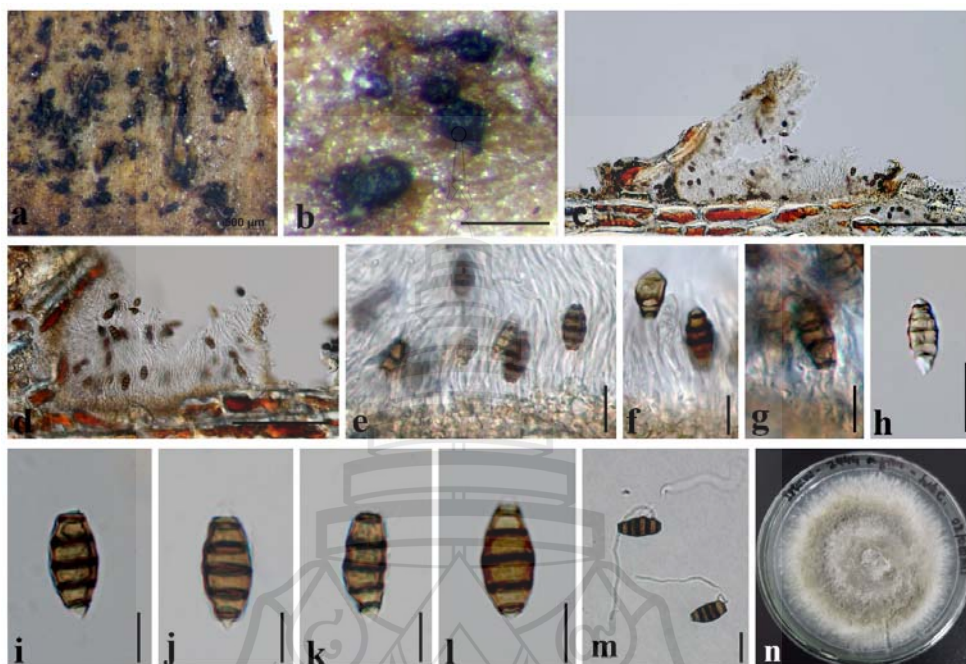
GenBank numbers: ITS: MN848165; LSU: MN848234; SSU: MN848240.

Known distribution (based on molecular data): Australia, Italy, New Zealand (Bonthond et al., 2018; this study)

Known hosts (based on molecular data): *Cupressus leylandii*, *C. macrocarpa*, *C. sempervirens* (Bonthond et al., 2018), *Picea excelsa* (this study)

Notes: In the phylogenetic analysis, our isolate KUMCC 16-0115 clusters with the type strain of *Seiridium neocupressi* CBS 142625. *Seiridium neocupressi* is phylogenetically and morphologically distinct from *S. cupressi* (Bonthond et al., 2018). The species described (from culture) by Bonthond et al. (2018) has larger conidia ($26.5\text{--}31 \times 8.5\text{--}10 \mu\text{m}$) compared to our specimen illustrated here ($22\text{--}28 \times 7\text{--}9 \mu\text{m}$). The morphological features of the conidiophores, conidiogenous cells, conidial septation and shape are similar in both collections. However, we did not observe any basal appendages attached to the conidia in our collection. *Seiridium neocupressi* is mostly recorded from

Cupressus sp. while our collection from Italy is on *Picea excelsa*. This is the first record of a *Seiridium* species from this host.



Note (a) Conidiomata on host. (b) Close up of conidiomata. (c, d) Section of conidioma. (e–g) Conidiophores with conidia. (h–l) Conidia. (m) Germinating conidia. (n) Culture on PDA. Scale bars: c, d = 1 mm, e, m = 50 μ m, f–l = 20 μ m.

Figure 6.16 *Seiridium neocupressi* (MFLU 15-0813, new host record)

Truncatella Steyaert

The genus *Truncatella*, typified by *T. truncata* was established by Steyaert (1949) to accommodate species distinct in having 3-septate, verruculose, pigmented conidia (Steyaert, 1949; Maharachchikumbura et al., 2012, 2015). It was previously placed in *Amphisphaeriaceae*, *Xylariales* (Lumbsch & Huhndorf, 2010), and later included in *Bartaliniaceae* by Senanayake et al. (2015) based on both morphological features and molecular evidence. Several studies have contributed to the phylogenetic understanding of *Truncatella*, such as its affinity to *Bartalinia* (Jeewon et al., 2002; Lee et al., 2006; Maharachchikumbura et al., 2012, 2014, 2016, 2017; Senanayake et al., 2015). The

sexual morph of *Truncatella* remains largely unresolved, however Senanayake et al. (2015) illustrated both the sexual and asexual morph for a new species *T. spartii*.

Truncatella spartii Senan., Camporesi & K.D. Hyde

Index Fungorum number: IF551196; Facesoffungi number: FoF 00685, Figure 6.17

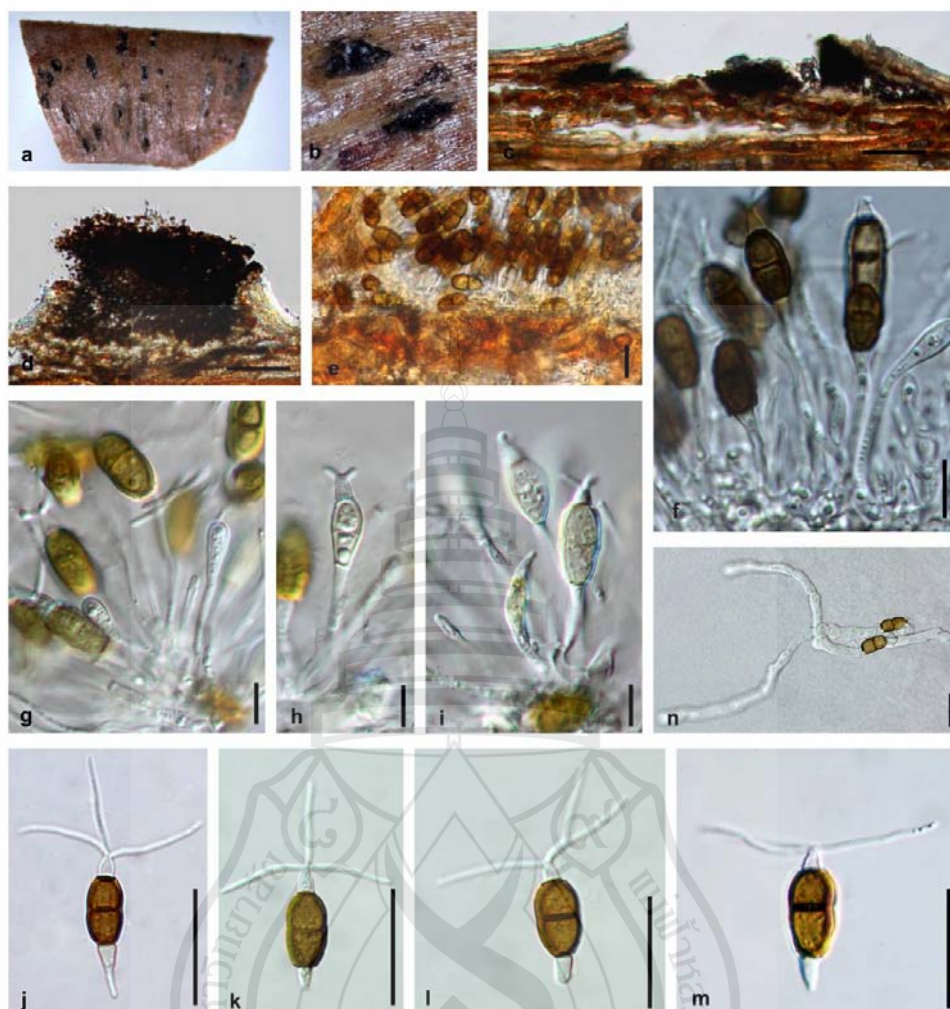
Saprobic on cone of *Piceae* sp., appearing as black, irregular lesions on the host surface. **Sexual morph:** See Senanayake et al. 2015. **Asexual morph:** *Conidiomata* (240–)320–370(–433) μm diam, 160–200 μm , ($\bar{x}$ = 340 $\times$ 180 μm , n = 8), pycnidial or acervular, semi-immersed to superficial becoming erumpent at maturity, globose with a flattened base, solitary to aggregated, black. *Conidiomata wall* consisting of outer light brown to hyaline cells of *textura angularis* and inner hyaline cells of *textura angularis* to *textura globulosa*. *Conidiophores* 2.5–4 μm long, hyaline, cylindrical, simple or branched. *Conidiogenous cells* 8.5–14.2 μm long hyaline, simple, filiform, annellidic, indeterminate, integrated. *Conidia* 17.5–24.8 $\times$ 5.3–7.2 μm ($\bar{x}$ = 21.5 $\times$ 6.5 μm , n = 30), broadly fusiform, 3-septate, slightly constricted at the mid septum, verruculose, median cells brown to yellowish brown, 10.5–15 μm long, thick-walled, globules present, basal cell hyaline, conical tapering towards the base with an acute apex, apical cell hyaline, bearing 3 appendages. *Appendages* 10–13.5 μm long, hyaline, simple, filiform, similar in length or shorter than conidia.

Culture characteristics: On PDA slow growing, attain 15 mm in 7 days at 18 °C, circular colony, serrate margin, from above white, reverse greyish white.

Material examined: Italy, Province of Forli-Cesena [FC], near Camposonardo - Santa Sofia, on dead cone attached to *Picea excelsa* (Pinaceae), 6 May 2013, Camporesi Erio, IT1226 (HKAS96302), living culture, KUMCC 15-0127.

GenBank numbers: ITS: KY474383.

Notes: This is the first record of *Truncatella sparti* on *Picea excelsa*. This species was introduced by Senanayake et al. (2015) from the host *Spartium* with both sexual and asexual morphs illustrated. *Truncatella spartii* is closely related to *T. hartigii* (Tubef) Steyaert, *T. helichrysi* (Severini) B. Sutton and *T. restionacearum* S. Lee & Crous but differs in its apical cell characteristics of the conidia (Lee et al., 2006; Senanayake et al., 2015).



Note (a) Appearance of conidiomata on cone of *Piceae* sp. (b) A close-up of conidiomata. (c, d) Cross section of conidioma. (e) Conidioma wall. (f-i) Developing conidia from conidiogenous cells. (j-m) Conidia. (n) Germinating spores. Scale bars: c, d = 50 µm, e-i, n = 10 µm, j-m = 20µm.

Figure 6.17 *Truncatella spartii* (HKAS96302, new host record)

6.4 Discussion

The use of conidial morphology has often been considered an important criterion in species identification (Sutton, 1980; Nag Raj, 1993) and its value in taxonomy has been discussed in Norphanphoun et al. (2015). In our phylogenetic analysis (Figure 6.7)

Seimatosporium sensu lato groups into five sub-clades (Clades A–E). Clade A consists of *Discostroma fuscillum* and the species *S. cornii*, *S. glandigenum* and *S. lichenicola* which have non-appendaged spores with coloured median cells. Clade B consists of *Discostroma stonaea* and 11 putatively named *Seimatosporium* species. *Seimatosporium quercina* groups within this second clade, along with *S. biseptatum*, *S. elegans*, *S. eucalypti*, *S. falcatum*, *S. grevilleae*, *S. hakeae*, *S. kriegeianum*, *S. leptospermi*, *S. mariae*, *S. obtusum* and *S. walkeri*. *Seimatosporium quercina* groups close to *S. walkeri*, also a 3-septate conidial species, except that the spores of *S. walkeri* lack an apical appendage. *Seimatosporium mariae* can be distinguished by having 5-septate conidia (Nag Raj, 1993). *Seimatosporium elegans*, *S. hakeae*, *S. leptospermi* and *S. grevilleae* are species with 4-septate conidia, while the remaining members in the clade have 3-septate conidia. While these species in the lower part of the clade have coloured median cells ranging from pale, to mid brown or brown (with the exception of *S. leptospermi*) (Nag Raj, 1993), those grouped in the upper part of the clade (*S. biseptatum*, *S. elegans*, *S. eucalypti*, *S. falcatum*, *S. kriegeianum* and *S. obtusum*) share the characteristic of having hyaline to yellowish or pale brown coloured conidia. Clade C consists of *Seimatosporium vacinii*, a species with 3-septate conidia that lack appendages. Clade D consists of four *Discostroma* strains and nine *Seimatosporium* strains, including the ex-epitype strain *S. rosae*. Clade E is represented by one *Discostroma* sp., *Sarcostroma lomatiae*, *Seimatosporium ficeae*, *S. foliicola* and *S. rhombisporum*. Clade F consists of the *Adiscosia* and *Discosia* species. It must however, be stressed that the taxon names used in the tree (Figure 6.7) are generally not linked to herbarium material, thus the names cannot be confirmed and therefore any conclusions are tentative. The taxa need recollecting and designating as epitypes or reference specimens (sensu Ariyawansa et al., 2014), before any certain conclusions can be made. These clades may require distinct genera, but more reliable data is needed before these can be introduced.

A key to identifying morphologically similar *Seimatosporium* species that have 3-septate conidia with appendages at both ends is given below.

1. Conidia 3-septate; with one apical or basal appendage.....2
 - 1a. Conidia 3-septate; with more than one apical or basal appendage.....
 -*S. hypericinum*
 2. Apical appendage always present.....3

2a. Apical appendage mostly absent.....	4
3. Conidia $6-9 \times 1-3 \mu\text{m}$, appendages $3-3.5 \mu\text{m}$ long.....	<i>S. quercina</i>
3a. Conidia $15.5-18 \times 5.5-6.5$, appendages $1-3 \mu\text{m}$ long.....	<i>S. monochaetioides</i>
4. Conidia more than $15 \mu\text{m}$ long.....	5
4a. Conidia less than $15 \mu\text{m}$ long.....	6
5. Appendages up to $30 \mu\text{m}$ long.....	<i>S. piceae</i>
5a. Appendages up to $10 \mu\text{m}$ long.....	<i>S. rosae</i>
6. Conidia less than $4.5 \mu\text{m}$ wide.....	<i>S. lonicerae</i>
6a. Conidia more than $4.5 \mu\text{m}$ wide.....	7
7. Median cells together $7-8 \mu\text{m}$ long.....	8
7a. Median cells together $6-9 \mu\text{m}$ long; appendages up to $30-35 \mu\text{m}$ long.....	<i>S. caudatum</i>
8. Appendages $14 \mu\text{m}$ long.....	<i>S. salicinum</i>
8a. Appendages $5 \mu\text{m}$ long.....	<i>S. grammitum</i>
8b. Appendages $7 \mu\text{m}$ long.....	<i>S. hysteroioides</i>

Further studies of the generic type and other species in *Seimatosporium*, using both morphological and molecular data, and establishing connections between asexual and sexual morphs is needed (Maharachchikumbura et al., 2015; Norphanphoun et al., 2015).

CHAPTER 7

CONCLUSIONS

7.1 Overall Conclusions

The checklist is the first checklist of microfungi on grasses (excluding bambusicolous fungi) that have been recorded in Thailand. In total, 172 species and 35 unidentified taxa have been recorded. They belong to the main taxonomic groups *Ascomycota*: 98 species and 28 unidentified, in 15 orders, 37 families and 68 genera; *Basidiomycota*: 73 species and 7 unidentified, in 8 orders, 8 families and 18 genera; and *Chytridiomycota*: one identified species in *Physodermatales*, *Physodermataceae*. Based on the checklist, *Puccinia*, *Sporisorium*, *Tilletia* and *Ustilago* (which are commonly referred to as the rusts and smuts) were the most reported genera, followed *Bipolaris*/*Cochliobolus*, *Curvularia*, *Cercospora*, *Colletotrichum* and *Fusarium*.

Based on morphology and phylogenetic data, three new species and nine host records from four grasses *Axonopus compressus*, *Imperata cylindrica*, *Thysanolaena latifolia* and *Zea mays* in northern Thailand were discovered. *Clonostachys Chiangmaiensis* on *Zea mays*, *Paraphaeosphaeria thysanolaenae* on *T. latifolia*, and *Pestalotiopsis compressa* on *A. compressus* are the novel taxa. *Cladosporium tenuissimum* was recorded for the first time on *A. compressus*, *I. cylindrica* and *Z. mays*. *Neopestalotiopsis asiatica* was recorded for the first time on both *T. latifolia* and *Zea mays*. *Diaporthe yunnanensis* and *Lasiodiplodia pseudotheobromae* are new host records on *T. latifolia*. *Striaticonidium synnematum* and *Phaeosphaeria musae* are new records for *A. compressus*. Several species were identified based on morphology, and ITS sequences, however further molecular analysis is required to confirm their identification. These include *Epicoccum poaceicola*, *Fusarium incarnatum* on *A. compressus*, *Alfaria terrestris*, *Ectophoma pomi* and *Fusarium lacertarum* on *I. cylindrica*, *Apiospora pseudospegazzini*, *Arthrimum euphorbiae*, *Botryosphaeria fabicerciana*, *Lasiodiplodia*

theobromae, *L. iraniensis* and *L. chiangraiensis* on *T. latifolia* and *Xylaria feejeensis* on *Zea mays*.

In the study of microfungi on grass litter two saprobic members *Dinemasporium pseudostrigosum* and *Robillarda africana* belonging to *Chaetosphaeriaceae* and *Sporocadaceae* in *Sodariomycetes* were collected from dead grasses in Chiang Mai province, Thailand. Both species were recorded for the first time in Thailand. This was also the first report of *R. africana* from Poaceae. A paraphaeosphaeria-like sexual morph of *Coniothyrium* was collected from Chiang Mai province and thus introduced as *Coniothyrium chiangmaiense*. A new species of *Neosetophoma*, *Neosetophoma poaceicola* was introduced and this is the first record of the sexual morph in this genus. The paraphaeosphaeria-like asexual morph from grass litter grouped with two strains of *Ophiosphaerella agrostidis* (MFLUCC 11–0152 & MFLUCC 12–0007). Therefore, this species was assigned as the asexual morph of *O. agrostidis* based on phylogenetic results. This is the first report of an asexual morph for *Ophiosphaerella agrostidis*. *Setophoma poaceicola* is a new species and also the first record of *Setophoma* from Thailand. *Epicoccum thailandicum* is another species introduced from dead grass.

Among the genera in *Phaeosphaeriaceae*, *Parastagonospora* is well noted for being pathogenic on plants. In this study, two saprobes on Poaceae were introduced from grass hosts in Italy and Russia. The sexual morph of a new *Parastagonospora* species, *P. elymi* was collected from dead stems of *Elymus repens* in Russia while an asexual morph was isolated from dead stems of *Dactylis glomerata* in Italy and introduced as *P. macrouniseptata*. *Pyrenophora trichostoma* is a well-known pathogen and the history, morphology and pathogenicity of the species was discussed in detail, with a description of a new host record on *Bromopsis inermis* in Russia (Rostov region).

7.2 Drawbacks and Limitations of This Study

7.2.1 Although we were able to record 176 taxa from over 300 samples of four grass hosts, the percentage of germination and obtaining pure cultures was less than 35%. In some samples the amount of spores was very less or not fertile.

7.2.2 With regard to some sample materials, they were not in good condition, as they lacked fungal structures for morphology observation or even to deposit as herbarium material.

7.2.3 Some samples had very less fruiting body structures, therefore it was not possible to utilize them for DNA extraction from contents.

7.2.4 During the dry season, the grass samples were more fragile and difficult to handle. However, in such conditions, incubation in a moist chamber was very helpful for coelomycetous genera. It was observed that the samples collected during this season hosted almost the same types of hyphomycetous species, hence, *Cladosporium* was recorded in the majority of the collections.

7.2.5 Contaminations by bacteria or mites were quite common in isolates from grasses, especially during isolation of a specific fungus.

7.2.6 On several occasions the sequences obtained were not of good quality (mostly the protein coding regions), therefore some isolates lacked molecular data for their classification and identification.

7.3 Future Work

In order to expand the knowledge of grass inhabiting microfungi in Thailand as well as around the world, the following are suggestions are made as future work:

7.3.2 The saprobic diversity of microfungi on grasses still lacks collections, molecular data in some taxonomic groups. Therefore, further collections are necessary.

7.3.3 The selected hosts can be viewed as model hosts for mycologists to further investigate fungi-host relationships, species richness and biogeographical diversity.

7.3.4 Studies can be developed to investigate the species richness among tall grasses as well as stout grasses.

7.3.5 The study and records in the diversity of saprobic fungi can be implemented to reveal what fungal groups are important or play a significant role in the degradation of grass litter.

7.4 Publications

- Chethana, K. W., Niranjan, M., Dong, W., Samarakoon, M. C., Bao, D. F., Calabon, M. S., . . . Devadatha, B. (2021). AJOM new records and collections of fungi: 101–150. *Asian Journal of Mycology*, 4, 113-260.
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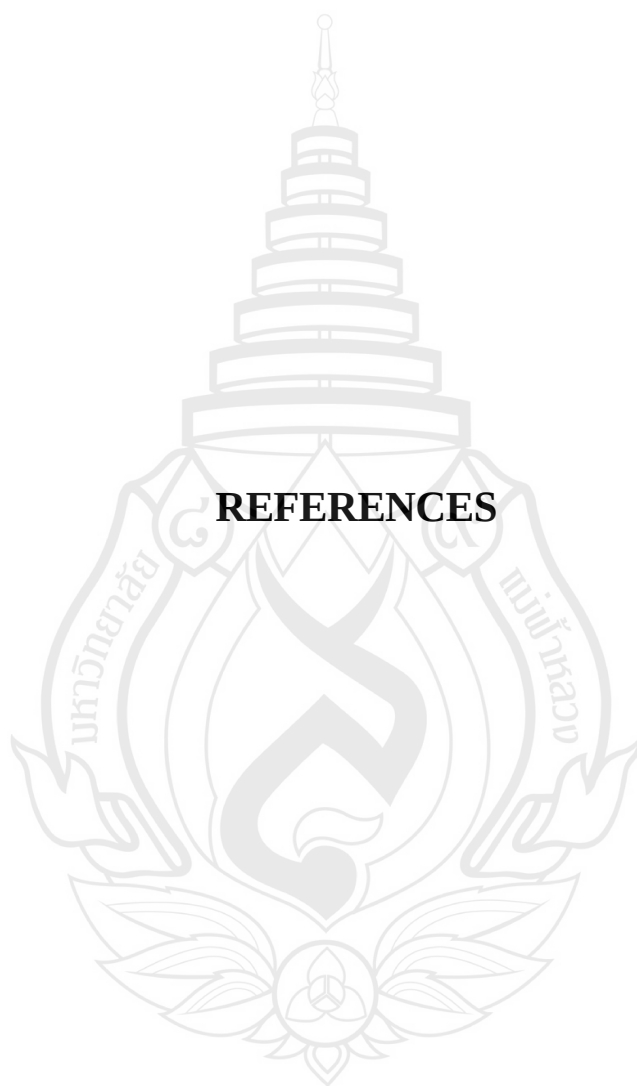
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APPENDIX A

CHEMICAL REAGENTS AND MEDIA

1. Potassium hydroxide (KOH) used in the rehydration of dried specimens. 5% aqueous solution

2. 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

3. Lactic acid which preserves fungal structures and for getting a true color image of the fungal spore and structures without staining. This is helpful for some of the darker colored organisms.

85% Lactic acid 100 ml

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 is a good general mounting medium that clears the material somewhat and allows particularly brilliant resolution with a microscope, and 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. 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 powder and bring volume to 1000 ml. Autoclave at 121°C for 15 minutes

7. Potato dextrose agar (PDA) used for fungal cultivation

Potato starch (from infusion) 4 g

Dextrose 20 g

Agar 15 g

Suspend 39 g of Potato dextrose agar in distilled water and mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder and bring volume to 1000 ml. Autoclave at 121°C for 15 minutes.

APPENDIX B

NOTES FOR OUTLINE OF SORDARIOMYCETES

Diaporthe Nitschke

Santos et al. (2011) distinguished four species of the *Diaporthe* taxa occurring on soybean throughout Croatia. This was based on morphological and molecular data determined through analyses of partial TEF 1-alpha (EF1- α) gene and ITS nrDNA sequence data. *Diaporthe phaseolorum* was described and its synonyms discussed. *Diaporthe phaseolorum* var. *caulivora* was raised to species status and the name *Diaporthe caulivora* was introduced to accommodate it. A species previously known as *Phomopsis* sp. 9 from earlier studies was reported for the first time on soybean, and formally described as *Diaporthe novem*. The well-known soybean pathogen *Phomopsis longicolla* was also studied and transferred to *Diaporthe longicolla* comb. nov.

Crous et al. (2011) characterized several pathogens occurring in the family Proteaceae, using morphology, culture characteristics, DNA sequence data of rRNA-ITS and LSU genes and in some cases additional genes such as TEF 1- α and CHS. Based on the results, *Diaporthe leucospermi* was described on *Leucospermum* sp. (Australia).

The identification of *Diaporthe* species associated with stem canker of sunflower (*Helianthus annuus*) in Australia was studied by Thompson et al. (2011) using morphology, DNA sequence analysis and pathology. The phylogenetic analysis revealed three clades that did not correspond with known taxa, and they were believed to represent novel species. *Diaporthe gulyae* sp. nov. was described for isolates that caused a severe stem canker. They also showed that there has been a broad misapplication of the name *D. helianthi*, to many isolates of *Diaporthe* found causing, or associated with, stem cankers on sunflower.

A study conducted by Gomes et al. (2013), using multi-locus DNA sequence data to investigate 243 isolates of the *Diaporthe* resolved 95 clades, while 15 new species and 14 new combinations were introduced. The study confirmed that several taxa had wide host ranges, while some were strictly host-specific.

Udayanga et al. (2014) conducted a study to revise the species of this genus causing melanose and stem end rot diseases of *Citrus* spp.. Three species of *Diaporthe* - *D. citri*, *D. cytospora* and *D. foeniculina* occurring on *Citrus* were characterized, using morphology and phylogenetic analyses of the complete nuclear ribosomal ITS regions and partial sequences of actin, beta-tubulin, calmodulin and TEF1- α in order to resolve species on *Citrus* and related other *Diaporthe* species. *Diaporthe cytospora*, *D. foeniculina* and *D. rudis* were epitypified, fully described and illustrated with a review of all synonyms based on molecular data and morphological studies. Newly designed primers were introduced to optimise the amplification and sequencing of calmodulin and actin genes in *Diaporthe*. Also discussed was the need for multigene phylogenies when distinguishing species of *Diaporthe* or describing new species.

***Lopadostoma* (Nitschke) Traverso**

Accepted genus of Xylariaceae that was first introduced by Nitschke (1867) as *Anthostoma* subg. *Lopadostoma* Nitschke and later elevated to generic rank by Traverso (1906). 12 species were recognized during a revision of this genus (Jaklitsch et al., 2014). Combined molecular phylogenetic analysis based on ITS, LSU, RPB2 revealed 10 distinct taxa within the genus which were also recognized at species level. Out of these 10, six species, *L. americanum*, *L. fagi*, *L. insulare*, *L. lechatii*, *L. meridionale* and *L. quercicola* were newly described. Two taxa were recognized based only on morphological analysis. A phylogenetic analysis based on nuLSU rDNA confirmed that the genus appeared as a monophyletic clade within the family Xylariaceae, with moderate bootstrap support (Jaklitsch et al., 2014). Also, the species *Phaeosperma ailanthei*, *Phaeosperma dryophilum* and *Sphaeria linosperma* were synonymized into *Lopadostoma* (Jaklitsch et al., 2014).

APPENDIX C

NOTES AND HERBARIUM STUDY FOR FAMILIES OF SORDARIOMYCETES

Amphisphaeriaceae G. Winter [as 'Amphisphaerieae'], Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1.2: 259 (1885)

Saprobic, hemibiotrophic or necrotrophic on leaves, twigs and branches of deciduous shrubs and trees, conifers or monocotyledons, appearing as slightly raised, black dots on host surface, often surrounded by a darkened area. **Sexual morph:** *Pseudostromata* when present made up of host cells and brown to black fungal hyphae, solitary, uni- to bi-loculate, hemisphaerical, initially, appearing as raised, pale brown areas, with small, black dots at the center, becoming dark at maturity, glabrous. *Ascomata* perithecial, scattered to clustered, immersed in host cortex, becoming raised, subglobose to lenticular, glabrous, dark brown to black, vegetative hyphae surrounding the locules, ostioles individual, central. *Papilla* erumpent through host surface, internally covered by hyaline, filamentous periphyses. *Peridium* of unequal thickness, thinner at the base, thicker towards the mid-upper section of the ascomata, comprising several layers of dark brown pseudoparenchymatous cells, outer layer of thick-walled, brown cells of *textura prismatica*, inner layer of flattened, hyaline cells of *textura prismatica*. *Hamathecium* comprising numerous, septate, filamentous paraphyses. *Asci* 8-spored, unitunicate, cylindrical, short pedicellate, apex rounded, with J+ or J- apical ring. *Ascospores* overlapping uniseriate, light to dark brown, ellipsoidal to fusiform, 1-septate. **Asexual morph:** Coelomycetous. *Conidiomata* solitary or aggregated, globose, dark brown. *Peridium* comprising thick-walled, septate, brown mycelium. *Conidiophores* dichotomously branched, septate, thick-walled, smooth, hyaline. *Conidiogenous cells* phialidic, elongated, wide at the base and narrow at the tip, thin-walled, hyaline. *Conidia* hyaline, 1-celled, smooth-walled, elongate to fusiform, narrow at both ends.

*Type: **Amphisphaeria** Ces. & De Not.*

Notes: The family *Amphisphaeriaceae* was introduced by Winter (1885) as ‘*Amphisphaerieae*’ and later established as *Amphisphaeriaceae* to accommodate the type genus *Amphisphaeria* (typified by *A. umbrina*. Ces. & De Not) and similar genera, viz. *Ohleria*, *Trematosphaeria*, *Caryospora*, *Winteria* and *Strickeria* (Winter 1887) The family is characterized by partially immersed or erumpent, perithecial ascomata, unitunicate, cylindrical asci, with J+ or J- apical rings and pale to dark brown, 1-septate ascospores (Kang et al., 1998; Senanayake et al., 2015). The species of the family were subsequently included in various other families (Clements & Shear, 1931; Arx & Müller, 1954; Müller & Arx, 1973), leading to a doubt of its exact taxonomic position (Munk, 1957; Kang et al., 1999b). Hence, *Amphisphaeriaceae* was reintroduced by Müller and von Arx (1962) based on the presence of a small iodine positive ring or disc in the apex of the asci and in having ascomata immersed under a clypeus (Kang et al., 1999b; Maharachchikumbura et al., 2015). Samuels et al. (1987) suggested that the genera linked to *Pestalotia*-like asexual morphs, *Amphisphaeria*, *Broomella*, *Discostroma*, *Lepteutypa* and *Pestalosphaeria* should be kept as *Amphisphaeriaceae* (*sensu stricto*). Hawksworth et al. (1995) listed *Amphisphaeriaceae* as a heterogeneous sexual morph family with 36 genera. Of this, Kang et al. (1999b) accepted only *Amphisphaeria*, *Broomella*, *Discostroma*, *Ellurema*, *Griphosphaerioma*, *Neobroomella*, *Blogiascospora*, *Lepteutypa*, *Paracainiella* and *Pestalosphaeria* based on the study of molecular and morphological data. As a result of various studies, previously included genera in *Amphisphaeriaceae* have been shifted to other taxonomic groups such as *Cainiaceae*, *Clypeosphaeriaceae*, *Phyllachoraceae*, *Pleurotremataceae* and *Xylariaceae* (Krug, 1978; Kang et al., 1998; Kang et al., 1999a). Senanayake et al. (2015) accepted only the type genus *Amphisphaeria* excluding all the other genera based on their morphology and phylogeny. Some were transferred to *Bartaliniaceae*, *Discosiaceae*, *Iodosphaeriaceae*, *Pestalotiopsidaceae* and *Phlogicylindriaceae*, while most genera were listed as *Amphisphaeriales* genera *incertae sedis*. *Amphisphaeriaceae* is currently classified in the order *Amphisphaeriales* (Senanayake et al., 2015) and is known to share a close relationship with *Cainiaceae*, *Clypeosphaeriaceae*, and *Hyponectriaceae* (Jeewon, 2001), as well as with *Apiosporaceae* (Crous &

Groenewald, 2013; Senanayake et al., 2015). As the family is presently monotypic, a family description only is provided.

Amphisphaeria Ces. & De Not., Comm. Soc. crittog. Ital. 1(4): 223 (1863)

Possible synonyms (see Index Fungorum 2015)

Notes: *Amphisphaeria* was introduced by Cesati and De Notaris (1863), with no generic type designated (Wang et al., 2004). Later Petrak (1923) proposed *A. umbrina* (previously introduced as *Sphaeria umbrina*) as the lectotype of this genus. The characterization of *Amphisphaeria* and subsequent epitypification of the generic type *A. umbrina*, and its current phylogenetic status has been reviewed in Senanayake et al. (2015). Most of the confusion regarding the genus arises from the lack of sexual-asexual morph connections (Kang et al., 1998). The asexual morph *Bleptosporium* has been linked to *Amphisphaeria* (Nag Raj, 1977; Hyde et al., 2011), but has not been phylogenetically proven. However, Senanayake et al. (2015) illustrated the sexual and asexual morphs of *Amphisphaeria sorbi*, the former being introduced in Lui et al. (2015).

Amphisphaeria species are thought to be widely distributed among both tropical and temperate regions having mostly been recorded in Europe, and others across the Americas, South East Asia and South India (Liu et al., 2015; Farr & Rossman, 2015). However, since the genus is quite confused, its distribution needs confirmation by species identification with molecular data.

Type species: ***Amphisphaeria umbrina*** (Fr.) De Not., Sfer. Ital.: 69 (1863)

Possible synonyms (see Index Fungorum, 2015)



Figure C1 *Amphisphaeria sorbi* (Material examined: ITALY, Province of Trento [TN], Dimaro, Folgarida, on branch of *Sorbus aucuparia* (Rosaceae), 2 August 2013, Erio Camporesi IT 1400 (MFLU 14-0797, holotype); Appearance of pseudostroma on host. b Close up of pseudostroma with ostioles. c Biloculate pseudostromata. d Section of ascoma. e Peridium. f Paraphyses. g-i Immature asci. j-l Mature asci. m-o Ascospores. p Sheath surrounding ascospore. Scale bars: a-c=not to scale, d-e=10 μ m, f=20 μ m, g-p=50 μ m.

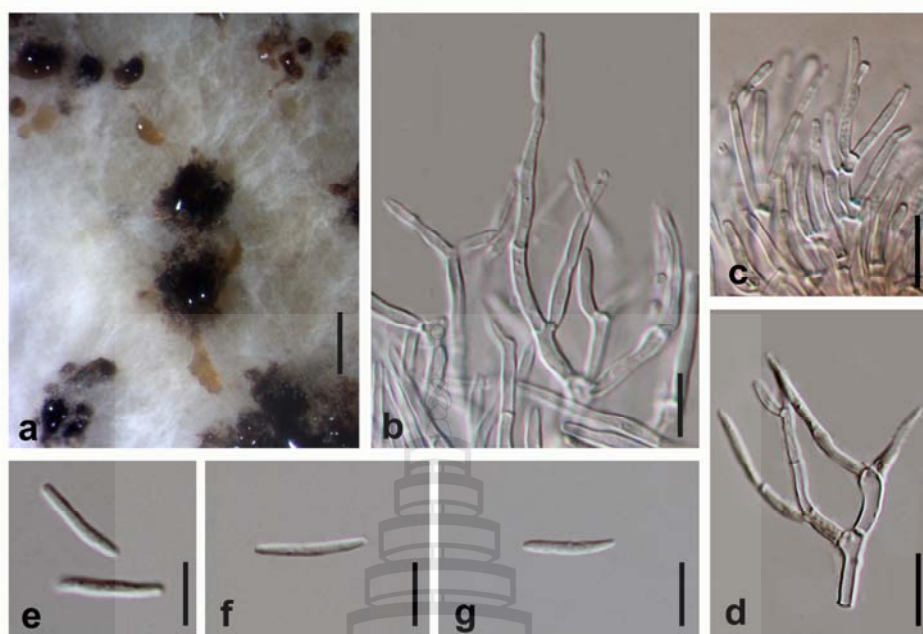


Figure C2 Asexual morph of *Amphisphaeria sorbi* (introduced in Senanayake et al. 2015); a Conidiomata on MEA. b–d Conidia attached to conidiophores. e–g Conidia. Scale bars: a=1000 μ m, b–g=20 μ m

Bartaliniaceae Wijayawardene, Maharachch. & K.D.Hyde, in Senanayake et al., Fungal Divers 73: 13 (2015)

Facesoffunginumber: FoF 00667

Saprobic, endophytic or pathogenic on leaves, twigs and branches of a variety of hosts. **Sexual morph:** Pseudostromata immersed, solitary, scattered. *Ascomata* black, immersed to semi-immersed, solitary or gregarious, globose to subglobose. *Ostiole* central, circular. *Papilla* periphysate. *Peridium* composed of an outer layer, of brown to dark brown textura angularis or textura prismatica cells with thin inner layer of hyaline textura angularis cells. Hamathecium comprised of numerous, hyaline, septate, filamentous paraphyses. *Asci* 8-spored, unitunicate, cylindrical, short pedicellate, apex rounded, with J+ or J- subapical ring. *Ascospores* overlapping uniseriate, pale brown to brown, ellipsoid to fusiform, 3–4-septate. **Asexual morph:** *Coelomyceteous*. *Conidiomata* acervular to pycnidial to irregular, solitary or gregarious, superficial to sub-immersed, unilocular, globose to subglobose, dark brown to black. *Ostiole* apapillate. *Conidiomata wall* comprised of thick outer wall of

dark brown cells of *textura angularis*, thin inner wall, composed of hyaline to sub-hyaline cells of *textura angularis*. *Conidiophores* present or reduced to conidiogenous cells, when present cylindrical, hyaline, sparsely septate, smooth-walled. *Conidiogenous cells* holoblastic, ampulliform, integrated or discrete, determinate, hyaline, smooth-walled. *Conidia* fusiform, straight to slightly curved, subhyaline to brown bearing apical appendage or both apical and basal appendages.

Type: ***Bartalinia*** Tassi

Notes: The family *Bartaliniaceae* (order *Amphisphaeriales*) was introduced by Wijewardena et al. in Senanayake et al. (2015) to accommodate the genera *Bartalinia*, *Broomella*, *Dyrithiopsis*, *Hyalotiella*, *Truncatella* and *Zetiasplonza* based on both morphological characters and phylogenetic analysis. It is phylogenetically closely related to the families *Discosiaceae* and *Pestalotiopsidaceae*.

Bartalinia Tassi, Bulletin Labor. Orto Bot. de R. Univ. Siena 3: 4 (1900)

Facesoffungi number: FoF 00659

Possible synonyms: *Hyalotia* Guba, Monograph of *Monochaetia* and *Pestalotia*: 292 (1961), *Pestalozzina* P. Karst. & Roum., in Karste, Fautrey & Roumeguère, Revue mycol., Toulouse 12(no. 47): 126 (1890)

Endophytic or saprobic on plants. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* pycnidial or variable, solitary to gregarious, subepidermal, erumpent at maturity, globose, unilocular, brown to black. *Conidiomata* wall two layered, outer layer comprising dark brown to black cells of *textura angularis*, inner wall comprising thin, hyaline cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* ampulliform, holoblastic, discrete, determinate, hyaline, generated from inner layer of the pycnidium wall. *Conidia* hyaline to pale brown, subcylindrical, trans-septate, with appendages, basal cell with truncate base, obconic, hyaline, with single unbranched appendage; 3 median cells subcylindrical, hyaline to pale brown, apical cell conical, almost hyaline, with three branched appendage.

Notes: *Bartilinia* was introduced by Tassi (1900) with *Bartalinia robillardoides* as the type species. Both Morgan-Jones (1972) accepted nine species for this genus. Sutton (1980) also accepted the same while emphasizing on the need for the taxonomic revision of the genus. Von Arx (1981) listed *Bartalinia* under

Seimatosporium. Nag Raj (1993) rejected this classification stating the differences in conidial appendages and retained the type *B. robillardoides* and five other species *B. bischoffiae* Nag Raj, *B. lateripes* (Ellis & Everh.) Nag Raj, *B. laurina* (Mont. ex Desm.) Nag Raj, *B. pistacina* (J.L. Maas) Nag Raj, *B. tamarindi* Nag Raj as belonging to the genus *Bartalinia*. New *Bartalinia* species have been added as a result of various studies (Anderson & Bianchinotti, 1996; Xi et al., 2000; Chi et al., 2002; Andrianova & Minter, 2007; Marincowitz et al., 2010; Senanayake et al., 2015) and currently 19 species epithets are listed in Index Fungorum (2015). The species of *Bartalinia* are characterized by having three to four septate conidia. A key to ten *Bartalinia* species including the six listed by Nag Raj (1993) has been provided by Andrianova and Minter (2007). In a study of appendaged coelomycetes, Crous et al. (2014) designated an epitype for *B. robillardoides*, collected on *Leptoglossus occidentalis*.

Species of *Bartalinia* have been recorded on a wide range of hosts - especially flowering shrubs and trees, and causing leaf spots on economically important plants across Europe, India, China, South East Asia, South America and Australia (Wong et al., 2003; Farr & Rossman, 2015).

Type species: *Bartalinia robillardoides* Tassi, Bulletin Labor. Orto Bot. de R. Univ. Siena 3: 4 (1900)

Possible synonym: *Seimatosporium robillardoides* (Tassi) Arx, Gen. Fungi Sporul. Cult., Edn 3 (Vaduz): 224 (1981)

Other genera included

Broomella Sacc., Syll. fung. (Abellini) 2: 557 (1883)

Type species: *Broomella vitalbae* (Berk. & Broome) Sacc., Syll. fung. (Abellini) 2: 558 (1883)

Notes: Li et al (2015) designated an epitype for the type species of *B. vitalbae* illustrating both sexual and asexual morphs, based on morphological and molecular studies. Several species of *Broomella* species have been linked to the asexual morph *Truncatella* (Shoemaker & Müller, 1963; Yuan et al., 1992; Kang et al., 1999).

Dyrithiopsis L. Cai, Jeewon & K.D. Hyde, in Jeewon, Cai, Liew, Zhang & Hyde, Mycologia 95(5): 912 (2003)

Type species: *Dyrithiopsis lakefluxianensis* L. Cai, Jeewon & K.D. Hyde, in Jeewon, Cai, Liew, Zhang & Hyde, Mycologia 95(5): 913 (2003)

Hyalotiella Papendorf, Trans. Br. mycol. Soc. 50(1): 69 (1967)

Type species: *Hyalotiella transvalensis* Papendorf, Trans. Br. mycol. Soc. 50(1): 69 (1967)

Truncatella Steyaert, Bull. Jard. bot. État Brux. 19: 293 (1949)

Type species: *Truncatella truncata* (Lév.) Steyaert, Bull. Jard. bot. État Brux. 19: 295 (1949)

Zetiasplozna Nag Raj, Coelomycetous Anamorphs with Appendage-bearing Conidia (Ontario): 996 (1993)

Type species: *Zetiasplozna caffra* Matsush. [as 'caffera'], Matsush. Mycol. Mem. 9: 29 (1996)

Key to the sexual genera of Bartaliniaceae

1. a. Ascomata solitary to aggregated, globose to subglobose, cylindrical-elongate or cylindrical-clavate asci with discoid J- apical ring, having 3-septate ascospores bearing simple, centric appendages at each end or at one end, with sometimes having warts.....*Broomella*

1. b. Ascomata aggregated, subglobose, cylindrical asci with discoid J+ subapical ring, having smooth walled, muriform ascospores (3-transverse, 1-2 longitudinal septa).....

.....*Dyrithiopsis*

Key to the asexual genera of Bartaliniaceae

1. a. Brown, dark brown or dark brown to black, immersed, pycnidial or acervular fruiting body, conidiophores reduced or not reduced to conidiogenous cells, spores bearing single apical appendage, branched, narrow and attenuated or broad and attenuated..... 2

1. b. Black pycnidial, immersed to erumpent fruiting body, conidiophores mostly reduced to conidiogenous cells, spores bearing several apical appendages, unbranched, broad and attenuated.....*Zetiasplozna*

2. a. Spores smooth walled, median cells hyaline or almost hyaline to pale brown, slightly constricted at septa 3
 2. b. Spores verruculose, median cells brown, often constricted at septa.... *Truncatella*
 3. a. Conidia 3–4-septate with single basal appendage..... *Bartalinia*
 3. b. Conidia 3-septate, rarely 4-septate with no basal appendage..... *Hyalotiella*

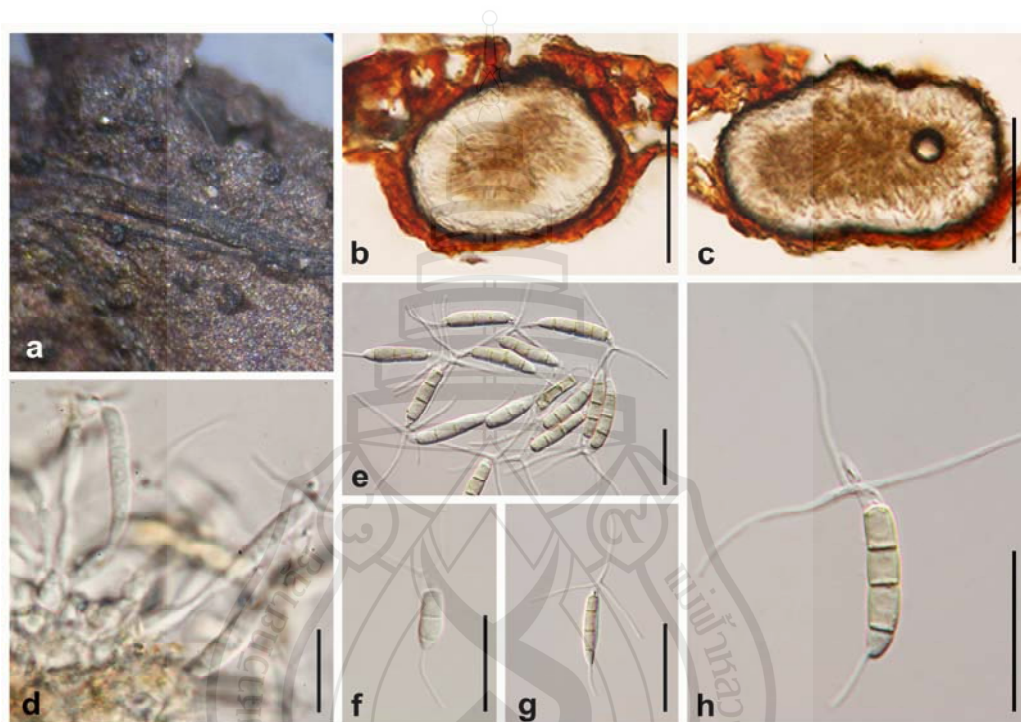


Figure C3 *Bartalinia robillardoides* (Material examined: THAILAND, Chiang Rai, Mae Fah Luang University grounds, on leaves of *Eucalyptus* sp., 30 June 2012, Nalin N. Wijayawardene, NNW 120630–1, MFLU 13–0084, holotype); a Conidiomata on host. b,c Section of conidioma. d Conidia arising from conidiogenous cells. e–h Conidia. Scale bars: a=not to scale, b,c=150 μm , d = 20 μm , e–h = 25 μm .

Cainiaceae J.C. Krug, Sydowia 30 (1–6):123 (1978) [1977]

Facesoffungi number: FoF 00687

Saprobic on dead grasses, bamboo and other monocotyledons, appearing as shiny black dots, slightly effuse from the substrate. **Sexual morph:** *Pseudostromata*

poorly developed or lacking, or sometimes clypeate, scattered, superficial, dome-shaped or slightly effuse, dark brown to black. *Ascomata* immersed, solitary or aggregated, globose to subglobose, coriaceous, brown, ostiolate. *Ostiolar* papilla short, internally lined with hyaline periphyses. *Peridium* one or two-layered, outer layer comprising of thick-walled, brown cells of *textura angularis* and inner layer comprising of hyaline thin-walled cells of *textura angularis*. *Hamathecium* comprising abundant, filamentous paraphyses, slightly constricted at the septa. *Asci* 8-spored, unitunicate, cylindrical to broadly cylindrical, short pedicellate, with a complex J+, cylindrical apical ring or series of rings. *Ascospores* uniseriate, overlapping uniseriate to biseriate, hyaline when young and dark brown at maturity, sphaerical to ellipsoidal, unicellular to 1-septate, slightly constricted at the septum, wall ornamented with longitudinal germ slits or germ pores, surrounded by a gelatinous sheath. **Asexual morph:** Coelomycetous. *Conidiomata* pycnidial, scattered, immersed, globose to subglobose, black. *Conidiophores* hyaline, denticulate, sympodially proliferating. *Conidiogenous cells* with 1–3 phialides, filiform, branched or simple, septate, hyaline. *Conidia* elongate fusiform, falcate to lunate, unicellular or septate, hyaline, with pointed ends.

Type: Cainia Arx & E. Müll.

Notes: The family *Cainiaceae* was introduced by Krug (1977) to accommodate the species with unique apical ring of the asci, which consisted of a series of rings, and ascospores with longitudinal germ slits. The genera *Arecophila*, *Atrotriquata*, *Cainia*, *Ceriphora*, *Reticulosphaeria* and *Ommatomyces* have been placed in this family at various times (Kohlmeyer & Kohlmeyer, 1993; Hyde et al., 1996). *Seynesia* was included based on phylogenetic analysis by Maharachchikumbura et al. (2015). The placement of *Arecophila*, *Cainia* and *Seynesia* in *Cainiaceae* was confirmed based on molecular analysis (Smith et al., 2003; Maharachchikumbura et al., 2015). The combined LSU and ITS gene analysis also confirmed the phylogenetic placement of *Atrotriquata* in *Cainiaceae* (Senanayake et al., 2015). Another genus, *Amphibambusa* was introduced from bamboo in Liu et al. (2015) and LSU gene analysis and morphology indicated that the genus belongs to *Cainiaceae*. Based on both morphological and phylogenetic data, Senanayake et al. (2015) accepted the genera *Amphibambusa*, *Arecophila*,

Atrotriquata, *Cainia* and *Seynesia* in Cainiaceae. The asexual morph of *Cainiaceae* from a pure culture of *Cainia desmazieresii* has been observed and was reported by Muller and Corbaz (1956) as *Rhabdospora*-like. However, later attempts at obtaining the asexual morph from *Cainia desmazieresii* in culture have not been successful (Kang et al., 1999a, b; Senanayake et al., 2015).

Cainia Arx & E. Müll., Acta bot. neerl. 4 (1): 111 (1955)

Facesoffungi number: FoF 00688

Saprobic or pathogenic on Poaceae and Cyperaceae, forming leaf or stem spots, appearing as shiny, black, scattered dots on host surface. **Sexual morph:** *Pseudoclypeus* indistinct, scattered, dome-shaped, effuse, dark brown to black, smooth, each containing one, or rarely several ascomata. *Ascomata* immersed, scattered, solitary or aggregated, ampulliform, coriaceous, with ostiolate. *Ostioles* short, internally lined with hyaline, filamentous periphyses. *Peridium* comprising an outer layer of thick-walled, brown, *textura angularis* cells and inner layer comprising of thick-walled, hyaline, *textura angularis* cells. *Hamathecium* comprising numerous, septate paraphyses, slightly constricted at the septa, *Asci* 8-spored, unitunicate, cylindrical to clavate, short pedicellate, apex rounded, with a complex, J+, dome-shaped to cylindrical series of rings. *Ascospores* overlapping uniseriate to biseriate, dark brown, ellipsoidal, 1-septate, constricted at the septum, wall with longitudinal striations, surrounded by a gelatinous sheath. **Asexual morph:** Coelomycetous. *Conidiomata* pycnidia, scattered, immersed, globose to subglobose, black. *Conidiophores* hyaline, denticulate. *Conidiogenous cells* with 1–3 phialides, filiform, simple or branched, septate, hyaline. *Conidia* elongate-fusiform to filiform, falcate to lunate, with holoblastic conidiogenesis, unicellular or septate, hyaline, sometimes with pointed ends (description of asexual morph is based on Krug 1977 and Kang et al. 1999a, b).

Notes: The genus was introduced by von Arx and Müller (1955). It was distinguished from Amphisphaeriaceae based on the complex structure of the apical apparatus and presence of germ slits in the ascospores and thus, accommodated in Cainiaceae. Senanayake et al. (2015) designated a reference specimen for the type *Cainia graminis*. Currently, five species epithets have been listed for *Cainia* in Index Fungorum (2016).

Type species: Cainia graminis (Niessl) Arx & E. Müll., Acta bot. neerl. 4(1):112 (1955)

Basionym = *Delitschia graminis* Niessl, Notiz. Pyr.: 48 (1876)

Other genera included

Amphibambusa D.Q. Dai & K.D. Hyde, in Liu et al., Fungal Divers. 72: 7 (2015)

Type species: Amphibambusa bambusicola D.Q. Dai & K.D. Hyde, Liu et al. Fungal Divers. 72: 7 (2015)

Arecophila K.D. Hyde, Nova Hedwigia 63(1-2):82 (1996)

Type species: Arecophila gulubiicola K.D. Hyde, Nova Hedwigia 63(1-2):91 (1996)

Atrotriquata Kohlm. & Volkm.-Kohlm., Syst. Ascom. 12(1-2):8 (1993)

Type species: Atrotriquata lineata Kohlm. & Volkm.-Kohlm., Syst. Ascom. 12(1-2):8 (1993)

Seynesia Sacc., Syll. fung. (Abellini) 2:668 (1883)

Type species: Seynesia nobilis (Welw. & Curr.) Sacc., Syll. fung. (Abellini) 2:668 (1883)

Basionym = *Pemphidium nobile* Welw. & Curr., Trans. Linn. Soc. London 26(1): 283 (1868) [1870]

Key to the sexual genera of Cainiaceae

1. Apical apparatus is a cylindrical complex sub apical ring, wedge-shaped or conical. 2
1. Apical apparatus consisting of several rings 4
2. Asci cylindrical, pedunculate, ascospores striate, bearing a single conical appendage emerging from outer sheath.....***Seynesia***
2. Asci cylindrical, ascospores striate, no appendage emerging from outer sheath 3
3. Ascospores fusiform to broad fusiform in shape.....***Amphibambusa***
3. Ascospores ellipsoidal in shape.....***Arecophila***
4. Apical apparatus wedge-shaped, ascospores having 5–7 longitudinal striations at each apex.....***Atrotriquata***
4. Apical apparatus dome-shaped to cylindrical or appearing as plates, ascospores bearing a germ slit at each end.....***Cainia***

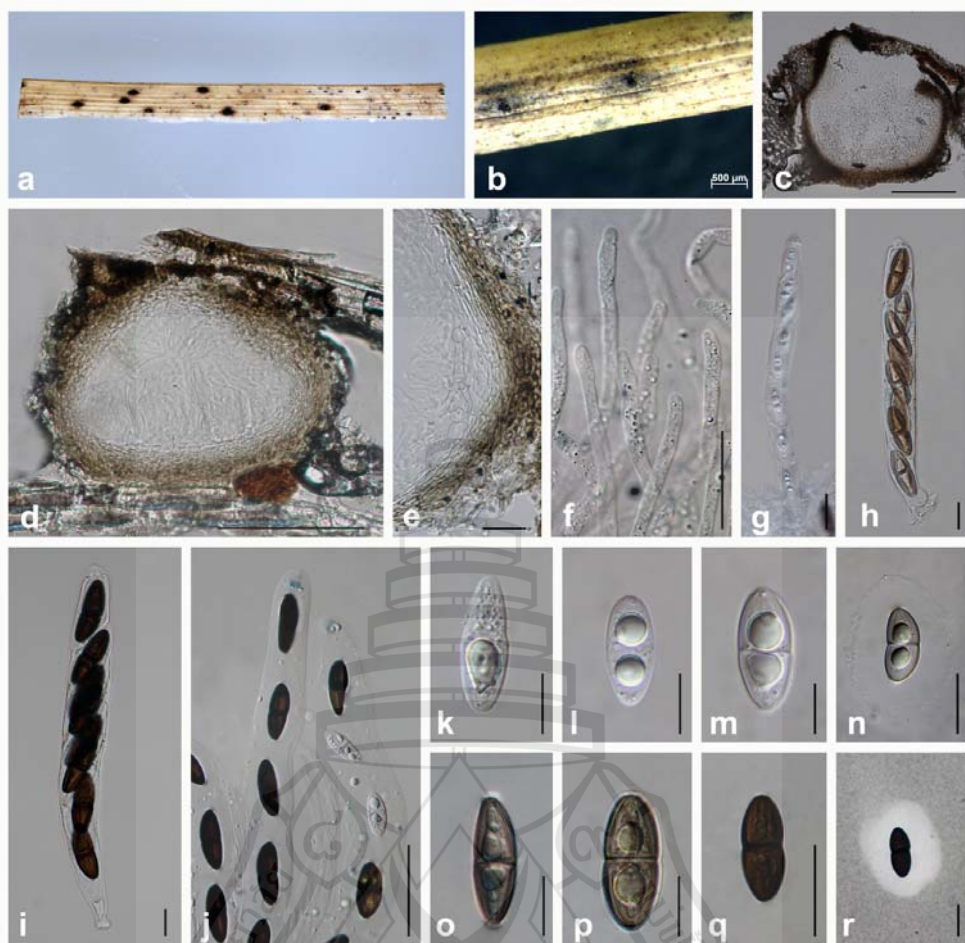


Figure C4 *Cainia graminis* (Material examined: ITALY, Province of Forlì-Cesena [FC], Santa Sofia, Corniolo, stem of *Lolium temulentum* L (Poaceae), 25 September 2013, Erio Camporesi, IT 1462, (MFLU 15–0724)) a Ascomata on host b Close up of ascomata c, d Vertical section of ascomata e Peridium f Paraphyses g–i Asci j J+ apical ring in Melzer's reagent k–q Ascospores r Sheath surrounding ascospore after treating with Indian ink. Scale bars: a = not to scale, c, g–j = 100 μm , d = 250 μm , e, f, o–r = 25 μm , k–n = 10 μm .

Cordanaceae Nann., Repert. mic. uomo: 498 (1934)

Facesoffungi number: FoF 01673

Saprobic or *pathogenic* on wood and leaves of various shrubs, deciduous and evergreen trees, conifers, bamboo and grasses in terrestrial and rarely in freshwater

habitats. **Sexual morph:** *Ascomata* perithecial, solitary or gregarious, superficial, globose to ovoid, sometimes with basal stroma, setose or glabrous, ostiolate, papillate, with periphyses. *Peridium* composed of 3–5 layers of cells of *textura prismatica*. *Hamathecium* comprising simple, unbranched, septate, hyaline paraphyses. *Asci* 8-spored, unitunicate, cylindrical, short pedicellate, with or without an inconspicuous, non-amyloid, apical ring. *Ascospores* uniseriate, ellipsoid to fusiform, 1-septate, light brown to brown, sometimes with pores at the ends. **Asexual morph:** Hyphomycetous. *Colonies* effuse, dark brown to black. *Conidiophores* erect, mononematous, macronematous, brown, branched or unbranched, with a fertile apex. *Conidiogenous cells* polyblastic, denticulate. *Conidia* brown to dark brown, obovate or ellipsoidal, 1-septate, smooth, with or without germ pores at the end of the conidia (description modified from Hughes, 1955, Müller & Samuels, 1982 and Hernández-Restrepo et al., 2014).

Type: ***Cordana*** Preuss

Notes: The family *Cordanaceae* was established by Nannfeldt (1984) with *Cordana* as the type genus. Based on molecular data, Hernández-Restrepo et al. (2015) introduced the order *Cordanales* to accommodate this family, which is easily distinguished from the species of its sister order *Coniochaetales* (*Coniochaetaceae*), in having a basal stroma, ascospores lacking germ slits and polyblastic asexual morphs (Hernández-Restrepo et al., 2015). The family contains a single genus thus only the family description is given.

Cordana Preuss, *Linnaea* 24: 129 (1851)

Facesoffungi number: FoF 01674

Possible synonyms: *Pseudobotrytis* Krzemien. & Badura, *Acta Soc. Bot. Pol.* 23:761, 1954, *Umbellula* E.F. Morris, *Mycologia* 47:602, 1955, *Porosphaerella* E. Müll. & Samuels, *Sydowia* 35:151, 1982

Notes: The genus *Cordana* was introduced by Preuss (1851), while describing the species *Cordana polyseptata* Preuss, *C. pauciseptata* Preuss and *C. pedunculata* Preuss. Later a fourth species *C. parvispora* Preuss was also described (Preuss, 1852), but in both publications, a type was not designated. Saccardo (1877) retained only *C. pauciseptata* in *Cordana* selecting it as the lectotype and classifying the other three *Cordana* species under *Acrothecium*. Kuntze (1891) disagreed with this classification

and renamed the genus as *Preussiaster* in honour of its first author. *Cordana pauciseptata* was reclassified as *Preussiaster pauciseptatus* (Preuss) O. Kuntze and the three species placed by Saccardo (1877) under *Acrothecium* were also added (Kuntze, 1891). However, Hughes (1955) stated that any three of the specimens originally described by Preuss (1851) should be the lectotype. After studying the type material of *Cordana pauciseptata*, Hughes (1955) re-described and illustrated it as *Brachysporium polyseptatum* (= *Brachysporium bloxami*), which resulted in *C. pauciseptata* being an older epithet for the species. However, this type material was reported to be lost (de Hoog, 1973) and an epitype was designated by Hernández-Restrepo et al. (2014).

Species of *Cordana* are characterized by brown, septate conidiophores with swollen conidiogenous zones, terminal and intercalary conidiogenous cells, and pale brown to brown, 1-septate or aseptate conidia (Hernández-Restrepo et al., 2015). Müller and Samuels (1982) linked *Porosphaerella cordanophora* to *Cordana pauciseptata*, the type species of *Cordana* using culture studies. Romero and Samuels (1991) published a second species in *Porosphaerella* viz. *P. setosa* A.I. Romero & Samuels, but no asexual morph was reported. Fernandez and Huhndorf (2004) established that *Porosphaerella borinquensis* was linked to *Pseudobotrytis terrestris* as the asexual morph formed *in vitro*. The genus is known to be cosmopolitan owing to its asexual morph. Molecular studies have shown that *Cordanaceae* is closely related to *Coniochaetales* (Réblová & Winka, 2000; Huhndorf et al., 2004; Réblová & Seifert, 2007; Hernández-Restrepo et al., 2014; Zelski et al., 2014; Maharachchikumbura et al., 2015). Hernández-Restrepo et al. (2014) accepted 19 species for this genus and provided a key for identification. *Cordana* species have been recorded across various temperate and tropical regions in the world, including Africa, South America, South East Asia and New Zealand.

Type species: Cordana pauciseptata Preuss, Linnaea 24: 129 (1851)

Possible synonyms: *Acrothecium pauciseptatum* (Preuss) Sacc., *Michelia* 1:74, 1877, *Preussiaster pauciseptatus* (Preuss) O. Kuntze, *Revis. Gen. Pl.* 2:867, 1891, *Porosphaerella cordanophora* E. Müll. & Samuels, *Sydowia* 35:151, 1982

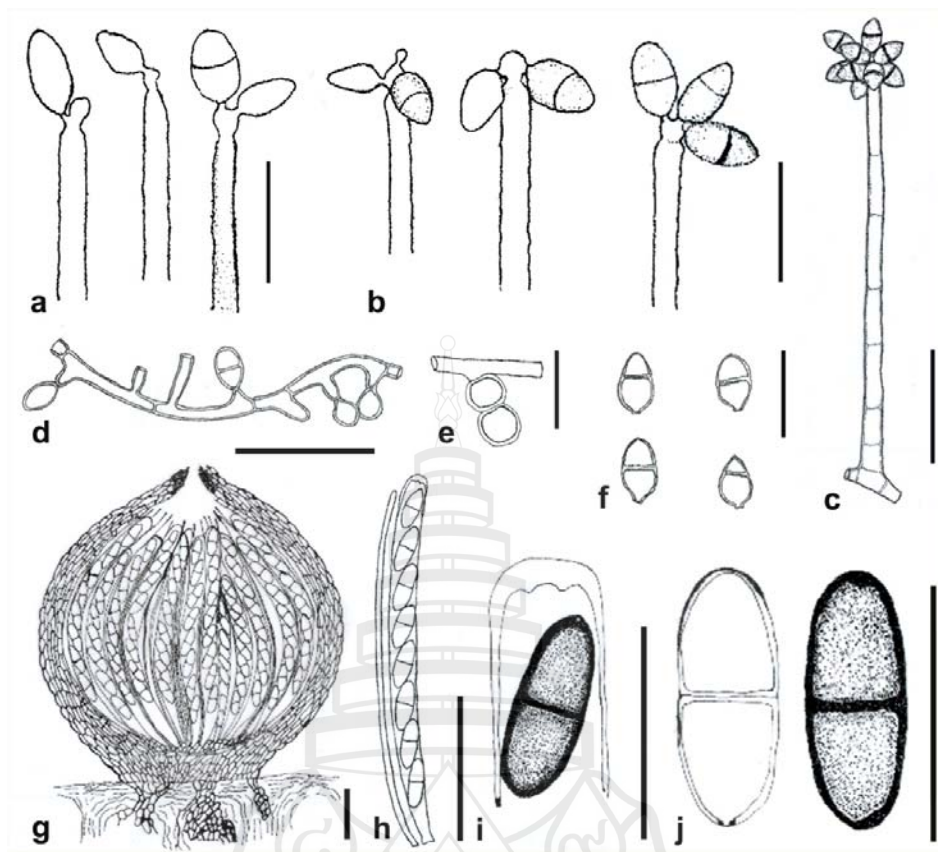


Figure C5 *Cordana pauciseptata* (redrawn from Hughes, 1955) a, b Conidiophores with developing conidia on MEA (DAOM 33990). c Conidiophore with proliferated conidia on PDA (DAOM 28664). d, e Hypha bearing chlamydospores on MEA (DAOM 33990) f Mature conidia (DAOM 33990). Sexual morph *Porosphaerella cordanaphora*, the first species reported to have a *Cordana* asexual morph (redrawn from Müller and Samuels 1982) g Vertical section of ascoma h Asci with ascospores i Nonamyloid apical ring of asci with a mature ascospore j Ascospores. Scale bars: a, b, e–j = 10 µm, c, d = 20 µm.

Lopadostomaceae Daranagama & K.D. Hyde, in Senanayake et al., Fungal Divers 73: 1 (2015)

Facesoffungi number: FoF 00071

Notes: *Lopadostomaceae* was introduced by Daranagama & K.D. Hyde in Senanayake et al. (2015) into the order *Xylariales* to accommodate the genera

Creosphaeria and *Lopadostoma*. *Creosphaeria* was introduced by Theissen (1910) with having soft, light coloured stromata. Petrini and Müller (1986) reported a *Libertella* asexual morph from culture of *Creosphaeria*, while Ju et al. (1993) observed the same type of conidia associated with the sexual morph. Jaklitsch et al. (2014) revised the genus *Lopadostoma* and reported a *Libertella* asexual morph as well. The species *L. americanum* Jaklitsch, J. Fourn., J.D. Rogers & Voglmayr, *L. fagi* Jaklitsch, J. Fourn. & Voglmayr, *L. linospermum* (Durieu & Mont.) Jaklitsch, J. Fourn. & Voglmayr, *L. meridionale* Jaklitsch, J. Fourn. & Voglmayr and *L. turgidum* (Pers.) Traverso, are known to have libertella-like asexual morphs. Ju et al. (1993) believed the two genera *Lopadostoma* and *Creosphaeria* should be represented separately as they are not similar in morphology with other Xylariaceae members. In the phylogenetic analysis conducted by Maharachchikumbura et al. (2015) and Senanayake et al. (2015) *Lopadostoma* placed separately as a monophyletic group outside *Xylariaceae* with *Creosphaeria sassafras* (Schwein.). Although they form separate monophyletic groups, the close relationship between *Lopadostoma* and *Creosphaeria* noted in Senanayake et al. (2015), is in agreement with Jaklitsch et al. (2014).

Sporocadaceae Corda (as ‘Sporocadeae’), Icon. Fungorum (Prague) 5: 34. 1842

= *Discosiaceae* Maharachch. & K.D. Hyde

Saprobic or *pathogenic* on plant leaves leaves of flowering plants and gymnosperms.

Sexual morph: *Ascomata* scattered, immersed to erumpent. *Ostiole* circular, papillate. *Peridium* comprising several layers of *textura prismatica* cells, brown at the base, dark brown outwardly. *Hamathecium* comprising numerous, hypha-like, flexuose, paraphyses. *Asci* 8-spored, unitunicate, obclavate to cylindrical, with a discoid, J+ subapical ring. *Ascospores* overlapping uniseriate or biseriate, obovoid to ellipsoid, transversally septate, longitudinal septum in mid cells, with or without vertical septa, hyaline. **Asexual morph:** Coelomycetous. *Conidiomata* stromatic, scattered to gregarious, subepidermal or subepidermal in origin, immersed to erumpent. *Peridium* composed of 3–4 layers of cells of *textura angularis*, outer 1–2 layers brown and inner 1–2 layers hyaline or pale brown. *Conidiophores* arising from the inner layer cells of basal stroma, absent or reduced to conidiogenous cells, when present long, septate and branched. *Conidiogenous cells* holoblastic, annelidic,

cylindrical or lageniform, hyaline. *Conidia* cylindrical to clavate, or fusiform, straight or curved, 2–4-euseptate, hyaline, pale olivaceous, or brown, bearing cellular, filiform or attenuated appendages.

Type: Seimatosporium Corda

= *Sporocadus* Corda

Notes: Members of the family *Sporocadaceae* are saprobes, endophytes, or foliar pathogens in tropical and temperate regions (Nag Raj, 1993; Tanaka et al., 2011). The genus *Sporocadus* was synonymized under *Seimatosporium*. The genera *Adisciso*, *Discosia*, *Discostroma*, *Sarcostroma* and the type genus *Seimatosporium* are placed in *Sporocadaceae* following the studies by Maharachchikumbura et al. (2015) and Senanayake et al. (2015). In addition, is the genus *Strickeria* which was included into the family by Jaklitsch et al. (2016) based on both morphology and phylogeny.

Seimatosporium Corda, in Sturm, *Deutschl. Fl.*, 3 Abt. (Pilze Deutschl.) 3(13): 79 (1833)

Saprobic on leaves, stem and bark of various plants, pathogenic on leaves of *Eucalyptus* spp. **Sexual morph:** *Discostroma*-like. **Asexual morph:** *Conidiomata* acervular, solitary to gregarious, superficial to immersed, unilocular, globose to subglobose, dark brown to black. *Peridium* composed of brown thin or thick-walled *textura angularis*. *Conidiophores* cylindrical, filiform, septate, branched, hyaline. *Conidiogenous cells* holoblastic, annellidic, integrated or discrete, hyaline, determinate. *Conidia* variable, cylindrical, fusiform or clavate or obovoid, (2 –)3(–5)-septate, aguttulate, medium cells brown, basal cell hyaline, with or without basal appendage, or with the apical cell provided with a single, cellular, simple or branched appendage, basal cell truncate, continuous or occasionally constricted at the septa.

Type species: Seimatosporium rosae Corda, in Sturm, *Deutschl. Fl.*, 3 Abt. (Pilze Deutschl.) 3(13): 79 (1833)

Notes: The genus *Seimatosporium* was introduced by Corda (1833) based on the type *S. rosae*. Von Arx (1981) treated *Bartalinia*, *Bartaliniopsis*, *Bleptosporium*, *Doliomyces* and *Hyalotia* as synonyms of *Seimatosporium*. This was not accepted by Nag Raj (1993) and furthermore is not supported by molecular data (Maharachchikumbura et al. 2015). *Seimatosporium* species have been linked to *Discostroma* sexual morphs through molecular data (Tanaka et al., 2011), however

the type of *Discostroma* lacks molecular data to confirm this. Therefore, both names have been retained until further studies based on fresh collections and molecular data are conducted (Maharachchikumbura et al., 2015).

Other genera included:

Adisciso Kaz. Tanaka et al., in Tanaka et al., *Persoonia*, Mol. Phyl. Evol. Fungi 26: 90 (2011)

Type species: Adisciso yakushimense Kaz. Tanaka et al., in Tanaka et al., *Persoonia*, Mol. Phyl. Evol. Fungi 26: 92 (2011)

Discosia Lib., *Pl. crypt. Arduenna*, fasc. (Liège) 4: no. 346 (1837)

Type species: Discosia artocreas (Tode) Fr., *Summa veg. Scand.*, Section Post. (Stockholm): 423 (1849)

Discostroma Clem., *Gen. fung.* (Minneapolis): 50 (1909)

Type species: Discostroma massarinum (Sacc.) Arx, *Genera of Fungi Sporulating in Pure Culture*, Ed 2: 131 (1974)

Sarcostroma Cooke, *Journal of the Quekett microsc. Club* 2: 267 (1871)

Type species: Sarcostroma berkeleyi Cooke, *Journal of the Quekett microsc. Club* 2: 267 (1871)

Strickeria Körb., *Parerga lichenol.* (Breslau) 5: 400 (1865)

Type species: Strickeria kochii Körb., *Parerga lichenol.* (Breslau) 5: 400 (1865)

Key to the sexual genera of *Sporocadaceae*

1. Ascospores arranged biserially.....**Adisciso**
1. Ascospores arranged uniseriately.....2
2. Ascospores hyaline.....**Discostroma**
2. Ascospores brown, end cells subhyaline.....**Strickeria**

Key to the asexual genera of *Sporocadaceae*

1. Conidiomata not stromatic, immersed, conidia subhyaline to pale brown... **Discosia**
1. Conidiomata stromatic, semi-immersed to erumpent, conidia brown.....2
2. Conidiophores unbranched or irregularly branched, conidiogenous cells with minute periclinal thickenings, conidia fusiform, 3–5-septate, occasionally 7-septate.....**Sarcostroma**

2. Conidiophores branched, conidiogenous cells without minute periclinal thickenings, conidia ellipsoid to clavate, 2–4-septate, occasionally 5-, 6-septate.....*Seimatosporium*

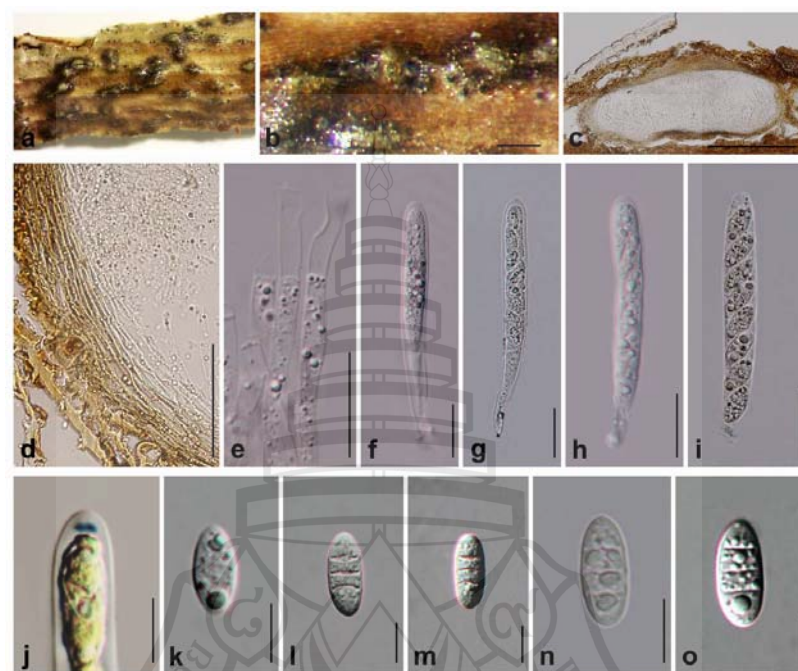


Figure C6 *Discostroma fuscillum* (Material examined: ITALY. Province of Forlì-Cesena [FC], Poggio Baldo - Predappio Alta, on dead stem of *Rosa canina*, 3 March 2013, E. Camporesi, IT 1093 (MFLU 15–0750)) a-b Ascomata on host surface c Section of ascoma d Peridium e Paraphyses. f-h Immature asci i Mature ascus j Ring-like apical apparatus of ascus in Congo red k-o ascospore. Scale bars: b, f-i = 200 μ m, c = 100 μ m, d-e, j = 50 μ m, f – l = 20 μ m, k-o = 30 μ m

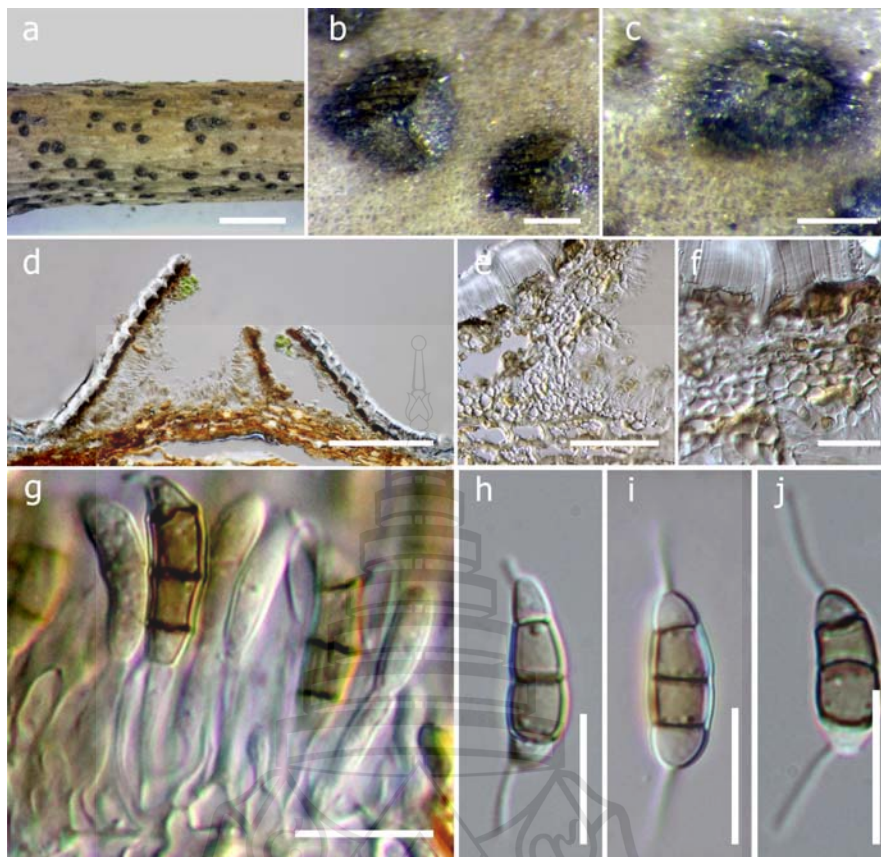


Figure C7 *Seimatosporium rosae* (Material examined – RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye Forestry, edge of ravine forest, on dying and dead branches of *Rosa kalmiussica* Chrshan. & Lasebna, 21 May 2014, T. Bulgakov T-056 (MFLU 14-0771, epitype)). a Stromatal habit in wood. b-c Fruiting bodies on host d Cross section of the stroma showing perithecia. e-f Peridium. g Conidiogenous cell with attached conidia. h-j Mature conidia. Scale bars: a = 2000 μ m, b-c = 200 μ m, d = 100 μ m, e = 50 μ m, f-j = 10 μ m.

APPENDIX D

NOTES AND HERBARIUM STUDY FOR REFINED FAMILIES OF SORDARIOMYCETES

Coniochaetales Huhndorf, A.N. Mill. & F.A. Fernández, *Mycologia* 96(2): 378 (2004) = *Cordanales* M. Hern.-Rest. & Crous

This order consists of two families *Coniochaetaceae* representing the genera *Barrina* A.W. Ramaley and *Coniochaeta* (Sacc.) Cooke, and *Cordanaceae* which accommodates the genus *Cordana* Preuss (Wijayawardene et al., 2018). *Cordanaceae* was previously classified in the order Cordanales, however based on molecular clock evidence Hongsanan et al. (2017) treated Cordanales to be a synonym of *Coniochaetales*. The phylogenetic tree presented here supports this placement.

Cordanaceae Nann., *Repert. mic. uomo*: 498 (1934)

Index Fungorum number: IF80640; Facesoffungi number: FoF01673, 23 known species.

Saprobic or pathogenic on wood of branches, twigs and leaves of various shrubs and trees, bamboo, grasses and in soil of terrestrial habitats, occasionally in freshwater habitats. **Sexual morph:** *Ascomata* perithecial, superficial, solitary or gregarious, globose to ovoid, setose or glabrous, with basal stroma, papillate. *Ostium* periphysate. *Ascomatal wall* comprising 3–5 layers of cells of *textura prismatica*. *Hamathecium* consisting of septate, hyaline, unbranched paraphyses. *Asci* 8-spored, unitunicate, cylindrical, with short pedicel, with or without a non-amyloid apical ring. *Ascospores* uniseriate, pale brown to brown, ellipsoid to fusiform, sometimes with pores at both ends, 1-septate. **Asexual morph:** *Colonies* effuse, dark brown to black. *Conidiophores* mononematous, macronematous, brown, septate, erect, branched or unbranched. *Conidiogenous cells* terminal or intercalary, polyblastic, denticulate. *Conidia* pale brown to brown, obovoid to ellipsoidal, smooth,

sometimes with germ pores at the ends, 1-septate or aseptate (description modified from Hughes, 1955; Müller & Samuels, 1982; Hernández-Restrepo et al., 2014).

Type genus: ***Cordana*** Preuss

Notes: *Cordanaceae* was introduced by Nannfeldt (1984) based on the type genus *Cordana*. Initially, Hernández-Restrepo et al. (2015) introduced the order *Cordanales* to accommodate the *Cordanaceae*, which was recognized as a monotypic, monophyletic sister group to *Coniochaetales* (Maharachchikumbura et al., 2016). However, in a study backed by a molecular clock evidence, *Cordanaceae* was found to share a common ancestor with *Coniochaetaceae* in *Coniochaetales* 77MYA (Hongsanan et al., 2017). While the family lineage had good statistical support, *Cordanales* did not have enough support and was therefore combined under *Coniochaetales* (Hongsanan et al., 2017; Wijayawardene et al., 2017) of which the stem age was 176MYA and had higher support as an order. *Cordanaceae* differ from the closely related *Coniochaetaceae* by morphology of their basally stromatic ascomata, septate ascospores and holoblastic-denticulate conidiogenesis. (Hernández-Restrepo et al., 2015).

Cordana Preuss, *Linnaea* 24: 129 (1851)

Index Fungorum number: IF7777; Facesoffungi number: FoF01674; 23 morphological species (Species Fungorum 2019), 11 confirmed species with molecular data (Hernández-Restrepo et al., 2014; Ai et al., 2019).

Type: ***Cordana pauciseptata*** Preuss

Notes: *Cordana* is a dematiaceous hyphomycetous genus introduced by Preuss (1851) together with three species, *C. polyseptata* Preuss, *C. pauciseptata* Preuss and *C. pedunculata* Preuss, without designation of a type species (Hernández-Restrepo et al., 2014). Saccardo (1877) and Hughes (1955) attempted to designate *C. pauciseptata* as the lectotype, however in both occasions the species was subjected to reclassification and later the type material was reported as lost (Hernández-Restrepo et al., 2014). Thus, Hernández-Restrepo et al. (2014) designated an epitype for this genus, produced a phylogram based on ITS sequences and provided a dichotomous key to the species. Based on cultural studies, Müller and Samuels (1982) suggested *Porosphaerella cordanophora* was the sexual morph of *Cordana pauciseptata*, and this was confirmed phylogenetically by Hernández-Restrepo et al. (2014). ITS

sequence data for eight species and LSU for some are available in GenBank (Réblová & Winka, 2000; Huhndorf et al., 2004; Réblová & Seifert, 2007; Hernández-Restrepo et al., 2014; Zelski et al., 2014; Vu et al., 2019).

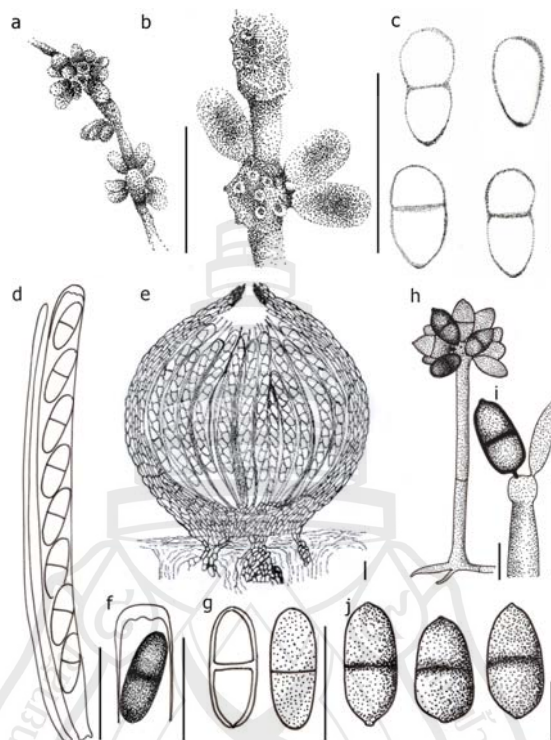


Figure D1 *Cordana mercadiana* (FMR 11828, a–c), drawings based on illustrations given in Hernández-Restrepo et al. (2014). a, b Arrangement of conidia on conidiophore and denticulate conidiogenous cells giving rise to conidia (based on SEM microphotographs provided in Hernández-Restrepo et al., 2014) c Conidia. Sexual morph *Porosphaerella cordanaphora*, and its *Cordana* asexual morph (d–i, redrawn from Müller & Samuels 1982) d Asci with ascospores e Vertical section of ascoma f Nonamyloid apical ring of asci with a mature ascospore g Ascospores h Conidiophore with conidia clustered at apex i Apical meristem protruding in conidiophore and developing conidia j Conidia. Scale bars: a = 50 μ m, b–j = 10 μ m.

Ecological and economic significance

Cordana species are widespread in temperate and tropical regions of the world, including Africa, South and Central America, Spain, South East Asia and New Zealand (Fernández & Huhndorf, 2004; Hernández-Restrepo et al., 2014; Zelski et al., 2014; Ai et al., 2019). Species occur on decaying plant matter, in soil or on other fungi (Hernández-Restrepo et al., 2014). They cause leaf spots on banana and *Canna* species (Ellis, 1976; Markovskaja, 2003; Cai et al., 2004; Hernández-Restrepo et al., 2014). The type, *C. pauciseptata* is a common saprobe reported on dead wood.

Chaetosphaeriales genera *incertae sedis*

Calvolachnella Marinc., T.A. Duong & M.J. Wingf., *Sydowia* 68: 203 (2016)

Index Fungorum number: IF815619; 1 species with sequence data.

Type species: ***Calvolachnella guaviyunis*** (Marinc., T.A. Duong, M.J. Wingf. & C.A. Perez) Marinc., T.A. Duong, M.J. Wingf.

Caudatispora J. Fröhl. & K.D. Hyde, *Sydowia* 47(1): 38 (1995)

Index Fungorum number: IF27569; 2 morphological species (Species Fungorum, 2019), 1 species with sequence data.

Type species: ***Caudatispora palmicola*** J. Fröhl. & K.D. Hyde

Erythromada Huhndorf, A.N. Mill., F.A. Fernández & Lodge, *Fungal Diversity* 20: 63 (2005)

Index Fungorum number: IF501320; 1 species with sequence data.

Type species: ***Erythromada lanciospora*** Huhndorf, A.N. Mill., F.A. Fernández & Lodge

Lasiosphaeriella Sivan., *Trans. Br. mycol. Soc.* 64(3): 443 (1975)

Index Fungorum number: IF2657; 5 morphological species (Species Fungorum 2019), 3 species with sequence data.

Type species: ***Lasiosphaeriella dennisii*** Sivan.

Rimaconus Huhndorf, F.A. Fernández, Joanne E. Taylor & K.D. Hyde, *Mycologia* 93(6): 1073 (2001)

Index Fungorum number: IF28573; 2 species with sequence data.

Type species: ***Rimaconus jamaicensis*** (Seaver) Huhndorf, F.A. Fernández, Joanne E. Taylor & K.D. Hyde

Coniochaetales genera incertae sedis

Cannonia Joanne E. Taylor & K.D. Hyde, Mycol. Res. 103(11): 1398 (1999)

Index Fungorum number: IF28356; 1 morphological species.

Type species: **Cannonia australis** (Speg.) Joanne E. Taylor & K.D. Hyde

Pseudogliomastix W. Gams, in Gams & Boekhout, Proc. Indian Acad. Sci., Pl. Sci. 94(2-3): 279 (1985)

Index Fungorum number: IF11172; 1 morphological species (Species Fungorum, 2019).

Type species: **Pseudogliomastix protea** (Sacc.) W. Gams

Coronophorales genera incertae sedis

Papulaspora Preuss, Linnaea 24: 112 (1851)

Index Fungorum number: IF9205; 33 morphological species (Species Fungorum, 2019), 7 species with sequence data.

Type species: **Papulaspora sepedonioides** Preuss

Pseudobambusicola Hern.-Restr. & Crous, MycoKeys 33: 9 (2018)

Index Fungorum number: IF824299; 1 species with sequence data.

Type species: **Pseudobambusicola thailandica** Hern.-Restr. & Crous

Diaporthomycetidae genera incertae sedis

Aquapteridospora Jiao Yang, K.D. Hyde & Maharachch., Cryptog. Mycol. 36(4): 474 (2015)

Index Fungorum number: IF551731; 2 species with sequence data.

Type species: **Aquapteridospora lignicola** J. Yang, K.D. Hyde & Maharachch.

Aquaticola W.H. Ho, C.K.M. Tsui, Hodgkiss & K.D. Hyde, Fungal Diversity Res. Ser. 3: 88 (1999)

Index Fungorum number: IF28389; 4 morphological species (Species Fungorum, 2019), 1 species with sequence data.

Type species: **Aquaticola hyalomura** W.H. Ho, K.M. Tsui, Hodgkiss & K.D. Hyde

Fusoidispora Vijaykr., Jeewon & K.D. Hyde, Sydowia 57(2): 272 (2005)

Index Fungorum number: IF28975; 1 species with sequence data.

Type species: **Fusoidispora aquatica** Vijaykr., Jeewon & K.D. Hyde

Platytrachelon Réblová, Mycologia 105(2): 466 (2013)

Index Fungorum number: IF800340; 1 species with sequence data.

Type species: **Platytrachelon abietis** (Réblová) Réblová

Proliferophorum G.N. Wang, H. Zhang & Senan., Fungal Diversity: 10.1007/s13225-019-00421-w, [128] (2019)

Index Fungorum number: IF555401; 1 species with sequence data.

Type species: ***Proliferophorum thailandicum*** G.N. Wang, H. Zhang & Senan.

Pseudoannulatascus Z.L. Luo, Maharachch. & K.D. Hyde, Phytotaxa 239(2): 179 (2015)

Index Fungorum number: IF551105; 1 species with sequence data.

Type species: ***Pseudoannulatascus biatriisporus*** (K.D. Hyde) Z.L. Luo, Maharachch. & K.D. Hyde

Pseudostanjehughesia J. Yang & K.D. Hyde, Mycol. Progr. 17(5): 609 (2017) [2018]

Index Fungorum number: IF821274; 2 species with sequence data.

Type species: ***Pseudostanjehughesia aquitropica*** J. Yang & K.D. Hyde

Phlogicylindriaceae Senan. & K.D. Hyde, Fungal Diversity 73: 35 (2015)

Index Fungorum number: IF551190; *Facesoffungi number*: FoF00681; 10 species.

Saprobic on leaves, twigs and branches of dicotyledons especially on *Eucalyptus* (Myrtaceae). **Sexual morph**: *Pseudostroma* solitary, black, scattered, immersed. *Ascomata* forming under pseudostroma, solitary, brown to black, scattered, globose, coriaceous, ostiolate, papillate. *Papilla* short, narrow, surrounding thickened. *Peridium* comprising outer, brown thick-walled cells of *textura angularis* and inner, hyaline, thin-walled cells of *textura angularis*. *Asci* 8-spored, unitunicate, cylindrical, short pedicellate, rounded at apex, with J+ discoid, subapical ring. *Ascospores* hyaline, overlapping uniseriate, fusiform, rounded at ends, 1-septate, slightly constricted at septa. **Asexual morph**: Coelomycetous. *Conidiomata* appearing as slimy, erect tufts of hyaline conidial masses, eustromatic, unilocular or multilocular, sporodochial, synnematus, indeterminate, turning brown with age. *Conidiophores* brown, smooth, branched. *Conidiogenous cells* annellidic, smooth, hyaline becoming light brown with maturity, subcylindrical, ampulliform with elongated necks, often having percurrent proliferations. *Conidia* cylindrical to subcylindrical, fusoid, hyaline, straight, smooth, rounded or subobtuse apex, truncate base, aseptate to 1-septate, guttulate.

Type genus: ***Phlogicylindrium*** Crous, Summerb. & Summerell

Notes: Senanayake et al. (2015) introduced the family Phlogicylindriaceae to accommodate the genera *Phlogicylindrium* and *Ciferriascosea*. *Phlogicylindrium* the asexual genus produces slimy erect flame-like tufts of hyaline conidial masses, and cylindrical, unicellular to 1-septate, hyaline conidia (Summerell et al., 2006). The sexual morph of *Phlogicylindrium* has not been reported. *Ciferriascosea* is a sexual genus with no reported asexual morph, and was included in Phlogicylindriaceae due to its phylogenetic affinity to *Phlogicylindrium* (Senanayake et al., 2015). The sexual genus *Idriellomyces* was introduced in Phlogicylindriaceae recently by Crous et al. (2018c) based on its distinct morphology and molecular data.

Ecological and economic significance of Phlogicylindriaceae

Phlogicylindrium eucalypti and *P. uniforme* were reported occurring in association with a *Mycosphaerella* spp. causing lesions on living leaves of *Eucalyptus* sp. (Summerell et al. 2006; Crous et al. 2011). Therefore, they possibly play a role as secondary invaders (Crous et al. 2011). *Phlogicylindrium tereticornis* was found on leaves of *Eucalyptus tereticornis* infected with circular to angular, brown leaf spots and red-purple margins (Crous et al. 2017a). All *Phlogicylindrium* species and *Idriellomyces eucalypti* have been reported on leaves from various *Eucalyptus* species in Australia. *Chitonospora* and *Ciferriascosea* species have been reported as saprobes from Africa and Europe.

Genera included in Phlogicylindriaceae

Ciferriascosea Senan., Bhat, Camporesi & K.D. Hyde, Fungal Divers 73: 35 (2015)

Index Fungorum number: IF551191; 2 species with sequence data.

Type species: ***Ciferriascosea rectamurum*** Senan., Bhat, Camporesi & K.D. Hyde

Notes: *Ciferriascosea* was introduced to accommodate two sexual morph species *Ciferriascosea fluctamurum* and *C. rectamurum* occurring on *Sparticum junceum* collected, from Italy (Senanayake et al., 2015). They are characterized in having immersed, black pseudostromata, ostiolate and papillate ascomata that produce asci with an indistinct J+ apical ring, and hyaline, 1-septate, fusiform ascospores with narrowly rounded ends (Senanayake et al., 2015). Both species of *Ciferriascosea* were introduced based on LSU and ITS sequence data.

Idriellomyces Crous, Persoonia 40: 369 (2018)

Index Fungorum number: IF825426; 1 morphological species with sequence data.

Type species: ***Idriellomyces eucalypti*** Crous

Notes: The genus *Idriellomyces* introduced by Crous et al. (2018c) is characterized in having branched conidiophores arranged as thick, erect synnemata, subcylindrical conidiogenous cells tapering towards the apex to a rachis and fusoid, hyaline, aseptate conidia. It is morphologically similar to the genus *Idriella* in Microdochiaceae but distinct in having pigmented conidiophores often in aggregated synnemata and in lacking chlamydospores (Crous et al., 2018c). The type species was found on leaves of *Eucalyptus obliqua* from Australia (Crous et al., 2018c).

Phlogicylindrium Crous, Summerb. & Summerell, Fungal Diversity 23: 340 (2006)

Index Fungorum number: IF510013; 7 morphological species with sequence data.

Type species: ***Phlogicylindrium eucalypti*** Crous et al.

Notes: All species of *Phlogicylindrium* have been reported from Australian *Eucalyptus* (Summerell et al., 2006; Crous et al., 2011, 2016a; Crous et al., 2019b; Farr & Rossman, 2019). *Phlogicylindrium dunnii* is characteristically differ from others *Phlogicylindrium* species by producing eustromatic, multilocular conidiomata with subcylindrical, 1-septate macroconidia and cylindrical aseptate microconidia within the same conidioma (Crous et al., 2019b). Here we provide a illustration of *P. uniforme* (Figure D2). All species of *Phlogicylindrium* are introduced based on LSU or ITS sequence data, while *rpb2* and *tefl* sequence data are available for *P. dunnii* and *P. tereticornis* (Crous et al. 2017a, 2019b).

Key to asexual genera of *Phlogicylindriaceae*

1. Conidia cylindrical..... ***Phlogicylindrium***
 1. Conidia fusoid..... ***Idriellomyces***

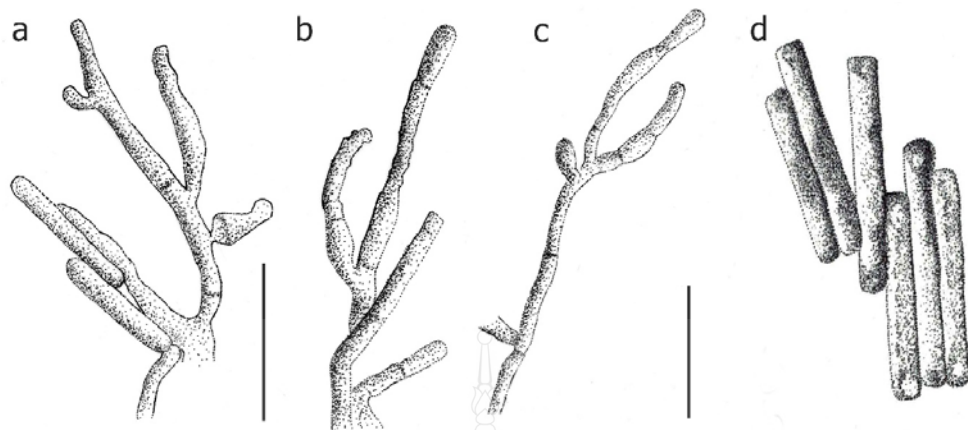


Figure D2 *Phlogicylindrium uniforme* (redrawn from Crous et al. 2011) a–c Conidiophores producing conidia d Conidia. Scale bars: a–c = 10 μ m.

Sporocadaceae Corda, Icon. Fungorum (Prague) 5: 34 (1842)

Index Fungorum number: IF81408; *Facesoffungi* number: FoF06111; more than 500 species.

Saprobic or *pathogenic* on leaves, twigs, branches, fruits of flowering plants and gymnosperms, as endophytes or parasitic on humans and animals. **Sexual morph:** *Ascomata* scattered or confluent, perithecial, immersed to erumpent, black to dark brown. *Ostiole* circular, papillate. *Peridium* comprising several layers of cells of *textura prismatica*, brown at the base, dark brown outwardly. *Paraphyses* numerous, hypha-like, flexuose, filiform. *Asci* 8-spored, unitunicate, obclavate to cylindrical, with or without a discoid, J+ subapical ring. *Ascospores* overlapping uniseriate or biseriate, obovoid to ellipsoid, transversally septate, longitudinal septum in mid cells, with or without vertical septa, hyaline, pale brown to brown. **Asexual morph:** Coelomycetous. *Conidiomata* scattered to gregarious, subepidermal or subepidermal in origin, immersed to erumpent, pycnidial, acervular, stromatic or synnematosus in *Synnemapestaloides*. *Conidiomata* wall composed of at least 3–4 layers of cells of *textura angularis* to *textura prismatica*, outer 1–2-layers brown and inner 1–2 layers hyaline or pale brown. *Conidiophores* arising from the inner layer cells of basal stroma, absent or reduced to conidiogenous cells, when present, long, septate and branched, hyaline, smooth. *Conidiogenous cells* holoblastic, annellidic, ampulliform,

cylindrical to subcylindrical, or lageniform, mostly hyaline. *Conidia* cylindrical to clavate, or fusiform, straight or curved, 2–4-euseptate, smooth or verruculose, median cells hyaline, pale olivaceous or pale brown to dark brown, bearing cellular, terminal cells mostly hyaline, appendages absent or if present, tubular, filiform, straight or flexuous, branched or unbranched (description based on Maharachchikumbura et al., 2016b; Liu et al., 2019a).

Type genus: Sporocadus Corda

Notes: Sporocadaceae typically includes genera of fungi that are appendaged coelomycetes and have been subjected to several taxonomic re-classifications (Nag Raj, 1993; Maharachchikumbura et al., 2016b; Liu et al., 2019a). The family was re-validated by Jaklitsch et al. (2016b) with four families derived from *Amphisphaeriaceae* viz. *Bartaliniaceae*, *Discosiaceae*, *Pestalotiopsidaceae* and *Robillardaceae* (Crous et al., 2015a; Senanayake et al., 2015), as synonyms of *Sporocadaceae* thus increasing the number of genera of *Sporocadaceae*. In addition to the phylogeny, the morphological characters of the asexual morph genera having acervular conidiomata that produce hyaline, pale or dark brown, septate conidia were taken into consideration. Jaklitsch et al. (2016b) and Wijayawardene et al. (2018a) accepted 22 genera under this family.

Subsequent studies involving *Sporocadaceae* were mostly based on ITS and LSU sequence data and therefore not adequate enough in resolving generic boundaries within the family (Jaklitsch et al., 2016b; Liu et al., 2019a). The most recent study by Liu et al. (2019a) provided a revision of this family complete with morphological descriptions and multi-gene phylogeny based on the LSU, ITS and *rpb2* sequence data, while further analysis using protein coding genes such as *tefl* or *tub2* for each genera were presented. As a result, the placement of 23 known genera were confirmed belonging to *Sporocadaceae* and seven new genera were included. Here, we follow the treatments and updated classification as presented by Liu et al. (2019a). The accepted genera are listed below with illustrations and a description for two new host records of *Sporocadaceae* members.

Ecological and economic significance of *Spodocadaceae*

Spodocadaceae members are ecologically important as they occur as saprobes, endophytes or pathogens, on leaves, twigs, branches and fruits on a wide range of host plants (Nag Raj, 1993; Hyde et al., 2017b; Liu et al., 2019a). In recent pathogen related studies several *Pestalotiopsis* species such as *P. neglecta* causing shoot blight in *Cryptomeria japonica* trees in China (Zhou et al., 2019), *Pestalotiopsis* grey blight disease on *Camellia sinensis* have been recorded (Wang et al., 2019a).

Several studies over the recent years have focused on the secondary metabolic production of species of *Bartalinia*, *Morinia* and *Pestalotiopsis* (Collado et al., 2006; Gangadevi & Muthumary, 2008; Liu et al., 2009; Liu et al., 2019a), while *Pestalotiopsis fici* has been mentioned to possess a higher ability in the production of bioactive compounds. Liu et al. (2019a) mentions that within the *Sporocadaceae* there is a high possibility of discovering such species that have the potential to produce metabolic compounds. Another interesting study by Wikee et al. (2019) mentions the potential use of *Pestalotiopsis* sp. in decolouration of dyes.

Genera included in *Spodocadaceae*

Allelochaeta Petr., Sydowia 9(1-6): 464 (1955)

Index Fungorum number: IF7095; 41 morphological species (Species Fungorum, 2019), 37 species with sequence data.

Type species: ***Annellolacinia dinemasporioides*** B. Sutton

Notes: *Allelochaeta* was considered a synonym of *Diploceras*, one of the five groups of fungi into which the *Seimatosporium* complex was split, by Nag Raj (1993). Based on a multi-gene phylogenetic study using the type species, Crous et al. (2018) resurrected this into genus level to accommodate the species previously belonging to *Discostromopsis* and *Vermisporium*. Members of this genus are characterized by mostly 3-septate, hyaline or pigmented, concolourous or versicolourous conidia, having branched or solitary, cellular or continuous appendages (Crous et al., 2018). Crous et al. (2018) further discusses the conidial septation, appendage types and pigmentation loss and gain during the evolution of *Allelochaeta* species.

Annellolacinia B. Sutton, Mycol. Pap. 97: 31 (1964)

Index Fungorum number: IF 7161; 2 morphological species (Species Fungorum, 2019)

Type species: ***Annellolacinia dinemasporioides*** B. Sutton

Notes: This coelomycete genus introduced by Sutton (1964) is characterized by its fusiform, aseptate conidia bearing a single, cellular, unbranched appendage at each end (Nag Raj, 1993). Wijayawardene et al. (2016b) tentatively placed it in *Discosiaceae* (now *Sporocadaceae*) based on its morphological similarities, and we follow the same. However, there is no culture or sequence data to confirm this placement. They have been reported on *Ananas* sp., *Carica papaya* and *Pandanus* sp. from African and Australian continents, China, India and South East Asia (Farr & Rossman, 2019).

Bartalinia Tassi, Bulletin Labor. Orto Bot. de R. Univ. Siena 3: 4 (1900)

Index Fungorum number: IF7327; 21 morphological species (Species Fungorum, 2019), 7 species with sequence data.

Type species: ***Bartalinia robillardoides*** Tassi

Notes: *Bartalinia*, established by Tassi (1900) is a genus characterized in producing conidia that are 3–4 septate (Nag Raj, 1993). They have a worldwide distribution, occurring as saprobes on various shrubs and trees, even monocots and are often associated with leaf spots (Wong et al., 2003; Maharachchikumbura et al., 2016b; Wijayawardene et al., 2017a; Phookamsak et al., 2019; Farr & Rossman, 2019). They have been recorded in freshwater as well (Nguyen et al., 2019). The genus was placed in the family *Bartaliniaceae* by Senanayake et al. (2015) which was later synonymized under *Sporocadaceae* by Jaklitsch et al. (2016b).

Broomella Sacc., Syll. fung. (Abellini) 2: 557 (1883)

Index Fungorum number: IF658; 14 morphological species (Species Fungorum, 2019), 2 species with sequence data

Type species: ***Broomella vitalbae*** (Berk. & Broome) Sacc.

Notes: *Broomella* species have been mostly reported from Asia and Europe (Farr & Rossman, 2019), as saprobes, from terrestrial habitats (Li et al., 2015d; Wijayawardene et al., 2018a). It was introduced by Saccardo (1883a). The sexual morph is characterized in having cylindric-elongate unitunicate asci, with J-, discoid apical ring, bearing 2-3-septate, ellipsoid-fusiform ascospores with brown, median

and hyaline, terminal cells bearing centric appendages at each end (Shoemaker & Müller, 1963; Li et al., 2015d; Wanasinghe et al., 2018; Liu et al., 2019a). The asexual morph is pestalotia-like and is yet to be confirmed by phylogeny (Wanasinghe et al., 2018; Liu et al., 2019a). Li et al. (2015d) provided an epitype for *Broomella vitalbe* illustrating both its sexual and asexual morphs.

Ciliochorella Syd., Annls mycol. 33(1/2): 62 (1935)

Index Fungorum number: IF7657; 7 morphological species (Species Fungorum, 2019), 3 species with sequence data.

Type species: ***Ciliochorella mangiferae*** Syd.

Notes: *Ciliochorella*, introduced by Sydow and Mitter (1935) consists of saprobic species reported from India, Japan, South America and Thailand (Tangthirasunun et al., 2015; Hyde et al., 2016b; Liu et al., 2019a; Farr & Rossman, 2019), from terrestrial habitats. Sutton (1980) mentioned *Ciliochorella mangiferae* as a common fungus found from both tropical and subtropical leaf litter.

Diploceras (Sacc.) Died., Mykol. Untersuch. Ber.: 342 (1915)

Index Fungorum number: IF8043; 12 morphological species (Species Fungorum, 2019), 1 species with sequence data

Type species: ***Diploceras hypericinum*** (Ces.) Died.

Notes: *Diploceras* was initially introduced as a subgenus of *Hyaloceras* by Saccardo (1892) and subsequently raised to generic rank by Diedicke (1915). Sutton (1975) re-classified this genus as a synonym of *Seimatosporium* after re-examining the type. Nag Raj (1993) resurrected *Diploceras* as a genus to accommodate *D. hypericinum*. Most other species previously classified as *Diploceras* were later included in *Allelochaeta* during a study based on multi-gene phylogeny by Crous et al. (2018). An emended description for this genus was provided by Liu et al. (2019a).

Disaeta Bonar, Mycologia 20: 299 (1928)

Index Fungorum number: IF8066; 1 morphological species with sequence data.

Type species: ***Disaeta arbuti*** Bonar

Notes: *Disaeta* was introduced by Bonar (1928) without mentioning the type species. The species is characteristic in producing 4-septate conidia, with bristle-like appendages (Bonar, 1928). Liu et al. (2019a) provided a representative strain for *D.*

arbuti isolated from *Acacia pycnantha* from Australia, along with an emended description and further citing the need for a taxonomic revision of this genus.

Discosia Lib. ex Durieu & Mont., Fl. d'Algérie, Cryptog. 1: 587 (1849)

Index Fungorum number: IF8072; 15 morphological species (Species Fungorum 2019), 9 species with sequence data.

Type species: ***Discosia artocreas*** (Tode) Fr.

Notes: Species of *Discosia*, mostly saprobes, have been recorded worldwide from terrestrial habitats on a various range of host plants (Farr & Rossman, 2019). Based on phylogenetic evidence, Liu et al. (2019a) synonymised the genus *Adiscio* under *Discosia*, the former which was introduced by Tanaka et al. (2011) to accommodate the sexual morph species of this genus. An epitype for the type species was designated by Liu et al. (2019a) as the original specimens were destroyed.

Distononappendiculata F. Liu, L. Cai & Crous (2019)

Index Fungorum number: IF828323; 3 morphological species with sequence data.

Type species: ***Distononappendiculata banksiae*** (Crous & Summerell) F. Liu, L. Cai & Crous.

Notes: *Distononappendiculata* species are characteristic in producing distoseptate conidia that lack appendages (Liu et al., 2019a). They are known to occur on leaves of *Banksia* sp. from Australia (Liu et al., 2019a).

Diversimediispora F. Liu, L. Cai & Crous (2019)

Index Fungorum number: IF828326; 1 morphological species with sequence data.

Type species: ***Diversimediispora humicola*** F. Liu, L. Cai & Crous.

Notes: *Diversimediispora* species produce conidia with versicolourous median cells where the second and third cells (from the apex) are darker, making it characteristically different from other genera in *Sporocadaceae* (Liu et al., 2019a).

Doliomyces Steyaert, Darwiniana 12(2): 169 (1961)

Index Fungorum number: IF11007; 3 morphological species.

Type species: ***Doliomyces senegalensis*** (Speg.) Steyaert

Notes: *Doliomyces* was initially placed in *Amphisphaeriaceae* (Kirk et al., 2008) but due to the lack of sequence data or cultures this placement was considered uncertain. Senanayake et al. (2015) listed *Doliomyces* in *Amphisphaeriales*, genera

incertae sedis. However, Wijayawardene et al. (2016b) classified this genus under *Bartaliniaceae* (now *Sporocadaceae*), based on its morphology and here, we too tentatively place *Doliomyces* in *Sporocadaceae*. The species are characterized in producing cylindrical to navicular, fusiform, 3–5-septate, conidia with dark brown median cells, bearing a single, unbranched, basal appendage and several branched, apical appendages (Nag Raj, 1993; Wijayawardene et al., 2016b). *Doliomyces* species have been found on several dicot plants from China, India and Kenya (Farr & Rossman, 2019).

Heterotruncatella F. Liu, L. Cai & Crous (2019)

Index Fungorum number: IF828340; 17 morphological species with sequence data.

Type species: ***Heterotruncatella lutea*** (H.J. Swart & D.A. Griffiths) F. Liu, L. Cai & Crous

Notes: *Heterotruncatella* was introduced by Liu et al. (2019a) to accommodate several new and previously described *Truncatella* species. Although morphologically similar, phylogenetically they were distinct from *Truncatella*. Most species have been recorded from Australia and Europe (Senanayake et al., 2015; Liu et al., 2019a; Farr & Rossman, 2019).

Hyalotiella Papendorf, Trans. Br. mycol. Soc. 50(1): 69 (1967)

Index Fungorum number: IF8588; 5 morphological species (Species Fungorum, 2019), 3 species with sequence data.

Type species: ***Hyalotiella transvalensis*** Papendorf

Notes: *Hyalotiella* was introduced by Papendorf and Du-Toit (1967). *Hyalotiella* species are characterized by their vase-shaped pycnidia, producing 3-septate, cylindrical conidia bearing more than one branched apical appendage but no basal appendages (Nag Raj, 1993; Li et al., 2015d; Senanayake et al., 2016). They occur as saprobes, and have been recorded from India, Italy, South Africa and South America (Nag Rai, 1993; Li et al., 2015d; Farr & Rossman, 2019).

Hymenopleella Munk, Dansk bot. Ark. 15(no. 2): 89 (1953)

Index Fungorum number: IF2416; 5 morphological species with sequence data.

Type species: ***Hymenopleella hippophaëicola*** Jaklitsch & Voglmayr

Notes: *Hymenopleella* is a sexual morph genus recorded from Africa, China and India (Jeewon et al., 2003a, Liu et al., 2019a). In the study by Liu et al. (2019a) the type species of *Dyrithiopsis* and *Neotruncatella* clustered together with *Hymenopleella* and therefore both genera were synonymized under the latter genus (Liu et al., 2019a).

Immersidiscosia Kaz. Tanaka, Okane & Hosoya, Persoonia 26: 94 (2011)

Index Fungorum number: IF519746; 1 morphological species with sequence data.

Type species: ***Immersidiscosia eucalypti*** (Pat.) Kaz. Tanaka, Okane & Hosoya

Notes: The genus was introduced by Tanaka et al. (2011) to accommodate *Immersidiscosia eucalypti* which has been recorded from France, Japan and Tunisia (Tanaka et al., 2011; Wijeyawardene et al., 2017a; Farr & Rossman, 2019).

Monochaetia (Sacc.) Allesch., Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1(7): 665 (1902)

Index Fungorum number: IF8971; 42 morphological species (Species Fungorum, 2019), 10 species with sequence data.

Type species: ***Monochaetia monochaeta*** (Desm.) Allesch.

Notes: Members of this genus have been reported worldwide, as saprobes, endophytes or pathogen, occurring on various hosts, especially on *Quercus* spp. (Maharachchikumbura et al., 2016b; de Silva et al., 2017; Wijayawardene et al., 2017a; Liu et al., 2019a; Farr & Rossman, 2019).

Morinia Berl. & Bres., Annuario Soc. Alpinisti Trident., 1887-88: 82 (1889)

Index Fungorum number: IF8997; 4 morphological species with sequence data.

Type species: ***Morinia pestalozzioides*** Berl. & Bres.

Notes: *Morinia* species are characterised in having transversely or muriformly septate conidia (Collado et al., 2006). They are recorded from terrestrial habitats (Wijayawardene et al., 2017a; Liu et al., 2019a). The genus *Zetiaspizna* was combined under *Morinia* based on phylogeny and morphological similarity (Liu et al., 2019a).

Nonappendiculata F. Liu, L. Cai & Crous, Stud. Mycol. 92: 358 (2018)

Index Fungorum number: IF828374; 1 morphological species with sequence data.

Type species: Nonappendiculata quercina F. Liu, L. Cai & Crous

Notes: Nonappendiculata, introduced by Liu et al. (2019a) produces 3-septate, fusoid conidia, bearing no appendages. It is known to occur on *Quercus* from Italy (Liu et al., 2019a).

Neopestalotiopsis Maharachch., K.D. Hyde & Crous, Stud. Mycol. 79: 135 (2014)

Index Fungorum number: IF809759; 36 species with sequence data.

Type species: Neopestalotiopsis protearum (Crous & L. Swart) Maharachch., K.D. Hyde & Crous

Notes: Neopestalotiopsis, established by Maharachchikumbura et al. (2014b) is widespread, occurring as saprobes or pathogens on various host plants (Maharachchikumbura et al., 2014b; Farr & Rossman, 2019). The genus is distinct from *Pestalotiopsis* morphologically in having versicolourous median cells.

Parabartalinia F. Liu, L. Cai & Crous, Stud. Mycol. 92: 358 (2018)

Index Fungorum number: IF828376; 1 morphological species with sequence data.

Type species: Parabartalinia lateralis F. Liu, L. Cai & Crous

Notes: This genus was introduced by Liu et al. (2019a) and is morphologically similar to *Bartalinia*, yet phylogenetically distinct. It is recorded from South Africa on *Acacia* sp. (Liu et al., 2019a).

Pestalotiopsis Steyaert, Bull. Jard. bot. État Brux. 19: 300 (1949)

Index Fungorum number: IF9272; over 250 morphological species (Species Fungorum, 2019), 73 species with sequence data.

Type species: Pestalotiopsis guepinii (Desm.) Steyaert

Notes: Pestalotiopsis was erected by Steyaert (1949). *Pestalotiopsis* species occur worldwide, as saprobes, endophytes or opportunistic pathogens on economically important plants as well as ornamental crops (Maharachchikumbura et al., 2014b, 2016b). The diversity and phylogenetic affinities based on ITS, *tub2* and *tefl* gene regions of this genus has been discussed comprehensively in several studies (Jeewon et al., 2003a; Maharachchikumbura et al., 2011, 2012, 2013, 2014a; Liu et al., 2019a).

Pseudopestalotiopsis Maharachch., K.D. Hyde & Crous, Stud. Mycol. 79: 180 (2014)

Index Fungorum number: IF809753; 16 morphological species with sequence data.

Type species: ***Pseudopestalotiopsis theae*** (Sawada) Maharachch., K.D. Hyde & Crous

Notes: *Pseudopestalotiopsis* species are widely distributed as saprobes or pathogens, occurring on leaves (Maharachchikumbura et al., 2014b, 2016a). They are characteristic in having conidia with concolourous, brown to dark brown or olivaceous median pigmented cells (Maharachchikumbura et al., 2014b).

Pseudosarcostroma F. Liu, L. Cai & Crous, Stud. Mycol. 92: 374 (2018)

Index Fungorum number: IF7657; 1 species based on molecular data.

Type species: ***Pseudosarcostroma osyridicola*** F. Liu, L. Cai & Crous

Notes: *Pseudosarcostroma*, introduced by Liu et al. (2019a) is phylogenetically related to *Broomella*, *Bartalinia*, *Truncatella*, *Parabartalinia*, and *Diversimediispora*. It shares morphologically similar characters with *Sarcostroma*, such as having conidia bearing a single appendage at each end, with an undulate or verruculose conidial wall (Liu et al., 2019a). It is recorded from France on *Osyris alba* (Liu et al., 2019a).

Robillarda Sacc., Michelia 2(no. 6): 8 (1880)

Index Fungorum number: IF22470; 38 morphological species (Species Fungorum, 2019), 5 species with sequence data.

Type species: ***Robillarda sessilis*** (Sacc.) Sacc.

Notes: The genus was introduced by Saccardo (1880b) and are characterized by their flexuous, narrow tubular, aseptate appendages and holoblastic conidiogenous cells, proliferating sympodially or percurrently near the apex (Crous et al., 2015a; Wijayawardene et al., 2016a). *Robillarda* species have been reported worldwide, as saprobes (Wijayawardene et al., 2017a; Farr & Rossman, 2019). Most recently, *Robillarda mangiferae* was introduced from leaf blight on mango in Yunnan, China (Phookamsak et al., 2019).

Sarcostroma Cooke, Journal of the Quekett microsc. Club 2: 267 (1871)

Index Fungorum number: IF7657; 28 morphological species (Species Fungorum, 2019), 9 species with sequence data.

Type species: ***Sarcostroma berkeleyi*** Cooke

Notes: Species of this genus are known to occur as saprobes, endophytes, pathogens on leaves in both tropical and temperate regions (Maharachchikumbura et al., 2016b; Norphanphoun et al., 2015; Farr & Rossman, 2019). *Sarcostroma* was resurrected to accommodate some several species characterised by fusoid conidia with four or more cells, having pigmented median cells and paler, thin-walled end cells, bearing an attenuated tubular apical appendage and a similar excentric basal appendage (Nag Raj, 1993). The holotype of the genus has been reported lost and epitypification is needed (Liu et al., 2019a).

Seimatosporium Corda, Deutschl. Fl., 3 Abt. (Pilze Deutschl.) 3(13): 79 (1837)

Index Fungorum number: IF9865; 77 morphological species (Species Fungorum, 2019), 9 species with sequence data.

Type species: ***Seimatosporium rosae*** Corda

Notes: *Seimatosporium* species are widely distributed, occurring as saprobes or pathogens (Norphanphoun et al., 2015; Maharachchikumbura et al., 2016b; Wijayawardene et al., 2017a; Wanasinghe et al., 2018). It was introduced by Corda (1833). They have been linked to *Discostroma* sexual morphs phylogenetically (Tanaka et al., 2011) but as there is a lack of sequence data for the type species, hence this requires further study.

Seiridium Nees, Syst. Pilze (Würzburg): 22 (1816)

Index Fungorum number: IF9868 38 morphological species (Species Fungorum, 2019), 20 species with sequence data.

Type species: ***Seiridium marginatum*** Nees

Notes: *Seiridium* are characterized in producing 6-celled conidia (Jeewon, 2003a; Maharachchikumbura et al., 2014b) and are widely distributed as saprobes and mainly plant pathogens (Tsopelas et al., 2007). The genus was established by Nees (1816). *Blogiascospora* and *Lepteutypa* have identified as the sexual morph (Senanayake et al., 2015). Jaklitsch et al. (2016b) epitypified *Seiridium marginatum* and confirmed its sexual morph as *Blogiascospora marginata* based on molecular data.

Seiridium marginatum Nees, Syst. Pilze (Würzburg): 23 (1816)

Index Fungorum number: IF827453; *Facesoffungi number:* FoF06881; Fig.

Saprobic on twigs, branches of dicotyledons. **Sexual morph:** Unknown. **Asexual morph:** *Conidiomata* pycnidial to sporodochial, mostly solitary, immersed, unilocular, conic or subglobose with flattened base, dark brown to black. *Conidiomata wall* comprising thin-walled cells of *textura angularis*, sometimes fusing at the base into the host tissue. *Conidiophores* septate, cylindrical, branched, hyaline or pale brown, thin-walled. *Conidiogenous cells* discrete, hyaline, cylindrical, smooth- and thin-walled, proliferating percurrently, with visible collarettes and minute periclinal thickenings. Conidia 22–28 × 7–9 µm ($\bar{x}$ = 26 × 8 µm), lunate to falcate, curved, 5-septate, rarely 4- or 6-septate, not striate, bearing one appendage, euseptate with no visible pores, basal cell obconic with a truncate base, hyaline, walls smooth, 3.5–4 µm; four median cells, smooth, cylindrical to doliform, brown to dark brown, and septa darker than the rest of the cells, second cell from base 5–6 µm long, third cell 5–6 µm long, fourth cell 5–6 µm long, fifth cell 4.5–5.5 µm long, apical cell conical, hyaline, smooth, 3.5–4 µm long, apical appendage single, mostly centric, up to 12 µm long.

Material examined: ITALY, Forli-Cesena Province, near Camposonardo - Santa Sofia, on dead and land cone of *Picea excelsa*, 15 April 2015, Erio Camporesi IT2444, MFLU 15-0813; living culture KUMCC 16-0115.

Notes: Our examined specimen, illustrated here, morphologically resembles *Seiridium spyridicola* as described in Nag Raj (1993). Based on separate analysis of molecular data consisting of ITS and LSU genes, our isolate clustered together with *S. spyridicola* strain CBS 142628, therefore it is identified as this species, based on morphology and phylogeny. Our collection of *S. spyridicola* is collected from Italy on *Picea excelsa*. This is the first record of a *Seiridium* species on this host.

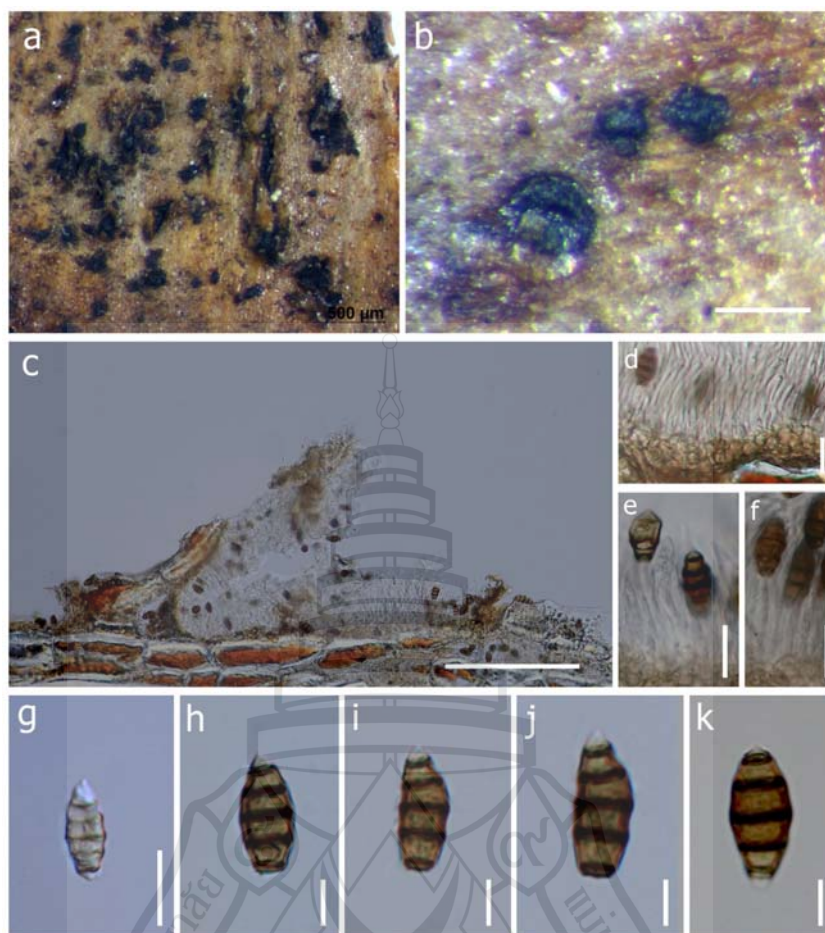


Figure D3 *Seiridium spyridicola* (MFLU 15-0813) a. Appearance on host, b. Close up of several conidiomata, c. Section of conidioma, d. Wall of conidioma, e, f. Conidiogenous cells giving rise to conidia, f. g. Conidiophores with conidia, h–k. Conidia (upside down). Scale bars: c = 1 mm, d = 100 µm, e–l = 20 µm.

Sporocadus Corda, Icon. fung. (Prague) 3: 23 (1839)

Index Fungorum number: IF10026; 49 morphological species (Species Fungorum, 2019), 13 species with sequence data.

Type species: Sporocadus lichenicola Corda.

Notes: *Sporocadus* was established by Corda (1839b) to include four species, but with no mention of the type. Hughes (1958) lectotypified *Sporocadus* based on *S. lichenicola*. The genus was once synonymised under *Seimatosporium* by Sutton

(1975) but later classified as a distinct genus by Brockman (1976) and Nag Raj (1993). Liu et al. (2019a) showed that both *Sporocadus* and *Seimatosporium* formed separate clades and that the species in *Sporocadus* clade all lacked appendages justifying it as a morphologically and phylogenetically distinct genus.

Strickeria Körb., Parerga lichenol. (Breslau) 5: 400 (1865)

Index Fungorum number: IF5283; 10 morphological species (Species Fungorum, 2019), 1 species with sequence data.

Type species: ***Strickeria kochii*** Körb.

Notes: *Strickeria* was introduced and described as a lichenised fungus by Körber (1865). It was often classified as a Dothideomycete and upon re-examination of the type material, it was placed under *Sordariomycetes incertae sedis* (Eriksson & Hawksworth, 1991; Lumbsh & Huhndorf, 2010a, b). Subsequently this genus was classified in *Xylariales* by Jaklitsch et al. (2016b).

Synnemapestaloides T. Handa & Y. Harada, Mycoscience 45:138 (2004)

Index Fungorum number: IF28803; 2 morphological species with sequence data.

Type species: ***Synnemapestaloides rhododendri*** T. Handa & Y. Harada.

Notes: *Synnemapestaloides* species are morphologically distinct in producing spores on synnemata (Watanabe et al., 2016). They are known to occur on *Juniperus* and *Rhododendron* (Liu et al., 2019a).

Truncatella Steyaert, Bull. Jard. bot. État Brux. 19: 293 (1949)

Index Fungorum number: IF10328; 11 morphological species (Species Fungorum, 2019), 1 species with sequence data.

Type species: ***Truncatella truncata*** (Lév.) Steyaert

Notes: *Truncatella* was established by Steyaert (1949) to accommodate distinct species having 3-septate, verruculose, pigmented conidia (Steyaert, 1949; Jeewon et al., 2002; Maharachchikumbura et al., 2016b; Tibpromma et al., 2017b). The sexual morph of *Truncatella* remains unresolved, however Senanayake et al. (2015) illustrated both the sexual and asexual morph for *T. spartii*. They occur worldwide as saprobes on various dicots and monocot plants (Farr & Rossman, 2019).

Xenoseimatosporium F. Liu, L. Cai & Crous, Stud. Mycol. 92: 411 (2018)

Index Fungorum number: IF828424; 1 morphological species with sequence data.

Type species: ***Xenoseimatosporium quercinum*** (Goonas. et al.) F. Liu, L. Cai & Crous

Notes: This genus was introduced by Liu et al. (2019a) to accommodate the previously described species *Seimatosporium quercinum* on *Quercus* from Germany (Goonasekara et al., 2016). It is phylogenetically close to the genera *Allelochaeta* and *Sarcostroma*. *Xenoseimatosporium* is distinct from *Seimatosporium* in producing allantoid and subcylindrical conidia with ragged appendages (Liu et al., 2019a).



APPENDIX E

NOTES FOR OUTLINE OF ASCOMYCOTA

Acarosporium Bubák & Vleugel ex Bubák (1911), *Sclerotiniaceae*, *Helotiales*, Leotiomyces, seven species, type specie *Acarosporium sympodiale* Bubák & Vleugel ex Bubák, sexual morph *Pycnopeziza* White & Whetzel ex Sutton, saprobes, terrestrial, distributed in Americas and Europe, see Johnston et al. (2014; nomenclature), no sequence data available.

Acrogynomyces Thaxt. (1931), *Laboulbeniaceae*, *Laboulbeniales*, Laboulbeniomyces, six species, type specie *Acrogynomyces eumicri* Thaxt, parasitic on African scydmaenid genus *Eumicrus*, See Thaxter (1931; morphology), no recent studies reported.

Amazonia Theiss. (1913) *Meliolaceae*, *Meliolales*, *Sordariomyces*, more than 70 species, type specie *Amazonia psychotriae* (Henn.) Theiss., asexual morph undetermined, pathogens causing black mildew disease, terrestrial, tropical and subtropical distribution, See Hosagoudar & Archana (2009; host distribution in India), Hosagoudar et al. (2010; new species, morphology), Hosagoudar and Robin (2011; new species, morphology), Hosagoudar (2013a, b; new species, morphology), LSU sequence data available for *A. acacia*.

Amphicytostroma Petr. (1921) *Gnomoniaceae*, *Diaporthales*, *Sordariomyces*, two species, type specie *Amphicytostroma tiliae* (Sacc.) Petr., sexual morph *Amphiporthe*, saprobes, terrestrial, Europe, see Rossman et al. (2015; nomenclature), no sequence data available.

Amphorulopsis Petr. (1959), family *incertae sedis*, order *incertae sedis*, *Sordariomyces*, one specie, type specie *Amphorulopsis polygonacearum* Petr. 1959, asexual morph undetermined, terrestrial, former Yugoslavia, no recent studies reported.

Amylis Speg. (1922), family *incertae sedis*, order *incertae sedis*, Sordariomycetes, one specie, type specie *Amylis memorabilis* Speg., asexual morph undetermined, saprobes, terrestrial, South America, no recent studies reported.

Anisomyces Theiss. & Syd. (1914), Gnomoniaceae, Diaporthales, Sordariomycetes, seven species, type specie *Anisomyces papilloideoseptatus* (Henn.) Theiss. & Syd., asexual morph undetermined, saprobes?, terrestrial, tropical America, no recent studies reported.

Anthostomaria (Sacc.) Theiss. & Syd. (1918), family *incertae sedis*, order *incertae sedis*, Sordariomycetes, one specie (type) *Anthostomaria apogyra* (Nyl.) Theiss. & Syd., asexual morph undetermined, lichenicolous, terrestrial, former USSR, no recent studies reported.

Anthostomellina L.A. Kantsch. (1928), family *incertae sedis*, order *incertae sedis*, Sordariomycetes, two species, type specie *Anthostomellina carpinea* L.A. Kantsch., asexual morph undetermined, saprobes, terrestrial, former USSR, no recent studies reported.

Apharia Bonord. (1864), family *incertae sedis*, order *incertae sedis*, Sordariomycetes, one specie, type specie *Hercospora nigrescens* Bonord., asexual morph undetermined, Europe, no recent studies reported.

Apiosporopsis (Traverso) Mariani 1911, family *incertae sedis*, order Diaporthales, Sordariomycetes, three species, type specie *Apiosporopsis saccardoana* Mariani (as '*saccardiana*'), asexual morph undetermined, saprobes, endophytes, terrestrial, Dominican Republic, Europe, India, Japan, see Osono and Masuya (2012; diversity on Betulaceae), Keča et al. (2015; diversity on Poplars), LSU and SSU sequence data available for *Apiosporopsis carpinea*.

Armatella Theiss. & Syd. 1915, family Armatellaceae, order Meliolales, Sordariomycetes, 20 species, type specie *Armatella litseae* (Henn.) Theiss. & Syd., asexual morph undetermined, epiphytes, terrestrial, worldwide distribution, see Hosagoudar and Archana (2009; distribution on Lauraceae), Hosagoudar et al. (2013; new species), Hongsanan et al. (2015, family placement), Maharachchikumbura et al. (2016; morphology and classification), no sequence data available.

Armatellaceae Hosag. 2003, order Meliolales, Sordariomycetes, one genus, type genus *Armatella* Theiss. & Syd. See Hosagoudar and Archana (2009,

distribution), Hongsanan et al. (2015, morphology and classification), Maharachchikumbura et al. (2016; morphology and classification).

Arniella Jeng & J.C. Krug (1977), family *Lasiosphaeriaceae*, order *Sordariales*, Sordariomycetes, 2 species, type specie *Arniella echoura* Jeng & J.C. Krug (as 'exoura'), asexual morph undetermined, coprophilous, USA, Venezuela, no recent studies reported.



APPENDIX F

CONTRIBUTIONS TO TAXONOMY AND PHYLOGENY OF DEMATIACEOUS COELOMYCETES

Herbarium illustrations

Dinemasporium nelloi W.J. Li, Camporesi & K.D. Hyde, in Liu et al., Fungal Diversity: 10.1007/s13225-015-0324-y, [18] (2015)

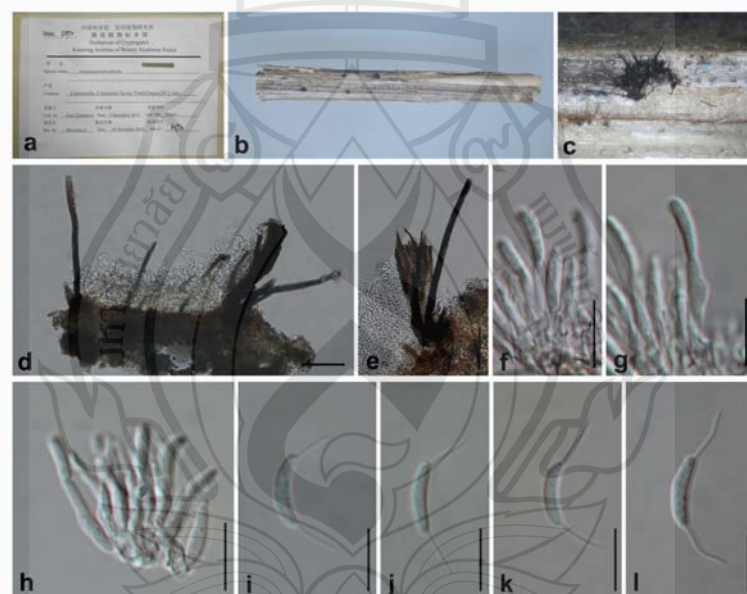


Figure F1 *Dinemasporium nelloi* (Material examined: ITALY, Province of Forlì-Cesena [FC], Castrocaro Terme, Converselle, on dead stem of *Dactylis glomerata*, 1 December 2012, E. Camporesi, KUN! HKAS 83970, isotype). a Herbarium label and material. b Conidiomata on specimen. c Close up of conidioma. d Vertical section of conidioma. e Setae f-h Conidia attached to conidiogenous cells. i-l Conidia. Scale bars: a-c = not to scale, d = 100 μ m, e = 50 μ m, f-l = 10 μ m.

Melnikia anthoxanthii Wijayaw., Goonas., Camporesi, D.J. Bhat & K.D. Hyde, sp. nov.



Figure F2 *Melnikia anthoxanthii* (holotype). a, b Conidiomata on host material. c, d Vertical sections of conidiomata. e–g Different stages of conidiogenesis. h–q Conidia. Scale bars: c, d=100 µm, e–g=10 µm, h–q=5 µm

Monochaetia monochaeta (Desm.) Allesch., Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1(7): 667 (1902) [1903]

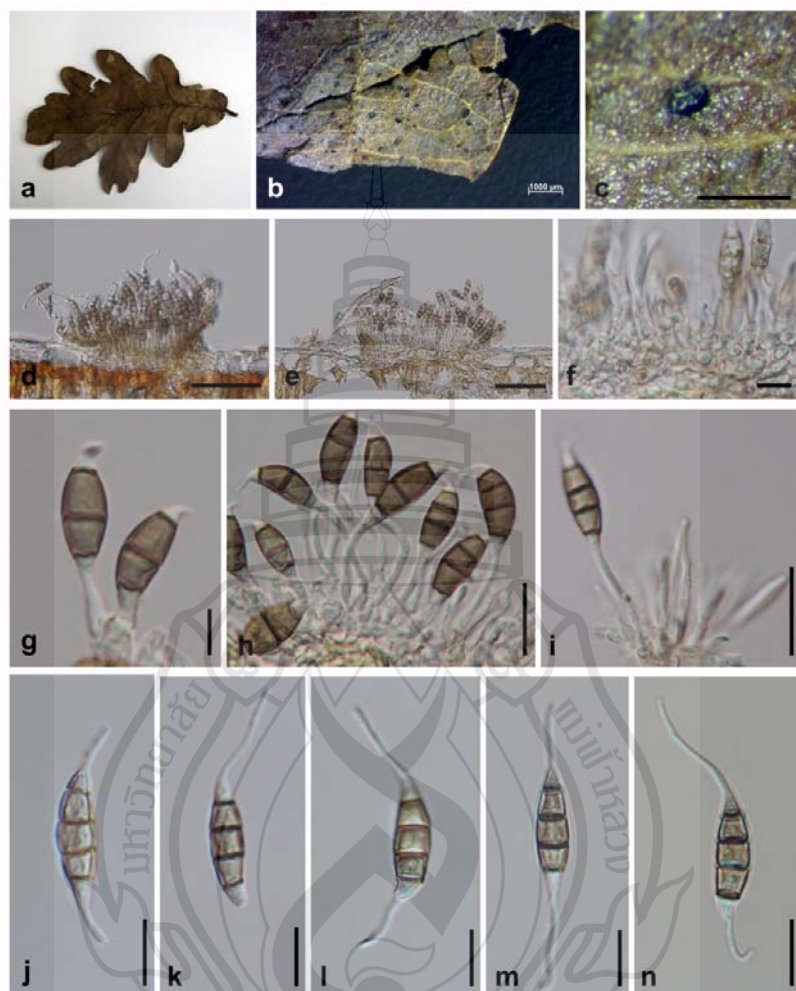


Figure F3 *Monochaetia monochaeta* (Material examined: Germany, on leaf of *Quercus robur*, 21 December 2013, René K. Schumacher, MFLU 16–0002). a Host specimen. b, c Conidiomata on host and close up of conidioma. d, e Vertical section of conidioma. f Section of conidioma wall. g–i Conidia arising from conidiogenous cells. j–n Conidia. Scale bars: a = not to scale, c = 100 μm , d, e, h, i = 50 μm , e = 100 μm , f, j–n = 10 μm , g = 20 μm .

APPENDIX G

NOTES ON GENERA DICHOTOMOHTHORA AND PHAEOSPHAERIOPSIS

Dichotomophthora Mehrl. & Fitzp. ex M.B. Ellis, Dematiaceous Hyphomycetes (Kew): 388 (1971)

Background

Dichotomophthora was introduced by Mehrlich and Fitzpatrick (1935) to accommodate the hyphomycetous species *D. portulacae*, causing leaf spots on common purslane (*Portulaca oleracea*) in Hawaii. However, it was not validly published and Rao (1966) attempted to validate it. Due to misapplications, this attempt was still unsuccessful and the name was considered invalid until Ellis (1971) validly published it based on the holotype specimen of *D. portulacae* (Marin-Felix et al., 2019). A second species *D. lutea* was introduced by De Hoog and van Oorschot (1983). The classification of *Dichotomophthora* in Pleosporaceae, order Pleosporales was confirmed by Marin-Felix et al. (2019) based on multi-gene phylogenetic analysis. In addition, two species *D. basellae* and *D. brunnea* were introduced, making the total number of accepted species as four for this genus (Marin-Felix et al., 2019). *Dichotomophthora* species have been commonly reported as plant pathogens on various hosts (mostly *P. oleracea*), as saprobes and soil inhabiting fungi and as a causative organism of human keratitis (Mehrlich & Fitzpatrick, 1935; Ellis, 1971; De Hoog & Oorschot, 1983; Klisiewicz et al., 1983; Alfieri et al., 1984; Baudoin, 1986; Pfeiffer et al., 1989; De Hoog et al., 2000; Eken, 2003; Soares & Nechet, 2017; Heidari et al., 2018; Marin-Felix et al., 2019; Farr & Rossman, 2022).

Classification – Ascomycota, Dothideomycetes, Pleosporales, Pleosporaceae

Type species – *Dichotomophthora portulacae* Mehrl. & Fitzp. ex M.B. Ellis 1971

Distribution – worldwide

Disease symptoms – crown and root rot, black stem, stem blight, leaf spots, foliar abscission, seed rot and damping-off.

The soil inhabiting *Dichotomophthora portulacae* is known for causing rot, blight and dieback of the stems and leaf spots in *Portulaca oleracea* (Merhlich & Fitzpatrick, 1935; Ellis, 1971; Klisiewicz et al., 1983; Mitchell, 1986; Baudoin, 1986). The disease symptoms are characterized by necrotic, oval to irregular, pale brown spots with darker borders (Kliseiwickz, 1985; Heidari et al., 2018). Klisiewicz et al. (1983) observed that the stems of purslane plants especially at the soil line, showed dark discolouration and constriction. The development of lesions was more frequently observed in parts of the stem that were in contact with the soil and the roots usually were infected by the same pathogen infecting the stems (Klisiewicz et al., 1983). Eken (2003) reported *D. portulacae* to cause crown and root rot of purslane. During experimental studies, symptoms of seed rot and post-emergence damping-off were observed within eight days, where all the seedlings died. In pathogenicity studies with plants, sunken, necrotic lesions became apparent on leaves and stems within forty-eight hours of inoculation. Later, the leaves turned yellow and dropped, while in the stems, dark lesions developed causing dieback (Eken, 2003). *Dichotomophthora portulacae* is more likely to cause death of its host plants when infected at a young age (Kliseiwickz et al., 1983). *Dichotomophthora lutea* was reported as a fungus that colonized wounded tissue surrounding the graft area of *Myrtillocactus geometrizans* (in a commercial greenhouse) where brown to black lesions occurred. The infection resulted in a grayish dry rot and gradually the collapsing of the tissue (Pfeiffer et al., 1989).

Hosts– *Dichotomophthora portulacae* was first reported from Hawaii, USA on *Portulaca oleracea* (Mehrllich & Fitzpatrick, 1935). Other hosts include *Basella rubra*, *Nicotiana glauca*, *Portulaca grandiflora* and *P. lutea* (Ellis, 1971; Raabe et al., 1981; De Hoog & Oorschot, 1983; Richardson, 1990; Jing et al., 2008; Camino-Vilaro et al., 2019; Marin-Felix et al., 2019; Farr & Rossman, 2022). *Dichotomophthora lutea* has been isolated from *Beta vulgaris* (Minter et al., 2001), from soil, on seeds, stems, leaves and roots of *Anredera cordifolia*, *Portulaca oleracea*, *Gymnocalycium mihanovichii* var. *friedrichii* and *Myrtillocactus geometrizans* (De Hoog & Oorschot, 1983; Miller, 1990; Soares & Nechet, 2017;

Heidari et al., 2018; Camino-Vilaro et al., 2019; Marin-Felix et al., 2019) in various regions, and from the seedbed of *Pinus radiata* in Italy (Heidari et al., 2018, Marin-Felix et al., 2019). *Dichotomophthora basellae* was isolated from leaves of *Basella alba* in Thailand (Marin-Felix et al., 2019). The location and substrate of *D. brunnea* is unknown (Marin-Felix et al., 2019).

Pathogen biology, disease cycle and epidemiology

Dichotomophthora portulacae is often the major pathogen on purslane (Farr & Rossman, 2022). It has been listed as an introduced plant pathogen into the United Kingdom (Jones & Baker, 2007). According to Klisiewicz (1985) the fungus produces sclerotia and conidia on both artificial media and dead plant tissues. The sclerotia help the fungus to survive in the soil and act as an inoculum for basal stem infection (Klisiewicz, 1985). Once the stem is infected by *D. portulacae* the plant may become more prone to damage by insects or larvae. When evaluated for its growth, reproduction (in culture media) and biological activity, the results showed that *D. portulacae* prefers a temperature range of 27–33 °C, and moist, humid conditions. This indicated that the fungus is less likely to infect plants where there is a lack of rain or moisture (Klisiewicz, 1985). Once infected, the disease is able to develop regardless of the environmental conditions, leading to rapid death especially in seedlings (Klisiewicz, 1985; Mitchell, 1986).

Dichotomophthora portulacae could also infect carpetweed (*Mollugo verticillata*) but experimental results showed only mild symptoms (Mitchell, 1986). In China, it was reported as an endophyte in Malabar spinach (*Basella rubra*) by Jing et al. (2008). The possibility of using *D. portulacae* as a biological control agent to control weed, especially in areas with heavy rain and high humidity has been mentioned and investigated to an extent (Rader 1948; Klisiewicz et al., 1983; Klisiewicz, 1985; Baudoin, 1986; Vink & Elsas, 2017). Alves et al. (2020) investigated the endophytic fungi obtained from *Jatropha curcas* for their bioactivity and among them two strains of *Dichotomophthora* showed promising antioxidant activities. Hence, it is likely that further studies focused on possible bioactive compounds of *Dichotomophthora* will lead to a better understanding of this genus.

Morphological based identification and diversity

Dichotomophthora species are characterized by their macronematous, mononematous, dichotomously branched, irregularly or unbranched conidiophores, and simple, ellipsoidal to cylindrical, subhyaline, pale brown to dark brown conidia with 1–5 septa. The sexual morph is not known (Ellis, 1971; Marin-Felix et al., 2019). The colonies of both *D. basellae* and *D. lutea* produce luteous, orange, diffusible pigments in culture (Marin-Felix et al., 2019). Several reports of *Dichotomophthora* have remained uncertain due to fungal or host misidentifications, such as *D. portulacae* being confused with *D. lutea* in previous studies (de Hoog & van Oorschot, 1983; Soares & Nechet, 2017; Vink & Elsas, 2017; Marin-Felix et al., 2019). Therefore, more collections are needed to investigate their diversity.

Molecular based identification and diversity

In Heidari et al. (2018) a combination of ITS and RPB2 sequence data was used to identify the pathogenic strains of *D. lutea* from Iran. The study by Marin-Felix et al. (2019) included various strains of *Dichotomophthora* isolated from different hosts and regions and a combination of the ITS, LSU, GAPDH and RPB2 molecular data. As a result, four *Dichotomophthora* species (including two new species) were confirmed. In our phylogenetic analysis presented we included two strains of *Dichotomophthora* sp. (DJS_770 and DJS_770) isolated by Soares and Nechet (2017) from *Anredera cordifolia*. Both isolates were mentioned to resemble *D. lutea* morphologically but their identification was not confirmed due to lack of molecular data at that time. They form a separate clade sister to the *D. lutea* strains. Although listed in Index Fungorum (2022), there was no description or molecular data available for the fungus named *D. cactacearum*.

Recommended genetic markers (genus level) – ITS

Recommended genetic markers (species level) – ITS, RPB2, GAPDH

Accepted number of species – There are five epithets listed in Index Fungorum (2022), however only **four** species are accepted with molecular data

Number of species with pathogenicity data – two

References – Mehrlich and Fitzpatrick (1935), Routien (1957), Rao (1966), Ellis (1971), de Hoog and van Oorschot (1983), Marin-Felix et al. (2019)

(morphology), Marin-Felix et al. (2019; phylogeny and accepted species numbers), Klisiewicz et al. (1983), Klisiewicz (1985), Baudoin (1986), Mitchell (1986), Pfeiffer et al. (1989), Eken (2003), Soares and Nechet (2017; pathogenicity)

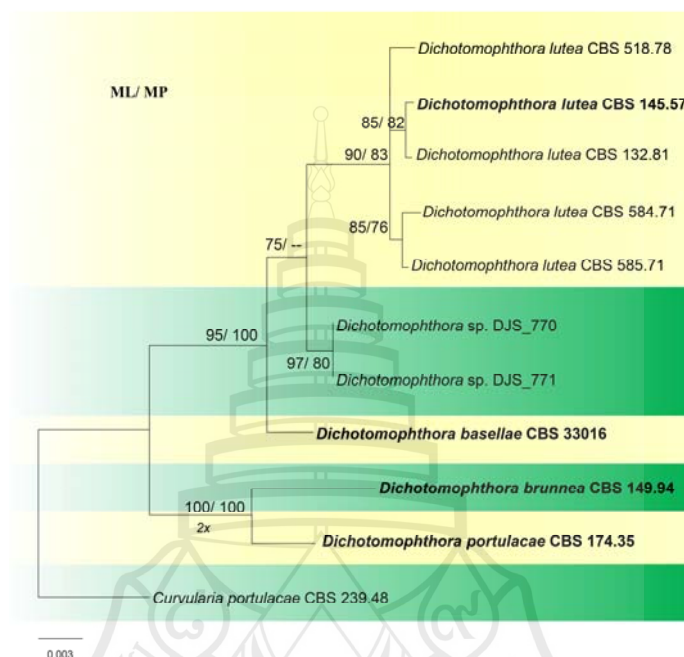


Figure G1 Maximum likelihood of *Dichotomophthora* species based on the concatenated ITS, LSU, GAPDH and RPB2 regions. Likelihood of the best scoring ML tree was -4658.194918. Estimated base frequencies were as follows; A = 0.264872, C = 0.163069, G = 0.213432, T = 0.358627; substitution rates AC = 1.743629, AG = 3.558942, AT = 1.890244, CG = 3.790110, CT = 6.675467, GT = 1.000000. The maximum parsimonious dataset consisted of a total 2784 characters, 707 constant, 2065 parsimony-informative and 12 parsimony-uninformative characters. The parsimony analysis of the data matrix resulted in the maximum of nine equally most parsimonious trees with a length of 3034 steps (CI = 0.711, RI = 0.735, RC = 0.522, HI = 0.289). ML and MP bootstrap support values over 75% are indicated. Type and Para-type strains are in bold. Scale bar indicates number of substitutions per site. The tree was rooted to *Curvularia portulacae* CBS 239.48.

Table G1 GenBank accession numbers of *Dichotomophthora* species used for phylogenetic analysis. Ex-type (T) /ex-paratype (PT) strains are in bold (N/A – sequences not available). Species confirmed with pathogenicity studies are marked with #.

Species	Strain	GenBank accession numbers			
		ITS	LSU	GAPDH	RPB2
<i>Curvularia portulacae</i>	CBS 239.48	MH856324	MH867878	KM083616	N/A
<i>Dichotomophthora basellae</i>	CPC 33016^T	LT990654	LT990626	LT990670	LT990640
<i>D. brunnea</i>	CBS 149.94^T	LT990653	N/A	LT990669	LT990639
<i>D. lutea</i> [#]	CBS 145.57^T	LT990647	NG_069497	LT990663	LT990634
	CBS 584.71	LT990648	LT990620	LT990664	LT990635
	CBS 585.71	LT990649	LT990621	LT990665	LT990636
	CBS 518.78	LT990650	LT990622	LT990666	N/A
	CBS 132.81	LT990651	LT990623	LT990667	LT990637
<i>D. portulacae</i> [#]	CBS 174.35^{PT}	LT990652	MH867137	LT990668	LT990638
<i>Dichotomophthora</i> sp.	DJS_771	MF196162	N/A	N/A	N/A
	DJS_770	MF196163	N/A	N/A	N/A

Phaeosphaeriopsis M.P.S. Câmara, M.E. Palm & A.W. Ramaley, in Câmara, Ramaley, Castlebury & Palm, Mycol. Res. 10(5): 51 (2003)

Background

Phaeosphaeriopsis was established by Câmara et al. (2003) to include several *Paraphaeosphaeria* species which could not be categorized as *Paraphaeosphaeria* based on their morphological characters and SSU molecular data. Câmara et al. (2003) transferred *Paraphaeosphaeria agavensis*, *Pa. glauco-punctata*, *Pa. nolineae* and *Pa. obtusispora* to *Phaeosphaeriopsis* and described a new species *P. amblyspora*. *Phaeosphaeriopsis* represents species that produce uni- or multi-loculate stromata, 4–5-septate, punctate or verrucose ascospores and have asexual morphs characterized by 0–3-septate, cylindrical, brown conidia or sometimes bacillar microconidia (Câmara et al., 2003; Phookamsak et al., 2014; Thambugala et al., 2014; Marin-Felix et al., 2011).

Classification – Ascomycota, Dothideomycetes, Pleosporales, Phaeosphaeriaceae

Type species – *Phaeosphaeriopsis glaucopunctata* (Grev.) M.P.S. Câmara, M.E. Palm & A.W. Ramaley (2003)

Distribution – worldwide

Disease symptoms – associated with leaf spots and leaf necrosis

Phaeosphaeriopsis glauco-punctata has been reported as the causative organism (as “*Paraphaeosphaeria glauco-punctata*” in earlier studies) of leaf spot and necrosis on *Ruscus* (Camara et al., 2001). The symptoms described are 2mm wide, discoloured lesions that gradually develop into larger brown necrotic lesions. When infection is severe, these lesions coalesce and form large necrotic regions sometimes leading to the complete leaf necrosis (Camara et al., 2001; Golzar & Wang, 2012).

Hosts –*Phaeosphaeriopsis glauco-punctata*, *P. triseptata* has been reported on various *Ruscus* spp. from Europe. *Phaeosphaeriopsis amblyspora* was introduced from dead leaves of *Yucca baccata* (Câmara et al., 2003). *Phaeosphaeriopsis agapanthi* was isolated from *Agapanthus precox*. *Phaeosphaeriopsis agavacearum* and *P. pseudoagavacearum* have been reported from *Agave* sp. (Crous et al., 2016a, b). *Phaeosphaeriopsis aloes* and *P. aloicola*, were both recorded from leaves of *Aloe* sp. in USA (Marin-Felix et al., 2001). *Phaeosphaeriopsis musae* was associated with leaf spots on *Musa* sp. (Arzanlou & Crous, 2006). *Phaeosphaeriopsis dracaenicola* is reported from *Dracaena loureiroi* in Thailand (Phookamsak et al., 2014) while *Phaeosphaeriopsis grevilleae* was collected on leaves of *Grevillea* sp. from Queensland, Australia (Marin-Felix et al., 2001). *Phaeosphaeriopsis beaucarneae* was isolated from dead leaves of *Beaucarnea recurvata* (Tennakoon et al., 2020). *Phaeosphaeriopsis omaniana*, *P. sansevieriae* and *P. triseptata* were isolated from leaves of *Dracaena serrulata*, *Sansevieria hyacinthoides* and *Ruscus aculeatus*, respectively (Thambugala et al., 2014; Al-Jaradi et al., 2020; Crous et al., 2021).

Pathogen biology, disease cycle and epidemiology

Phaeosphaeriopsis consists mostly of saprobic species. However, *Phaeosphaeriopsis glaucopunctata* has been reported to cause leaf spot and necrosis in *Ruscus aculeatus* (Câmara et al., 2003; Golzar & Wang, 2012). *Phaeosphaeriopsis agapanthi* on *Agapanthus precox* and *P. dracaenicola* on *Dracaena lourieri* have been associated with causing necrotic leaf spots on these hosts (Phookamsak et al., 2014; Crous et al., 2016). *Phaeosphaeriopsis musae* is assumed to be a weak

pathogen or secondary invader (Arzanlou & Crous, 2006) as it has often been found associated with leaf lesions of *Mycosphaerella fijiensis*, *Sphaerulina musae* and *Cordana musae*. The first report of *P. glaucopunctata* causing leaf spot and foliage necrosis on *Ruscus aculeatus* in Australia was reported by Golzar and Wang (2012).

Morphological based identification and diversity

Phaeosphaeriopsis species produce uni- or multi-loculate stromata, scattered, semi-immersed to erumpent, globose to subglobose, ostiolate ascomata, cylindrical to broadly fusoid or clavate asci and 4–5-septate, yellowish to light brown, verrucose ascospores. The asexual morph is coniothyrium-like or phaeostagonospora-like (Câmara et al., 2003; Quaadvlieg et al., 2013; Thambugala et al., 2014; Marin-Felix et al., 2001). Morphologically they are similar to *Paraphaeosphaeria* species but can be distinguished based on the different number of septa in ascospores (4–5-septate vs. 2-septate ascospores) and asexual morphs (Câmara et al., 2003). Only few species have both their sexual and asexual morphs described e.g. *P. beaucarneae* isolated from *Beaucarnea recurvata* (Tennakoon et al., 2020). For species such as *P. agapanthi*, *P. aloes*, *P. grevilleae*, *P. pseudoagavacearum*, *P. sansevieriae* and *P. triseptata*, only the asexual morph is known and for example in *P. aloicola* and *P. obtusispora* only the sexual morph is known.

The asexual morphs of *P. amblyspora* and *P. agavensis* produce hyaline, bacillar conidia from simple phialides. The asexual morphs of *P. glauco-punctata*, *P. obtusispora*, and *P. nolinae* produce brown conidia from percurrently proliferating conidiogenous cells. In *P. nolinae* the conidia are 3-septate and in *P. glauco-punctata* often 1-septate while those in *P. obtusispora* are usually non-septate (Câmara et al., 2003; Thambugala et al., 2014).

Thambugala et al. (2014) mentioned the unlikeliness of *P. phacidiomorpha* belonging to *Phaeosphaeriopsis* due to its different asci characters, hyaline ascospores and *Microsphaeropsis*-like asexual morphs. This requires further investigation. Thus, *Phaeosphaeriopsis* is a genus that requires more collections in order to establish its taxonomic boundaries, sexual-aseexual connections and diversity.

Molecular based identification and diversity

Thambugala et al. (2014) accepted seven species in *Phaeosphaeriopsis* based on multi-gene analysis of LSU, SSU, ITS and RPB2 regions. Here, *P. musae* was excluded. However, in our phylogenetic analysis the type strain of *P. musae* was included and resulted in grouping with other species of the genus. Marin-Felix et al. (201) accepted 15, including four new species in *Phaeosphaeriopsis* based on multi-gene analysis consisting of ITS, LSU, RPB2, TEF1 and TUB2 genes. Currently there are twenty species listed in Index Fungorum (2022).

Phaeosphaeriopsis pseudoagavacearum is closely related to *P. agavacearum* (Marin-Felix et al., 2001) and were both recorded from *Agave* sp. However, both *P. aloes* and *P. aloicola* which were isolated from *Aloe* sp. are phylogenetically distant, with *P. aloes* being more related to *P. obtusispora* and *P. aloicola* to *P. agapanthi*. This fact is mentioned in Marin-Felix et al. (2001) and similar results can be observed in our phylogenetic analysis presented as well. The sequence available as RPB2 for the type strain of *P. musae* (CPC 1123 = CBS 120026, GenBank Accession No. GU354) is incorrect and should be RPB1. All accession numbers and details of species utilized for the phylogenetic analysis are given.

Recommended genetic markers (generic level) – LSU

Recommended genetic markers (species level) – ITS, RPB2, TEF

Accepted number of species – There are 20 epithets listed in Index Fungorum (2022) and all except *P. vectis* have molecular data.

Number of species with pathogenicity data – one

References – Câmara et al. (2003), Thambugala et al. (2014), Marin-Felix et al. (2001; morphology, phylogeny and accepted species numbers), Golzar and Wang (2012; pathogenicity)

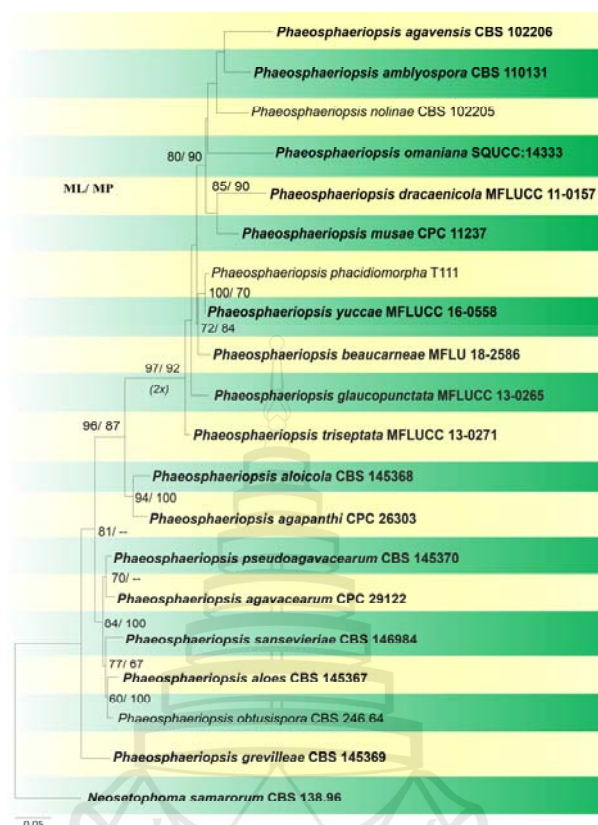


Figure G2 Maximum likelihood of *Phaeosphaeriopsis* species based on the concatenated ITS, LSU, RPB2 and TEF regions. The maximum parsimonious dataset consisted of 4 constant, 335 parsimony-informative and 123 parsimony-uninformative characters. The parsimony analysis of the data matrix resulted in the maximum of two equally most parsimonious trees with a length of 24 steps (CI = 0.25, RI = 0.00, RC = 0.42, HI = 0.15). ML and MP bootstrap support values over 60% are indicated. Type and epitype strains are in bold. Scale bar indicates number of substitutions per site. The tree was rooted to *Neosetophoma samarorum* CBS 13.6. Likelihood of the best scoring ML tree was -125.662246. Estimated base frequencies were as follows; A = 0.245606, C = 0.2343, G = 0.26123, T = 0.254233; substitution rates AC = 1.356002, AG = 3.20, AT = 1.2506, CG = 0.3613, CT = .31121, GT = 1.000000.

Table G2 GenBank accession numbers for accepted species of *Phaeosphaeriopsis*.

Ex-type/ex-epitype strains are in bold (N/A – sequences not available).

Species confirmed with pathogenicity studies are marked with #.

Species	Isolate	GenBank accession numbers			
		ITS	LSU	RPB2	TEF
<i>Neosetophoma samarorum</i>	CBS 13.6 ^{ET}	MH6256	MH415	KF25216	KF25311
<i>Phaeosphaeriopsis agapanthi</i>	CPC 26303 ^T	KX22260	KX22311	MK54004	MK54015
<i>P. agavacearum</i>	CPC 2122 ^T	KY13430	KY13520	KY1351	MK54015
<i>P. agavensis</i>	CBS 102206 ^T	KY00635	KY0066	KY0065	N/A
<i>P. aloes</i>	CBS 14536 ^T	MK535	MK540030	MK54000	MK540153
<i>P. aloicola</i>	CBS 14536 ^T	MK5360	MK540031	MK54001	MK540154
<i>P. amblyospora</i>	CBS 110131 ^T	MH6251	N/A	N/A	N/A
<i>P. beaucarnea</i>	MFLU 1-256 ^T	MT321	MT32113	N/A	MT3256
<i>P. dracaenicola</i>	MFLUCC 11-015 ^T	KM43423	KM43423	KM43430	KM434301
<i>P. glaucopunctata</i> [#]	MFLUCC 13-0265 ^{ET}	KJ52243	KJ5224	N/A	MG5201
<i>P. grevilleae</i>	CBS 14536 ^T	MK5361	MK540032	MK54002	MK540155
<i>P. musae</i>	CPC 1123 ^T	DQ54	GU30162	N/A	GU3403
<i>P. nolinae</i>	CBS 102205	KY0063	KY0066	KY0066	N/A
<i>P. obtusispora</i>	CBS 246.64	KY00644	JX6111	KY006	N/A
<i>P. omaniana</i>	SQUCC:14333 ^T	MT0540	MT054	N/A	N/A
<i>P. pseudoagavacearum</i>	CBS 14530 ^T	MK5362	MK540033	MK54003	MK540156
<i>P. sansevieriae</i>	CBS 1464 ^T	MZ06443	MZ06445	MZ0204	N/A
<i>P. triseptata</i>	MFLUCC 13-021 ^T	KJ52245	KJ5224	KJ52245	MG5201
<i>P. yuccae</i>	MFLUCC 16-055 ^T	KY55442	KY55441	N/A	MG52020
<i>P. phacidiomorpha</i>	T111	FJ46242	N/A	N/A	N/A

APPENDIX H

ONE STOP SHOP: BACKBONES TREES FOR IMPORTANT PHYTOPATHOGENIC GENERA – CERCOSPORA

Cercospora Fresen. ex Fuckel, Hedwigia 2(15): 133 (1863)

Cercospora includes pathogens, saprobes and endophytes. They are widely distributed, occurring on numerous flowering plants as well as grasses and other monocots, mostly causing leaf spots. The well-known asexual morph, which is hyphomycetous, are among the largest groups of plant pathogenic fungi causing leaf spots, leading to diseases on many economically important crops (Agrios, 2005; To-Anun et al., 2011; Groenewald et al., 2013; Guatimosim et al., 2016; Park et al., 2017). These include species like *Cercospora apii* (on celery) and *C. beticola* (on sugarbeet) (Braun et al., 2013; Guatimosim et al., 2016). Comparatively only a few sexual morphs have been studied (Hyde et al., 2013). One such pathogen *C. cf. sigesbeckiae*, is an ascomycete fungal pathogen known to infect various plant families, including economically valuable crops such as soybean causing ‘Cercospora leaf blight’ – a disease characterized by leaf bronzing (Albu et al., 2016, 2017). Some other species identified as causative organisms of the leaf blight are *C. kikuchii* and *C. cf. flagellaris* (Soares et al., 2015). The yield losses related to this disease have often been reported from Gulf South and other soybean-growing regions in the United States and South America (Alemida et al., 2005; Wrather et al., 2006; Cai et al., 2009; Hershman, 2009; Geisler et al., 2013; Albu et al., 2017). Other notable reports include *Cercospora* leaf spots on species of *Hibiscus* which are the most common and destructive of the *Hibiscus* diseases, often resulting in complete crop loss (Park et al., 2017) and more than 200 fungal species in association with various diseases of ‘kenaf’ worldwide (Park et al., 2017).

Classification— Dothideomycetes, Dothideomycetidae, Capnodiales,
Mycosphaerellaceae

Type species— ***Cercospora apii*** Fresen., Beitr. Mykol. 3: 91 (1863)

Distribution—Worldwide

Disease symptoms—Leaf blights, spots.

Hosts— Wide host range including plant genera in Amaranthaceae, Apiaceae, Asteraceae, Arecaceae, Chenopodiaceae, Convolvulaceae, Cryptogammaceae, Cucurbitaceae, Cyatheaceae, Dennstaedtiaceae, Dioscoreaceae, Euphorbiaceae, Fabaceae, Gunneraceae, Hydrangeaceae, Lamiaceae, Lygodiaceae, Musaceae, Myrtaceae, Onagraceae, Plumbaginaceae, Poaceae, Pteridaceae, Scrophulariaceae, Solanaceae, Thelypteridaceae and Urticaceae (Farr & Rossman, 2019).

Morphological based identification and diversity

Initially the name *Cercospora* widely applied to all kinds of dematiaceous hyphomycetous asexual morphs characterized by holoblastic conidiogenesis and even partly associated as having “*Mycosphaerella*”-like sexual morphs (Hyde et al., 2013; Groenewald et al., 2013). Later, *Cercospora* was confined to species similar to the type species, *C. penicillata*, characterized by pigmented conidiophores, thickened and darkened conidiogenous loci and colourless conidia formed singly (Ellis, 1971, 1976). Chupp (1954) published a worldwide monograph of this group which listed 1,419 species. A vast number of studies related to *Cercospora* have been based on morphology or confined to specific regions or hosts (Phengsintham et al., 2013a, b). Hence, more than 3,000 species of *Cercospora* were described (Pollack, 1987), often led by the fact that they were host-specific at a genus or family level of the host plants (Crous & Braun, 2003; Groenewald et al., 2005). However, based on morphological features of the structure of conidiogenous loci and hila, absence or presence of pigmentation in conidiophores and conidia, Crous and Braun (2003) revised the generic circumscription of *Cercospora*, resulting in the reduction of the number of species to 659. Braun et al. (2013) did a series of publications related to *Cercospora* and its allied genera in *Mycosphaerellaceae*, along with the illustration and description of sexual morphs where possible (Braun et al., 2014).

Molecular based identification and diversity

Cercospora is known to be monophyletic (Stewart et al., 1999; Hyde et al., 2013). Groenewald et al. (2013) provided a comprehensive phylogenetic analysis of

360 isolates of the genus which included protein-coding genes, such as translation elongation factor 1-alpha (EF-1 α), actin (ACT), calmodulin (CAL), and histone 3 (HIS). This provided a solid basis for the identification of *Cercospora* species, indicating most of them as host-specific (Park et al., 2017). The genomes for several species – *Cercospora arachidicola*, *C. aff. canescens*, *C. cf. sigesbeckiae*, *C. sojina* and *C. zea-maydis* have been published, out of which *C. cf. sigesbeckiae* and *C. sojina* are important soybean pathogens (Albu et al., 2017). The mating type genes of some apparently asexual *Cercospora* species have been characterised (Groenewald et al., 2013), of which *C. beticola*, *C. zea-maydis* and *C. zeina* have been discovered to be heterothallic, while only one mating type was discovered to be present in populations of *C. apii* and *C. apiicola* (Groenewald et al., 2006, 2010). *Cercospora* still lacks an ideal DNA barcode useful for distinguishing between its closely related species (Groenewald et al., 2013; Albu et al., 2017).

Recommended genetic markers (genus level)—LSU, ITS

Recommended genetic markers (species level)—ITS, ACT, CAL, HIS, *tefl*

Accepted number of species: There are over 3100 species epithets in Index Fungorum (2020) under this genus. However, only 134 have DNA sequence data in GenBank.

References: Groenewald et al. (2013), Albu et al. (2017; morphology, phylogeny), Guatimosim et al. (2016; morphology, phylogeny)

Table H1 Gene regions and primers

Genus	Gene regions	Primers		Reference
		Forward	Reverse	
<i>Cercospora</i>	ITS	ITS5	ITS4	White et al. (1990)
	LSU	LROR	LR5	Vilgalys and Hester (1990)
	ACT	ACT-512F	ACT-783 R	Carbone and Kohn (1999)
	TEF1	EF-728F	986R	Carbone and Kohn (1999)
		EF2Fd		Groenewald et al. 2013
	cmdA		EF-2	O'Donnell et al. 1998
		CAL-228F	CAL-737R	Carbone and Kohn (1999)
	H3	H3-1a	H3-1b	Glass & Donaldson (1995)

Table H2 DNA barcodes for accepted species of *Cercospora*. Ex-type/ex-epitype/ex-neotype/ex-lectotype strains and voucher strains are in bold. Species confirmed with pathogenicity studies are marked with #.

Species	Isolate no	GenBank accession numbers							
		ITS	<i>tef1</i>	ACT	CAL	HIS	<i>tub2</i>	RPB2	GAPDH
<i>Cercospora achyranthis</i>	CBS 132613	JX1435 23	JX1432 77	JX1430 31	JX1427 85	JX1425 39	—	—	—
	CPC 10091	JX1435 24	JX1432 78	JX1430 32	JX1427 86	JX1425 40	—	—	—
<i>C. agavicola</i>	CBS 117292	AY647 237	AY966 897	AY966 898	AY966 899	AY966 900	—	—	—
<i>C. alchemillicola</i>	CPC 5259	JX1435 25	JX1432 79	JX1430 33	JX1427 87	JX1425 41	—	—	—
<i>C. althaeina</i>	CBS 248.67	JX1435 30	JX1432 84	JX1430 38	JX1427 92	JX1425 46	MH4963 40	—	MH4961 70
<i>C. apii</i>	CBS 116455	AY840 519	AY840 486	AY840 450	AY840 417	AY840 384	MH4963 43	—	MH4961 73
<i>C. apiicola</i>	CBS 116457	AY840 536	AY840 503	AY840 467	AY840 434	AY840 401	—	—	—
<i>C. armoraciae</i>	CBS 250.67	JX1435 45	JX1432 99	JX1430 53	JX1428 07	JX1425 61	MH4963 51	—	MH4961 81

Table H2 (continued)

Species	Isolate no	GenBank accession numbers							
		ITS	<i>tefl</i>	ACT	CAL	HIS	<i>tub2</i>	RPB2	GAPDH
<i>C. beticola</i>	CBS 116456	AY840 527	AY840 494	AY840 458	AY840 425	AY840 392	MH4963 55	KT21 6555	MH4961 85
<i>C. bizzozzeria</i>	CBS 258.67	JX1435 46	JX1433 00	JX1430 54	JX1428 08	JX1425 62	MH4963 68	—	MH4961 98
<i>C. cf. brunkii</i>	CBS 132657	JX1435 59	JX1433 13	JX1430 67	JX1428 21	JX1425 75	—	—	—
<i>C. campisilii</i>	CBS 132625	JX1435 61	JX1433 15	JX1430 69	JX1428 23	JX1425 77	—	—	—
<i>C. canescens</i>	CPC 15871	JX1435 67	JX1433 21	JX1430 75	JX1428 29	JX1425 83	—	—	—
	CBS 111133	AY260 065	DQ835 084	DQ835 103	DQ835 130	DQ835 157	—	—	—
<i>C. capsici</i>	CBS 132622	JX1435 68	JX1433 23	JX1430 77	JX1428 31	JX1425 85	—	—	—
<i>C. celosiae</i>	CBS 132600	JX1435 70	JX1433 26	JX1430 80	JX1428 34	—	—	—	—
<i>C. cf. chenopodii</i>	CBS 132594	JX1435 72	JX1433 28	JX1430 82	JX1428 36	JX1425 90	—	—	—
	CCTU 1060	KJ8864 38	KJ8862 77	KJ8859 55	KJ8857 94	KJ8861 16	MH4963 71	MH51 1862	MH4962 01
<i>C. chinensis</i>	CBS 132612	JX1435 78	JX1433 34	JX1430 88	JX1428 42	JX1425 96	—	—	—
<i>C. cf. citrulina</i>	CBS 119395	EU514 222	JX1433 35	JX1430 89	JX1428 43	—	—	—	—
	MUCC 576	JX1435 79	JX1433 37	JX1430 91	JX1428 45	—	—	—	—
<i>C. coniogrammes</i>	CBS 132634	JX1435 83	JX1433 41	JX1430 95	JX1428 49	JX1426 03	—	—	—
<i>C. convolvulicola</i>	CCTU 1083	KJ8864 41	KJ8862 80	KJ8859 58	KJ8857 97	KJ8861 19	MH4963 74	MH51 1865	MH4962 04
<i>C. conyzae-canadensis</i>	CCTU 1119	KJ8864 45	KJ8862 84	KJ8859 62	KJ8858 01	KJ8861 23	MH4963 77	MH51 1868	MH4962 07
<i>C. corchori</i>	MUCC 585	JX1435 84	JX1433 42	JX1430 96	JX1428 50	JX1426 04	—	—	—
<i>C. cf. coreopsidis</i>	CBS 132598	JX1435 85	JX1433 43	JX1430 97	JX1428 51	JX1426 05	—	—	—
<i>C. cylindracea</i>	CCTU 1081	KJ8864 49	KJ8862 88	KJ8859 66	KJ8858 05	KJ8861 27	MH4963 81	MH51 1872	MH4962 11
<i>C. delaireae</i>	CBS 13259	JX1435 87	JX1433 45	JX1430 99	JX1428 53	JX1426 07	—	—	—

Table H2 (continued)

Species	Isolate no	GenBank accession numbers							
		ITS	<i>tef1</i>	ACT	CAL	HIS	<i>tub2</i>	RPB2	GAPDH
<i>C. dianellicola</i>	CBS 143453	MG386 075	—	MG674 152	MG674 153	—	—	—	—
<i>C. dispori</i>	CBS 13260	JX1435 91	JX1433 49	JX1431 03	JX1428 57	JX1426 11	—	—	—
<i>C. cf. erysimi</i>	CBS 115059	JX1435 92	JX1433 50	JX1433 50	JX1428 58	JX1426 12	—	—	—
<i>C. euphorbia esieboldiana</i>	CBS 113306	JX1435 93	JX1433 51	JX1431 05	JX1428 59	JX1426 13	—	—	—
<i>C. fagopyri</i>	CBS 132623	JX1435 94	JX1433 52	JX1431 06	JX1428 60	JX1426 14	—	—	—
<i>C. cf. nicotianae</i>	CBS 131.32	DQ835 073	DQ835 099	DQ835 119	DQ835 146	—	—	—	—
	CBS 132632	JX1436 31	JX1436 31	JX1431 44	JX1428 98	—	—	—	—
<i>C. cf. flagellaris</i>	CCTU 1159	KJ8864 93	KJ8863 32	KJ8860 10	KJ8858 49	KJ8861 71	MH4963 88	MH51 1879	MH4962 18
	CBS 132653	JX1436 03	JX1433 61	JX1431 15	JX1428 69	JX1426 23	MH4963 90	MH51 1881	MH4962 20
	CCTU 1204	KJ8865 05	KJ8863 44	KJ8860 22	KJ8858 61	KJ8861 83	MH4963 99	MH51 1890	MH4962 29
	CBS 132667	JX1436 04	JX1433 62	JX1431 16	JX1428 70	JX1426 24	MH4964 01	MH51 1892	MH4962 31
	CCTU 1172	KJ8865 01	KJ8863 40	KJ8860 18	KJ8858 57	KJ8861 79	MH4964 09	MH51 1900	MH4962 39
	CCTU 1154	KJ8864 89	KJ8863 28	KJ8860 06	KJ8858 45	KJ8861 67	MH4964 10	MH51 1901	MH4962 40
<i>C. gamsiana</i>	CCTU 1074*	KJ8864 26	KJ8862 65	KJ8859 43	KJ8857 82	KJ8861 04	MH4964 46	MH51 1937	MH4962 76
<i>cf. gossypii</i>	CCTU 1070	KJ8864 67	KJ8863 06	KJ8859 84	KJ8858 23	KJ8861 45	MH4964 52	MH51 1943	MH4962 82
<i>C. cf. helianthicola</i>	MUCC 716	JX1436 15	JX1433 74	JX1431 28	JX1428 82	JX1426 36	—	—	—
<i>C. cf. ipomoeae</i>	CBS 132639	JX1436 16	JX1433 75	JX1431 29	JX1428 83	JX1426 37	—	—	—
<i>C. iranica</i>	CCTU 1137	KJ8865 13	KJ8863 52	KJ8860 30	KJ8858 69	KJ8861 91	MH4964 55	MH51 1946	MH4962 85
<i>C. kikuchii</i>	CBS 128.27	DQ835 070	DQ835 088	DQ835 107	DQ835 134	DQ835 161	—	—	—
<i>C. lactucae-sativae</i>	CBS 132604	JX1436 21	JX1433 80	JX1431 34	JX1428 88	JX1426 42	—	—	—

Table H2 (continued)

Species	Isolate no	GenBank accession numbers							
		ITS	<i>tef1</i>	ACT	CAL	HIS	<i>tub2</i>	RPB2	GAPDH
<i>C. malayensis</i>	KUS-F27687	KR400 012	KY082 663	KY082 664	KY082 665	KY082 666	—	—	—
<i>C. cf. malloti</i>	MUCC 575	JX1436 25	JX1433 84	JX1431 38	JX1428 92	JX1426 46	—	—	—
<i>C. mercurialis</i>	CBS 550.71	JX1436 28	JX1436 28	JX1431 41	JX1428 95	JX1426 49	—	—	—
<i>C. cf. modiolae</i>	CPC 5115	JX1436 30	JX1433 89	JX1431 43	JXJX14 2897	JX1426 51	—	—	—
<i>C. cf. nicotianae</i>	CBS 131.32	DQ835 073	DQ835 099	DQ835 119	DQ835 146	DQ835 173	—	—	—
	CBS 132632	JX1436 31	JX1433 90	JX1431 44	JX1428 98	JX1426 52	—	—	—
<i>C. olivascens</i>	CBS 253.67	JX1436 32	JX1433 91	JX1431 45	JX1428 99	JX1426 53	—	—	—
<i>C. cf. physalidis</i>	CBS 765.79	JX1436 33	JX1433 92	JX1431 46	JX1429 00	—	—	—	—
<i>C. pileicola</i>	CBS 132607	JX1436 34	JX1433 93	JX1431 47	JX1429 01	JX1426 55	—	—	—
<i>C. plantagininis</i>	CBS 252.67	DQ233 318	DQ233 342	DQ233 368	DQ233 394	DQ233 420	MH4964 61	—	MH4962 91
<i>C. polygonacea</i>	CBS 132614	JX1436 37	JX1433 96	JX1431 50	JX1429 04	JX1426 58	—	—	—
<i>C. pseudoche nopodii</i>	CBS 136022	KJ8865 16	KJ8863 55	KJ8860 33	KJ8858 72	KJ8861 94	MH4964 64	MH51 1954	MH4962 94
<i>C. punctiformis</i>	CBS 132626	JX1436 38	JX1433 97	JX1431 51	JX1429 05	JX1426 59	—	—	—
<i>C. cf. resedae</i>	CBS 118793	JX1436 39	JX1433 98	JX1431 52	JX1429 06	JX1426 60	—	—	—
<i>C. cf. richardiicola</i>	CBS 132627	JX1436 40	JX1433 99	JX1431 53	JX1429 07	JX1426 61	MH4964 65	MH51 1955	MH4962 95
<i>C. ricinella</i>	CBS 132605	JX1436 46	JX1434 05	JX1431 59	JX1429 13	JX1426 67	—	—	—
<i>C. rodmanii</i>	CBS 113123	DQ835 076	AF1461 36	DQ835 122	DQ835 149	DQ835 176	—	—	—
<i>C. rumicis</i>	CCTU 1123	KJ8865 21	KJ8863 60	KJ8860 38	KJ8858 77	KJ8861 99	MH4964 66	MH51 1956	MH4962 96
<i>C. rumicis</i>	CPC 5439	JX1436 48	JX1434 07	JX1431 61	JX1429 15	JX1426 69	—	—	—
<i>C. samambai</i>	CPC 24673	KT037 514	KT037 474	KT037 596	KT037 463	KT037 555	—	—	—
<i>C. senecionis walkeri</i>	CBS 132636	JX1436 49	JX1434 08	JX1431 62	JX1429 16	JX1426 70	—	—	—

Table H2 (continued)

Species	Isolate no	GenBank accession numbers							
		ITS	<i>tef1</i>	ACT	CAL	HIS	<i>tub2</i>	RPB2	GAPDH
<i>C. cf. sigesbecki</i>	CBS 132675	JX1436 55	JX1434 14	JX1431 68	JX1429 22	JX1426 76	—	—	—
<i>C. sojina</i>	CBS 132615	JX1436 59	JX1434 19	JX1431 73	JX1429 27	JX1426 81	—	—	—
<i>C. solani</i>	CCTU 1043	KJ8865 23	KJ8863 62	KJ8860 40	KJ8858 79	KJ8862 01	MH4964 69	MH51 1959	MH4962 99
<i>C. sorghicola</i>	CCTU 1173	KJ8865 25	KJ8863 64	KJ8860 42	KJ8858 81	KJ8862 03	MH4964 71	MH51 1961	MH4963 01
<i>Cercospora</i>	CBS	AY752	AY752	AY752	AY752	AY752	—	—	—
<i>a sp. P</i>	116365	141	176	204	235	266	—	—	—
<i>Cercospora</i>	JZG-2013	JX1437 14	JX1434 73	JX1432 27	JX1429 81	JX1427 35	—	—	—
<i>a sp. P</i>	CCTU 1197	KJ8865 40	KJ8863 79	KJ8860 57	KJ8858 96	KJ8862 18	MH4964 72	MH51 1962	MH4963 02
<i>a sp. G</i>	CCTU 1015	KJ8865 28	KJ8863 67	KJ8860 45	KJ8858 84	KJ8862 06	MH4964 73	MH51 1963	MH4963 03
<i>a sp. G</i>	CCTU 1090	KJ8865 36	KJ8863 75	KJ8860 53	KJ8858 92	KJ8862 14	MH4964 76	MH51 1965	MH4963 06
<i>a sp. G</i>	CBS 115518	JX1436 81	JX1434 41	JX1431 95	JX1429 49	JX1427 03	MH4964 80	—	MH4963 10
<i>a sp. T</i>	CCTU 1148	KJ8865 41	KJ8863 80	KJ8860 58	KJ8858 97	KJ8862 19	MH4964 88	MH51 1976	MH4963 18
<i>C. uwebaruniana</i>	CCTU 1200	KJ8864 08	KJ8862 47	KJ8859 25	KJ8857 64	KJ8860 86	MH4964 89	MH51 1977	MH4963 19
<i>C. vignigena</i>	CBS 132611	JX1437 34	JX1434 93	JX1432 47	JX1430 01	JX1427 55	—	—	—
<i>C. violae</i>	CBS 251.67	JX1437 37	JX1434 96	JX1432 50	JX1430 04	JX1427 58	MH4964 92	—	MH4963 22
<i>C. zeae-maydis</i>	CBS 117757	DQ185 074	DQ185 086	DQ185 098	DQ185 110	DQ185 122	—	—	—
<i>C. zebrina</i>	CCTU 1225	KJ8865 50	KJ8863 89	KJ8860 67	KJ8859 06	KJ8862 28	MH4964 95	MH51 1981	MH4963 25
	CCTU 1239	KJ8865 51	KJ8863 90	KJ8860 68	KJ8859 07	KJ8862 29	MH4965 04	MH51 1987	MH4963 34
<i>C. zeina</i>	CBS 118820	DQ185 081	DQ185 093	DQ185 105	DQ185 117	DQ185 129	—	—	—
<i>C. cf. zinniae</i>	CBS 132624	JX1437 56	JX1435 18	JX1432 72	JX1430 26	—	—	—	—
	CCTU 1003	KJ8865 52	KJ8863 91	KJ8860 69	KJ8859 08	KJ8862 30	MH4965 05	MH51 1988	MH4963 35

Figure H1 The most parsimonious tree generated by MP analysis of combined ITS, *tef1*, *act*, *cal*, *his*, *tub2*, *rpb2* and *gapdh* sequence data of *Cercospora* species is presented. Related sequences were obtained from previous publications and GenBank. Eighty-eight strains are included in the analysis comprising 4242 characters including gaps, of which 3044 characters are constant, 464 characters are parsimony-uninformative and 734 are parsimony-informative. The parsimony analysis of the data matrix resulted in the maximum of 84 equally most parsimonious trees with a length of 2886 steps (CI = 0.556, RI=0.700, RC = 0.389, HI = 0.444) in the first tree. The tree was rooted with *Septoria provencialis* (CBS 118910). Tree topology of the MP analysis was similar to the ML and BYPP analyses. ML and MP bootstrap support values $\geq 70\%$ and BYPP ≥ 0.95 (ML/ MP/ BYPP) are shown respectively near the nodes. Ex-type strains are in bold.

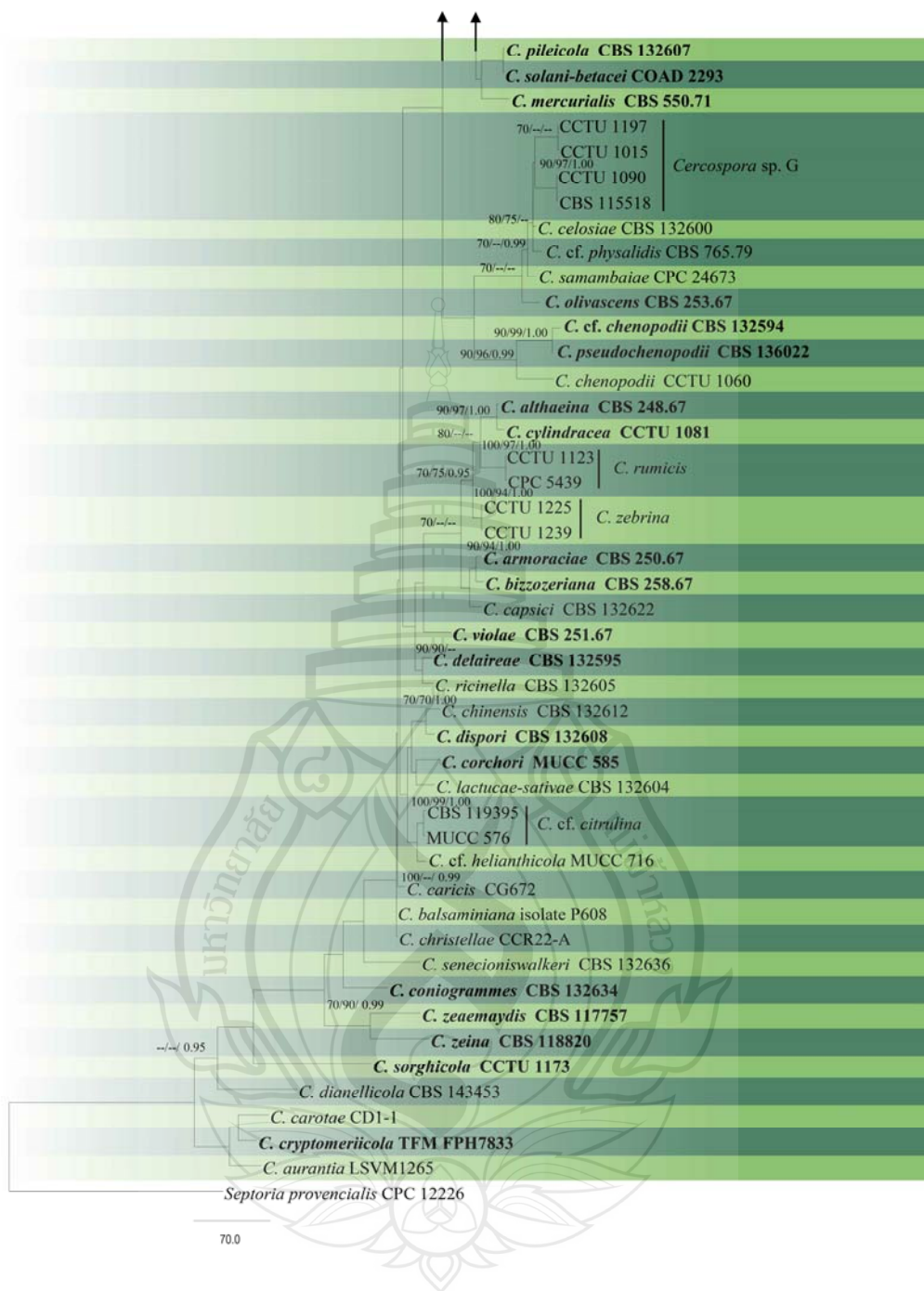


Figure H1 (continued)

APPENDIX I

CONTRIBUTION TO INTRODUCING A NEW PLEOSPORALEAN FAMILY SUBLOPHIOSTOMATACEAE FAM NOV. TO ACCOMMODATE SUBLOPHIOSTOMA GEN NOV.

Introduction

Pleosporales is the largest order of *Dothideomycetes* (Kirk et al., 2008; Schoch et al., 2009; Zhang et al., 2012; Hyde et al., 2013; Wijayawardene et al., 2014; Li et al., 2016). However, the order was invalidly introduced by Luttrell (1955) and subsequently validated by Barr (1987a), based on the family Pleosporaceae and its type species *Pleospora herbarum* (Pers.) Rabenh. (Barr 1987b). The classification of these families was mainly based on morphological characters, along with some support from molecular data (Schoch et al., 2006, 2009; Hyde et al., 2013; Wijayawardene et al., 2014; Li et al., 2016). Wijayawardene et al. (2014) listed 39 families under *Pleosporales*, and 48 as *Pleosporales* genera *incertae sedis*. The order *Pleosporales* consists of two suborders – viz. *Massarineae* and *Pleosporineae*. The suborder *Massarineae* currently comprises 12 families (Tanaka et al., 2015; Li et al., 2016) and *Pleosporineae*, 10 families (Ariyawansa et al., 2015).

Species of *Pleosporales* are highly diverse, occurring as saprobes on dead plant materials in both aquatic and terrestrial habitats, while a large proportion, especially the asexual morphs, inhabit living plants as pathogens (Kruys et al., 2009; Zhang et al., 2009b, 2012; Manamgoda et al., 2011; Boonmee et al., 2012; Hyde et al., 2013; Woudenberg et al., 2013). Therefore it is necessary to collect additional taxa from diverse localities and isolate them, extract DNA to resolve taxonomic placements and phylogenies linked with morphology. In this paper we introduce a

sexual genus *Paralophiostoma* (*Pleosporales*, genera *incertae sedis*), based on its type *Paralophiostoma thailandicum*, complete with illustrations and descriptions.

Taxonomy

Paralophiostoma thailandicum Phookamsak, Hongsanan, Goonas. & K.D. Hyde, *sp. nov.*

Index Fungorum Number: IF557973; *Facesoffungi* number: FoF 09403

Etymology:—The specific epithet “*thailandicum*” refers to the country from which the holotype was collected

Holotype:—MFLU 11-0158

Saprobic on bamboo or grass. **Sexual morph:** *Ascomata* 450–600 µm high, 135–185 µm diam. ($\bar{x} = 505 \times 160$ µm, $n = 10$), solitary to gregarious, scattered, immersed to semi-immersed or erumpent, visible as dark ellipsoidal spots on the host surface, uni-loculate, coriaceous, black, subglobose, hemisphaerical to lenticular, glabrous, ostiole central, with crest-like opening. *Peridium* 13–30 µm wide ($\bar{x} = 21.7$ µm, $n = 30$), thick at the upper lateral part and thinner at the base, composed of several layers comprising of outer thick, dark brown layers of cells of *textura angularis* and inner hyaline cells of *textura angularis* to *textura epidermoidea*. *Hamathecium* comprising 0.6–1.5 µm wide, dense, septate, branched cellular pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 85–105 × 8.5–10.5 µm ($\bar{x} = 95 \times 9$, $n = 30$), 8-spored, bitunicate, fissitunicate, cylindric-clavate, rounded at the apex, with a small bulbous pedicel. *Ascospores* 20.4–25.5 × 3.5–5.6 µm ($\bar{x} = 23.7 \times 4.5$ µm, $n = 30$), overlapping uni to bi-seriate, hyaline, fusiform, 1-septate, constricted at the septum, each cell swollen near the septum, becoming acute at the ends, having the same number of guttules, smooth-walled, surrounded by a mucilaginous sheath. **Asexual morph:** Ceolomycetous, produced on bamboo pieces on WA after 8 weeks, appearing as black, punctiform, globose structures, covered by grey to dark grey vegetative mycelium. *Conidiomata* solitary or aggregated, pycnidial, obpyriform. *Conidiomata* wall 5.8–8 µm wide, comprising of outer layer of brown cells of *textura globulosa* to *textura angularis* and inner layer of hyaline cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 1.8–4.2 µm long,

hyaline, integrated. *Conidia* $5.3 \times 3.2 \mu\text{m}$, hyaline, aseptate, smooth-walled, subglobose to oblong.

Material examined:—THAILAND, Chiang Mai Prov., Chom Tong District, Doi Inthanon, on dead stem of grass, 16 November 2010, R. Phookamsak, RP0038 (MFLU 11-0158, **holotype**), ex-type living culture MFLUCC 11-0207, KUMCC, *ibid.* on dead branch of bamboo, 6 October 2010, R. Phookamsak, RP0081 (MFLU 11-0201), living culture MFLUCC 11-0165; on dead stem of *Thysanolaena maxima* Kuntze, 6 October 2010, R. Phookamsak, RP0088 (MFLU 11-0208), living culture MFLUCC 11-0172; on dead stem of *Thysanolaena maxima*, 16 November 2010, R. Phookamsak, RP0090 (MFLU 11-0210), living culture MFLUCC 11-0174; on dead stem of *Thysanolaena maxima*, 16 October 2010, R. Phookamsak, RP0101 (MFLU 11-0221), living culture MFLUCC 11-0185; THAILAND, Chiang Rai Prov., Muang District, Khun Korn Waterfall, on dead stem of *Thysanolaena maxima*, 21 June 2011, R. Phookamsak, RP0128 (MFLU 11-0245), living culture MFLUCC 12-0006.

Notes:—*Paralophiostoma thailandicum* closely resembles *Lophiostoma macrostomum*, *L. semiliberum*, *Lophiopoacea paramacrostoma*, *L. winteri*, *Pseudolophiostoma vitigenum* and *Vaginatispora fuckelii* in Lophiostomataceae in having globose to subglobose ascomata, with slit like openings, a peridium comprising of brown to hyaline cells of *textura angularis*, cylindric-clavate asci and smooth-walled, mostly 1-septate, hyaline, guttulate ascospores. Yet these species differentiate in their ascomata, peridium and ascospore dimensions.

In addition, *Lophiostoma macrostomum* have 3–5-euseptate ascospores with terminal appendages (Thambugala et al., 2015). In *L. semiliberum* the peridium consists of lightly pigmented to reddish brown cells, while in *L. paramacrostoma* and *Vaginatispora fuckelii*, it is composed of two strata. The ascospores of both *L. semiliberum* and *L. paramacrostoma* lack a sheath. The ascospores of *Vaginatispora fuckelii* have globose appendages at both ends (Thambugala et al., 2015). The asexual morph of *L. semiliberum* is coelomycetous but is different in having septate, branched, hyaline conidiophores and ellipsoid to obovoid, hyaline conidia (Tanaka & Harada, 2003; Thambugala et al., 2015). *Lophiopoacea winteri* and *Pseudolophiostoma vitigenum* produce 5-septate and 2-euseptate ascospores

respectively, which have thin sheaths, drawn out at the ends (Thambugala et al., 2015).



Figure I1 *Paralophiostoma thailandicum* (MFLU11-0158, holotype). a. Appearance of ascomata on host. b. Section of ascoma. c. Peridium. d. Pseudoparaphyses. e–i. Asci. j. Appearance of mucilaginous sheath surrounding young ascospore (stained in Indian ink). k–r. Ascospores. s. Conidiomata on bamboo pieces on WA after eight weeks. t. Section of conidioma. u. Conidioma wall. v, w. Conidiogenous cells stained in congo red. x, y1–y6. Conidia. Scale bars: a, s = not to scale; b = 100 μ m; c, d, t = 20 μ m; e–o = 10 μ m; p–r = 5 μ m; t, v, w, y1–y6 = 5 μ m; u, x = 25 μ m.

APPENDIX J

CONTRIBUTION TO MORPHOLOGICAL APPROACHES IN STUDYING FUNGI

Preparing fungaria specimens

Fungarium specimens are a vital source of information of all discovered fungal species. They are very important in all aspects of fungal systematics as well as an important historical record or archive. Not only do they provide details of morphology pertaining to a fungus but also gives us an idea with regards to its diversity, host and geographical distribution, especially in the case of plant pathogens. Proper preparation, storage and handling of fungal specimens will ensure that they remain in good condition for use by future researchers.

Herbarium specimens provide a permanent, verifiable record of the occurrence of a taxon at a particular time and place. Therefore, they provide the most reliable means of documenting our plant and fungal biodiversity. The specimens designated as types are the basic raw materials used in identification and classification of the species both unknown and known. If they are dried culture herbarium, they can be used as evidence to link between their sexual or asexual morphs, especially in the absence of molecular evidence. Well prepared herbarium could even be utilized to obtain molecular data if the fungus is unable to grow on artificial media. Any books or papers published about fungi should always be backed up by vouchered specimens. These specimens should be given a herbarium code which will enable the tracking of the specimen in available online databases. There will be arguments about names of specimens, so the best practice is to have your printed information backed by a specimen lodged in a Herbarium.

Rules and recommendations

Handling guidelines

Careful handling of specimens will minimize the risk of physical damage to them:

- Specimens should always be kept horizontal and flat and must never be bent; hold both sides of the sheet when handling specimens; when carrying specimens, place them on a sheet of cardboard.
- Never shuffle specimens as the edges of sheets may cut underlying specimens.
- Look through specimens by stacking and unstacking each specimen individually with the plant facing up. Do not page through specimens like pages in a book, or stack specimens with the plant facing downwards.
- Never rest an object on a specimen.
- Specimens can be damaged by sunlight, dust, wind, and moisture, and should always be protected when not in use. If specimens are left out of a cupboard, they must be covered with a cardboard sheet.
- When storing specimens, do not pack them tightly onto one shelf; do not overfill genus and species covers.
- When replacing specimens in the cupboard, make sure the sheets are all aligned, as protruding edges may be damaged.

(Victor, J.E. Herbarium essentials: the Southern African Herbarium user manual Pretoria : Southern African Botanical Diversity Network, 2004, p41)

Condition surveying:

Older herbarium specimens may have been stored previously in poor conditions or been damaged from careless handling. In order to identify the types of damage to specimens in the RBGE Herbarium collection, we carried out a condition survey during imaging and databasing of mounted herbarium specimens. This allowed us to prioritize specimens for repair, and estimate the cost in terms of staff time and materials. The most common problems are:

- Non-archival materials eg cellophane, sellotape, many types of polythene
- Surface dirt eg soot, dust
- Stains eg from pesticides
- Paper problems eg flimsy, distorted or torn sheets

- Loose attachments eg loose stapes or stitches; dried out glue
- Insect damage

From fresh material

Fresh specimens, if moist should be dried well before being deposited as herbarium specimens. The packaging will depend on the size of the specimen but should ideally follow the rules and regulations of the Herbarium you wish to deposit. Flat specimens like leaves, can be stored in envelopes, while specimens such as seeds, stems, twigs or mushrooms can be stored in boxes.

Woody specimens (bark, branches, stems or twigs) and lichens

- usually air dried, using a 60 or 100 watt desk lamp. If material is thick oven dry at 28-30 °C

Fruits, cones, and seeds

- if a pathogen on a fruit, the diseased part (lesion) can be cut and air dried at room temperature
- for cones and seeds, the parts with the fungus can be cut out separately and dried as in woody specimens, while small seeds can be dried in whole

Leaf and inflorescence

- they should be kept pressed between papers, ideally in a drying press. The drying press can be kept under the sun or in a place facing hot air

Mushroom specimens

- using a dryer machine/ desiccator at 27-30 °C

Herbarium slides:

- permanent slides containing various morphological features of the fungus (section of fruiting body, asci, spores etc.) can be included with the herbarium specimen
- these are very useful in case the specimen bears a limited amount of the fungus or have rare characteristics

From cultures

There are several published studies on how to dry culture specimens for fungal herbarium. One technique is drying your cultures in the lid of the petri dishes you used to grow your isolate. To do this, cultures for drying are grown in water agar with 2.5% glycerol. When doing this drying method, you need to use plastic Petri dishes as

the cultures tend to stick to the glass dishes. Then, you allowed the culture to dry up in a dessicator with silica crystals for 3-5 days. The dried culture plates, a flat circular media with the fungus, can be placed in an archival-paper envelope and can be deposited, along with the herbarium package, and preserve in fungarium collection.

